Aquaculture

Farming Aquatic Animals and Plants

Second edition

Edited by

John S. Lucas

Adjunct Professor in the Centre for Marine Studies
School of Biological Sciences
University of Queensland
Brisbane, Australia

Paul C. Southgate

Professor of Aquaculture
School of Marine & Tropical Biology
James Cook University
Townsville, Australia

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Preface to the Second Edition

The rapid growth of aquaculture continues at a faster rate than predicted a decade ago. Total global production by 2007 had increased by two-thirds over the production reported in the first edition of this book. This has been possible because of new technical developments, rapid expansion of some new and existing industries, and diversification in the species utilised by aquaculture. These exciting developments provide the basis for this second edition, which includes a major revision of production statistics and chapter contents, seven new chapters and a more diverse international authorship and coverage. There are contributors from 12 countries, and aquaculture in many more countries is considered. With the increasing importance of China as the major source of aquaculture products, there is greater consideration of aquaculture in that country. There are three new Chinese authors contributing to this edition. Sadly, Professor C. K. Tseng, who contributed Macroalgae in the first edition, is now deceased. He is considered to be the 'father of Chinese mariculture' for his great achievements in marine science and outstanding leadership in that country over many years.

We express our sincere gratitude to the authors for their commitment in contributing chapters and, in some cases, for their understanding and patience. We also express our gratitude to our wives, Helen and Dawn, for their contributions and support. We trust that you will find this new edition both helpful and stimulating.

John S. Lucas
Paul C. Southgate
June 2011

Preface to the First Edition

This textbook seeks to convey to its readers the contributors' enthusiasm for aquaculture and their accumulated knowledge. The contributors are recognised internationally in their fields. While it is not possible to comprehensively cover the ranges of aquaculture theory, practices and cultured organisms in one textbook, it is our earnest hope that this text will give readers a broad understanding of these topics.

The first part of the text introduces aquaculture with a series of 'theory and practice' topics, ranging from traditional topics such as ponds and pumps to contemporary environmental issues, nutrition physiology and genetic engineering. The second part of the text consists of chapters dealing with specific organisms, or groups of organisms, which illustrate the variety of culture methods used in aquaculture. It also provides examples of biological and other factors that make these organisms suitable for culture. The aquatic animals and plants treated in the text are but a small proportion of the hundreds of commercially cultured species; however, they constitute the most significant commercial components of world aquaculture production. They include the four major groups of cultured organisms – fish, crustaceans, bivalve molluscs and seaweeds; the three broad categories of aquatic environments – fresh, brackish and seawater; and the broad latitudinal zones – temperate, subtropical and tropical regions.

We express our sincere gratitude to the authors for their commitment in contributing chapters and, in some cases, for their understanding. Mr Michael New, President, European Aquaculture Society, Past-President, World Aquaculture Society, kindly assisted by reviewing Chapters 1 and 23. We also wish to express our gratitude to our wives, Helen and Dawn, for their substantial contributions.

John Lucas
Paul Southgate
April 2003

List of Contributors

Dr Peter Appleford
Department of Sustainability and Environment
Victoria
Australia

Dr Nasser Ayaril
Research and Development
National Prawn Company
Kingdom of Saudi Arabia

Associate Professor Andrew Barnes
Aquatic Animal Health
University of Queensland
Australia

Dr Ram C. Bhujel
Aquaculture and Aquatic Resources Management
Asian Institute of Technology
Thailand

Professor Michael Borowitzka
Biological Science and Biotechnology
Murdoch University
Australia

Professor Claude Boyd
Department of Fisheries and Allied Aquacultures
Auburn University
USA

Dr Allan Bremner
Allan Bremner and Associates
Coolum Beach
Australia

Dr Tomás Cabrera
Instituto de Investigaciones Científicas
Universidad de Oriente
Venezuela

Dr Laura Castell
School of Marine and Tropical Biology
James Cook University
Australia

Professor Yongxu Cheng
Key Laboratory of Aquatic Genetic Resources and Ecology
Shanghai Ocean University
P. R. China

Dr Simon Cripps
Dorset Wildlife Trust
Dorchester
UK

Professor Sena De Silva
Network of Aquaculture Centres in Asia Pacific
Bangkok
Thailand

Professor Rex Dunham
Department of Fisheries and Allied Aquacultures
Auburn University
USA

Professor Nigel Forteath
School of Human Life Sciences
University of Tasmania
Australia

Dr David Francis
Australian Institute of Marine Science
Townsville
Australia

Professor Gideon Hulata
Agricultural Research Organization
Institute of Animal Science
Israel

Dr Nathanael Hishamunda
Development and Planning Service
FAO Fisheries and Aquaculture
Italy

Dr Darryl Jory
Divisions of Marine Biology and Fisheries
University of Miami
USA

Mr Daniel Knop
Chief editor 'KORALLE'
Sinsheim
Germany

Dr Sagiv Kolkovski
Marine Aquaculture
Western Australia Department of Fisheries
Australia

Dr Martin Kumar
SARDI Aquatic Sciences
South Australia
Australia

Mr Jonathan Moorhead
School of Marine and Tropical Biology
James Cook University
Australia

Professor Leigh Owens
School of Biomedical and Molecular Sciences
James Cook University
Australia

Dr Nicholas A. Paul
School of Marine and Tropical Biology
James Cook University
Australia

Associate Professor John Purser
National Centre for Marine Conservation and Resource Sustainability
University of Tasmania
Australia

Professor Qingjun Shao
College of Animal Sciences
Zhejiang University
China

Mr Yitzhak Simon
Ministry of Agriculture and Rural Development
Israel

Dr Rohana Subasinghe
FAO Fisheries and Aquaculture Department
Rome
Italy

Dr Victor Suresh
United Research (Singapore) Pty Ltd.
Singapore

Professor Clem Tisdell
School of Economics
University of Queensland
Australia

Professor C. K. Tseng
Chinese Academy of Science
P. R. China
(deceased)

Professor Craig Tucker
National Warmwater Aquaculture Center
Mississippi State University
USA

Dr John Tucker
Indian River Institute, Inc.
Florida
USA

Dr Giovanni Turchini
School of Life and Environmental Sciences
Deakin University
Australia

Dr Chaoshu Zeng
School of Marine and Tropical Biology
James Cook University
Australia

1
Introduction

John S. Lucas

1.1 WHAT IS AQUACULTURE?

Give a person a fish and you feed them for a day; teach them how to grow fish and you feed them and their descendants for their lifetimes.

(somewhat modified from a Chinese proverb)

Aquaculture continues to develop rapidly, especially through its growth in Asia. World aquaculture production is increasing much more rapidly than animal husbandry and capture fisheries, the other two sources of animal protein for the world's population. There is widespread recognition that seafood production from capture fisheries is at or near its peak, and that aquaculture will become increasingly important as a source of seafood production, and ultimately the main source. There is widespread public interest in aquaculture. This is the context in which this textbook is written and we trust that it will convey some of the excitement of the rapidly developing discipline of aquaculture.

The term 'seafood' is used inclusively in this textbook, i.e. for all animal and plant products from aquatic environments, including freshwater, brackish and marine environments[1]. The term 'shellfish', according to common usage, describes aquatic invertebrates with a 'shell'. In this way, bivalve and gastropod molluscs, decapod crustaceans and sea urchins are combined, while recognising the extreme diversity of morphology and biology within this grouping. The two groups that overwhelmingly constitute shellfish are the bivalves (oysters, mussels, clams, etc.) and decapod crustaceans (shrimp, crayfish, crabs, etc.). The other major group of aquatic animal that is cultured is fish, also known as finfish. 'Fish farming' is used in the sense of aquaculture of fish, crustaceans, molluscs, etc., but not plants.

There are many different forms of aquaculture and, at the outset of this book, it is important to establish what aquaculture is, what it isn't and what distinguishes it from capture fisheries.

> The definition of aquaculture is understood to mean the farming of aquatic organisms, including fish, molluscs, crustaceans and aquatic plants. Farming implies some form of *intervention* in the rearing process to enhance production, such as regular stocking, feeding and protection from predators. Farming also implies individual or corporate *ownership* of stock being cultivated.
>
> *(FAO, 2006)*

> For statistical purposes, aquatic organisms that are harvested by an individual or corporate body that has owned them throughout their rearing period contribute to *aquaculture* while aquatic organisms that are exploitable by the public as a common property resource, with or without appropriate licences, are the harvest of *fisheries*.
>
> *(FAO, 2006)*

The two essential factors that together distinguish aquaculture from capture fisheries are:

- *intervention* to enhance the stock;
- *ownership* of the stock.

[1]With the exception of the final chapter, Chapter 26 The Next 20 Years, which focuses on edible products ('Food fish') and food security. The terms used in Chapter 26 are defined in its Introduction.

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Thus, a structure to which fish are attracted and caught (e.g. a fish-aggregating device (FAD) floating in the open ocean) may be owned, but this does not confer ownership of the stock of attracted fish. Furthermore, the FAD facilitates capture, but does not enhance the fish stock that is being captured. This is capture fisheries production. Hatchery production of juvenile salmon is aquaculture: they are owned by the hatchery and may be sold as fingerling fish. Their ultimate capture, after being released into rivers to which they eventually return to breed, is a fishery. The released fingerlings enhance the stock, but they become a common property resource. The same situation applies where hatchery-reared fish fingerlings are sold to fishing clubs and local government bodies to be stocked into lakes and dams to improve recreational fishing.

Hydroponics, the cultivation of terrestrial plants with their roots in dilute nutrient solutions instead of soil, isn't aquaculture. Hydroponics is an alternative method for growing terrestrial plants.

Activities constituting aquaculture production, according to FAO (2006), are:

- hatchery rearing of fry, spat, postlarvae, etc.;
- stocking of ponds, cages, tanks, raceways and temporary barrages (e.g. dams) with wild-caught or hatchery-produced juveniles to be reared to market size;
- culture in private tidal ponds (e.g. Indonesian 'tambaks');
- rearing molluscs to market size from hatchery-produced spat, transferred natural spatfall or transferred part-grown animals;
- stocked fish culture in paddy fields;
- harvesting planted or suspended seaweed;
- valliculture (culture in coastal lagoons).

1.2 ORIGINS OF AQUACULTURE AND AGRICULTURE

There were a number of independent origins of small-scale agriculture and a substantial variety of crops were domesticated for farming. Small-scale agriculture appears to have first developed about 10 000 years BC in the Fertile Crescent of south-west Asia, corresponding roughly to the modern-day region of Syria, Israel and Iraq, as human populations changed from hunting–gathering to cultivating crops that included wheat, barley, lentils, chickpeas, etc. (Fig. 1.1). Farming of cereals and other crops spread to adjacent regions. Subsequently, the farming of cereal crops arose independently on other major landmasses. Rice cultivation began in Asia about 7000 years ago. Sorghum and millet cultivation, and maize cultivation, developed somewhat later in Africa and America, respectively. These changes from hunting–gathering to farming cereal crops caused profound changes in lifestyle, from a nomadic to a settled existence. They resulted in greatly increased productivity from the land for human consumption and increased human populations per unit land area as a consequence. Whether quality of life improved in the early farming communities is debatable: diet became less varied and conditions became more favourable for disease.

The origins of aquaculture are much later. Culture of common carp (*Cyprinus carpio*) was developed thousands of years later in China, where the carp is a native species (Fig. 1.2). The first aquaculture text is attributed to a Chinese politician, Fan Lei, and is dated about 500 BC (Ling, 1977). Fan Lei attributed the source of his wealth to his fish ponds: so his fish culture was more than a hobby. However, in Africa, America and Australia, aquaculture was not practised until it was introduced in recent centuries.

The late origin of aquaculture compared with agriculture and its failure to develop in some continents is partly because humans are terrestrial inhabitants. We cannot readily appreciate the parameters of aquatic environments and there are environmental factors that may profoundly affect aquatic organisms, such as:

- very low solubility of O_2 in water;
- high solubility of CO_2 in water;
- pH;
- salinity;
- buffering capacity;
- dissolved nutrients;
- toxic nitrogenous waste molecules;
- turbidity;
- heavy metals and other toxic molecules in solution;
- phyto- and zooplankton concentrations;
- current velocity.

These can only be rigorously measured with modern instrumentation.

Many of the diseases that afflict aquatic organisms are quite unfamiliar to us. Furthermore, virtually all the animals used in aquaculture are poikilotherms (their body temperature is variable and strongly influenced by environmental temperature) ('cold blooded'). Their metabolic rates, and all functions depending on metabolic rate, are profoundly influenced by environmental temperature in ways that we do not experience as 'warm-blooded' mammals.

The difficulties of appreciating the influences of these environmental factors still apply today, causing aquacul-



Fig. 1.1 The court bakery of Ramses III. From the tomb of Ramses III in the Valley of the Kings, twentieth dynasty. (*The Oxford encyclopedia of ancient Egypt*, copyright expired.)



Fig. 1.2 The common carp (*Cyprinus carpio*). (Photograph by Piet Spaans.)

ture programmes to have a relatively longer development period than other forms of food production. 'Even when tested technologies are adopted, the construction of physical facilities (particularly pond farms), solution of site-specific problems, the building up of the productivity of the system and, above all, attainment of skills by workers take considerable time' (Pillay, 1990). In agriculture we are much more readily able to appreciate the parameters influencing the success or otherwise of the output, and we have a very long history of attaining the skills needed.

A further major consequence of the late origin of aquaculture is that there has been relatively little genetic selection for many species and this is compared with the highly selected plants and animals of agriculture. Modern agriculture is based on organisms that are vastly different from their wild ancestors, and in many cases their wild ancestors no longer exist. This selection for desirable traits took place steadily and without any scientific basis over thousands of years of domestication. It was more intense last century with scientific breeding programmes. Modern

agriculture would be totally uneconomic and the current world population would starve without these domesticated and genetically selected agricultural plants and animals. Much of aquaculture, by contrast, is based on plants and animals that are still 'wild'. There are, however, species that have been subject to strong selection, hybridisation, and molecular and genomic techniques (Chapter 7), such as:

- common carp;
- Atlantic salmon;
- rainbow trout;
- tilapia species;
- channel catfish.

Their breeding is based on broodstock that differ substantially from their ancestors in their genetics. Many other aquaculture species are based on wild broodstock obtained from natural populations. In some cases the life cycle has not yet been 'closed', i.e. the species has not been reared to sexual maturity and then spawned on a regular basis under culture conditions. Until the life cycle is closed, there is minimal potential for selective breeding.

1.3 AQUACULTURE AND CAPTURE FISHERIES PRODUCTION

Fishing activities, whether they are spearing individual fish, collecting shellfish from a rocky shore or coral reef, using a cast net, or capturing schools of fish with huge nets from factory trawlers that ply the world's oceans, are all hunting–gathering regardless of the degree of technology. As capture fisheries production currently exceeds aquaculture production, hunting–gathering activities remain the principal source of seafood. These fisheries suffer problems that are fundamental to hunting–gathering:

- variable recruitment and consequent unpredictability of stock size;
- difficulties in assessing stock size and its capacity for exploitation;
- difficulty in regulating exploitation to match the stock size;
- relatively low productivity.

The natural productivity of the world's water masses, fresh, brackish and marine, is huge, but finite; and a finite amount of plant and animal products can be harvested by fishing. For instance, the mean harvest from oceans that can be obtained for human consumption or processed for use in fish meal is ca. 2.5 kg per hectare of ocean surface per year. Furthermore, this huge but finite amount of harvest is within our current fishing capacity. Many of the world's major capture fisheries range from being heavily exploited to heavily overexploited, and production from capture fisheries has reached a plateau of ca.90 million t/year[2], around which it now fluctuates annually (Fig. 1.3). Global capture fisheries production increased to 93 million tonnes in 1994 and since then has fluctuated between 89 and 97 million t/year, with an overall mean increase of <1% per year.

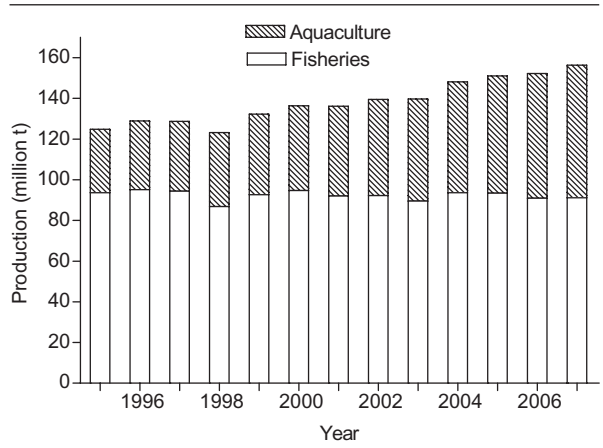


Fig. 1.3 Global production of capture fisheries and aquaculture per year from 1995 to 2007.

There are two further factors in capture fisheries production. About one-third of capture fisheries production is used to make fish meal, i.e. dried fish products, based on sardines, anchovy, fish wastes, etc. Fish meal is used as a source of animal protein and lipids in feeds in agricultural animal husbandry, e.g. pig feeds, but it is also extensively used in feeds for aquaculture (section 9.10). Thus, the effective annual production from global capture fisheries for direct human consumption is in the order of 60 million t/year. The other 30 million tonnes subsequently finds its way into the human diet by indirect processes.

A further factor that does not appear in FAO fisheries statistics is the substantial proportion of capture fisheries that is bycatch (non-target catch) and discards (e.g. under commercial size). A high proportion of these die. Alverson *et al.* (1994) estimated a mean of 27 million t/year of bycatch and discards from global commercial fisheries. They considered this conservative as it didn't include data on some invertebrate fisheries, and recreational and subsistence fisheries. Bycatch and discards vary according to

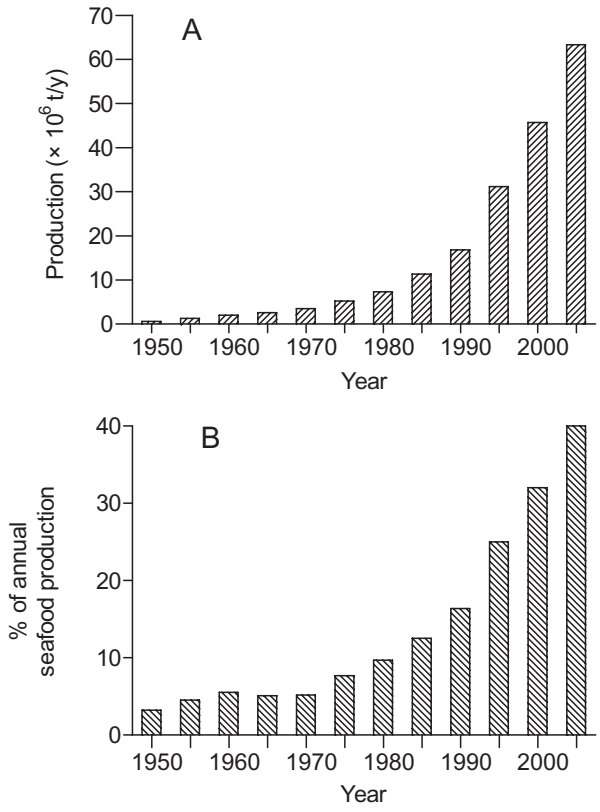


Fig. 1.4 A. Global production of aquaculture per year from 1950 to 2007. **B.** Aquaculture production as a percentage of global seafood production per year.

the nature of fishery and other factors. Alverson *et al.* (1994) rated marine shrimp trawl fisheries wherever they occur as consistently being among the highest bycatch fisheries. In view of the increase in world fishery effort since 1994, it is reasonable to assume that this wastage amounts to about 30 million t/year, i.e. a third again on top of the 90 million t/year global fishery.

In contrast to capture fisheries, aquaculture production of animals and plants grew at a mean rate of 8.1% over the same period (Fig. 1.4A). The increase in global aquatic animal and plant supply in recent decades has come largely from aquaculture. In 2007, aquaculture contributed 42% of total global seafood production (Fig. 1.4B) and it will continue to increase in relative importance. It is clear that further increases in supply from aquatic environments will come largely from aquaculture. Unlike capture fisheries, aquaculture is not limited by the natural productivity of the world's water masses. It is therefore not surprising that aquaculture production has been increasing and will inevitably overtake global capture fisheries production. In view of the fact that the percent contribution of aquaculture to global seafood supply seems to be increasing exponentially (Fig. 1.4B), this may happen sooner rather than later.

Aquatic plants (very predominantly seaweeds) contribute substantially to aquaculture and capture fisheries production, especially to the former (section 13.2.1). Aquatic plant productions from aquaculture and capture fisheries were ca. 14.9 million and 1.1 million tonnes, respectively, in 2007 (FAO Fishstat Plus, 2009)[2]. The relative proportions by weight and value of fish, molluscs, crustaceans and plants from aquaculture in 2007 are shown in Fig. 1.5. Fish constitute about half the weight and value of aquaculture production. Plants and shellfish each constitute about 20% of the weight. There are, however, major changes between relative weights and relative values of plants and shellfish. Molluscs decline in relative value. Plants decline even more in relative value and together molluscs and plants constitute a bit less than a quarter of the value of aquaculture production. Crustaceans (mainly marine shrimp) show a very large increase in value to almost a quarter of the value. Fish remain at about half the value of aquaculture production. This has significant implications for countries such as China.

Food security for the world's population, especially in poorer countries, is a major factor constraining international organisations such as the FAO (Chapter 26). To put aquaculture and fisheries production into the perspective of providing animal protein for the world's current population: global production of slaughtered meat from livestock (pork, beef, chicken and lamb) is in the order of 250 million t/year compared with about 60 million t/year from capture fisheries (for direct human consumption) and 52 million tonnes from aquaculture (excluding aquatic plants). This 110 million tonnes from aquaculture and fisheries is pre-slaughtered weight, and slaughtered weight (after removal of viscera, heads and shells) is probably around 50–60%. This value is not easy to estimate as it varies markedly with the kind of animal and country of consumption. Consequently, seafood makes up about 20% of all animal protein production per year. Aquaculture is a modest 7% of all animal protein production/year based on these calculations. With fisheries production/year almost

[2]Unless otherwise stated, all production and value data given for aquaculture and capture fisheries in this textbook are derived from the FAO online site Fishstat Plus 2009. The software may be downloaded and installed from http://www.fao.org/fishery/statistics/software/en.

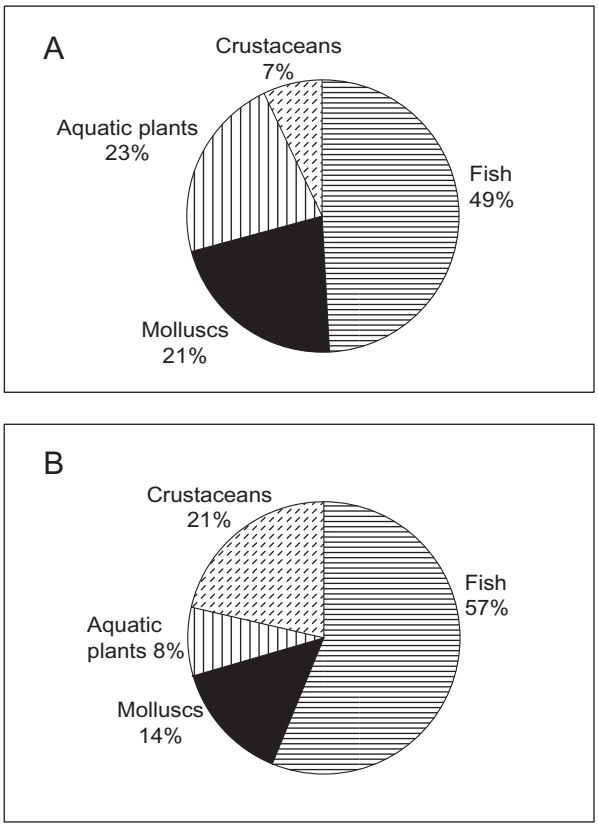


Fig. 1.5 Relative proportions of fish, molluscs, crustaceans and aquatic plants in global aquaculture production in 2007. **A**. By weight. **B**. By value.

static and unlikely to increase much, and, while livestock production is increasing at about 2% per year, aquaculture is increasing at 8% per year. This roughly estimated value (7%) of aquaculture's contribution to world animal protein production is an increase from 3.5% in 1993, estimated by New (1997).

1.4 THE 'BLUE REVOLUTION'

The rapid increase in aquaculture production in the 1990s led to suggestions that aquaculture was undergoing a 'Blue Revolution' that would transform the productivity of marine and other aquatic environments with new technology (e.g. Holmes, 1996; Entis, 1997) (Fig. 1.6). This envisaged a revolution in productivity similar to the 'Green Revolution' in agriculture during the decades following World War II. The Green Revolution occurred 'where concentrated research developed the basis for the agricultural practices in use today (e.g. mechanisation, heavy fertilisation, heavy pesticide use, irrigation, genetically improved stocks, advanced feed formulations)' (Hopkins, 1996).

The great, almost exponential, increase in the quantity of animal and plant aquaculture production in the two decades from 1987 to 2007 came mainly from increases in freshwater and marine aquaculture. There was a greater absolute increase in the marine environment in total aquaculture production, which includes considerable aquatic plant production (Fig. 1.7A). In terms of specifically animal production, however, and hence protein production directly for human consumption, the increase was substantially greater from freshwater aquaculture (Fig. 1.7B). The annual rate of production of aquatic animals in freshwater environments increased by 25 million tonnes over these two decades compared with an increase of 15 million tonnes in the rate of annual production in the marine environment. This clearly establishes freshwater as the major environment of aquatic animal production (Fig. 1.7). The large increase in seaweed production in the marine environment is important as a source of income, but it is of low value as an immediate source of protein for human consumption.

There is a further very important statistic about the growth in aquaculture production over recent decades: it was particularly driven by growth of the industry in China (Table 1.1). Furthermore, dividing between developing countries (FAO classification), which include China, and developed countries, 50 out of the 51 million t/year increased rate of global aquaculture production over the period 1987 to 2007 came from developing countries (Table 1.1). Aquaculture production in these countries increased at the remarkable rate of 9.0% per year over these two decades (Table 1.1). Compared with this, the 1.9% per year growth of aquaculture production in the developed countries was modest. The 'Blue Revolution' in aquaculture, like the 'Green Revolution' in agriculture, occurred primarily in developing countries. China alone showed an increase of about 35 million t/year over the period 1987 to 2007 at the even more remarkable rate of 10.1% per year (Table 1.1). It was the driving force of aquaculture expansion in the developing countries and the world. By 2007, China accounted for almost two-thirds of global aquaculture production (Table 1.1). In view of the fact that the growth in aquaculture production of aquatic animals in particular occurred predominately in freshwater systems, which are often anything but blue, it might be appropriate to call the huge increase in aquaculture the 'Brown Revolution'.



Fig. 1.6 Sea Station 3000 is a fully submersible 3000 m^3 seacage (24 m diam × 16.5 m tall). In operation the seacage is submerged to below 10 m depth, ca. 800 m off the Kona Coast of the Big Island of Hawaii. It is stocked with up to 70 000 *Seriola rivoliana* (see cover photograph). The Offshore Site Manager is standing on the spar of the semi-submerged structure. (Photograph from Kona Blue Water Farms, Inc. Kona Kampachi®.)

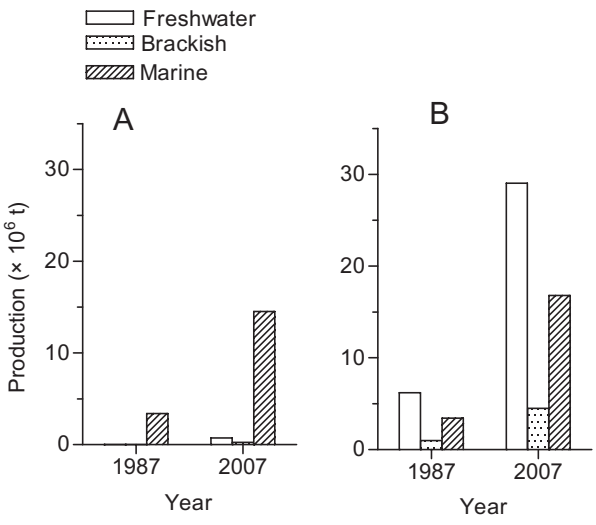


Fig. 1.7 Increases in global aquaculture production between 1987 and 2007 by environment. **A**. Increase in plant production. **B**. Increase in animal production.

It would be misleading, however, to see aquaculture developing purely as an expansion of pond culture. For example, there are a number of sophisticated developments in disease control (section 19.5.4), genetics (section 7.8) and feed formulation (section 8.8).

The 10 major countries in terms of quantity of aquaculture production in 2007 are shown in Table 1.2. All except Chile are Asian countries and, according to FAO classification, all except Japan are developing countries. The huge gap between China and the following countries is notable.

Although the developing Asian countries have aquaculture industries of high-value products, such as shrimp and scallops, for lucrative export markets, a high proportion of aquaculture in these countries continues to be from traditional pond culture of freshwater fish, especially carps and other cyprinids (Chapter 14). Fig. 1.8 shows that carps together with other cyprinids are by far the most important fish by weight in global production. Cyprinid production is a magnitude greater than the next two major groups of fish in aquaculture, tilapia and salmonids: ca. 20 million t/year versus ca. 2 million t/year for each of the latter two. Cyprinids are the most valuable fish in total global production, but have substantially lower value per unit weight

Table 1.1 Global aquaculture production in 1987 and 2007 by developing and developed countries. Data for China are also shown.

		Global aquaculture production		
	Population (1999) (millions)	1987 (000t)	2007 (000t)	Mean % increase/year
World	5978	13962	65190	8.0%
Developing countries	3664	10855	60676	9.0%
% of world total		77.7%	93.1%	
Developed countries	2314	3106	4515	1.9%
% of world total		22.2%	6.9%	
China	*1243*	*6011*	*41173*	*10.1%*
% of world total		*43.1%*	*63.2%*	

Table 1.2 The top 10 countries in terms of weight of total aquaculture production in 2007.

Sequence	Country	% of world production	Asian country	Develping country
1	China	63.2	Y	Y
2	India	6.1	Y	Y
3	Indonesia	4.8	Y	Y
4	Philippines	3.4	Y	Y
5	Vietnam	3.4	Y	Y
6	Korean Republic	2.1	Y	Y
7	Thailand	2.1	Y	Y
8	Japan	2.0	Y	
9	Bangladesh	1.5	Y	Y
10	Chile	1.3		Y

than salmonids and are only twice as valuable as the latter: ca. US$19 billion/year versus ca. US$10 billion/year for salmonids.

These freshwater fishes are part of the reason for China's percentage of global aquaculture output being ca. 50% by value compared with 70% by weight in 2007. Further factors are that China provides more than 70% of the world's seaweed production and two-thirds of the molluscs (mainly bivalves). Both are of relatively low value (Fig. 1.5).

Freshwater fish are often cultured together as complementary species (polyculture; section 2.3.5). These fish species are herbivores, omnivores and detritivores, feeding low in the food chain and requiring little supplementary input of feeds. This aquaculture often involves simple ponds, basic technology and low stocking densities. The production is often enhanced by using inexpensive organic fertilisers such as farm animal faeces and crop residues to feed the cultured fish, and to promote primary production in the ponds (integrated culture; section 2.3.6). In rural communities where animal protein is scarce and is prohibitively expensive from other sources, cultured fish form the major, if not the exclusive, source of animal protein.

A number of factors are responsible for the outstanding increase in aquaculture production in China (Cen and Zhang, 1998). However, the major factor has been a great increase in the surface area of ponds, other freshwater environments and shallow coastal environments committed to aquaculture. Listed below is a series of important factors.

- The Chinese government deliberately embarked on a large-scale programme to develop aquaculture.

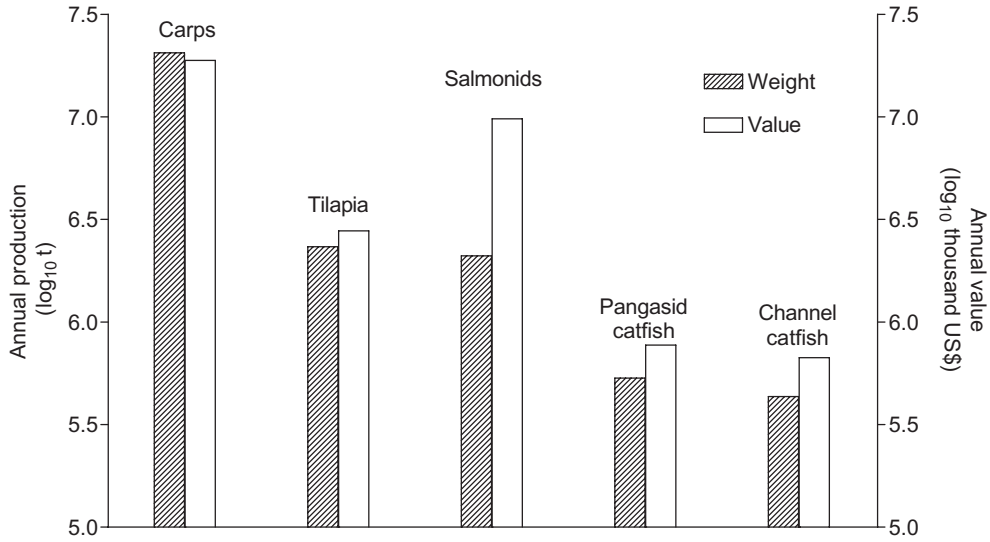


Fig. 1.8 Annual production of major groups of fish. Note that both *Y*-axes are logarithmic and there are magnitudes of differences.

- China has identified huge areas of potential sites for aquaculture and is prepared to use them to a large extent, i.e. 2.6 million ha of suitable coastal sites and 17.5 million ha of inland freshwater sites.
- Mariculture utilisation of shallow sea and mudflat areas was 25.1% in 1995 compared with 3.9% in 1978, and offshore areas used for mariculture have been extended offshore from 10 m to 50 m depth.
- Utilisation for aquaculture of inland waters was 25.5% in 1995 compared with 4.3% in 1978 (this 4.3% already consisted of 100 500 ha).
- Productivity per unit area, although still generally low, has been increased through research, extension and better technology.
- The number of species in culture has greatly increased, including high-value species for international markets such as marine shrimp, crabs, scallops, abalone, eels and rainbow trout.
- Sixty exotic species were introduced from abroad, 20 new species have been domesticated from wild populations and about 30 species have been improved through hybridisation and stock selection programmes.
- Huge numbers of fingerlings and juveniles are produced from many large hatcheries.

Unlike the Green Revolution, where technological developments played a major role in increased productivity, the outstanding growth of aquaculture in China has not depended on major technological developments. The growth has resulted in particular from upscaling by increasing the areas devoted to ongrowing aquaculture, increasing hatchery output and increasing the number of species cultured.

The result of this deliberate programme to promote aquaculture is that China leads the world in being one of the few, or perhaps the only country, in which aquaculture production substantially exceeds fisheries production. China has already achieved the anticipated stage when the balance of production between aquaculture and fisheries shifts to the former. Aquaculture production is more than double fisheries production.

It may not seem surprising that China, as the country with the largest population, is the greatest aquaculture producer. Some production versus population size data are presented in Table 1.1. China's aquaculture production is ca. 36 kg/person/year. Mean production for all developing countries is ca. 17 kg/person/year and for developed countries it is <2 kg/person/year. Taken at face value these figures are misleading. For instance, China produces a lot of seaweed (see above), which is not much used for human consumption, and, like other Asian countries, it exports high-value aquaculture products to developed countries.

In considering production versus consumption, there is a further complication when the data for total production

Table 1.3 Annual production of food fish[1] (t, million) from aquaculture and capture fisheries in the major regions of the world. Food fish consumption/person/year is also included.

	Africa	North America	Central America	Asia	Europe	Oceania	World
Total food fish production	8.0	6.8	10.9	82.3	15.9	1.6	134.6
Non-food use	−1.2	−0.9	−12.6	−10.1	−3.5	−0.4	−28.8
Imports	2.5	5.0	1.3	12.5	15.4	0.5	37.3
Exports	−1.8	−3.1	−3.8	−14.6	−12.8	−0.9	−38.8
Total food supply[2]	7.6	8.0	4.0	70.2	15.2	0.8	106.7
Consumption (kg/person/year)	8.3	24.1	8.7	17.8	20.7	24.6	16.4

[1]Food fish in this table refers to fish, crustaceans and molluscs destined for human consumption (see Chapter 26).

[2]Total food supply is the summation of the positive (production and imports) and negative (non-food use and exports) values. (FAO Fisheries and Aquaculture statistics.)

of edible sea food ('food fish') from aquaculture together with capture fisheries are considered (Table 1.3). As the Asian region includes the major aquaculture producers, it has by far the greatest production, but the European and North American regions have relatively greater imports. Despite the very high production of seafood in Asia, when the total available supplies of edible seafood are related to population sizes, the consumption in kg/person/year is greatest in Europe and North America (Table 1.3). These affluent regions also consume the greatest amounts of animal products from animal husbandry. Affluence supports high consumption of animal protein from both land and sea. The African countries, which on average have a low intake of animal products/person/year from animal husbandry, also have a low consumption of seafood.

These differences are further discussed in Chapter 26.

There is another contrast between aquaculture in the developing Asian countries and in developed countries. Over 60% of fish production in developed countries is based on high market-value species of carnivorous fish (Fig. 2.8). These fish are reared in monoculture at high stocking densities and need inputs of high-protein feeds. High-protein feeds are expensive and usually require fish meal as sources of animal protein and lipids for the carnivorous diet. Thus, this form of aquaculture uses low market-value products from fisheries as feed to increase their value in the final products of culture. This is inefficient (section 2.3.1).

A variety of factors have affected particular aquaculture industries, e.g. section 16.9, but environmental issues are a major factor consistently limiting the growth of aquaculture industries in developed countries (Chapter 4).

All agriculture and aquaculture needs environmental modifications on various scales to enhance production. For instance, grain farming such as wheat farming has irreversibly destroyed the environment in countless millions of hectares around the globe, with:

- native vegetation clear-felled;
- soil structure destroyed;
- soil eroded;
- extinctions or near extinctions of native species dependent on the original environment;
- introductions of exotic and pest species.

On the basis of contemporary environmental awareness it is very unlikely that grain farming would be permitted or even considered on this scale. However, the environmental damage has occurred over hundreds and thousands of years, is irreversible, and the alternative to grain farming is that the world population starves.

There have been fisheries for tens of thousands of years. However, since global fisheries have reached the point that it is no longer possible to extract more products (Fig. 1.3), it is inconceivable that they are not having widespread impacts on all aquatic ecosystems. These profound impacts are difficult to determine, but the *Sea Around Us* project is attempting this. 'Broadly, the work of the project is aimed at a reappraisal of fisheries, from the benign activity that many interested people still perceive them to be, to a realization that *they have become the driver for massive loss of biodiversity in the ocean* (italics added) (Pauly, 2007). There have been studies (e.g. Myers and Worm, 2003) showing the flow-down effects to lower trophic levels of overfishing predatory fish, so that the community structure is changed. Environmentally sound aquaculture may be a better alternative to fisheries for more than just increased seafood production.

Agriculture and fisheries are part of our heritage, but aquaculture, especially in the developed countries, is a latecomer and has to make its way in regulatory circumstances that are much more rigorous. Agriculture and fisheries are accepted despite their massive environmental damage because we depend on them as necessary sources of food. Aquaculture must develop within the constraints of comprehensive, sometimes unreasonable, environmental regulations in developed countries. It also has to develop within the constraints of existing stakeholders who may lodge objections to the proposed aquaculture development, e.g. for interfering with recreational boating, for noise that is unacceptable to nearby coastal communities. If the aquaculture farm is a long-standing fixture before the marina is proposed or the coastal community begins to develop in the region, these objections are more difficult to sustain, but this usually isn't the case. Many proposed projects founder on the number of permits and approvals required, legal and administrative costs, and the duration of processes in obtaining the final approval to go ahead with the project. There is widespread recognition in developed countries that aquaculture is the future if we are to meet the growing demand for seafood. It seems, however, that the governments of some developed countries are prepared to import increasing amounts of cultured seafood rather than facilitate growth of the industries within their countries. The greatest rate of increase in aquaculture production in developed countries, 6.5% per year, occurred some time ago in the decade 1966–1976. It was down to increasing by 1.9% per year over the period 1997–2007 (Table 1.1). This seems very low compared with the developing countries, although it is not much less than the 2% increase per year in livestock production. The rate of aquaculture growth in developed countries over the next decade will be interesting. It will strongly depend on government policies, for example:

- to what extent they are content to accept increasing seafood imports;
- to what extent they seek to enhance within-country production.

If it is the latter, they will need to do more to 'take the brakes off' and promote new aquaculture ventures. Major growth of aquaculture will not occur in the face of government indifference and over-regulation. In developing countries, the aquaculture industry growth has involved governments making decisions to facilitate aquaculture and particular aquaculture industries (section 1.8.1).

1.5 AN ALLEGORY

Short-tail lums are moderate-sized herbivores that weigh up to 50 kg. Some of their meat is sold on the local market, but most is packaged, frozen and exported to Asian markets where it is highly valued. Harvesting is based on shooting lums at night when they are active. The lums are fixed in a spotlight and shot by professional hunters riding on the backs of four-wheel-drive vehicles. However, as the populations were harvested at unsustainable levels it was necessary to drive further and longer to find lums. Furthermore, the remaining short-tail lums were vehicle shy and the vehicles had to drive faster and travel further into the bush, smashing down swathes of bushes and small trees. Some similar-sized carnivorous mammals were also shot because of limited time for careful identification. In fact, as the lums became increasingly rare, the strategy became to shoot first and check later when moving mammals were sighted. Increasing numbers of the non-target mammals were shot in this way, but they were of little commercial value. The short-tail lum industry became marginally profitable and numbers of hunters left for other employment.

Amateur lum hunters were affected by the diminishing populations and sent a delegation to raise the issue with the appropriate government Minister. It was proposed that reserves for short-tail lum protection be established. Some biologists, however, pointed out that the rates of recovery of short-tail lum populations, and the other diminished mammal populations, would not be limited by their rates of reproduction. Population recoveries would be limited by the long period required for recovery of their environment.

Independently, a farm to raise short-tail lums was proposed in the region. Careful planning was made for the lum farm to minimise environmental impacts and to meet all the requirements for a new animal production venture. The lums would be farmed at relatively high density and fed a comprehensive diet of grain, pelleted feeds and chaff, instead of grazing over a large area. The economic viability of the proposal was demonstrated. The short-tail lum breeding stock would be obtained from other farms with semi-domesticated stock. The proposal went to the Department of Primary Industries, Enterprise and Environment after two years of planning and discussion with local, state and national government bodies to meet the criteria for the new development. The Department published the proposal and invited submissions from the various stakeholders.

When asked, most people recognised that farming lums was the only solution to maintain or obtain more lum

products. However, they did not want a nearby farm (NIMBY – Not In My Backyard). Protest meetings were organised, including the remaining professional hunters and a visiting habitat activist. Deputations were made to the Department and local politicians.

The bases for oppositions were:

- short-tail lums are noisy animals and active at night;
- the smell of short-tail lum faeces would be pervasive when the wind blew from that direction;
- the local abattoir with its unpleasant odour would increase operations;
- hectares of pristine countryside could possibly be destroyed if the mode of farming changed with a new permit;
- semi-domesticated lums would get out through the fences and mate with the remaining wild lums, weakening the genetic pool of the latter in this area;
- professional hunters would suffer further financial hardship through being in competition with lum farmers.

The application was refused by the Department of Primary Industries, Enterprise and Environment in the face of vigorous opposition.

Meanwhile, two species of flies and three species of beetles, which lay their eggs specifically in short-tail lum faeces or in the ground beneath them, were on their way to extinction in the region due to scarcity of lum faeces. A bird species and a lizard species that depend on these insects and their larvae for food also faced extinction. None of these animals had been studied and their decline towards extinction in the region was passing unnoticed. What didn't pass unnoticed resulted from the reduced numbers of one of the non-target mammals. Substantial numbers of this mammal, which preys on rodents, were incidentally shot and consequently populations of native rats began to expand rapidly due to reduced predator pressure. The rats' food became scarce in their natural environment and they began searching in new environments. Adjacent residential areas were inexplicably invaded by plagues of native rats.

1.6 DIVERSITY OF AQUACULTURE

Freshwater cyprinid fish and seaweeds dominate world aquaculture production. However, as well as these two large groups and the modest number of aquaculture species treated in this textbook, a huge diversity of species are cultured. FAO provides quantity and commercial value data on aquaculture production of some hundreds of species of fish, shellfish and algae that are cultured for human consumption and other uses. Even these three very broad categories of organisms do not encompass the whole range of aquacultured species. In addition to aquaculture for human consumption, there are international and local industries producing live feeds for hatcheries, e.g. dried brine shrimp cysts and live 'blood worms'. There is a huge worldwide aquaculture industry for ornamental fish, especially tropical freshwater and marine fish (Chapter 25). Other aquarium-related industries produce aquarium plants and freshwater and marine invertebrates, including coralline algae/coral-encrusted substrates ('living rock') for tropical marine aquaria. Pearls and sponges are cultured and sold for their traditional uses.

In particular, the Asian countries with large and traditional aquaculture industries are involved in culturing a wide diversity of species. Cen and Zhang (1998) indicate that at least 110 species (including introduced species) are cultured in China. These include freshwater and marine fish, marine shrimps, prawns, crabs, bivalves, gastropods, a variety of seaweeds, and special products such as sea cucumbers, sea urchins, bullfrogs and soft-shell turtles (Chapter 20). Aquaculturists in Taiwan have developed techniques for larval culture of more than 90 species of freshwater and marine fish (Liao *et al.*, 2001). In Japan in 1997, 284 hatcheries together produced fingerlings of 88 fish species (Fushimi, 2001). Fingerlings of 73 species, amounting to 168 million fingerlings, were released into the environment in stock enhancement programmes.

1.7 STOCK ENHANCEMENT

This raises another aspect of the diversity of aquaculture: links between aquaculture and fisheries. Stock enhancement of restricted freshwater environments for subsequent fishing (either recreational or commercial) is a standard procedure. Stock enhancement of marine populations in an attempt to improve fishery catches is an important aspect of Japanese aquaculture (Fig. 1.9). Stock enhancement of marine populations is also practised in Taiwan, and to a limited extent in countries such as Canada, France, Norway, the UK and the USA. Stock enhancement programmes with 'seed' from aquaculture involve predominantly fish, but they are also used for shellfish such as abalone, scallops and marine lobsters. These restocking programmes are not unequivocally successful and the best that can be shown for many is increasing fisheries that are approximately correlated with increasing input to the population of cultured 'seed' (Fig. 1.10). The results of stock enhancement programmes for heavily overfished abalone stocks in Japan illustrate the variable successes of restocking programmes (Hamasaki and

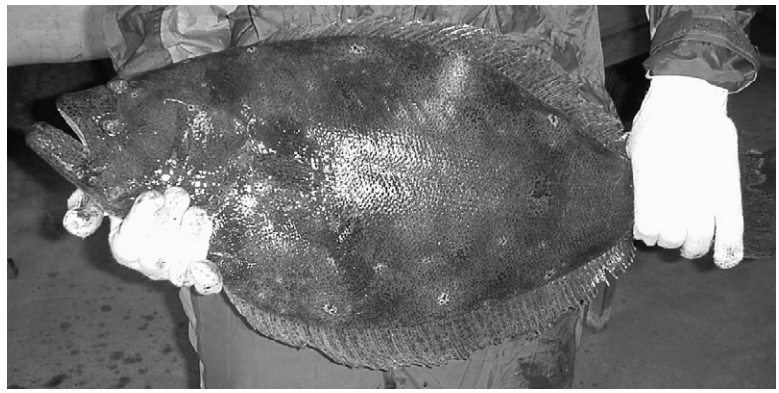


Fig. 1.9 Japanese flounder (*Paralichthyes olivaceus*) broodstock used to produce seed for stock enhancement (photograph by Dr Kotaro Kikuchi, Abiko Research Laboratory).

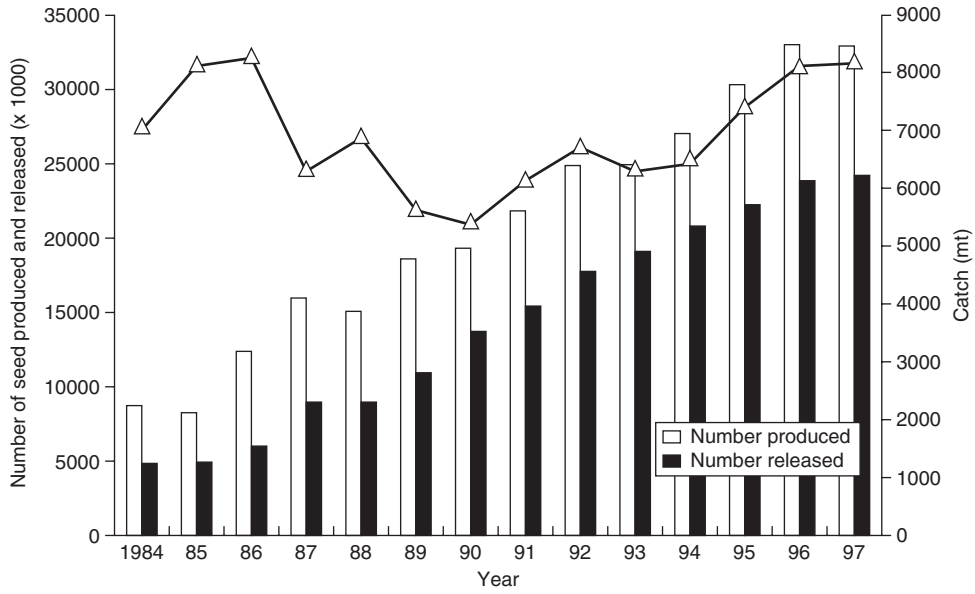


Fig. 1.10 Annual fluctuations in the catch of Japanese flounder (*Paralichthyes olivaceus*) versus 'seed' production and release in stock enhancement. Note upturn in declining fishery from 1990. Reprinted from Fushimi (2001) with permission from Elsevier Science.

Kitada, 2008). From surveys at 13 locations, the overall recapture rates ranged from 1.4 to 23.8%, the released abalone contributed from 6.9 to 83.5% of total catches, and the economic efficiency was estimated to range from 0.4 to 6.2. Economic efficiency is important in that it considers the increased value of the fishery from stock enhancement versus the hatchery costs of producing juveniles and seeding the fished populations (Hilborn, 1998). This variability in abalone restocking data was between locations in particular, but also between years of release. The abalone restocking programmes began in the late 1960s, but annual catches in Japan have declined from ca. 6500 tonnes in 1970 to 2000 tonnes in the mid-1990s (Hamasaki and Kitada, 2008).

Stock enhancement involves a more sophisticated approach than rearing large numbers of juveniles and releasing them into the vicinity of the fishery stock; other factors are very important in the success of the process (Le Vay *et al.*, 2007). These factors include genetic diversity of broodstock, sufficient habitat availability for the new releases, and the population dynamics and level of recruitment limitation of the fished stock. There is no point in stocking a fishery where there are inadequate habitats for new releases or where the fished stock is not limited by the level of natural recruitment. Conditioning and selection during culture in the hatchery may be a critical phase in maximising the physiological and behavioural fitness of juveniles after release.

1.8 NEW DEVELOPMENTS IN AQUACULTURE

Unlike animal husbandry, which is now based on a stable group of species, aquaculture is not stable in terms of the variety of species cultured and where they are cultured. As well as its rapid growth, this is an exciting aspect of aquaculture to consider what new developments will occur. For instance, bluefin tuna are very valuable and the southern and northern bluefin tuna fisheries are overfished to the extent that there is concern in some quarters that there are insufficient reproductive stocks for the populations to recover. Developing culture methods for these rapid-swimming oceanic fish that specifically feed on moving prey, and closing their life cycle in captivity, are two of the most challenging targets in aquaculture, but there is progress in techniques for culture on an experimental scale. There is potential for lucrative farming industries in the Southern and Northern hemispheres when commercial farming technology is developed. Two other valuable and very challenging animals to culture from eggs and to close the life cycle are spiny and rock lobsters, with their 8+ month plankton larval period (section 22.7), and freshwater eels, with their 10+ month planktonic period in the ocean (section 18.10.1). Both are currently caught for culturing after metamorphosis in the wild. Three 'holy grails' of aquaculture are to culture bluefin tuna and these lobsters and freshwater eels throughout their life cycles in captive conditions, removing the current reliance on capturing developing stages from the field, and giving the potential for genetic selection.

There are a number of other valuable species of marine fish for which the techniques of culture are in the early phases of development (Table 18.1). It is likely that some of these will become major aquaculture industries as the techniques required for commercial-scale farming are achieved.

1.8.1 Pangasid catfish

Pangasid catfish have joined the ranks of the major groups of fish species that are farmed (Figs 1.8, 1.11). The development and assessment of this industry is reviewed by Phuong and Oanh (2009). Vietnam is recorded as producing about 850 000 t/year and the sheer size of the industry is illustrated by the great number of staff processing fillets for one company (Fig. 1.12).

There are a number of pangasid species in the Mekong Delta rivers, and some were traditionally reared on a small scale. 'Basa' (Vietnamese name for *Pangasia bocourti*) was cultured in floating cages in the Delta (Phuong and Oanh, 2009).

Pangasid catfish have a number of favourable attributes for commercial aquaculture, and were 'waiting to be adopted', for the following reasons:

- Broodstock mature several times a year and can be strip spawned.
- Good numbers of large eggs are produced and hatchery techniques for rearing of eggs and larvae are relatively straightforward.
- Fingerlings grow rapidly in the hatchery to 3 cm when they may be transferred to farms for grow-out.
- There is rapid growth to market size, 2 kg, in 12 months.
- Farming techniques are inexpensive and straightforward: floating net cages, fenced enclosures and earthen ponds.
- They can be fed on inexpensive diets, such as rice bran and soybean meal.
- They tolerate low DO conditions.
- They are relatively disease free.



Fig. 1.11 *Pangasius hypophthalmus* catfish ('tra'). (Photograph by Karelj.)



Fig. 1.12 Workers trimming basa fillets. (Photograph courtesy of Vihn Hoan Corp.)

- There is good market acceptance: they can be processed to produce fillets of pale, mild and bone-free flesh, which can be prepared in a variety of ways.

However, there was insignificant production of these catfish until the mid-1990s (Fig. 1.13) and then phenomenal growth in the Mekong Delta of Vietnam when the favourable attributes of pangasid catfish for commercial aquaculture were realised (Phuong and Oanh, 2009). This growth came essentially from three developments:

- Establishing reliable hatchery and nursery methods in 2000, instead of being dependent on collecting seed stock from the wild was probably the key breakthrough.
- A move from pond, cage and pen modes of grow-out culture to focus on pond culture with 95% of total production coming from ponds in recent years and large farms having up to 26 ponds (Phuong and Oanh, 2009).
- Recognition of the economic potential of pangasid aquaculture by the Vietnamese government, which established supportive policies such as giving bank loans to

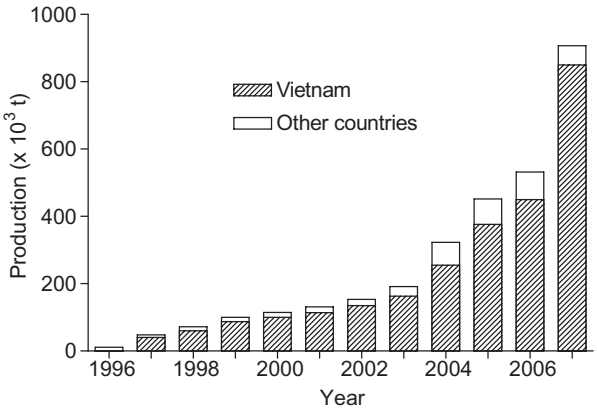


Fig. 1.13 Growth of pangasid catfish farming as production per year in Vietnam and in other countries.

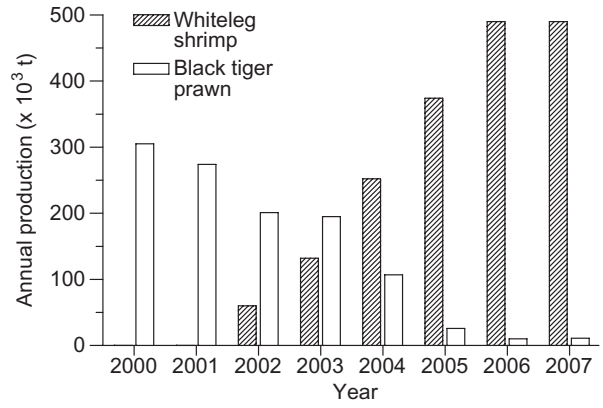


Fig. 1.14 Decline in annual production of black tiger (*Penaeus monodon*) and growth of whiteleg shrimp (*Litopenaeus vannamei*) production in Thailand from 2000 to 2007.

expand production and to improve processing infrastructure; providing technical training; and involving universities and other research organisations.

Post-harvest processing of the catfish is to international standards (Fig. 1.12) and production is mainly for export to a large number (ca. 80) and wide range of countries, to the chagrin of some local fishing and aquaculture industries. Vietnam has been accused of dumping in the USA and the catfish industry lobbied for a 39% tariff on pangasid catfish imports. The Vietnamese names 'basa' (*Pangasia bocourti*), and 'tra' (another commonly cultured species, *P. hypophthalmus*) (Fig. 1.11) are not good marketing names in some overseas countries (although used). Thus, a variety of other, more attractive, marketing names have been used, including 'sea sole', 'grouper', 'cream dory', 'white river cobbler' and 'white Mekong grouper'. Although the bulk of production is tra, it is regarded as having lesser flesh quality than basa. The latter attracts a high price and is sometimes given a different name where the market discriminates.

1.8.2 Whiteleg shrimp

In the initial decades of marine shrimp farming, the black tiger prawn (or giant tiger prawn), *Penaeus monodon*, was the preferred species[3]. In 2000, global production of *P. monodon* was 623194 t. Global production of whiteleg shrimp, *Litopenaeus vannamei*, was only 2310 t in that year. The relative amounts of these two species have since changed dramatically to 560704 and 1884061 t, respectively. Thailand, the major global producer of marine shrimp, is a case in point. The whiteleg shrimp was introduced into Thailand and other countries of Asia in the late 1990s. Thailand did not record production of any whiteleg shrimp until 2002 and then its production expanded while that of the black tiger declined precipitously (Fig. 1.14). Some advantages of *L. vannamei* are outlined in section 21.2.3.

[3]Members of the decapod family Penaeidae are general called 'shrimp' and they are identified as 'marine shrimp' in this book (Chapter 21); but, confusingly, the official name of some species, i.e. in statistical records, is 'prawn'.

1.9 CONCLUSIONS

Aquaculture continues to grow in importance for seafood production while fisheries production shows little change. Aquaculture's increase as a percentage of total seafood production seems to be exponential and it seems inevitable that it will become the major source of seafood in the not too distant future. In terms of growth in animal production, which is particularly important as a direct food source ('food fish'), there has been most increase in production of freshwater species. This growth is mainly in the low-value fish species such as carp and related species. There will continue to be most growth in the developing countries where the political and cultural environments are more conducive to new and expanding ventures in aquaculture. As well as future expansion in quantity and value of aquaculture products, it is likely that significant new industries will develop based on new species. The very

rapid developments of pangasid aquaculture in Vietnam and of whiteleg shrimp aquaculture in Thailand are examples of the potential for new industries.

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2
General Principles

Peter Appleford, John S. Lucas and Paul C. Southgate

2.1 INTRODUCTION

2.1.1 General aspects

A large number of aquaculture practices are not treated systematically in any other chapter of this book and hence they are considered here. There are differences in the structures used, the intensities of culture, the degree of water exchange and the factors to be considered in selecting suitable species and farm sites for aquaculture. To a considerable extent they are inter-related (Fig. 2.1). Choosing a site for an aquaculture venture will be strongly influenced by, among other things, the intensity of culture, the amount of water exchange required and the biological characteristics of the selected species (e.g. life cycle stages, diet, growth rate). The method of culture will depend on the availability and characteristics of farm sites. Culture structures, site and species selected for aquaculture will depend on the economics of the venture. The aquaculture venture will fail, despite having a species that is very suitable for culture and good technology on a good farm site, if there is insufficient demand for the product and if it cannot be sold profitably (Chapter 12).

Many aquaculture ventures have failed because at least one of these inter-related components has not been properly considered before undertaking the venture.

2.1.2 Aquaculture systems

Owing to the great diversity of aquaculture operations, the description of types of aquaculture systems may be complex and sometimes confusing to the novice. Usually culture systems are classified according to three criteria:

1. Type of culture structure. Culture structure describes what encloses or supports the aquaculture organisms. Broadly, aquaculture structures include ponds, tanks, raceways, cages, pens, racks, long-lines and floats.
2. Water exchange. Water exchange describes the amount of water exchanged or the control over water flow to the system. Broadly, the levels of water exchange are static, open, semi-closed and recirculating (closed).
3. Intensity of culture. Intensity of culture reflects the number of aquaculture organisms per unit area or water volume and also the ability of the natural productivity to support the crop. Broadly, the intensity of culture is described as intensive, semi-intensive or extensive.

These criteria will be described in more detail in sections 2.2, 2.3 and 2.4.

The type of system used for aquaculture production is a combination of the above criteria. For example, there may be a pond system that is:

- extensive and static: to grow major carp in ponds in China and India;
- semi-intensive and semi-closed: to grow silver perch in ponds in Australia;
- semi-closed and intensive: to grow shrimp in ponds in Asia and the Americas;
- open and intensive: to grow Atlantic salmon in seacages in Norway and Canada.

2.2 STRUCTURES USED FOR AQUACULTURE

Although a particular kind of structure will be considered under each heading, one structure is rarely used for the whole lifespan of a cultured species, except in some extensive culture. For instance, the intensive culture of a

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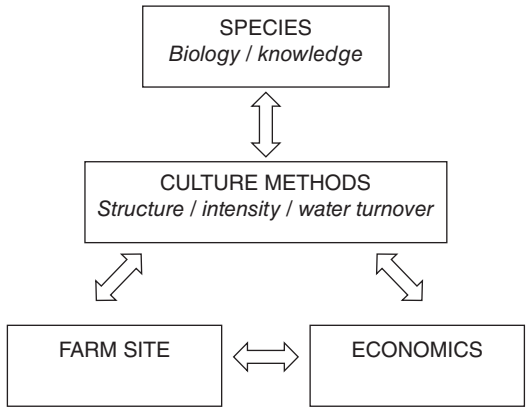


Fig. 2.1 The inter-relationships between cultured species, culture methods, farm site and economics in an aquaculture venture.

particular fish species from gametes to harvest may successively involve the following structures:

- a small fertilisation tank;
- an embryonic development tank with gentle flow-through of water;
- a larval tank with gentle circulation;
- a fingerling tank with gentle flow-through of water;
- a pond for growth of fingerlings to large juveniles;
- a cage for grow-out to harvest.

2.2.1 Ponds

Ponds are broadly defined as earthen impoundments for holding aquatic species. Ponds are the oldest aquaculture structure because of the simplicity of basic pond culture in freshwater. Pond culture can be undertaken with nothing more than convenient natural ponds. Purely harvesting from natural ponds is not aquaculture – it lacks the component of enhancing production; but enhancing production may involve nothing more than adding crude organic fertilisers or removing predators or competitors of the cultured species.

A pond may be a simple hole in the ground (sunken pond) or an enclosed waterway in a valley or stream bed where only one or two walls are constructed (barrage ponds), or it may be above ground (embankment pond). Embankments may also be used to divide a large freshwater or brackish water mass into adjacent subunits, e.g. ponds of varying shapes according to the natural topography. Embankment ponds may be any size or shape, but rectangular ponds are most common as they limit wasted space between ponds. Ideally, water input and discharge from ponds are facilitated by gravity to minimise construction and operating costs. Ponds are most commonly used for culture of fish and crustaceans.

Cheap simple ponds are the most widely used freshwater and brackish water aquaculture systems. The main requirements for ponds are:

- a reliable supply of good-quality water (preferably gravity fed);
- relatively impermeable soils for construction;
- well-structured soils with good organic matter content to support pond ecosystems;
- gravity drainage.

Ponds in general are cheaper to construct per unit area than tanks and cages, and may be inexpensive to run, depending on pumping costs. Ponds tend to have the lowest stocking densities of the culture structures; however, density varies according to whether the system is extensive, semi-intensive or intensive. Ponds are generally maintained with a light algal bloom to minimise water exchange. More detail on pond culture techniques is given in sections 3.4 and 21.4.

2.2.2 Pond site requirements

Assessment of the soil is a very important consideration in aquaculture pond site selection, development and management. It is the soils at the site that will determine the natural productivity of the pond, water-holding capacity, fertilisation requirements, water quality and construction.

Poor site selection will result in ponds that cannot be managed to suit the requirements of the cultured animals or that require an unacceptable level of inputs and maintenance to do so. The main soil properties important for aquaculture use are of three types:

1. Physical: texture, strength, stability and water-holding ability.
2. Physicochemical: ion exchange capacity, acid–alkaline reaction, leaching effects and absorptive/binding capacity.
3. Biological: organic matter and nutrient transforming biomass.

More information on the properties of soil is available from texts on aquaculture pond construction (e.g. Boyd, 1991; Yoo and Boyd, 1994).

In some circumstances, particularly with small ponds and in areas where the soil is porous, the ponds may be



Fig. 2.2 Two series of ponds beside a central drainage channel at the marine shrimp farm of National Prawn Company (NPC), Saudi Arabia. The structure in the lower left-hand of the photograph is the upper rim of a large bin for storing feed pellets. (Photograph by courtesy of NPC, Saudi Arabia.)

lined with impermeable sheeting, e.g. thick plastic sheeting. Soil substrates may then be added to these lined ponds to provide the functions of soil.

2.2.3 Pond layout

In large pond farms, there is a carefully planned layout of ponds and embankments to include channels between ponds for inflow of water and outflow of effluent water. There may be tens to hundreds of ponds on a large farm, with the surface area of each pond ranging from ca. 0.5 to 10 ha (e.g. Figs 2.2, 17.2 and 21.7).

Ponds are generally constructed in one of four main configurations or layouts, each with its advantages and disadvantages:

1. Series. Ponds are constructed consecutively so that water flows through each pond prior to its discharge. This layout allows easy movement of stock from one pond to another and maximal use of water. A disadvantage of constructing ponds in series is the decrease in water quality through consecutive ponds and the inability to isolate individual ponds, preventing the isolation of disease.
2. Parallel. In a parallel pond layout, water is distributed from a common supply channel into each pond individually. Effluent water is collected into a common drainage channel. Stock movement is more difficult and water usage increases; however, this is offset by superior control over the water exchange for individual ponds and simpler water quality management.
3. Radial. Constructing ponds in a radial design is uncommon and is only employed when there is a specific production planning purpose. In a radial layout, ponds are constructed so that each inner pond feeds into multiple outer ponds or larger outer ponds in a concentric pattern. This pond layout may be employed when there is a continual supply of smaller animals (e.g. fingerlings) and, as the animals grow and require more space, they are moved to the outer ponds until they are eventually harvested.
4. Inset. In an inset pond layout, one pond is placed inside another larger pond. This method is used when a nursery pond(s) is set inside a grow-out pond.

2.2.4 Pond design

There are many pond designs, reflecting their range of uses. The most complicated pond design is one in which water must be delivered to the pond by pumping. This is usually because the pond is above the level of the water source. The following sections describe various components of ponds with pumping. The description is particularly applicable to shrimp ponds, but there are general

features that are applicable to all such ponds (Yoo and Boyd, 1994).

2.2.4.1 Size and shape

Ponds are usually rectangular to allow for the maximal utilisation of land. Typically, the length to width ratio is 2–3 : 1. The factors governing the size of the pond are the biological requirements of the animals and access to the pond for husbandry. For example, if a pond is to be harvested by seine netting then a recommended maximal width is 20 m. Similarly 20 m is about the maximum reach of many feed dispensers. If, however, the cultured animals come to the edge to feed and can be harvested using a collection sump, the pond may be much larger. A disadvantage of larger ponds with greater biomass and higher feed inputs is that they may be more difficult to manage.

Probably the greatest disadvantage of a rectangular pond is poor water circulation. Circular ponds have been trailed for high-value intensive crops with good results; however, the trade-off is less pondage per unit farm area (Wyban and Sweeney, 1989).

2.2.4.2 Walls

Wall design requires consideration of both wall height and wall slope. Wall height is governed by the depth of the pond, wave height and freeboard. Freeboard is the additional height above normal water and wave height to allow for extreme storm events resulting in raised water levels or greater wave height. Depth of ponds is generally in the range of 0.8 to 1.8 m. These depths allow adequate light penetration for primary productivity and enough depth to reduce temperature fluctuations while decreasing the chance of both oxygen and temperature stratification.

The photosynthetically productive depths of ponds are limited by the penetration of light through the water and, consequently, ponds are generally shallow compared with their surface area. This is because, unlike smaller culture systems, in which aeration can be used to promote gas exchange, and, unlike systems with continuous water flow, the pond water mass depends on gas exchange at the surface for both oxygen uptake and carbon dioxide removal at night. Gas exchange and diffusion are slow processes and it is possible to have strong stratification of dissolved oxygen through the pond water column. This stratification increases with water depth and is therefore a limiting factor on water depth, despite some aeration systems for ponds (section 3.4.3).

Sunlight penetration can also warm the water, creating higher temperatures at lower pond depths. If a pond is too deep, warming will occur to a certain depth. This may create a thermocline with water below that depth substantially cooler than water above that depth. The creation of thermoclines can result in anoxic, cold water layers that can invert and result in substantial stock losses.

Settlement of the wall must also be considered when constructing the pond. Settlement generally varies from 5% to 10%, depending on the soil type used for construction. It is recommended that 10% additional height be allowed to take account of settlement (Yoo and Boyd, 1994).

Wall slope depends on the stability of the soil at the site, the erosion protection and what is deemed as acceptable maintenance. Working with stable soils to achieve minimal erosion, an internal wall slope of between 1 : 2 (vertical–horizontal) and 1 : 3 is recommended, and the external wall slope should be between 1 : 2.5 and 1 : 3.5. In practice, both slopes are often decreased to ca. 1 : 1 (internal) and 1 : 2 (external) to reduce the capital cost of construction. This also increases maintenance costs. If erosion protection is included, e.g. vegetation above the water line and stone below the water line, slopes can be reduced further. Wall reinforcing can allow the use of vertical walls, although this is more expensive.

Walls must also have a top width to allow appropriate access to the pond. Top widths depend on the height of the wall and the activity to be conducted around the pond. Recommended top widths (Yoo and Boyd, 1994) are related to wall height, but generally do not consider the activity around the pond. Farm machinery is often used around the pond during normal operation, and these vehicles will require a top width greater than that recommended.

2.2.4.3 Floors

The main considerations in pond floor design are pond drainage and harvesting. If a pond is to be drained to collect culture stock, the bottom must be smooth to prevent water collecting in hollows. A bottom gradient of 0.3% to 0.6% is used to effectively drain water to the end of the pond or to a central drain (Wang and Fast, 1991). Additional channels (30–50 cm wide and 10–20 cm deep) may be cut to facilitate drainage. At the lower end of the drainage system there may be a harvest sump, usually about 10–20 cm deep and occupying 0.5–1.0% of the total pond area.

2.2.4.4 Water supply (inlets) and drainage system (outlets)

Inlets and outlets are important components of ponds and need to be secure, easy to operate and adequately sized. The exchange of water through a pond is controlled via

the inlet. Inlets may be pipes, channels or sluices (an artificial channel for conducting water). Flow through the inlets can be regulated using valves (pipes) or boards (channels and sluices) or by the use of pumps.

In many ponds, water must be filtered to prevent the inclusion of organisms that may be predators, competitors or vectors for disease. To do this, the water is screened using either a simple nylon sock or bag over a pipe or a screen/filter box in the channel or sluice. Socks and screens must be sized appropriately to remove anticipated predators (section 21.4.5, Fig. 21.9).

Outlets serve a number of functions and are used to:

- adjust and hold the water level;
- drain or partially drain the pond;
- drain water from specific levels of the pond;
- provide an overflow in a continually flowing pond;
- screen and collect culture stock.

Outlets are usually pipes or weir gates, which are also known as 'monks'. Pipes are cheap, simple and convenient for small ponds; however, they are not adequate for larger ponds. Weir gates are vertical control boxes attached to waste pipes or channels (Fig. 2.3). They are a combination of screens to regulate animal movements, boards to regulate water level and flow, and pipes or channels to move the water from the pond. Landau (1992) and Yoo and Boyd (1994) provide detailed descriptions of pond outlets.



Fig. 2.3 A weir gate or 'monk' controlling effluent water from a shrimp culture pond in Saudi Arabia. Grooves in the concrete walls hold screens to regulate animal movements (at rear) and boards (at front) that regulate water level and flow. Water passing through the screens and over the boards is drained away from the pond in a channel. (Photograph by courtesy of Nasser Ayaril.)

2.2.5 Tanks and raceways

Tanks are second to ponds as the most commonly used structures for aquaculture. Tanks are generally situated above ground on a solid base and may be used indoors or outdoors. Tanks have the advantage of allowing the use of land normally unsuitable for aquaculture, as the water is contained within the structures with no contact to the surrounding soils. There is a wide range of dimensions and sizes of tanks, corresponding to their wide range of uses in culturing microalgae, macroalgae and various life cycle stages of fish and invertebrates. They range in size from tens of litres to hundreds of cubic metres.

Raceways are basically elongated tanks in which water enters at one end and exits at the other. They generally consist of elongate, narrow and shallow systems with continuous water flow. The limited cross-section of the raceway together with the strong flow rate are designed to keep continuous unidirectional flow along the raceway. It is a particularly suitable system for some fish, such as salmonids, that live in shallow streams and swim against the current (Fig. 2.4). Raceways generally require large amounts of water per unit volume of the system. Freshwater raceways, the most common form, need to be sited near a freshwater spring or a substantial stream that does not dry up. As with other aquaculture, they need an unpolluted source of water. A potential problem with raceways is the deterioration of water quality along their length.

Tanks are most commonly used for culturing the early developmental stages of fish, bivalves and crustaceans, and for culturing high-value fish species. Raceways are also used for culturing high-value fish. As with ponds, there is a variety of tank systems ranging from flow-through systems, in which the tank system is simply a confinement for the animals, to recirculating tank systems, in which the water is used, treated and re-used while maintaining a high density of animals. The majority of raceways are flow-through.

2.2.5.1 Structure

Tanks and raceways may be constructed from a variety of materials but are most commonly constructed from concrete or synthetics, such as fibreglass. The major considerations in material selection are:

- strength;
- abrasiveness to the animals;



Fig. 2.4 Concrete raceways for culturing rainbow trout in Iran. (Photograph by courtesy of Dr Rohana Subasinghe.)

- cost;
- possible chemical residues;
- shape.

The shape must allow good water flow to maintain uniform water quality and prevent build-up of wastes in 'dead' places. The shape of tanks varies greatly from hemispherical to flat-bottom and square. Circulation and self-cleaning ability of tanks is a function of shape, depth and water exchange. Tanks that are circular in the horizontal plane with conical bottoms are generally considered to provide the best circulation and self-cleaning properties, for the lowest flow or exchange rates. The flow of water in raceways, being linear from inlet to outlet, facilitates cleaning and more uniform water quality. In abalone culture flat-bottomed, rectangular shallow tanks are combined with high exchange rates.

2.2.5.2 Inlets and outlets

As discussed for ponds, the major function of the inlet for tanks and raceways is to regulate water exchange. Generally, water flow into tanks is regulated using a valve, whereas flow to raceways may be regulated by either valves or sluices.

Inlets in tanks or raceways, however, must also provide water movement. The delivery of water into the tank or raceway must be such that it facilitates an even movement of water throughout the structure, maintaining uniform water quality. In raceways this is often achieved by delivering water at multiple points along the input end of the raceway either through perforated pipes or over spillways. Maintaining uniform water quality may also be assisted by having multiple inlets along the length of the raceway.

Water in tanks is generally supplied through pipes and the number of input points, combined with directional inlet(s), will govern water movement and assist with aeration in some systems.

The functions of outlets from tanks and raceways include:

- maintaining water level;
- retaining cultured animals;
- allowing drainage of the structure;
- removal of wastes.

Outlets for tanks usually consist of a vertical or horizontal pipe, a screen to retain stock (or a collection trap for stock) and pipes to direct water either back to a sump or to waste. However, given the diversity of tank systems and the flow design for these systems, there are many outlet designs.

2.2.6 Cages

Originally, the cages used for aquaculture consisted of poles or stakes driven into the sediment of shallow lakes or bays with netting stretched around them. These are still in use and are referred to as net pens or hapas (section 16.6.4). Modern cages are floating structures with a net



Fig. 2.5 Cage farming tilapia in the Philippines. Note that drums are used as floatation for the cages. (Photograph by courtesy of Dr Rohana Subasinghe.)

suspended below. They may be square, rectangular (Fig. 2.5) or round (Figs 2.6 and 26.9). Floating cages may be small and of limited strength or they may be many thousands of cubic metres in volume and designed for use in the open ocean (Figs 1.6 and 18.17). Cages are used for fish culture in their grow-out phase, that is the months or years up to their market size (Table 18.1).

Cages are intermediate to tanks and ponds in capital cost, and are relatively cheap to operate, requiring maintenance but no pumping. Cages located in open systems allow no control over water quality and therefore require a good site with adequate exchange of high-quality water. If sited correctly, intensive farming at stocking densities up to 15–40 kg/m^3 can be achieved. Being in the ambient water, cages have problems with:

- fouling of the meshes by species such as weeds, bivalves and sponges, which reduce the flow of water through the cage;
- predators, e.g. seals, birds;
- parasites;
- diseases, which are difficult to manage, as cultured stock are difficult to observe and diseases are not easily treated except with medicated feeds;
- algal blooms.

Cages are typically sited in protected inshore ocean locations, such as bays, lochs and fjords. Cages may, however, also be used in large freshwater lakes and ponds

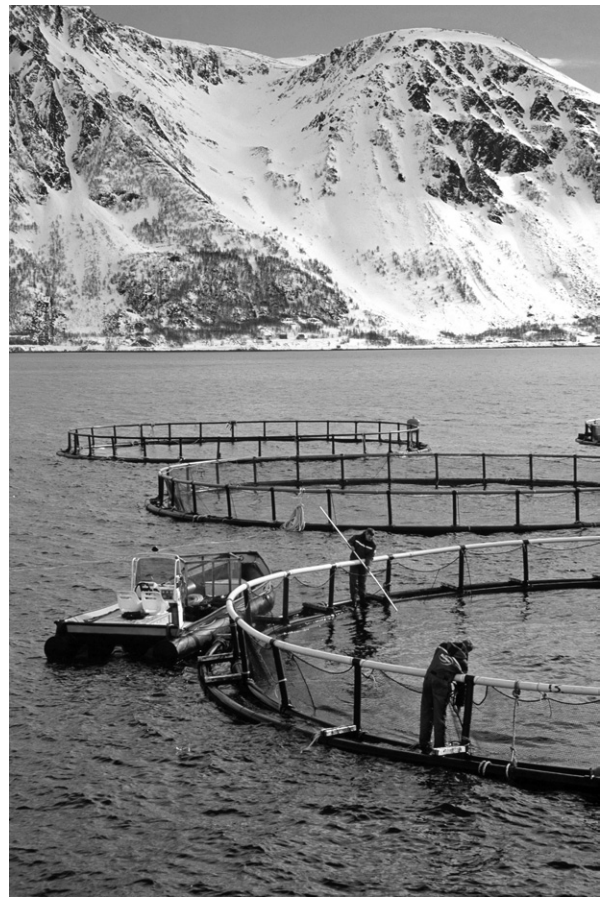


Fig. 2.6 Large circular cages for Atlantic salmon farming in a fjord in Norway. (Photograph by courtesy of Dr Rohana Subasinghe.)

(Fig. 14.5). Seacages are generally used in groups, either individually or linked together, and anchored to the substrate. In these groups, they can be serviced and the fish fed and managed from a base facility on the adjacent shore or from a proximate floating facility.

Cages may be used in combination with ponds, where the cage is used to contain the culture species and the pond is used to manage water quality. This technology has now progressed to the use of 'floating raceways' where the cultured species is maintained in a raceway floating within a larger water body that provides for water quality management. These types of systems are being used in 'irrigation' districts where water previously stored in large reservoirs solely to irrigate crops now has multiple uses through fish culture. This has resulted in increased water

use efficiency per unit of water through horticultural and aquaculture production.

The design of cages varies depending upon their use and location.

1. Ponds. These cages are small (approximately 4×6 m and 1.5 m deep) and are secured to stakes driven into the bottom of shallow ponds. The cages are located within a very 'friendly' environment and are accessed easily from the pond wall or a floating walkway.
2. Lakes, estuaries or protected bays. These cages tend to be much larger (10–40 m diameter) and are secured to the bottom of the waterway by anchoring lines. The forces associated with these environments are greater than those found in ponds as a result of tidal currents, wind-generated waves (<1 m) and swells. Again, these cages are easily accessed from shore or by a short trip in a small powerboat.
3. Open seas. These cages must undergo the rigours of oceanic swells and wind-driven waves in excess of 4 m. The cages are very large, and may be 50 m or more in diameter. Often located far offshore, these cages require attached working areas.

Irrespective of the use of the cage, there are structures that are common features. The aims of these structures are to hold and protect culture stock, and to maintain the position, size and shape of the cage.

1. Float. The float keeps the cage at the surface of the water and helps maintain the shape of the cage in the vertical and horizontal planes. Floats may be large-diameter rubber hose or high-density polyethylene tubes or pipes.
2. Collar. The collar maintains the shape of the cage in the horizontal plane. It may simply be a ring of metal placed at the bottom of the cage to weigh the cage down in the required shape or it can be a complicated design involving flotation and weights.
3. Nets. There are several types of nets for cages.
   (a) Main net. This net holds the cultured stock. It must resist ripping by objects and predators, and be able to hold the biomass when nets are lifted for harvest. The mesh must be designed to prevent cultured fish being caught in the netting. Besides holding the fish, this net must also permit adequate water flow through the cages to maintain water quality.
   (b) Predator net. This net keeps sea-borne predators away from the cultured stock to reduce predation. It is placed outside the main net.
   (c) Covering net. This net is placed over the top of the cages to prevent birds landing, fouling, scavenging and preying on cultured stock.
   (d) Jump net. This net projects vertically out of the water around the main net, preventing fish escaping.
4. Moorings. These are used to secure the cage at the selected site. Moorings are often specific to the type of cage and for larger cages are generally specified by the manufacturers.

Beveridge (1996) provides further details on cages and cage culture.

2.2.7 Pens

Pens, enclosures or hapas are used in shallow water, typically in ponds, to create a restricted environment for culture of fish and some crustaceans. They are not usually large, being in the order of tens of square metres or less. The walls of the enclosures may be closely spaced stakes, such as bamboo stems or mangrove branches, or wire and other mesh. This system of culture is practised mainly in developing countries. One interesting exception to the shallow water pen is the use of mesh fences or walls to enclose bottom-dwelling scallops. These pens are of sufficient height to prevent the scallops from swimming over the wall. They may use floats to allow the mesh to rise and fall with the tide (Quayle and Newkirk, 1989). Similar enclosures may also be used for culture of gastropod molluscs.

2.2.8 Substrates, racks and suspended culture

Surfaces are used, as would be expected, for culture of bottom-dwelling and attached species. This category is taken to apply to species that are grown in the field for most of their culture, rather than in tanks with artificial surfaces, e.g. abalone culture that occurs totally in tanks.

The techniques used in this category are quite varied, reflecting the diverse range of organisms that are cultured and their varying environmental requirements.

Some bivalves attach permanently to hard substrates. Their larvae settle and metamorphose on attractive substrates, which are put out in the field at appropriate places and times to obtain juveniles. The juveniles may then be grown on the original substrate or are removed to another culture system. The secondary culture systems may be:

- mesh trays on horizontal racks above the substrate in the intertidal zone;



Fig. 2.7 Raft culture of blue mussels (*Mytilus edulis*) on Loch Etive, Scotland.

- suspension in/on various kinds of nets, ropes, trays and baskets, hanging down at intervals from a horizontal long-line on the surface;
- suspension on vertical ropes below rafts (Fig. 2.7).

More on these techniques and their application to bivalve mollusc culture can be found in Chapter 23.

Other benthic organisms, e.g. seaweeds and sponges, may be grown tied to lines stretched above the substrate.

2.3 INTENSITY OF AQUACULTURE

Intensity of aquaculture describes the various densities of organisms per unit volume or per unit area. It is meaningful in comparisons between the levels of culture of a species or related species. It is, however, meaningless in terms of comparisons of densities of organisms from different groups. For instance, culture of tilapia at 100 kg/m^3 of water in a recirculating system is considered to be intensive culture; culture of shrimp at 50 individuals per m^2 (1–2 kg/m^3) in ponds is considered to be intensive culture. There are, however, characteristics of different intensities of culture that are not specific to groups, and the consideration of culture intensity will be based on these.

Intensity of culture will broadly consider the inputs into the system to maintain adequate growth of the cultured organisms. It comes under the term 'intensity' because the greater the intensity (or density) of cultured organisms the greater the requirement for inputs into the system.

2.3.1 Natural aquatic ecosystems

All ecosystems, natural and artificial, need some form of energy input to sustain them. Even if there is perfect recycling of organic matter, there will be loss of energy through metabolism and that energy must be replaced.

Natural aquatic ecosystems typically consist of primary producers, various levels of consumers (primary, secondary, etc.) and decomposers. They are self-supporting with recycling of nutrients and input of energy from the sun. They are characterised by long and complex food webs. There are very large energy losses in this food chain. Energy transfer from one level to another in the food chain is in the order of 10% as a 'rule of thumb'. The following is a hypothetical example of six levels in a food chain:

1. Sun's energy + inorganic nutrients →
2. Phytoplankton (e.g. flagellates and diatoms) →
3. Zooplankton and filter feeders (e.g. copepods, invertebrate larvae, bivalves) →
4. Larger zooplankton (e.g. fish larvae, planktivorous fish) →
5. Juvenile fish →
6. Large carnivorous fish.

Thus, in the example consisting of six levels in a food chain: for levels (1) to (6):

(1) 100% → (2) 10% → (3) 1% → (4) 0.1% → (5) 0.01% → (6) 0.001%

This is a very crude approximation, but it is clear that the final stage of this food chain, for example the large carnivorous fish, has magnitudes less energy (and food) available to it than lower levels in the food chain. The energy lost at each step (approximately 90%) is lost through:

- metabolism (the metabolic energy required to keep the organism functioning, e.g. locomotion, feeding, digestion of food, circulation of body fluids, etc.);

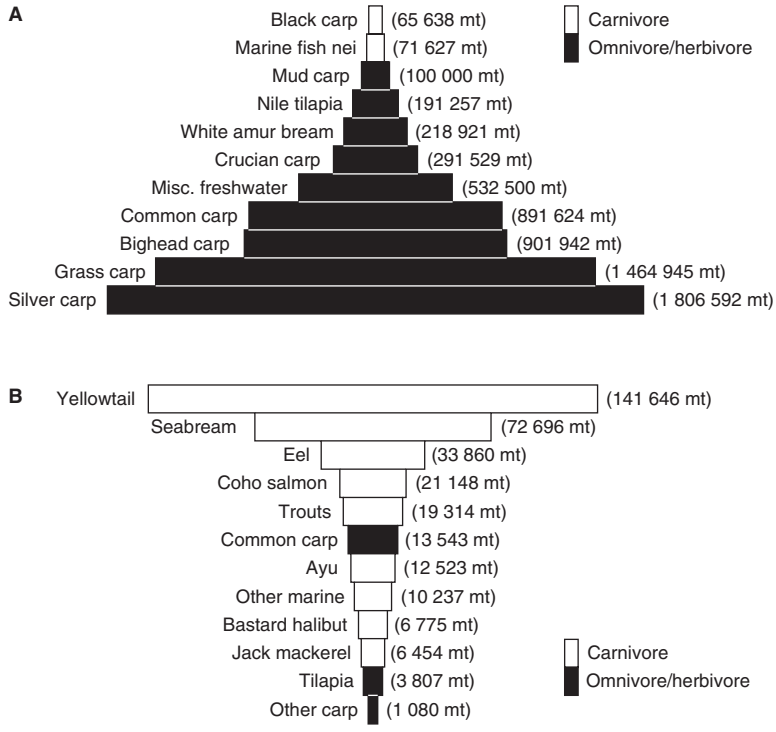


Fig. 2.8 Production pyramid of major farmed fish according to feeding type. **A.** China in 1993; **B.** Japan in 1993. The widths of the bars are relative, not absolute, production. Based on data from FAO (1995), modified from Tacon (1996).

- metabolic wastes excreted and faeces released by the organism;
- energy expenditure on reproduction;
- energy released into the environment as heat, because metabolic processes are not 100% efficient.

These substantial declines in energy with each step in the food chain have an obvious implication for aquaculture; it is more efficient to culture primary producers or animals that feed at a low stage in the food chain. This is an important factor in developing nations, where aquaculture products are valued as a major source of animal protein and are cultured for food security rather than export trade. With the exception of aquaculture of species such as shrimp, which are directed at international markets, most aquaculture in developing countries is derived from extensive or semi-intensive culture based on species that feed low in the food chain (Fig. 2.8A). Not only is it inefficient to produce carnivorous species through a multilevel food chain, but productivity will be very poor per unit area or volume from such extensive or semi-intensive systems.

In many developed nations, however, the demand is for aquaculture products that are high in the food chain. In fact, the largest demand is for top or near-top carnivorous fish and shrimp, as these are regarded as the 'quality' products (Fig. 2.8B). These carnivores must be fed high-protein feed, typically with fish meal as a major source of protein. Thus, one kind of less valuable seafood is used to produce a more valuable seafood, with the consequent loss of energy.

2.3.2 Intensive aquaculture systems

In intensive aquaculture systems all the nutrition for the culture stock comes from introduced feeds, with no utilisation of natural diets. Intensive systems may be in:

- ponds (e.g. for shrimp in tropical/subtropical regions);
- cages (e.g. for marine fish culture in temperate waters) (Fig. 2.9);
- raceways (e.g. for trout species in temperate regions);
- tanks (e.g. for eels in Japan).



Fig. 2.9 Underwater view of a seacage used for culturing Atlantic salmon (*Salmo salar*) in Norway. Note the two figures standing on the floating ring of the cage. (Photograph by Norway Marine Research, courtesy of Dr Rohana Subasinghe.)

The peak stocking density achieved in each case depends upon being able to maintain the water quality conditions required by the cultured organism. Generally, stocking densities are lowest in ponds, followed by cages and with greatest densities achieved for raceways and tanks.

Intensive aquaculture systems are a complete contrast to natural systems. They are characterised by:

- very simple food chains: feed → cultured organisms;
- low energy losses from feed input, with high food conversion ratios from specialised artificial feeds;
- no recycling of energy and totally non-self-supporting;
- the requirement for high inputs of energy (e.g. feed, nutrients, aeration, filtration, pumping);
- high yields per unit area or volume.

Water quality is usually maintained by high water exchange rates and, in some cases, by mechanical means. In intensive culture in indoor tanks, particulate waste removal, gas exchange and oxygen production are all undertaken by mechanical means. In outdoor intensive systems with a soil substrate and phytoplankton there is settlement of particulate wastes, decomposition by bacteria and gas exchange enhanced by mechanical aeration. Stocking density (mass of culture stock per volume or area of water, expressed as kg/m^3 or kg/ha) in intensive systems varies greatly with the type of system and the cultured organism, but is always relatively high.

2.3.3 Extensive aquaculture systems

Intensive and extensive aquaculture systems are contrasted in Table 2.1. Extensive aquaculture differs markedly, largely being part of a natural ecosystem and depending upon it for maintenance of water quality and most of the animals' food and other requirements. An extensive aquaculture system, therefore, has limited inputs to maintain animal growth and survival, i.e. it may have some basic organic fertilisers, such as animal and plant wastes, but no aeration, etc. These systems usually have a low stocking density, generally <500 kg/ha, and the natural productivity of feed (plants and animals) within the system and natural gas exchange is sufficient to support the cultured organisms. Extensive aquaculture is used for pond culture and for organisms grown on or in various substrates, e.g. bivalve and seaweed culture. Seaweed culture differs, of course, from animal culture in terms of food requirements, but the seaweeds are dependent on the culture environment for inorganic nutrients.

Apart from seaweeds and bivalves, a considerable amount of extensive aquaculture is of low-value fish, such as carps and tilapia. This is possible because of the low costs of production associated with extensive culture. Furthermore, the production statistics per country and species show that this is the major source of aquaculture production in the world.

Extensive versus intensive aquaculture is rather like the difference between

Table 2.1 Comparison of the general characteristics of extensive and intensive aquaculture systems.

	Extensive aquaculture	Intensive aquaculture
Establishment costs	Low	High
Culture systems used	Natural water bodies and simple containment structures	Fabricated culture systems, including tanks, pond systems, raceways, seacages, etc.
Technology level	Low	High
Degree of control over the environment, nutrition, predators, competition and disease agents	Very low	High
Typical source of seed stock (plant or animal)	From nature	From domesticated broodstock (possibly genetically selected)
Operating costs, stocking rates and production levels	Low	High
Dependence on local climate and water quality	High, may be some crude adjustment of pH with lime and related substances	Low to very low
Monitoring of water quality	Nil	Undertaken regularly
Food source for cultured animals	Natural food organisms; often some input of animal and plant wastes	Pelletised, fabricated feeds, which must be nutritionally complete
Production (kg/ha/year)	Low (per unit area or volume) (20–500)	High: maximising output of product in minimum surface area, water volume and time (5 000–100 000+)[1]
Production costs	Low to very low	High to very high

[1]The highest values are extrapolations from square metres.

- feed-lot cattle, which are totally dependent on the feed-lot environment and the feed provided (intensive culture); and
- free-range cattle roaming through natural vegetation on a large cattle station or ranch, feeding on natural pasture and with minimal husbandry (extensive culture).

In each case there is a harvest of beef, but the harvest per hectare is magnitudes higher from the feed-lot system. None the less, both systems are profitable. Profitability depends on the difference between total cost of production and total value of product.

2.3.4 Semi-intensive aquaculture systems

There is no abrupt cut-off point between extensive and intensive aquaculture (Table 2.2). Semi-intensive aquaculture is used as an approximation to describe the middle ground. Semi-intensive aquaculture systems rely to an extent on natural productivity; however, there is more supplementation of the natural system. Supplementation may take many forms, including:

- addition of aeration to maintain dissolved oxygen levels;
- addition of inorganic or organic fertilisers to improve natural productivity;
- addition of prepared feeds (supplemental feeding).

Semi-intensive culture is almost exclusive to ponds and allows for an increase in the stocking density within the pond.

Further comparisons of the three levels of culture intensity are given in Tables 16.2 and 21.2.

2.3.5 Polyculture

In some semi-intensive and extensive culture systems, such as ponds, the target species will share the environment with other related species, e.g. a target fish with other fish or a target shrimp with crabs. Depending on the degree of niche overlap, these other species may compete with the target species for food, habitat, etc. On the other hand, they may facilitate its growth and health through supporting activities, e.g. a detritivore removing benthic waste

Table 2.2 Examples of extensive, semi-intensive and intensive grow-out aquaculture showing the continuity between levels.

Harvest rate (kg/ha)	Examples of cultured animal and structure	Culture methods	Level of aquaculture
90	Freshwater crayfish, large-mouth bass – dam	Simple stocking	Extensive
>10 000	Marine mussels – raft/longline	No supplementary feeding, suspended in water currents to filter feed	Extensive
900	Carps, catfish, tilapia – pond	Some supplementary feeding and pond fertilisation, possible emergency aeration	Semi-intensive
6 000	Shrimp – pond	Almost complete diet, regular water exchange, aeration	Intensive
15 000	Sea bream, sea bass, Atlantic salmon – cage	Complete diet	Intensive
>60 000[1]	Eels, barramundi – battery tank systems in warehouse	Complete diet, recirculation and rigorous water quality control	Intensive

[1]Extrapolated.

from the environment before it creates anoxic conditions in the lower water column.

Polyculture describes the deliberate culture together of complementary species, i.e. species occupying complementary niches, especially in regard to their food and feeding (Fig. 2.10). Polyculture increases production per unit area or volume of the pond by maximising the utilisation of all nutritional niches within the pond. A greater proportion of the primary production is utilised as the target species are linked to more of the food webs than a single species, resulting in increased productivity. They also have the effect of reducing the complexity of the food webs because they 'cover' a high proportion of primary production at its sources with their feeding. In these ways, polyculture systems may average fish production of ca. 3 t/ha/year in ponds in which there is heavy addition of manure fertiliser (Billard, 1999). With supplemental feeding there may be yields up to 16 000 kg/ha/year. Carp polyculture is described in greater detail in section 14.5.2.

A new form of polyculture that is being researched is the use of effluent from, for example, marine shrimp ponds for the culture of complementary species. The effluent, rich in nutrients and sediment and potentially an environmental hazard, may be passed through further ponds containing animals or plants that extract the nutrients and sediment from the effluent. There may be bivalves, which filter the water of sediment, microalgae and some bacteria and macroalgae, which extract nutrients from the water, or even mangroves for the production of wood. The objective is not only to restore the quality of the effluent water, to the extent that it can be recycled into the aquaculture system, but also to produce a commercial crop from the effluent-treating organisms, e.g. using a bivalve of commercial value.

This developing form of polyculture differs from traditional polyculture in that the complementary species are isolated in different parts of the system.

Similar technology is also being utilized in some countries to 'upgrade' the quality of recycled water to increase its value as a resource in a cost-neutral fashion. Aquaculture recycled water, only previously usable for plantation watering, can be used for horticulture activities.

2.3.6 Integrated agri-aquaculture systems

Aquaculture was traditionally developed on an integrated basis, and much aquaculture in Asian countries still operates in this way. Integration involves:

- growing a variety of aquatic species in a single body of water;
- water re-use for successive aquaculture species or other crops;
- integration of aquaculture with other farm production or by-products.

Traditional integrated aquaculture usually occurs on a family farm in which there are plant crops, animal rearing

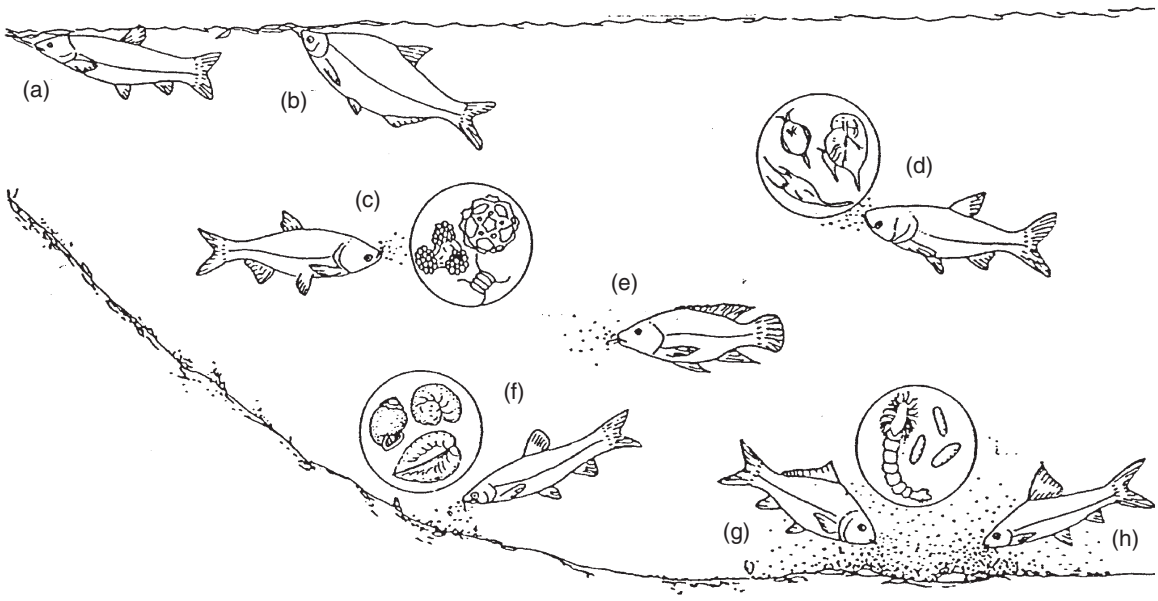


Fig. 2.10 Natural food resources utilised by major fish species cultivated in Chinese carp polyculture ponds. Grass carp (a) and wuchang fish (b) feed upon terrestrial vegetation and aquatic macrophytes; silver carp (c) graze upon phytoplankton; bighead carp (d) consume zooplankton; tilapias (e) feed upon both kinds of plankton, green fodders and benthic organic matter; black carp (f) feed on molluscs; and common carp (g) and mud carp (h) consume benthic invertebrates and bottom detritus. Reproduced from Zweig (1985) with permission from the Royal Swedish Academy of Sciences.

and aquaculture. Aquaculture is extensive and the cultured animals are low in the food web. Ponds are fertilised with faeces from pigs, ducks, etc., whatever is reared or used on the farm (Fig. 2.11). Wastes from crops, e.g. stubble from cereal crops, may be added to the ponds as food for benthic feeders and detritivores. Ponds are not drained but re-used for successive aquaculture crops. In some cases, the aquaculture animals (fish and freshwater crustaceans) are added to fields where the crop, typically rice, is grown partially submerged in water. The cultured animals feed on the plant waste materials and grow, and are harvested after the crop, when the field is drained. A double crop, plant and animal, is obtained from the field.

Modern aquaculture in developed countries has disintegrated aquaculture. The move has been towards developing monoculture systems for high-value species with single use of water. However, the limited availability of water and realisation of the global importance of water resources has led to a move towards aquaculture integration in countries where monoculture industries have developed.

Integrated agri-aquaculture systems (IAASs) have been variously defined (Cohen, 1997; Edwards, 1998). The basic premise of IAASs is the multiple use of water for both traditional terrestrial farming and aquaculture in a profitable and ecologically sustainable manner. As a result, integrated agri-aquaculture embraces a diversity of practices, systems and operations.

Water is one of the world's most precious and poorly utilised natural resources. Integration of farming practices to enhance productivity and water-use efficiency will contribute to the ecologically sustainable development of natural resources. IAASs allow for irrigated farming systems and the multiple use of the same water, typically for fish production first and then for irrigation.

Presently, IAASs most commonly occur in countries with very limited water resources. In many developing countries of the world and in Israel, IAASs are highly



Fig. 2.11 Fish (tilapia) culture integrated with pig production in Thailand. The pigs are in the shed on the left and their faeces are washed into the tilapia pond through pipes. (Photograph by courtesy of Dr Victor Suresh.)

developed and make optimal use of the available water. In Israel, fresh surface water and brackish groundwater have been utilised in integrated farming of a variety of fish species with a variety of land crops (Cohen, 1997). In Asian countries, fish, rice crops and ducks have been integrated to better utilise available water, land and nutrients (Huazhu *et al.*, 1994).

In developed countries, such as the USA, Australia and those in Europe, IAAS technology has generally been limited to small-scale systems linked to irrigation farming. Opportunities commonly include use of traditional farm water storage facilities (dams), irrigation channels, ground and surface water and inland saline groundwater. With advances in technology such as floating raceways, larger-scale systems are now being trialled.

2.4 STATIC, OPEN, SEMI-CLOSED AND RECIRCULATING (CLOSED) SYSTEMS

2.4.1 Static systems

As outlined in Chapter 1, much global aquaculture production uses traditional pond culture methods. These ponds are static, with no exchange of water during the culture period. There may be some topping up to offset evaporation.

Static pond culture is usually extensive because of major problems in maintaining water quality under conditions of a large biomass of cultured animals per unit volume of static water (Table 2.3). Increasing biomass requires increasing inputs of fertilisers and supplementary feeds to maintain productivity. This, in turn, requires management for such water quality problems as unacceptable levels of nitrogen compounds and low DO levels at night. With supplementary aeration it may be possible to maintain DO with a higher biomass and achieve greater productivity (section 2.4.4). Aerators are, however, often not available or feasible in rural regions where static pond culture is employed.

2.4.2 Open systems

In these systems the environment is the aquaculture farm, i.e. the culture organisms are confined or protected within the farm in a vast amount of water (e.g. a lake or an ocean) so that water quality is maintained by natural flows and processing. There is no artificial circulation of water through or within the system. The following are two examples of open systems:

1. Cage systems are classified as open systems when they are placed within a large body of water such as an ocean or an estuary. In these cage systems the .fish are generally at high density and artificial feed is supplied. Water quality is, however, maintained by natural currents and tides. Therefore, these are intensive open systems (Table 2.3).
2. Bivalve culture on racks or long-lines placed in the open water is an open system. Natural currents and

Table 2.3 Example of aquaculture in combinations of different levels of culture intensity together with open, static, semi-closed and recirculating (closed) systems.

	Culture intensity		
System	Extensive	Semi-intensive	Intensive
Open	Long-line culture of scallops, table oysters in mesh baskets	Enclosures within freshwater lakes for culturing fish, such as tilapia	Seacage culture of fish, such as sea bream, sea bass, Atlantic salmon
Static (ponds)	Carp polyculture in ponds , milkfish culture in lakes	Freshwater fish polyculture with aeration	Saltwater crocodiles
Semi-closed	Mud crab culture in tidal ponds in mangroves	Pond culture of shrimp at 20 animals/m^2	Pond culture of shrimp at ca. 50 animals per m^2
Closed	X	X	Indoor culture of high-value fish

X, very uncommon.

tides maintain water quality. The bivalves gain their food by filtering phytoplankton from the water flowing past. These are extensive open systems (Table 2.3).

Open systems tend to have low operating costs, as there is no requirement for pumping. Capital costs vary greatly depending on the type of culture, with bivalve culture systems generally of low cost and intensive fish cage culture systems of high cost. Generally, sites for open water culture are not available for freehold purchase and must be leased from the appropriate government agency (e.g. oyster leases). Ideal locations for open water culture are also often popular for other uses, such as boating, and user conflict can result.

Open systems are prone to problems that either do not apply to or are more difficult to mitigate than in other culture systems. A major problem associated with site selection for open systems is lack of control over water quality. The quality of water depends on local factors and cannot be moderated. It is therefore essential that the farmer is aware of all extremes of water quality occurring at the site, i.e. water temperature, salinity, algae blooms, etc., prior to developing an aquaculture facility. Seasonal variations in environmental factors may result in large variations in growth rates, and local differences in the environment may also cause major differences in growth and survival. Climate change has now introduced an additional variable to the location of new farms and the operation of existing farms. Historical water quality data, previously used to indicate the likely future water quality, may be of limited use. Scenario testing, i.e. potential changes in water quality that may occur with climate change, will be required to inform choices of farm location and management. Possible seawater rise with climate change may also impact on estuarine or delta-based industries. For example, the Vietnam catfish industry may have a more saline water source in the future.

Open systems are also more prone to predation. Predation can be controlled through the addition of some protective devices; however, methods of predator control in most countries must be non-destructive and are generally expensive to operate and maintain. Predation may also extend beyond wild animals to human interference or poaching. Restricting disease introduction to the system and management of disease outbreaks is more difficult in open systems; however, more intensive systems are most likely to have major disease events. Although interfering with aquaculture equipment and stock is an offence in most countries, enforcement is difficult, and the responsibility to protect equipment and stock often falls to the system operator.

2.4.3 Semi-closed systems

Semi-closed systems involve ponds, tanks and raceways where culture water is confined in discrete units. These systems fall distinctly between static and open systems in terms of water exchange with adjacent water sources. There is a degree of water exchange in semi-closed systems that is substantially greater than in static systems and much less than in open systems. Another major difference between semi-closed and open aquaculture systems is that the culture water is continuously or frequently brought to the farm. The water source may be freshwater, brackish or marine. Characteristics of freshwater and brackish water

Table 2.4 Freshwater sources for semi-closed culture.

Water source	Advantages	Potential problems
Lakes and reservoirs	Large volumes available	Susceptible to climactic changes and pollution Predators, competitors and pathogens may be present
Streams or shallow springs	High oxygen content	Highly variable supply and water quality as a result of climatic changes Susceptible to pollution Predators, competitors and pathogens may be present
Deep springs	Quite constant supply Sediment free	Low oxygen Supersaturation with N_2 gas Sulphides
Wells	Quite constant supply Sediment free	Yields difficult to predict High pumping costs May deplete groundwater Supersaturation with N_2 gas

Table 2.5 Brackishwater source for semi-closed culture.

Water source	Advantages	Potential problems
Estuary	Reliability May rely on tides for water exchange in small ponds	Seasonal variation in salinity Tidal affect on pumping period (if pump is sited above low-tide level) Variable suction lift with tidal range Susceptible to pollution Predators and competitors will be present Pathogens may be present High sediment load

sources are outlined in Tables 2.4 and 2.5 and aspects of marine water supply are outlined in section 2.7.

Water is drawn from a reliable source and flows to and through the farm, driven by gravity, tidal exchange or pumping. In these systems, water is exchanged to maintain water quality. As the farm is not located within a natural aquatic environment, there is a degree of control over water quality, but only to the extent that water flow can be increased, decreased or stopped. If the source of water becomes contaminated or of unacceptable quality, the farmer can only stop the water flow, leaving the stock in water with deteriorating quality. The benefits of semi-closed systems vary from:

- enhancing production from ponds by exchanging some water while maintaining some reliance on natural processes of the pond ecosystem, to
- complete reliance of water quality on water exchange (e.g. raceways) resulting in a large increase in production.

In the latter, water use per unit of production is extremely high. In these systems water may make a single or multiple passes through the culture structures.

If water is exchanged by pumping, the costs of pumping may be high, depending on the height water has to be pumped and the volume of water exchanged (section 2.5.1).

In large semi-closed systems with semi-intensive to intensive culture (Table 2.3), water flow is generally high to very high. It is generally recommended that water exchange is in the range 5–10% per day for semi-intensive ponds (Boyd, 1991) and up to 30–40% per day for intensive ponds (Yoo and Boyd, 1994). An exchange rate of 1% per day is equal to 100 m^3/ha per metre of average pond depth (Yoo and Boyd, 1994). That is, an exchange rate of 5% per day in a 1-ha pond of average depth 2 m requires a total of 1000 m^3 of water per day. In addition to this, water loss from evaporation and seepage must be consid-

Table 2.6 General characteristics, advantage and potential problems of recirculating aquaculture systems in indoor conditions.

Characteristics	Advantages	Potential problems and disadvantages
Limited water exchange outside system	Rigorous control is possible	High capital costs
Rigorous monitoring and control of crucial water quality parameters	Unaffected by weather	High running costs (pumping, filtration, maintenance, etc.)
High densities of culture animals	Predators and competitors are easily excluded	Requires very careful management (no buffering capacity)
High yield per unit volume and per unit surface area of water	Pathogens are less likely to infect	A pathogen outbreak will be severe
	Efficient feeding	
	Minimal environmental impacts	
	Requires minimal water	

ered. Boyd (1991) provides further details on determining exchange rates.

2.4.4 Recirculating (closed) systems

Recirculating systems are usually characterised by minimal connection with the ambient environment and the original water source (Table 2.6). These systems have minimal exchange of water during a production cycle, hence the description as 'closed' systems. Water is added to offset the effects of evaporation or incidental losses or, more frequently, to maintain water quality. Some water is discharged and replaced each day in most recirculating tank systems with intensive culture (Losordo, 1998a, 1998b). This arises from aspects of the regular maintenance system, such as removing accumulated solids from filters. Water quality in completely closed tank systems with intensive culture is much more difficult to maintain than in systems in which there is a regular 5% or more replacement per day (Losordo, 1998a, 1998b). Even with some limited water exchange each day, water quality within a recirculating tank system will only be maintained by artificial manipulation. Losordo (1998a, 1998b) and Losordo *et al.* (2001) reviewed these systems and their critical considerations, status and future.

The cost of construction and production in intensive recirculating tank systems has limited the commercial development of these systems for grow-out production. However, the possibility of high yields with year-round production close to markets drives their development. Some of the advantages and potential disadvantages of recirculating systems are outlined in Table 2.6.

The artificial means of waste processing and some typical components used in recirculating systems are shown in Fig. 2.12. Feed input, animal metabolism, wasted feed and faeces production all impact upon water quality. Parameters that require regulating in an intensive recirculating tank system are:

- particulate matter (settleable, suspended and fine waste solids) in the system resulting from feed and faeces;
- nitrogenous wastes (un-ionised ammonia, ionised ammonia, nitrite and nitrate, which are often expressed as NH_3-N, NH_4-N, NO_2-N and NO_3-N, respectively);
- dissolved gases (O_2, CO_2 and N_2);
- pathogens;
- pH;
- alkalinity.

For more detail on each of these aspects of water quality refer to Chapter 3.

At high stocking densities without recirculation technology, a water exchange in excess of 100% per hour would be required to maintain water quality during maximal production. This is an unsustainable amount.

In recirculating tank systems, water quality is maintained by pumping the culture water through specialised filtration and aeration equipment. The components of intensive recirculating tank systems are described below. Some further descriptions of the various components of these systems are provided by Losordo *et al.* (2001).

2.4.4.1 Waste solids filtration

The method used for removal of solid wastes depends on the type of waste. Settleable solids sink and may be removed by gravity and flow on a continuous basis or by siphoning on a regular basis. Alternatively, settleable solids can be kept in suspension with continuous agitation from aeration, water flow and stock movement, and removed using a mesh screen or allowed to flow into settlement basins or tanks. Settleable solids can also be

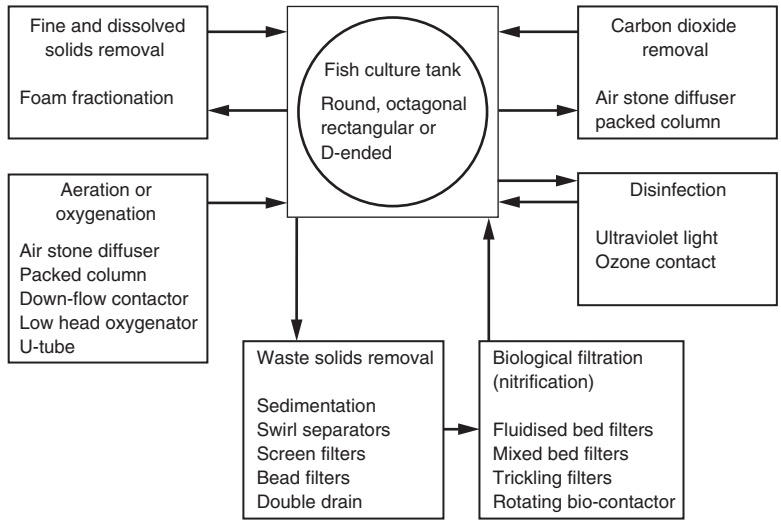


Fig. 2.12 The water treatment processes and typical components of a major recirculating system for intensive culture. Reproduced from Losordo *et al.* (2001) with permission of the World Aquaculture Society.

removed by centrifugal force using a swirl separator or hydrocyclone.

Suspended solids that will not settle out by gravity are generally removed from recirculating tank systems using the following mechanisms:

1. Screen filters are made of a fine mesh through which water flows and the suspended solids are retained on the screen. The screen filters are then periodically or continuously cleaned. Types of self-cleaning screen filters include rotating screen filters and rotating drum filters.
2. Particulate filters include sand filters and expandable granular media filters. Particulate filters also require cleaning, a process known as 'backwashing'.
3. Foam fractionators (or protein skimmers) are used to remove very fine suspended solids and dissolved organic compounds (DOCs) that cannot be removed by mechanical filtration in recirculation systems. Air is injected into the bottom of the vertical fractionator column, usually through a venturi system, forming fine bubbles which rise up to create a foam at the surface. This waste foam is then collected and removed. Foam fractionation works through the physical adsorption (bonding) of the DOCs to fine bubbles as they rise through the fractionator column. The efficiency of the removal of DOCs and fine suspended solids depends on the size of the air bubbles (the smaller the better) and contact time (the longer the better). Increased contact time is usually achieved with a counter-current design.

2.4.4.2 Ammonia-N and nitrite-N removal

There are numerous technologies available for removing ammonia-N from water (denitrification), including air stripping, ion exchange, biological filtration and removal by algae. Air stripping requires the water pH to be adjusted to 10 prior to stripping and then readjusted to culture levels near 7 prior to water re-entering the rearing tanks. Ion exchange technology is costly. For these reasons, biological filtration is still the most widely used method. Biological filters are available in a variety of forms including trickle filters, submerged filters, rotating biological contactors, packed tower filters and fluidised bed filters. The objective of biological filters is to optimise the surface area available for denitrifying bacterial attachment and flow through the filter across the bacteria.

2.4.4.3 Dissolved oxygen

Aeration is the dissolving of oxygen from the atmosphere into water. It is an important aspect of recirculating tank systems, as it is necessary to maintain dissolved oxygen at the required level for the cultured animals. It is also impor-

tant to maintain dissolved carbon dioxide below 20 mg/L to ensure favourable pH, reduce stress and maximise growth. Several types of aeration devices are commonly used in recirculating tank systems, including diffuser aerators, mechanical aerators, vertical pump aerators and packed column aerators.

Oxygenation is the process of pure oxygen transfer to water and is used when oxygen consumption is greater than the capacity to transfer oxygen through aeration using air (which is only 21% oxygen). Oxygen can be supplied as compressed oxygen gas and liquid oxygen from commercial sources or it can be produced on-site by oxygen generators. The choice is generally one of cost and reliability of electricity supply to produce oxygen on site. As the transfer of gas to water using diffusers is poor (less than 40% for oxygen), specialised components to deliver oxygen to recirculating tank systems have been developed. These components can effectively transfer more than 90% of oxygen to the water. A variety of oxygenation devices are available.

2.4.4.4 Pathogens

Continuous disinfection of water in a recirculating tank system can help to limit the introduction and spread of disease, and the build-up of pathogenic bacteria, e.g. *Vibrio* species, to levels at which they cause mortality in the stock. Ultraviolet (UV) irradiation and ozonation are two methods of continuous disinfection used in recirculating tank systems. This section has dealt primarily with recirculating tank systems used for intensive culture, but larger-scale recirculation systems are now being developed for pond culture. In ponds with intensive culture, e.g. shrimp culture, it is difficult to operate without regular water exchange. However, pond systems in which there is very limited or no water exchange throughout a growing season are now being developed (section 21.10.5).

2.5 PLUMBING AND PUMPS

This section falls within the scope of aquaculture system engineering. Further detailed information on aspects of these topics may be obtained from an appropriate text, e.g. Wheaton (1977), Huguenin and Colt (1989) and Lawson (1994). Wheaton (1977) is still regarded as the classic text in this field.

2.5.1 Pipes

2.5.1.1 General

Pipes, channels and pumps are basic components of most aquaculture systems, except those that are open and static. Water can be conducted around an aquaculture facility using either pipes or open channels. The advantages of pipes include the ability to pump water at pressure and up a pressure gradient. The disadvantages of pipes compared with open channels include larger construction costs per unit water flow, and larger frictional losses causing higher pumping costs and lower achievable rates of water flow. However, channels cannot deliver water at pressure or up a pressure gradient. As such, pipes are more commonly used for the delivery of water, except when very large flow rates are required, and channels are employed largely as drains. Often a combination of pipes and channels are used where large pipes are used to 'lift' the water a short distance up the pressure gradient and into distribution channels. This provides the advantage of pumping from estuaries, rivers and oceans onto the farm, and avoids the costs associated with lengthy piping systems.

The aim of pipe selection is to obtain the optimum size of pipe (usually expressed as internal diameter in mm), the correct pipe material, and the best pressure ratings to meet the pumping pressure and the flow capacities required for the facility.

When selecting pipes for a given flow there is a choice between:

- smaller pipes with higher flow velocity and pressure; and
- larger pipes with lower flow velocity and pressure.

The advantage of using smaller pipes is that the capital cost of construction is much reduced, with the cost of pipes increasing rapidly with pipe diameter. The disadvantages of using smaller pipes are that they require greater water velocities for a given water volume and produce higher frictional forces and pumping costs. Higher frictional forces also result in larger pressure heads to be pumped against, increasing the strength (or class) of pipe required and, to an extent, increasing the capital cost of construction. Selection of smaller-diameter pipes during initial construction also greatly reduces the ability to expand the capabilities of the system in the future.

In addition, the higher the flow rate the greater the possibility of water hammer occurring (see section 2.5.1.5). As a rule, it is better to have the water velocity as low as possible within the constraints of the capital costs of construction.

If the flow rate and water velocity required are known, then the required pipe can be determined. Manufacturers of thermoplastic piping will provide charts, known as nomograms, to facilitate the correct choice of pipe by

cross-referencing flow rate and water velocity. Pipes, however, are manufactured in discrete sizes and pressure-class ratings (i.e. the maximum pressure a pipe can work under), not all of which are readily available or provide the same selection of fittings. In general, the velocity criteria will not provide a clear decision on the best pipe to use and will result in a choice of two sizes. The wise decision is to be conservative and select the larger pipe.

Another conservative measure in system design is to duplicate pumps, major plumbing and other components of the system. Even in the best-maintained systems there may be abrupt equipment failures. Duplication allows the problem component to be by-passed without having to shut the system down while repairs or replacements are effected. Furthermore, there are a number of routine maintenance activities that can be conducted without otherwise having to shut the whole system down. Some of these activities are:

- servicing pumps and other components in the system;
- back-flushing filters to remove sediment or otherwise renewing filter surfaces;
- clearing intake pipes of bio-fouling;
- flushing general plumbing to clear out accumulated sediment.

2.5.1.2 Frictional losses

Frictional losses are due to factors such as flow type, flow rate or velocity, pipe length and the roughness of the internal surface of the pipe. In aquaculture systems, flow is turbulent, creating internal eddies that collide with the side of the pipe, causing increased internal friction and, as a result, increased energy losses. Flow rate depends on the water requirements of the facility, while flow velocity results from the required flow and the pipe diameter. Pipe length is a result of facility layout and design. Roughness of the internal surface of the pipe is due to sand scour factors, scale formation, sediment deposit and bio-fouling. Frictional losses will also vary with salinity and temperature, but these changes are only slight.

Frictional losses are generally measured in head (elevation units, m) or pressure difference. Frictional losses for pipes are available in nomograms and flow charts from the manufacturer for standard conditions. Wheaton (1977) and Yoo and Boyd (1994) provide further information on how each of the above factors affects friction and, therefore, how decisions on pipe size and flow rates may affect the operating of the system.

2.5.1.3 Fittings

Plumbing fittings are the pieces that:

- join pipes or in-line components to the line;
- redirect and split the flow;
- change the pipe diameter.

All these fittings provide resistance to the flow and must be allowed for in the calculation of friction within the system.

Losses due to fittings depend on:

- type of fittings or transition (e.g. bends, couplings);
- abruptness of flow change;
- materials and condition of inner surface;
- velocity of water movement.

In addition to fittings, there may be dramatic head losses from in-line components (e.g. UV sterilisers and sand filters). These effects on flow rate are given by the manufacturers and are described by Wheaton (1977) and Yoo and Boyd (1994).

2.5.1.4 Choice of plumbing materials

For the majority of applications, and in particular for delivery of water over extended distances with pipe diameter of less than 200 mm, the choice of plumbing materials comes down to two types of general-purpose, cost-effective thermoplastics: rigid PVC (polyvinylchloride) and high-density polypropylene. For special applications, e.g. in outdoor areas exposed to high UV levels, high temperature (>50 °C) or high abrasion due to water-borne sand, and in high-impact areas, especially adjacent to large pumps, acrylonitrile butadiene styrene (ABS) pipe can be used. When the internal diameter exceeds 200 mm, and especially in high-impact areas, concrete and steel tend to be used as these are more durable and cost-effective.

2.5.1.5 Two common problems in plumbing design

Two of the more common problems in plumbing design are water hammer and bio-fouling.

Water hammer is a transient pressure phenomenon caused by rapid stopping or changing in direction of flow (Wheaton, 1977; Yoo and Boyd, 1994). The change in flow creates a wave of back-pressure, resulting in a loud bang, hence water ‘hammer’. It is the noise that may be heard in domestic plumbing when taps are turned on. The pressure wave is added to the existing pressure in the system and has the ability to rupture plumping or permanently

damage piping, pumps and in-line equipment. The magnitude of the pressure wave is related to the velocity of water in the pipe, pipe length and rate of change of flow.

The risk of water hammer increases with:

- water velocity;
- the length of uninterrupted pipe;
- the abruptness of flow change.

As a result, it is recommended that water velocity in pipes be kept below 1.2 m/s. It is also important to be aware of high-risk areas in plumbing design. These include design parameters creating flow change at the end of a long length of uninterrupted pipe.

In aquaculture systems, water hammer often originates from rapid opening of a line resulting in a high-velocity flow down the pipe. This water eventually encounters a 90° bend or, perhaps, a mostly closed valve, resulting in a sudden change in flow and water hammer. Rapid opening of a line can result from either turning on a valve too quickly or having a pump switch on. In these circumstances it is important to turn valves on slowly. Similarly, the use of soft-start pumps reduces the risk of water hammer from pumps starting. The risk of water hammer can also be mitigated by ensuring that the receiving pipe is full of water, so reducing the resultant flow from the pump, or ensuring that there are no sharp bends or closed valves in the path of the flow. As with opening a valve too quickly, closing a valve in a flowing line too quickly will enhance the risk of water hammer. This is easily corrected by identifying and marking critical valves or using valves that require several turns to shut them, e.g. gate valves, thus extending the time of closing.

The adverse impact of bio-fouling organisms, algae, sponges, barnacles and other shelled animals has been indicated for cages. Bio-fouling also occurs on the inner surfaces of aquaculture plumbing. It is a major factor influencing performance in seawater systems, but has a lesser effect on freshwater systems. Bio-fouling roughens surfaces and thereby increases the friction on the inner surfaces of pipes and fittings. It also blocks protective grills and pipe cavities. Considerable effort is required to reduce bio-fouling in seawater systems, and it can only be controlled by regular maintenance. It is important during maintenance to remove both the fouling organisms and their calcareous shells, if present. Many methods, e.g. flushing with freshwater and hot water, and anoxia (by having stagnant water in the plumbing for a considerable period), simply kill the organism but do not remove the shells. The most common and effective method of controlling bio-fouling is to remove the fouling organisms by forcing a rigid structure through the pipe under pressure. These devices are known as 'pigs' or 'snakes', and access for pigs or snakes into and out of the system must be taken into account during system design, e.g. by having T-junctions instead of 90° elbows. Under pressure, the pigs or snakes scrape the internal surface of the pipe, removing both organisms and exoskeletons.

2.5.2 Open channel design

When high water flows are required either into or out of a system, and there is no need to have a pressurised flow, as water can flow by gravity, it is cost effective to replace pipes with open channels. The advantages of open channels over closed pipes have already been considered, but there is a further advantage of the high flow capability allowing them to handle high transient flows (i.e. burst pipes and emergency draining of tanks or ponds). There are also the advantages of ease of access for routine cleaning and disinfection, and potential to be used as a harvest sump.

Drains are generally rectangular, semicircular or U-shaped. They are generally earthen or concrete. Concrete is often used in earthen channels to protect areas vulnerable to erosion, such as the portion of the channel immediately after the delivery pump. Concrete channels are also used for tank systems, both indoors and outdoors. The volume of water that can be moved by a channel is related to the size, shape, construction material and slope of the channel. Further information on how each of the above factors affects the maximum flow for a channel design may be obtained from Yoo and Boyd (1994).

2.5.3 Pumps

An important aspect of aquaculture systems is the correct choice of pump. Although this task is best performed by qualified personnel, it is important to know the types of pumps available and understand factors affecting pump selection. Pumps and pumping are covered in detail by Wheaton (1977) and Yoo and Boyd (1994).

The variety of pumps used in aquaculture can generally be classified into two groups:

1. Mechanically driven (positive displacement and rotodynamic pumps).
2. Air driven (airlift and air/water ballast pumps).

The majority of pumps used for water delivery in aquaculture systems are rotodynamic pumps. These pumps

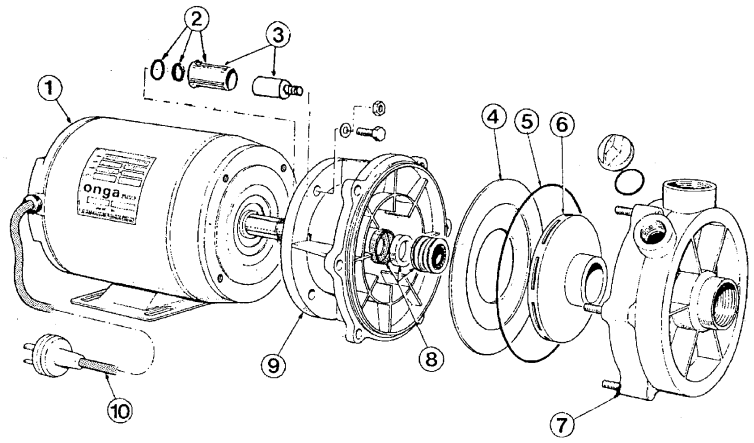


Fig. 2.13 A diagram showing the assembly and major components of a centrifugal pump. The major components include: (1) motor; (2/3) shaft sleeve assembly; (4) baffle; (5) 'O' ring; (6) impeller; (7) casing; (8) seal; (9) yoke; (10) power lead. Reproduced with permission of Onga Pty Ltd.

work by continuously imparting energy to the water using a rotating impeller, propeller or rotor. There are three types of rotodynamic pumps.

2.5.3.1 Centrifugal (radial flow) pumps

Water enters the impeller axially (along the axis of rotation) and exits radially in centrifugal pumps. The pressure is due to the centrifugal force of the impeller on the water and the water being contained by the casing of the pump. Centrifugal pumps have a high-lift/low-volume characteristic, being able to pump against heads >15 m. They also have the best suction lift characteristics; however, they perform best if located as near to the water source as possible. Although centrifugal pumps will draw water, they will do so only if they are primed, that is, if the line on the suction side and the pump are full of water.

2.5.3.2 Axial flow pumps

In axial flow pumps, water enters and leaves along the shaft axis (axially). The pressure is produced by the action of propeller blades (vanes) directly on the water. Axial flow pumps are used for low pressure head, generally <7 m, and high volume rate, >1 m^3/s. A limitation of these pumps is that they will not draw water, so the propeller must always be submerged in the water.

2.5.3.3 Mixed flow pumps

In mixed flow pumps, water enters axially and discharges axially and radially. The pressure developed is due to a combination of centrifugal force and lift due to propellers. These pumps are suitable for medium pressure head (5–15 m) and flow rate applications.

2.5.3.4 Pump components

Pumps are an indispensable part of an aquaculture system, and understanding their structure and maintenance is important for aquaculturists. There are two main elements to a rotodynamic pump. These are the rotatory element (impeller or propeller on the shaft) and the stationary elements (casing, stuffing box and shaft bearings).

Centrifugal pumps (Fig. 2.13) have the following components:

1. Casing. The casing converts water velocity leaving the impeller into pressure head. Two types of casings are used in centrifugal pumps. Volute casings spiral out from the centre, increasing casing volume with the spiral, resulting in a decrease in the velocity of water flow and the creation of pressure. Diffuser casings direct water through a set of diffuser vanes. The diffuser allows the conversion into pressure of a greater amount of energy imparted on the water, resulting in higher efficiency.
2. Shaft and bearings. The shaft transmits torque of the motor to the impeller. The shaft is supported by bearings to centre the shaft and impeller both radially and axially and to allow for the rotation of the shaft. Bearings may be either sealed or water lubricated.

3. Mechanical seals (or stuffing box). The mechanical seals prevent water from leaking around the shaft, which can result in deterioration of the bearings, shaft and motor. Seals are usually rings cut in soft plastic and fitted tightly around the shaft and shaft sleeve. Seals are cheap and readily replaced. It is essential to have spare seals in stock for crucial pumps.
4. Impellers. There are three types of impellers and the impeller best suited to the pump is generally governed by the requirements of water to be pumped. Open impellers have vanes that are attached to a central hub and supported by ribs. A small area of faceplate results in large clearances, which allow water-borne solids to be pumped. However, open impellers are not very efficient because they allow slippage of the impeller through the water. Semi-closed impellers have a complete faceplate to which the vanes are attached. This means that the efficiency of the pump is increased, but they do not handle solids as well as open impellers do. Enclosed impellers have a complete faceplate attached to each side of the vanes. Enclosing the water reduces slippage and thus increases the efficiency of the impeller. However, the small pathways for water movement result in a poor ability of these pumps to handle solids within the water.

Axial flow pumps are simpler in construction than centrifugal pumps, basically being propellers inside a pipe with a motor-driven shaft. As described above, these pumps are best for low head, although multistage pumps can create head of up to 40 m, and they are used for high-volume pumping. The low head pressure of these pumps means that they are not used for pipe distribution, their main use being for discharge into open channels or directly into ponds.

2.5.3.5 Pump selection

Apart from, or as well as, using the services of an expert, aquaculturists are required to select the type, make and model of pump, and alternative impeller size and speed, that will suit their system. This decision is aided by performance data for pumps supplied by the manufacturers. For a given pump body, data are presented for a number of different impeller sizes and pump speeds (Wheaton, 1977; Yoo and Boyd 1994).

Manufacturers will generally provide pump performance data in the form of head/flow capacity (*H–Q*) curves. *H–Q* curves are the graphical representation of the flow output of the pump against the total head (friction and elevation forces) against which the pump is acting. The shape of the *H–Q* capacity curve is critical. The curve must decrease uniformly from the high-head/low-flow to the low-head/high-flow conditions. If the curve has a flat spot or decreases abruptly at low flow the pump may not work smoothly in this range. In addition to the *H–Q* curve, the manufacturer's information will also often include efficiency data, allowing decisions about whether the selected pump will operate near its maximal efficiency in the system. The 'brake power' of the pump may also be represented. Brake power represents the power required to operate the pump at a certain flow and head. From this it is possible to determine the operating costs of different pumps by multiplying the operating time (hours) by the brake power for the pump and then multiplying this value by the kilowatt per hour charge of the electricity supplier.

2.5.3.6 Net positive suction head

The net positive suction head (NPSH) and total dynamic head (TDH) are required to determine the size of pump required (Wheaton, 1977; Yoo and Boyd, 1994). The latter will be treated in section 2.5.3.7.

In order for rotodynamic pumps to work, the water must be drawn to the pump under pressure. NPSH is the pressure on the suction side of the pump. The NPSH present within a system is the available NPSH (NPSH-A). The minimum NPSH required for a pump to operate effectively is the required NPSH (NPSH-R). NPSH-A can be calculated using the following formula:

$$\text{NPSH-A} = H_a + H_s - H_{fs} - H_{vap}$$

where H_a is atmospheric head (pressure), the pressure of the atmosphere on the surface of the water pushing the water to the pump. H_s is static suction head, the elevation difference between the pump and the water supply. It is negative if the water source is below the pump and positive if the water is above the pump. H_{fs} is the sum of the frictional losses from the pipe and plumbing on the suction side of the pump. H_{vap} is the vapour pressure of the fluid.

However, the values required for calculating NPSH-A are not all fixed:

- H_{fs} varies with flow rate and bio-fouling.
- H_s varies with height of tide, river, reservoir.
- H_a varies with weather conditions.

For this reason NPSH-A must be calculated for a range of conditions and be greater than NPSH-R for all of them, with a safety margin of at least 1 m. Failure to do so is the

single greatest source of pumping problems. If NPSH-R is greater than NPSH-A, the pump will produce little or no flow or will cavitate because of vaporisation.

2.5.3.7 Total dynamic head

TDH is also referred to as system head, differential head, generated head and total head. Total dynamic head is the height difference between the water source and the outlet plus the frictional losses:

$$\text{TDH} = H_{st} + H_{ft} + H_v$$

where H_{st} is the total static head, the difference between elevation of discharge and water supply; H_{ft} is the total frictional head of pipes, fittings and in-line components on the suction and discharge sides of the system; and H_v is the velocity head.

When calculating TDH, it is important that the range of flows used in the system is included, as frictional losses in pipes and fittings and the velocity head are proportional to water flow and velocity. Static head will also vary with the height of water in the water source, e.g. with tide height. Determining TDH over the range of flows and static head will enable the 'working range' of the pump to be determined.

A pump may be selected after establishing the flow rate, TDH and NPSH-A requirements.

2.6 SITE SELECTION AND DEVELOPMENT

The primary objective of aquaculture system design is to ensure that a facility is productive in a reliable and cost-effective manner. As a result, the design of a system must allow for:

- maximal growth and survival of the cultured species;
- optimal market price for the product;
- minimal operating/maintenance costs.

Prior to designing a system, it is important to recognise for whom and what the system is to be designed. There are three major users of an aquaculture system: the target species, the operators of the system and the owners. To accommodate the target species, the design must allow the animal to grow and survive optimally. In order to facilitate this, the operators need a system with minimal operating and maintenance requirements. It must also be reliable. The owners require that the system be cost-effective to construct and operate. An appropriate design needs to:

- meet the species' requirements;
- allow flexibility;
- provide for low maintenance;
- allow for the use of inexpensive materials without compromising quality;
- incorporate back-up systems where possible;
- allow for future expansion.

As seen earlier in this chapter, aquaculture systems vary immensely (outdoor ponds, indoor tanks, seacages, longlines, etc.), but similar forms of aquaculture production have facilities in common. For instance, a semi-closed tank or pond system will include:

- water supply;
- culture structure;
- aeration;
- power;
- buildings;
- layout.

Within the above constraints, the final system choice and design, as always, is a balance between species and user requirements, available capital for construction and site constraints. When designing an aquaculture facility it is important to consider the layout and design of the farm to minimise construction and operating costs, and to ensure reliable operation and production. As a result, the following issues need to be considered at the earliest stages of site selection and planning.

The general site selection considerations and designs of systems for culturing particular species or groups are covered in other chapters, e.g. Chapters 13–25. In this chapter only farm layout will be discussed in detail.

2.6.1 Movement of stocks around the site

Handling stock causes stress, reducing growth and increasing susceptibility to disease. It should therefore be minimised. Movement handling can be reduced by effective farm design. Movement can be facilitated by linking culture sites. Ponds can be linked via canals or by building ponds within ponds. Seacages have been designed in which cages are sewn together for stock transfer. Tank systems can also be connected through the overflows to allow stock to swim from tank to tank as required. Alternatively, stock handling can be reduced by growing animals in a single structure. It must be noted, however, that if the animals require handling for other husbandry reasons, e.g. grading, then using a single structure is of limited value.

2.6.2 Production patterns

When designing a facility, it is important to know the stages of production and the changes in the load (amount of product) within the various components of the system over the crop cycle and throughout the year. For example, if a system is stocked once a year, the load on a system at stocking is vastly different from the load at final production. As a result, the water and system requirements at the start of the crop will differ from those at the end of the crop.

It is also important to know whether the quantity and quality of water available throughout the year will vary. Consideration must be given to how these will be managed in relation to the crop cycle. It may be necessary to construct a dam and reservoir to enable excess water to be stored for drier months or when the required flow rates are higher.

Therefore, it is necessary to try to define the pattern of production as accurately as possible at the outset, allowing the sizing of components and the determination of project costs and site layout. Similarly, it is important to be able to vary pumping capacity to reduce excess capacity and therefore operational costs at times of low water requirement.

2.6.3 Water flows

Apart from ensuring the quantity and quality of water available to the system, the distribution of water around the site must also be considered. It is generally appropriate to utilise the shortest routes for water delivery and removal to save capital costs and, if piping is used, to reduce pressure losses due to friction and therefore pumping costs. In addition, it is preferable that the water supply system be controlled from a centralised point. In a pond system this may be as simple as being able to turn the water supply to a pond on or off without travelling to the pump house. Where possible, water flows to tanks, raceways and ponds should be able to be adjusted individually.

2.6.4 Use of existing topography: water head/pump capacity

All requirements of the farm are important when considering the topography. Topography can be utilised to reduce pumping costs, collect and store water from natural watersheds and provide sites for buildings.

'Water head' is the ability or the potential for water to fall, i.e. water will drop from a higher point to a lower point through gravity with no requirement for pumping. The provision of pumping is expensive in both capital construction and running costs. It is important to use water head efficiently in farm design. If using ponds or raceways, the design must utilise the slope of the land so that water may be supplied and drained or drained by gravity, i.e. let the water 'fall' down rather than pumping it up. The greater the height that water must be pumped and the greater the distance of piping, the greater the operating costs without improving productivity.

2.6.5 Utilisation of land and/or water

As land or water is generally expensive to purchase or lease, it is important to minimise non-productive space wherever possible. Minimisation of wasted space is as important for recirculation systems as it is for pond farms or marine leases. In general, it is considered prudent to minimise the distances between culture structures (ponds, tanks, raceways, long-lines) and the size of car parks or working areas and buildings.

However, although it is important to minimise unproductive space, there must be sufficient working space to allow:

- access to culture structures and pipework for routine activities;
- vehicle access to work areas;
- access to pumps and treatment systems for maintenance and adjustment for routine work (e.g. repairing equipment and cleaning/drying nets) and for non-routine work or emergencies to be conducted;
- animal husbandry (e.g. grading) and product processing (e.g. harvesting, slaughtering, packing);
- a buffer zone around the aquaculture facility, including an 'environmental protection zone'.

It is important to locate harvesting and processing facilities as close as possible to the harvest site. This will minimise transport requirements, but more importantly it will aid in maintaining product quality, especially in hot climates. Processing facilities must be kept away from hatchery facilities to prevent contamination of the hatchery area by diseases from the on-grown stock (section 2.7.2).

Apart from the areas directly related to growing the product, areas for the delivery, storage and handling of goods such as feeds, fertilisers and chemicals must be considered. Thus, road design must meet all access requirements while minimising construction costs. Consideration must be given to the loads entering and exiting the property as well as the types of vehicles used. Although small vehicles may be used on the farm, transport companies, e.g. feed companies, may deliver using much larger vehicles. When deliveries are purchased in bulk, sufficient

space must be available to store and handle the goods. In conjunction with access it is important to ensure that the working yard and turning area for the delivery of bulk supplies and the transport of harvested product is sufficient.

Expansion is often the target for aquaculture ventures, and the ability to expand facilities and operations beyond the initial development must be considered during the site selection and layout design. Where possible, adjacent land and/or water must be available to lease or buy in the future. Alternatively, if land or water is cheap enough, it can be purchased to allow for future expansion. The site must be engineered so it can be easily modified for increased capacity. For example, consider using larger pipes initially to avoid replacement costs later.

Although it may be thought of as 'unproductive space', a 'buffer zone' may be required as part of the licensing requirements from environmental agencies for some forms of aquaculture. A buffer zone around a pond farm may be required as protection against escapees, predators and vermin. Spare ground is also needed for disposal of the pond substrate wastes between crops. In seacage and high-biomass bivalve aquaculture, e.g. mussel rafts, there is the potential for heavy fouling of the benthos beneath the facilities (section 4.3.1; section 23.5.2). Pollution can be minimised if the cages and rafts can be moved to new areas within the lease.

2.6.6 Minimisation of construction costs

When constructing buildings of similar grades, it is generally cheaper to build larger buildings and then divide them into smaller sections and rooms. However, one needs to consider which activities can be conducted within close proximity to each other. Similarly, larger ponds are cheaper to build per unit area than smaller ponds. The manageable size of the pond, however, and the optimum size for growth performance of the cultured species must be considered.

The specification of buildings (i.e. the strength and sizing of materials) and pond walls (i.e. wall height, width and internal and external slopes) is also important. Costs of construction increase rapidly if over-specification occurs. As such, it is important to consider the appropriate specifications for the required tasks.

2.7 HATCHERY SYSTEMS

Hatcheries are those aquaculture facilities associated with reproduction, larval rearing and supply of juveniles to farms. A single hatchery often supplies a large number of farms, although many aquaculture industries (e.g. the oyster industry) still rely heavily on natural recruitment as a source of culture stock; natural recruitment can be unpredictable and unreliable. As well as providing independence from natural recruitment, other benefits of hatchery production include the potential for genetic improvement of culture stock (Chapter 7) and the reduced potential for conflict with capture fisheries.

Hatcheries have similar site requirements to nursery and grow-out sites (section 2.6). Of particular importance is the quality of intake water, which must be free from industrial, urban and agricultural contaminants. The water intake pipe is usually screened to remove foreign items such as seaweeds from intake water. Intake water should also have low levels of suspended solids, to minimise filtration requirements and bacterial contamination, and a temperature as close as possible to that to be used for larval rearing to minimise water heating/cooling costs. A hatchery should also be able to pump water regardless of the state of the tide and should be located close to the farms it supplies.

Marine aquaculture hatcheries may be positioned to use either an oceanic or estuarine water supply. Oceanic water is generally considered to be of higher quality, but such sites may be subject to high wave action and erosion that may lead to problems with water supply. Use of such sites may also result in public resistance on aesthetic grounds. Estuarine water sources are subject to rapid changes in temperature and salinity, which can result from heavy rainfall. Rainfall may also increase levels of suspended solids and bacteria in intake water. Both factors affect water quality and will impact on larval well-being. Freshwater hatcheries generally use either groundwater (e.g. from an aquifer using a well or bore) or surface water (e.g. streams, rivers and lakes) as water sources. It is important that marine hatcheries also have a reliable source of freshwater for cleaning and washing.

2.7.1 Water treatment in hatcheries

Water used for larval rearing is usually treated to reduce particulate matter and bacteria, and heated/cooled to optimise water temperature. Water treatment in hatcheries usually includes the following steps:

1. Water storage. Water is usually stored in large tanks (e.g. 20–100 000 L) before being pumped into the hatchery. This also allows suspended solids to settle from the water column.
2. Coarse filtration. Water is passed though filtration equipment (e.g. sand filter), which removes larger particles and filters water to around 20 μm.

3. Heating. Water may be heated before use. This may be done by heating a common water source or 'header tank', from which larval culture tanks are supplied. However, water may also be heated using heat exchange systems or larval culture tanks may be heated individually using immersion heaters.
4. Fine filtration. Before entering larval culture tanks, water is usually passed through fine filtration equipment (e.g. cartridge filters), which filters the water to ca. 1 µm. This is generally achieved using a series of filters of different sizes in sequence (e.g. 10 µm, 5 µm and 1 µm). Water used for live food culture (e.g. microalgae; section 9.3.1) is filtered to finer levels of 0.2–0.45 µm.
5. Ultraviolet (UV) treatment. Water to be used for larval culture and live food culture may also be passed through a UV steriliser to reduce levels of bacteria. Water must always be filtered to remove suspended particles before being passed through a UV steriliser because particulate matter is the major substrate for bacteria in culture water.

Other equipment used to manage water quality in hatcheries and recirculating water systems are included in section 2.4.4.

General considerations with regard to water quality and water treatment in freshwater hatcheries for salmonids are given in section 15.3.2.

2.7.2 General layout of hatcheries

Although hatcheries differ considerably in layout according to the type of animal propagated, they have a number of common design requirements. These include:

- an area for holding broodstock;
- a dedicated spawning area;
- dedicated food production area(s);
- a dedicated larval rearing area;
- an area for early nursery culture.

Hygiene is a major concern in aquaculture hatcheries. Disease can be very costly to a hatchery and may result in the loss of live feed cultures as well as larvae, juveniles and broodstock. Hatcheries must be designed to minimise the possibility of disease transfer from one area of the hatchery to another and to facilitate quarantine of a particular area if required. To achieve this, each part of a hatchery should be separated from the others. For example, live foods are usually cultured well away from areas housing culture stock (broodstock or larvae). Larval culture and broodstock holding areas are similarly separated. As well as disease considerations, the isolation of broodstock holding areas minimises noise and other disturbances that may stress the broodstock and impact on reproductive output. Appropriate hatchery design can help minimise the risk of disease and facilitate hatchery efficiency and productivity (Fig. 2.14). Some important design considerations include:

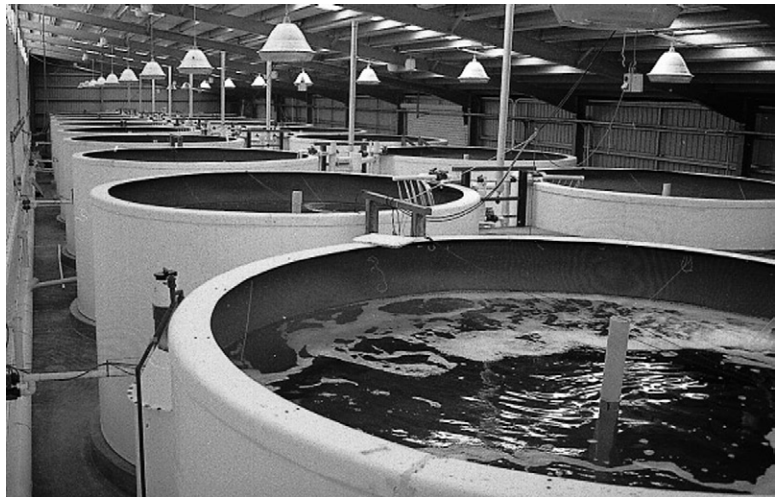


Fig. 2.14 A large, well-designed fish hatchery/nursery system (Good Fortune Bay Fisheries, Queensland, Australia).

- separate access to all hatchery areas;
- antiseptic foot baths between areas to minimise risk of disease transfer;
- larval tanks raised off the ground to allow access for washing or sterilising floor areas;
- well-drained floors to minimise standing water;
- air lines, water supply and power located overhead to allow easy access.

2.7.3 Hatchery management

Perhaps the two most important management factors in hatcheries are water quality and feeding regimen. Both influence the rate of growth and the health of the larvae.

Water entering larval culture tanks should be free from competing organisms and predators and have low levels of suspended sediment and bacteria. Both are achieved by filtration, as outlined in section 2.7.1. Water should also be of an appropriate salinity and temperature. The quality of water in larval culture tanks declines over time as a result of larval attrition, metabolic waste products and uneaten food. Maintenance of water quality parameters within an acceptable range is achieved by regular water changes. Water changes generally occur every 2–3 days in static culture systems, such as those used for bivalve larvae (section 23.4.2), or more frequently in flow-through larval culture tanks used with fish and crustaceans (e.g. section 18.7). Items used in maintaining larval culture tanks include nets, sieves, hoses and brushes for cleaning, etc. It is good practice to have one set of equipment for each tank to minimise the potential for disease transfer between tanks. If this is not possible, equipment must be sterilised (usually in a chlorine solution, then rinsed free of chlorine and dried) after every use.

2.8 SELECTING A NEW SPECIES FOR CULTURE

Which species will I grow? What type of system will I use to grow the selected species? Criteria for the selection of new species for commercial aquaculture were outlined by Avault (1996, 2001).

2.8.1 Selecting an appropriate species

The choice of aquaculture species is often a balance between biological knowledge and economics (Fig. 2.1). The biological knowledge required to allow successful culture of the species is diverse and will be outlined in the following section.

Prior to selecting a species for culture, it is important to consider the economics of the species. There is a wide range of uses of aquaculture products and, therefore, there is a myriad of potential aquaculture species. Aquaculture is much more than production of protein for human consumption, although this is the most frequent objective of new culture ventures. Other production objectives of aquaculture include:

- industrial products, e.g. agar/alginate;
- pharmaceutical products, e.g. UV-resistant compounds;
- augmenting wild stocks for conservation, wild fisheries or recreational fisheries;
- ornamental species for the aquarium industry;
- crocodile hides;
- food/feed components for culture organisms, e.g. phytoplankton and zooplankton.

Thus, the characteristics of the selected species will vary according to the production objectives. The use of the product may determine the market price, and this will in turn influence the economic viability of the venture.

2.8.2 Requirements of a suitable culture species

Aquaculture projects frequently fail as a result of an inadequate understanding of all facets of the biology and economics of the target species, or by selection of species that do not have the appropriate characteristics. Avault (1996) listed the following issues to be considered when selecting an aquaculture species.

2.8.2.1 Water temperature and water quality

Each species has specific requirements for various water quality parameters. These parameters include temperature, dissolved oxygen, salinity, pH and ammonia/nitrite/nitrate nitrogen (section 3.3). It is important to understand not only the tolerance ranges for these parameters, but also the optimum levels for growth, survival and reproduction.

2.8.2.2 Growth rate

Species showing rapid growth to reach market size in a short period of time are preferred for aquaculture. Historically it has been considered inappropriate to culture an animal that requires more than 2 years to reach market size. However, growth rate must be considered in relation to risk and economics. Risk is associated with the chance of losing the product to disease or system failure during the grow-out period. If, however, the risk of product losses is low or the product is of substantially higher value than the product from similar species, or both these desirable conditions pertain, then species with slower growth may be cultured effectively.

2.8.2.3 Feeding habits

A species' feeding habits can greatly influence the profitability of culture. Feeding habits can generally be divided according to production phase, i.e. hatchery/nursery, juvenile and grow-out phases. In general, feeding habits change markedly after the hatchery/nursery phase, with the juvenile and grow-out animals having similar feeding habits. Bivalves, however, have similar feeding habits throughout their life cycle. Feeding habits include the foods and the position in the water column in which the animal feeds. Feed requirements affect feeding costs, with animals feeding higher in the food chain generally requiring more expensive diets (section 2.3). The ability to accept an artificial diet is also of importance, as live feeds are expensive to produce in terms of space, labour and consumables (section 9.5). At the other end of the spectrum, extensively cultured grazing gastropods and filter-feeding bivalves feed from their environment without supplementation. The position in the water column at which the animal feeds can affect the type of aquaculture system that can be used to culture the animal. For example, if a fish is a benthic feeder, it is unlikely that it can be effectively cultured in a floating cage.

2.8.2.4 Reproductive biology

A reliable source of juveniles or 'seed' is fundamental to all aquaculture ventures. Seed must be available in the required quality and quantity. Although some aquaculture industries have been developed using wild-caught seed, e.g. table oysters and milkfish, it is highly desirable that the target species can be bred in captivity (section 2.7). In addition to being able to reproduce in captivity, it is also desirable that a culture species has high fecundity, either a large number of eggs per spawning or multiple spawnings per season. High fecundity helps offset the high costs of maintaining and spawning broodstock.

Pangasid catfish have many desirable characteristics for aquaculture, which are now exploited for a very large industry, but the breakthrough in their large-scale culture came with reliable spawning and hatchery techniques (section 1.8.1).

2.8.2.5 Hardiness

To achieve an acceptable level of production, cultured species will experience conditions that differ considerably from their natural environment. In culture, animals will generally experience social crowding, poorer water quality and handling. All these parameters will create stress. The cultured species should be able to adapt to these stresses and maintain high survival and optimal growth. It is important to consider the effects of these stresses on all stages of the life cycle. Combined with the ability to adapt to the stresses of culture is an ability to resist disease. The likelihood of disease occurrence and proliferation is higher in culture than in the wild, and a species susceptible to suffering mass mortalities from low levels of disease is not desirable.

2.8.2.6 Marketing

Prior to undertaking an aquaculture venture, it is important to thoroughly investigate the markets for the target species. As previously described, there is a range of uses for aquaculture products, all of which may be considered when selecting the target species. Some species may also have several marketing opportunities. For example, certain fish species may be sold as fingerlings for restocking into the wild, or they may be grown to plate size or grown to a larger size for filleting. A table oyster may be sold to oyster farms as seed or sold after grow-out as adults in the shell or shelled and smoked. It is important to identify the best market(s) for the product and access the market value of the product. A decision on the economics of culture can then be made.

2.8.2.7 Economics

At all stages of selecting an aquaculture species, the underlying aim of aquaculture is profit (Chapter 12). Thus, all the costs of production, including stock, feed, electricity, interest on money borrowed, labour, etc., and the returns from sale of the product must be estimated with a costs and return analysis. Economists have produced a variety of software that allows prospective aquaculture farmers to undertake all the appropriate economic calculations. The economic feasibility of the culture can be determined by combining market analysis and biological feasibility.

There may be alternative production systems for rearing a species. It may be possible to match the type of production system, and its associated costs, to the species and its market price to achieve profitability. For example, growing fish in an intensive, recirculating tank system may cost US$4–8 per kilogram to grow to market size. The market price for this species would need to be significantly higher than the production cost to ensure economic feasibility. If not, then the farmer must investigate an alternative, cheaper method of production, e.g. semi-intensive pond culture, which may reduce production costs per kilogram of market size fish.

It is not just a matter of having a positive balance of profit to costs. If the rate of return on the investment in an

aquaculture venture is not greater than the prevailing standard rate of return from investments, then the venture is effectively unprofitable. The committed funds would be better used for a lower-risk investment (section 12.2).

2.8.3 Compromise

The ideal culture species possesses all the above characteristics; however, few if any species are ideal, and generally there is some compromise in terms of these characteristics.

Often the choice of a species for aquaculture production will lead to two situations:

1. A species has an existing market and/or can be marketed quite easily. In this situation the farmer may enter an established market and the decision to culture is based upon the economic attractiveness. If there is biological information to allow complete control over the life cycle, then, given economic feasibility, successful culture is nearly assured. If the understanding of the life cycle is not complete, problems in culture may be experienced. This may lead to failure, even though a ready market exists.
2. Biological feasibility of culture is high, but there is no ready market or market price is low. In this situation, the decision is based upon the fact that the control of the life cycle is complete rather than on economic attractiveness. In this case there is a need to establish a market, which may involve considerable cost. If a market is established, given that culture is economically feasible, the venture must be successful.

If a product does not suit a market, culturing the product will be unsuccessful; however, an alternative to changing the species may be a matter of changing the market niche or undertaking an innovative marketing campaign. This approach has been exemplified for a number of fish species, e.g. pangasid catfish (section 1.8.1), tilapia in Asia (Chapter 16) and channel catfish in the USA (Chapter 17).

2.9 DEVELOPING A NEW CULTURED SPECIES

If the aquaculture species of choice is cultured widely in the region and culture techniques are well developed, then development of the culture species is relatively simple and the major concerns regarding success will be economic. In this situation, establishing culture is a matter of applying existing technologies to the chosen farm site. Some trial and error with husbandry may be required; however, provided that the training of employees is satisfactory, success is likely. If the species is cultured widely throughout other regions, then development may be more difficult. Difficulties may arise from population differences, with the species requiring more technological development, or from regional climate differences impacting upon traditional or developed culture technologies. Development of culture for such a species should begin at the pilot trial of the development protocol detailed below.

If the target species is a new species to the aquaculture industry, or if it is proposed to culture the species under very different conditions (e.g. intensive vs. extensive), then it is recommended that the following development protocol be used to determine the suitability of the species.

2.9.1 Stages of development

Many species fail at various points during the following development protocol. The key is not to make large investments in species destined for failure.

2.9.1.1 Screening

The aim of this stage is to make an informed decision about the culture potential of the species. During this stage, as much information as possible should be collected: life history, general ecology and husbandry. This information should then be considered in respect of the proposed site.

Provided the species looks promising, any legal constraints to culture should be investigated, e.g. permits and translocation issues. This will inform prospective farmers of the legal possibility of culturing the animal. Given that culture initially looks to be feasible from biological, site and legal perspectives, potential markets should be assessed. In assessing potential markets, the farmer should determine whether the species is consumed regionally or globally, and the present demand for the product. If a ready market does not exist, the farmer may wish to investigate if a potential market niche exists.

Finally, after determining a potential market for the species, the economics of culture should be assessed. It is wise at this stage to produce some preliminary figures on the potential returns on investment. These figures should be realistic and not work on the lowest possible production costs and highest possible production rates and market price. By adopting mid-range values, the reality of culturing this animal is more likely to be reflected. Potential investors should err on the conservative side. Determining the return on the investment should incorporate all eco-

nomic aspects, including capital investment in land purchase and infrastructure, depreciation of assets and interest rates on borrowed money, as well as annual production costs. In addition, the potential return on the invested capital if it was used in an alternative investment strategy should be calculated (Chapter 12). Software programs are available commercially to produce the appropriate economic information.

Based upon the desirable characteristics for a culture species, the biological potential of culture, the availability of a market and the economics of culture, the target species may be accepted or rejected at this stage. If the species is accepted, development should proceed by moving to the research stage.

2.9.1.2 Research trials

Once a species has progressed through screening, research into the biology and husbandry of the animal should be undertaken. Research provides the information required for the development of appropriate culture systems and husbandry techniques (Fig. 2.15). During the research trials it is necessary to perform controlled studies to investigate:

- environmental requirements, including water quality parameters (pH, salinity, nitrogenous wastes, alkalinity, etc.), temperature and photoperiod; it is important that the ranges of these parameters providing optimal and significant growth, as well as lethal levels, are determined;
- optimal stocking densities;
- reproductive physiology and broodstock husbandry;
- nutrition and growth of larvae, juveniles and adults. The nutritional requirements for both live and artificial diets, feeding techniques and expected growth rates should be determined.

The species may prove to be undesirable on the basis of one or more of the above areas and be rejected.

2.9.1.3 Pilot trial

Using the information derived from this research, a pilot trial can be conducted. Farmers consider this as taking culture from the laboratory into the 'real world'. In this trial, the culture structure, e.g. tanks, ponds, cages and raceways, is small and a low number of animals used. This trial will provide semi-commercial values for and/or information on:

- survival;
- potential yields;
- food conversion ratios;
- water quality problems;
- handling difficulties;
- economics of culture.

It is important at this stage to determine impediments to larger-scale culture. If problems are indicated, the farmer should either perform more research, or modify techniques and conduct further pilot trials.

2.9.1.4 Commercial trial

The commercial trial is a scale-up of the pilot trial. During commercial trials the farmer should use full-sized culture units and larger numbers of animals. At this stage a site should be secured and some capital invested in infrastructure development.

The commercial trial provides information on production costs and profits, and husbandry of large numbers of animals. The commercial trial may identify difficulties in animal husbandry or cost dynamics associated with the larger culture systems.

At this time some preliminary market development should be undertaken. However, it should be noted that it is difficult to establish firm markets before production is established.

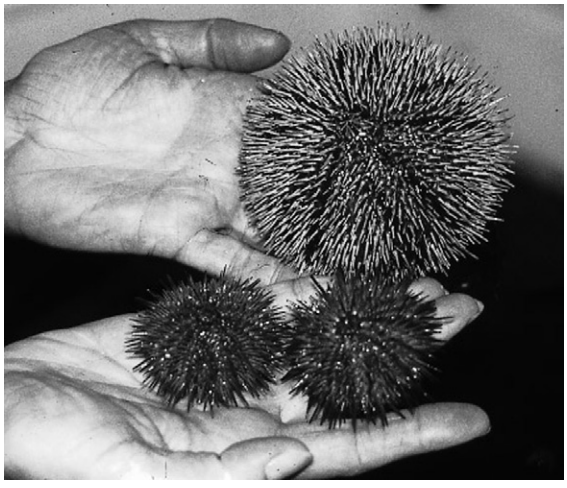


Fig. 2.15 While a ready market exists for sea urchins in South-East Asia, where they are prized for their gonads, further research is required to develop reliable and economic culture methods.

2.9.1.5 Full-scale production

Full-scale production involves the farmer developing the full number of culture units. It is important that other aspects of the culture units, such as structure size and shape, flow dynamics and husbandry, are not modified from those used in the commercial trial. Full production requires substantial capital and infrastructure development and a suitable site. Under-capitalisation has been a major problem in developing aquaculture industries.

As production expands, firm markets should be established.

2.9.2 Timescales for development

The timescale for development of a culture species is important to all prospective farmers. In the above protocol the length of time spent at each stage depends on many factors including:

- how much information is available on the target species;
- husbandry difficulties;
- the existence of markets;
- available capital.

As a rule of thumb the following should be expected:

1. Screening. Screening should last at least 2 years.
2. Research. Research should last at least 5 years for species previously not studied; often longer, particularly if information is scarce and the species is difficult to culture.
3. Pilot trial. A pilot trial should take place over at least two growing seasons.
4. Commercial trial. A commercial trial should take place over at least two growing seasons.

Failure to observe this protocol is a common mistake in aquaculture development. It is often costly and results in the failure of many new aquaculture ventures. Prospectuses for new companies often do not include a development protocol, and many ventures proceed with insufficient information about culture requirements of the target species. Many ventures targeting new species move directly to commercial trials or attempt full-scale production. Omitting pilot trials, even if the species is well known, or not correcting major problems before moving to commercial trials often results in the construction of inappropriate aquaculture facilities, leading to economic failure. However, sequential development, as outlined above, provides a sound basis for decision-making and appropriate progression of aquaculture ventures.

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3
Water Quality

Claude Boyd

3.1 INTRODUCTION

Production of fish, marine shrimp, and other species by aquaculture depends upon a supply of high-quality water. In cases of aquaculture projects sited where water quality is naturally impaired or polluted, the water quality limitations must be overcome or the aquaculture projects will fail. Water quality also declines in aquaculture systems because of waste accumulation and, thus, water quality management must be applied to avoid stress and mortality of aquaculture species, and to assure efficient production. Furthermore, the discharges from aquaculture facilities contain nutrients, organic matter, and suspended solids that pollute receiving water bodies. Many governments require aquaculture facilities to implement practices to minimize pollution. The purpose of this chapter is to provide a concise and practical discussion of aquaculture water quality management.

3.2 WATER QUALITY VARIABLES

Rainfall, the ultimate source of freshwater, is saturated with nitrogen, oxygen, carbon dioxide, and a few other gases. It sweeps dust, pollutants, and other particles from the air, and these substances dissolve in rain drops. Rainwater either evaporates, becomes storm runoff, or infiltrates into the earth to become soil moisture or groundwater. Storm runoff suspends and dissolves mineral and organic matter from the land surface. Suspended particles result from erosion favored by paucity of vegetative cover, disturbed or loosely aggregated soil, heavy rainfall and steep slopes. Factors favoring solution of minerals by storm runoff are high temperature, relatively soluble rocks and minerals, and long exposure time with watershed surfaces. Storm runoff enters streams, ponds, and lakes, where turbulence and velocity decrease, allowing sedimentation of suspended matter. The retention time of water increases as it flows downstream, allowing more time for dissolution of minerals, concentration by evaporation and biological effects on water quality. Streams enter estuaries where riverine water mixes with seawater. In estuaries, water quality is influenced particularly by volume of freshwater inflow, tidal action and flushing rate with the sea. The high concentration of cations in estuaries and the sea aids flocculation and sedimentation of suspended mineral matter. Seawater has a very long residence time, and its quality tends to be stable.

Water passing through the land surface infiltrates until it reaches an impermeable layer and stands in formations called aquifers. This water usually is depleted of dissolved oxygen (DO) and saturated with carbon dioxide while passing through the root zone. It stands in intimate contact with minerals in geological formations for a long time. Conditions for solubility of minerals are good in aquifers, and the major factor determining the composition of groundwater is the solubility of minerals in the formations.

3.2.1 Solids

The weight of dissolved solids in natural waters results primarily from inorganic substances in ionic form. There is a lesser amount of dissolved organic matter. Natural water also contains soil particles, particulate organic matter, and micro-organisms in suspension.

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Total solids (TS) are the weight of the residue remaining after a water sample is evaporated completely. The weight of the residue after complete evaporation of a filtered sample is the total dissolved solids (TDS). The dry weight of the material retained on the filter is the total suspended solids (TSS). Thus TDS + TSS = TS. The weight loss following ignition at 500 °C of the TS residue is the total volatile solids (TVS). Ignition loss from the residue on the filter used for the TSS analysis is the particulate organic matter (POM) (Eaton *et al.*, 2005). Concentrations of TDS and TSS are reported most frequently. The TDS consists primarily of major ions, and TSS is composed of clay particles, micro-organisms, and detritus. Concentrations of solids typically are given in mg/L, which is equivalent to parts per million (ppm).

Salinity of water is the total concentration of dissolved ions, and TDS and salinity are of similar concentration in most waters. Salinity and TDS vary from a few mg/L in rainwater to hundreds of thousands of mg/L in closed basin lakes.

Rainwater may contain only 1 or 2 mg/L TDS in rural, inland regions, but near cities, industrialized areas or coastal regions, concentration may be 10–30 mg/L. In humid regions, surface water varies in TDS concentration from 25–50 mg/L in places with thin, highly leached soil to 250–500 mg/L in areas with fertile or calcareous soils. In semi-arid regions, evaporation concentrates ions and surface waters often contain 500–1000 mg/L TDS. Even higher TDS concentrations are common in arid regions. Concentrations of ions also can vary greatly in groundwater depending upon the nature of the soil through which the water infiltrated and the composition of the geological formation containing the aquifer. Water infiltrating through sandy soil into sand or gravel formations often has TDS concentrations <50 mg/L. Water infiltrating through calcareous soil or held in a limestone formation may have 200–500 mg/L TDS. In some regions, formations contain salt deposits or ancient seawater, and aquifers yield saline water and TDS concentrations from 1000 mg/L to >50 000 mg/L.

Freshwater is usually considered to have a TDS concentration <1000 mg/L. Thus, not all inland waters are freshwater. Many arid regions have saline surface waters and groundwater may be saline even in humid regions.

It is more common to speak of salinity than TDS in discussions of marine waters. Salinity normally is expressed in parts per thousand (1‰ = 1000 mg/L). The average salinity of seawater is 34.5‰, while river water seldom has more than 0.5‰. River water mixes with seawater in estuaries and salinity varies from near that of river water in the upper ends of estuaries to near that of seawater at the lower ends. Freshwater input dilutes estuaries, and salinity is greater in the dry season than in the wet season. In poorly flushed estuaries near the end of the dry season, salinities may be higher than those of seawater. Salinity also varies with daily tidal action at a given place in an estuary. High salinity water is denser and flows beneath lower salinity water. Thus, many estuaries have salt wedges extending considerable distances upstream beneath the lower salinity water at the surface.

3.2.2 Specific conductance

Ability of water to convey an electrical current increases with increasing ionic concentration. Specific conductance is an index of the degree of mineralization of water. Specific conductance is reported in micromhos/cm (μmhos/cm). Rainwater usually has a specific conductance of 10–20 μmhos/cm or less. In humid, inland regions, specific conductance is seldom >500 μmhos/cm in surface water, but values >5000 μmhos/cm may be found in surface water in arid regions. The specific conductance of seawater is ca. 50 000 μmhos/cm. The proportionality between specific conductance and TDS concentration varies from 0.55 to 0.9 depending upon the source of water (Eaton *et al.*, 2005). In many waters, the TDS concentration is about 0.7 × specific conductance, e.g., 1500 μmhos/cm ≈1000 mg/L TDS.

3.2.3 Major ions

The cations, calcium, magnesium, sodium and potassium, and the anions, chloride, sulfate, bicarbonate and carbonate, account for most of the dissolved solids in inland surface water, groundwater, and seawater. In humid regions, the dominant ions in surface freshwater usually are calcium, magnesium, and bicarbonate (Table 3.1). In arid regions, evaporation concentrates ions resulting in precipitation of carbonate, and waters may contain much greater proportions of sodium, potassium, chloride, and sulfate than in other waters (Table 3.1). Seawater is especially high in sodium and chloride (Table 3.1). Groundwater often is similar to surface water in proportions of major ions. However, it also may exhibit unusual ionic proportions. For instance, some groundwaters in coastal areas have a high concentration of bicarbonate with low concentrations of calcium and magnesium resulting from a natural water-softening process within aquifers that once contained seawater. Some groundwaters are deficient in potassium and magnesium for unexplained reasons. In addition to major ions, freshwaters may contain several mg/L of largely undissociated silicic acid.

Table 3.1 Major ion concentrations (mg/L) for three ponds, trace element concentration in typical freshwater bodies, and chemical composition of normal seawater. From Boyd (1990).

Variable	Pond in acidic soil in humid region	Pond in basic soil in humid region	Pond in arid region	Normal seawater
Major ions:				
Bicarbonate/carbonate ($HCO_3^-/CO_3^=$)	11.6	136	244	142
Chloride (Cl^-)	2.6	29	7.6	19000
Sulfate ($SO4^=$)	1.4	28	64	2700
Calcium (Ca^{++})	2.7	41	53	400
Magnesium (Mg^{++})	1.4	9.1	15	1350
Potassium (K^+)	2.6	1.2	10	380
Sodium (Na^+)	1.4	2.2	34	10500
Bromide (Br^-)	–	–	–	65
Trace elements:	Range for typical freshwater			
Iron (Fe)	Trace to 1.0			0.01
Manganese (Mg)	Trace to 0.25			0.002
Zinc (Zn)	0.04–0.08			0.01
Copper (Cu)	0.01–0.02			0.003
Boron (B)	0.01–1.0			4.6
Cobalt (Co)	0.0005–0.0015			0.0005
Molybdenum (Mo)	0.0002–0.0008			0.01

3.2.4 Minor inorganic constituents

Water also contains ammonium, ammonia, nitrate, nitrite, phosphate, iron, manganese, zinc, copper, boron, cobalt, molybdenum, and other substances. Typical concentrations of some selected trace elements in surface freshwaters in the United States also are provided in Table 3.1. These concentrations are greater than those found in normal seawater. Low pH favors solubility of trace metals. Trace metals form ion pairs, hydrolysis products, and chelates with dissolved organic matter. The total concentration of trace metals is much greater than the concentration of the ionic form. Freshwaters tend to have greater concentrations of trace metals because they generally have a lower pH and more dissolved organic matter than seawater.

3.2.5 Dissolved organic matter

Dissolved organic matter in natural waters ranges in concentration from <1 to >20 mg/L. Tannins, lignins, and other humic substances originate from vegetative material. Water bodies receiving runoff from forested watersheds may be deeply stained the color of tea or coffee by humic substances. In aquaculture systems, dissolved organic matter also originates from manures, feed, and decay of dead plankton.

3.2.6 Particulate matter

Water bodies with disturbed watersheds typically have high concentrations of suspended soil particles and TSS concentrations may be 500 mg/L or higher. Elevated concentrations of particulate organic matter are more common in water bodies with moderate or high nutrient inputs and an abundance of plankton. In aquaculture ponds, TSS concentration often ranges from 20 to 60 mg/L. Particles comprising TSS usually are about half organic matter; thus, POM concentrations are usually 10–30 mg/L.

Phytoplankton is the source of much of the suspended organic matter in aquaculture ponds and POM concentration is used as an indicator of phytoplankton abundance. A better indicator is chlorophyll *a* concentration which increases as a function of increasing phytoplankton biomass.

3.2.7 Dissolved gases

The atmosphere contains 20.946% oxygen, 78.084% nitrogen, 0.934% argon, 0.038% carbon dioxide, and small percentages of a few other gases. Each gas has a characteristic absorption coefficient in water and the amount dissolved is also directly proportional to the partial pressure of the gas. Atmospheric gases enter water until equilibrium is reached in which the pressure of each gas in

Table 3.2 Solubility of oxygen (O_2) (mg/L) as a function of water temperature and salinity in moist air at 1 atmosphere barometric pressure (1013 kPa). Reproduced from Colt (1984).

Temp (°C)	Salinity (‰)								
	0	5	10	15	20	25	30	35	40
0	14.602	14.112	13.638	13.180	12.737	12.309	11.896	11.497	11.111
5	12.757	12.344	11.944	11.557	11.183	10.820	10.470	10.131	9.802
10	11.277	10.925	10.583	10.252	9.932	9.621	9.321	9.029	8.747
15	10.072	9.768	9.473	9.188	8.911	8.642	8.381	8.129	7.883
20	9.077	8.812	8.556	8.307	8.065	7.831	7.603	7.382	7.167
25	8.244	8.013	7.788	7.569	7.357	7.150	6.950	6.754	6.565
30	7.539	7.335	7.136	6.943	6.755	6.572	6.394	6.221	6.052
35	6.935	6.753	6.577	6.405	6.237	6.074	5.915	5.761	5.610

water is equal to its pressure in the atmosphere. The equilibrium concentration of oxygen in water is given in Table 3.2 for sea level pressure (1013 kPa = 760 mmHg) and different temperatures and salinities. The equilibrium (saturation) concentration of dissolved oxygen (DO) decreases with both increasing temperature and rising salinity. The DO concentrations in Table 3.2 may be adjusted for other atmospheric pressures:

$$DO_s = DO_t \times BP/1013$$

where DO_s = DO concentration at saturation (mg/L) at a measured barometric pressure; DO_t = DO concentration at saturation at sea level (mg/L) from Table 3.2; BP = measured barometric pressure (kPa).

For example, DO_s for freshwater at 28 °C and 900 kPa is:

$$DO_s = 8.244 \times 900/1013 = 7.324 \text{ mg/L}$$

The total pressure holding oxygen in water at greater depths is barometric pressure plus the weight of the water column (hydrostatic pressure). Hydrostatic pressure in freshwater is equal to 97.7 kPa/m depth at 25 °C. Values for other temperatures and salinities are provided by Colt (1984). At 2 m depth, total pressure would be 1207.7 kPa when atmospheric pressure is 1013 kPa. The value for DO_s at 2 m would be:

$$DO_s = 8.244 \times 1207.7/1013 = 9.83 \text{ mg/L}$$

This is more than 2 mg/L greater than the DO concentration in surface water at the same temperature and barometric pressure.

Because of biological activity, DO concentration in natural waters seldom is at saturation. The percentage DO saturation is calculated from the equation:

$$\%\ \text{DO saturation} = \frac{DO_m}{DO_s} \times 100$$

where DO_m = measured DO concentration (mg/L). Surface freshwater at 25 °C and 1013 kPa containing 10 mg/L DO would be at 121% saturation.

When surface water is below 100% saturation with DO, oxygen diffuses from the air to the water through the surface. The converse occurs when water is supersaturated with DO. Oxygen transfer between air and water is accelerated by waves and other disturbances that increase surface area. Mixing the surface layer with deeper water continuously re-establishes the surface, preventing it from becoming saturated with oxygen faster than oxygen can move into the underlying mass of water. Wind is the most important natural factor influencing oxygen exchange at the surface of ponds and lakes. Turbulence created by flowing water favors oxygen exchange; transfer is particularly great where water cascades over rocks.

Saturation concentrations for carbon dioxide and nitrogen in freshwater at several temperatures are provided in Table 3.3, and more complete data on the solubility of these gases are provided by Colt (1984). The methods used above to calculate saturation concentrations and percentage saturation of DO apply equally to nitrogen and carbon dioxide.

3.2.8 Water temperature and light

Water has a large capacity to store heat and water temperature in lakes often lags air temperature. However,

aquaculture facilities have relatively small volumes of water and water temperature closely follows air temperature. Organic solids absorb heat and surface waters of ponds with plankton blooms become warmer than those of ponds with clear water.

Table 3.3 Solubilities of carbon dioxide (CO_2) and nitrogen (N_2) (mg/L) as a function of water temperature and salinity from moist air at 1 atmosphere barometric pressure (1013 kPa). Reproduced from Colt (1984).

Temperature (°C)	Salinity (‰)			
	0	10	20	30
Carbon dioxide				
0	1.09	1.03	0.98	0.93
10	0.75	0.71	0.67	0.64
20	0.54	0.51	0.49	0.48
30	0.40	0.38	0.37	0.35
40	0.31	0.30	0.29	0.28
Nitrogen				
0	23.04	21.38	19.85	18.42
10	18.14	16.93	15.81	14.75
20	14.88	13.96	13.09	12.28
30	12.58	11.85	11.17	10.52
40	10.89	10.29	9.73	9.20

Water bodies receive solar radiation at their surface and surface water warms faster than deeper water. The density of water is at a maximum at ca. 4 °C. Surface water becomes lighter as it warms and may become so much lighter than deeper water that the two layers do not mix, resulting in thermal stratification. The surface layer above the thermocline, in which temperature changes rapidly, is the epilimnion and the deeper layer below the thermocline is the hypolimnion (Fig. 3.1). Thermal stratification will form in most water bodies of more than 2 or 3 m in average depth. Water bodies that are sheltered from the wind are especially susceptible to stratification.

Thermal stratification persists until wind mixing is strong enough to overcome the density difference between the two layers or until surface water cools, resulting in a decline in its density. In the temperate zone, water bodies stratify and destratify annually, but in the tropics some lakes may stratify for longer periods. Aquaculture ponds are usually shallow, and they stratify and destratify on a daily basis (Table 3.4) rather than seasonally or at longer intervals.

Solar radiation is the source of light for photosynthesis. Light intensity quickly diminishes with increasing depth and density of suspended particles. The particles absorb and reflect light, increasing the quenching rate and limiting the depth at which photosynthesis may occur. In stratified water bodies, photosynthesis is restricted to the epilimnion. In unstratified eutrophic ponds, most photosynthesis will occur in the upper 1 m layer.

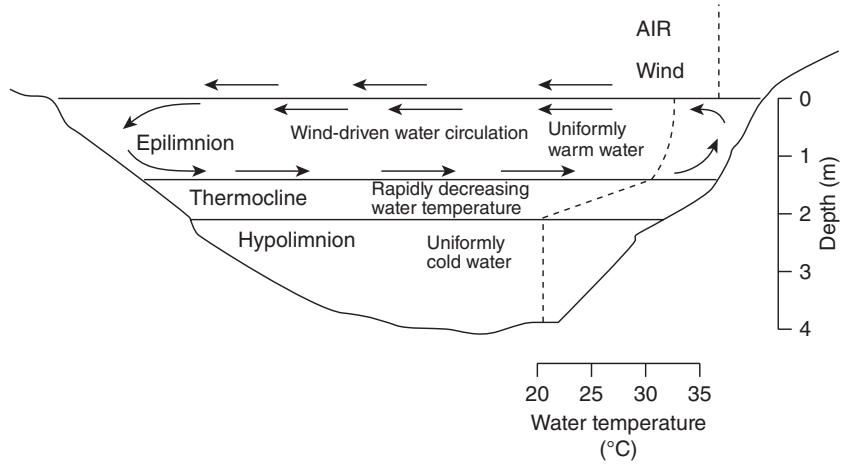


Fig. 3.1 Thermal stratification of a small lake. Reproduced from Boyd (2000) with permission from Springer Science+Business Media B.V.

Table 3.4 Daily thermal stratification and destratification in a typical warmwater aquaculture pond. During the day air warms and the surface water warms faster than the deeper water. During late afternoon and night the air cools and surface water cools until the pond destratifies. Approximate temperatures based on Boyd (2000).

	Temperature (°C)		
	6 AM	3 PM	6 AM
Air	25 →	33 →	25
Water			
0 m	26 →	31 →	26
0.5 m	26 →	29 →	26
1 m	26 →	27 →	26
1.5 m	26 →	26 →	26

The simplest method for evaluating light penetration in water is to measure transparency with a Secchi disk (Fig. 3.2). The average of depths of disappearance and reappearance of the disk is the Secchi disk visibility. Compensation depth (depth where photosynthesis and respiration are equal) corresponds roughly to twice the Secchi disk visibility. Natural, unpolluted lakes may have Secchi disk visibilities of 5–10 m, but aquaculture ponds often have values <75 cm.

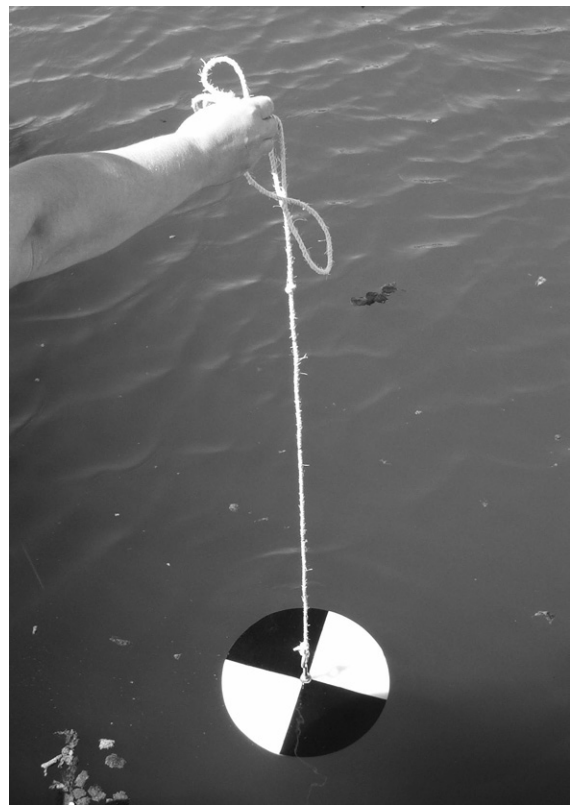


Fig. 3.2 A Secchi disk is lowered into pond water to determine the turbidity. Using graduations on the rope (or handle), the turbidity is measured by noting the depth at which the disk is no longer visible.

3.2.9 pH

The pH is the negative logarithm of the hydrogen ion concentration:

$$\mathrm{pH} = -\log(\mathrm{H}^+)$$

where (H^+) = molar concentration of hydrogen ion. The pH concept originated in the ionization of water:

$$\mathrm{H_2O} \leftrightarrow \mathrm{H}^+ + \mathrm{OH}. \quad K_w = 10^{-14}.$$

K is the equilibrium constant for a reaction. It provides a measure of the equilibrium position. When K is large ($>10^2$), products of the reaction are favored. When K is small, reactants are favored. In this reaction K_w is very small (10^{-14}), favoring the reactant H_2O.

Hydrogen ion concentration and pH of pure water are calculated as follows:

$$(\mathrm{H}^+)(\mathrm{OH}^-) = 10^{-14}, \text{ and } (\mathrm{H}^+) = (\mathrm{OH}^-)$$

$$(\mathrm{H}^+)^2 = 10^{-14}$$

$$\mathrm{H}^+ = \mathrm{OH}^- = 10^{-7}\ \mathrm{M}$$

$$\mathrm{pH} = -\log(10^{-7}) = -(-7) = 7.$$

The pH concept allows hydrogen ion concentration to be expressed as a number between 0 and 14 rather than exponentially or as a small decimal fraction.

Hydrogen ion causes acidity, and pH expresses the degree of acidity. Pure water is neutral, neither basic nor acidic, because $(H^+) = (OH^-)$. Waters with pH below 7 are acidic; those with pH above 7 are basic (or alkaline). Acidity increases as pH declines below 7 and declines as pH rises above 7. The opposite is true for basicity.

3.2.10 Carbon dioxide, bicarbonate, and carbonate

Rainfall naturally is acidic because it is saturated with carbon dioxide (CO_2):

$$H_2O + CO_2 \leftrightarrow HCO_3^- + H^+. \quad K = 10^{-6.35}$$

Carbon dioxide depresses the pH of rainwater to about 5.7. The lower pH values sometimes observed in rainwater are caused by sulfuric acid and other strong acids resulting from air pollution.

Carbon dioxide in rainwater dissolves limestone[1] and silicate minerals[1]:

$$CaCO_3 + CO_2 + H_2O \leftrightarrow Ca^{2+} + 2HCO_3^-$$

$$CaCO_3 \cdot MgCO_3 + 2CO_2 + 2H_2O \leftrightarrow Ca^{2+} + Mg^{2+} + 4HCO_3^-$$

$$CaSiO_3 + 2CO_2 + 3H_2O \leftrightarrow Ca^{2+} + 2HCO_3^- + H_4SiO_4.$$

Reaction of CO_2 with minerals is the source of bicarbonate (HCO_3^-) in water, for no measurable concentration of HCO_3^- results from the reaction of CO_2 with water. In fact, water supersaturated with carbon dioxide may have pH as low as 4.5. At pH values between 4.5 and 8.3, water contains CO_2 and HCO_3^- and the proportions of these two substances establish the pH (Fig. 3.3).

The CO_2 concentration is negligible at pH 8.3 and above, and HCO_3^- will be in equilibrium with carbonate (CO_3^{2-}) instead of carbon dioxide:

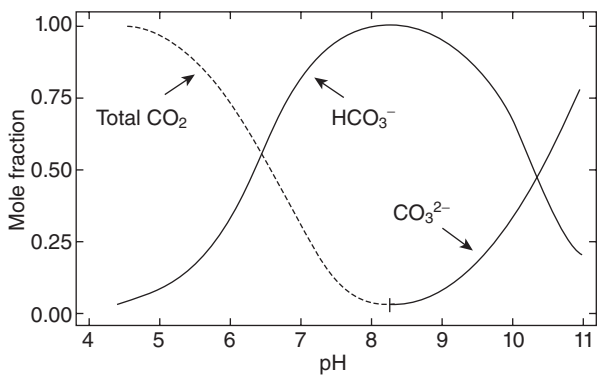


Fig. 3.3 Mole fractions (decimal fraction of all moles present) of CO_2, HCO_3^-, and CO_3^{2-} at different pH values. Reproduced from Boyd (2000) with kind permission from Springer Science+Business Media B.V.

[1]$CaCO_3$ is calcitic limestone and $CaCO_3 \cdot MgCO_3$ is dolomitic limestone, but most limestone is not pure calcite or pure dolomite. $CaSiO_3$ is a representative silicate mineral.

$$HCO_3^- \leftrightarrow H^+ + CO_3^{2-} \quad K = 10^{-10.33}.$$

The proportions of CO_2, HCO_3^- and CO_3^{2-} are related to pH (Fig. 3.3), and only two of them coexist at a given pH. The two components form a buffer to resist pH change. If H^+ is added to water at pH 7.5, it will react with HCO_3^- to produce more CO_2, and pH will not change much as long as HCO_3^- is available. If OH^- is added to water of pH 7.5, it will react with H^+, and more CO_2 will react with water to produce more HCO_3^- and H^+. This avoids a sharp rise in pH as long as CO_2 is present. No CO_2 is present at pH 8.3 and above, and HCO_3^- and CO_3^{2-} act as a buffer by reacting with added H^+ and OH^-.

Carbon in bicarbonate can be used in photosynthesis. As plants remove CO_2, bicarbonate reacts with H^+ and releases more CO_2. Bicarbonate is a source of CO_2 when pH is above 8.3:

$$2HCO_3^- \leftrightarrow CO_2 + CO_3^{2-} + H_2O.$$

3.2.11 Total alkalinity and total hardness

Total alkalinity is the concentration of titratable bases expressed as equivalent $CaCO_3$. In most natural waters, HCO_3^-, and CO_3^{2-} are the predominant bases. The alkalinity titration is done with standard acid, usually 0.02 N sulfuric acid, to an endpoint of pH 4.5. To illustrate, suppose 10.00 mL of 0.02 N H_2SO_4 are used to titrate a 100-mL sample to pH 4.5. Total alkalinity is calculated as follows:

$$(10.00 \text{ mL } H_2SO_4)\left(0.02 \frac{\text{meq}}{\text{mL}}\right) = 0.2 \text{ meq } H_2SO_4$$

$$= 0.2 \text{ meq total alkalinity}$$

$$(0.2 \text{ meq TA})\left(50 \text{ mg} \frac{CaCO_3}{\text{meq}}\right) = 10 \text{ mg } CaCO_3$$

[meq, milliequivalents]

$$\text{Total alkalinity} = 10 \text{ mg } CaCO_3 \times \frac{1{,}000 \frac{\text{mL}}{\text{L}}}{100 \text{ mL sample}}$$

$$= 100 \frac{\text{mg}}{\text{L}} CaCO_3.$$

Total alkalinity of natural water is seldom above 50 mg/L in areas with acidic soils. Areas with neutral or basic soils, especially where soils contain limestone, usually have waters with 50–200 mg/L total alkalinity. Higher concentrations often are found in arid regions. Groundwater from limestone formations may contain up to 500 mg/L total

alkalinity, but some groundwater may be very low in alkalinity. The average total alkalinity of seawater is 116 mg/L.

Total hardness is the concentration of divalent cations expressed as equivalent $CaCO_3$. In most waters, the major divalent cations are calcium and magnesium. Total hardness may be calculated from divalent cation concentration, as illustrated for a water containing 20 mg/L Ca^{2+} and 4 mg/L Mg^{2+}:

$$20\frac{\text{mg}}{\text{L}}\text{Ca}^{2+}\times\frac{100\text{ mg}\dfrac{\text{CaCO}_3}{\text{mmole}}}{40\text{ mg}\dfrac{\text{Ca}^{2+}}{\text{mmole}}}=50\text{ mg}\frac{\text{CaCO}_3}{\text{L}}$$

$$4\frac{\text{mg}}{\text{L}}\text{Mg}^{2+}\times\frac{100\text{ mg}\dfrac{\text{CaCO}_3}{\text{mmole}}}{24.31\text{ mg}\dfrac{\text{Mg}^{2+}}{\text{mmole}}}=16.5\text{ mg}\frac{\text{CaCO}_3}{\text{L}}$$

$$\text{Total hardness}=66.5\text{ mg}\frac{\text{CaCO}_3}{\text{L}}.$$

Total hardness also can be measured by titration with the chelating agent ethylenediaminetetraacetic acid (EDTA).

In humid areas, total hardness concentrations in water normally are similar to those of total alkalinity. Hardness may be considerably higher than total alkalinity in arid regions. Naturally softened groundwaters have lower hardness than alkalinity. The total hardness of seawater exceeds 6000 mg/L.

3.2.12 Nutrients

Plants require carbon, hydrogen, oxygen, nitrogen, sulfur, phosphorus, calcium, magnesium, potassium, sodium, iron, manganese, zinc, copper, and possibly other elements. However, nitrogen and phosphorus are usually the two most important nutrients regulating plant productivity in aquatic ecosystems.

Phosphorus occurs in water primarily as phosphate ion ($H_2PO_4^-$ or HPO_4^{2-}) and in combination with organic matter. In sediment, phosphorus forms relatively insoluble compounds with iron and aluminum under acidic conditions and with calcium under basic conditions. The phosphorus concentration in water in equilibrium with sediment phosphorus usually is less than 10 µg/L. Most phosphorus in water is bound in plankton biomass or adsorbed on suspended soil particles. Total phosphorus concentrations are typically 10–20 times greater than concentrations of soluble phosphorus.

Nitrogen occurs in water in several forms: molecular nitrogen (N_2), ammonia (NH_3), ammonium (NH_4^+), nitrite (NO_2^-), nitrate (NO_3^-), and organic nitrogen. Organic nitrogen may be dissolved or particulate, and particulate organic nitrogen may be in living organisms or in their remains.

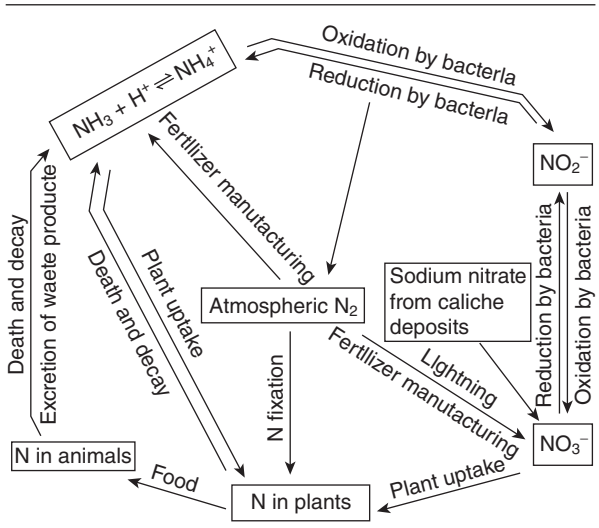


Fig. 3.4 The global nitrogen cycle. Reproduced from Boyd (1990) with kind permission from Springer Science+Business Media B.V.

The solubility of molecular nitrogen in water is presented in Table 3.3. In unpolluted waters, concentrations of ammonia nitrogen and nitrate-nitrogen seldom exceed 0.25 mg/L, and nitrite is often undetectable. Total nitrogen concentration may reach 1 mg/L. However, polluted water and water in aquaculture facilities may contain much greater concentrations of the various nitrogen fractions.

Nearly all aspects of the nitrogen cycle (Fig. 3.4) occur in a lake or pond. Some blue-green algae and bacteria can fix molecular nitrogen, and when they die and decompose, nitrogen is recycled. Rainfall contains nitrate because electrical activity in the atmosphere oxidizes molecular nitrogen to nitrate. Ammonia nitrogen is oxidized to nitrate-nitrogen by nitrifying bacteria in a two-step process:

$$2\text{NH}_4^+ + 1.5\text{O}_2 \rightarrow \text{NO}_2^- + 2\text{H}^+ + \text{H}_2\text{O}$$

$$\text{NO}_2^- + 0.5\text{O}_2 \rightarrow \text{NO}_3^-.$$

In anaerobic zones, denitrifying bacteria reduce nitrate to molecular nitrogen as illustrated below using methanol as a carbon source:

$$6\text{NO}_3^- + 5\text{CH}_3\text{OH} \rightarrow 5\text{CO}_2 + 3\text{N}_2 + 7\text{H}_2\text{O} + 6\text{OH}^-.$$

Ammonia also diffuses from water to the atmosphere, especially when pH is 8 or above.

Plants use ammonium and nitrate for synthesizing amino acids and proteins. When plants die, their remains are decomposed by micro-organisms with mineralization of ammonia to the surrounding environment. However, considerable nitrogen is contained in organic matter that accumulates in the bottoms of aquatic systems.

Both nitrogen and phosphorus are limiting factors in most aquatic ecosystems including aquaculture systems. Nevertheless, excessive nitrogen and phosphorus concentrations in some aquatic systems lead to eutrophication and undesirably dense phytoplankton blooms dominated by blue-green algae.

3.2.13 Effects of micro-organisms on water quality

Phytoplankton abundance is related to availability of light and nutrients. In water bodies of low nutrient status and clear water, underwater weeds will flourish. Waters with a greater availability of nutrients will develop phytoplankton blooms unless they are light limited because of humic substances or clay turbidity.

Plants produce organic matter and release oxygen through photosynthesis:

$$6CO_2 + 6H_2O \xrightarrow[\text{light and nutrients}]{} C_6H_{12}O_6 + 6O_2.$$

Organic matter from photosynthesis is the base of the food web (Fig. 3.5) in water bodies. All organisms respire, and much of the oxygen produced in photosynthesis is used in respiration by the pond biota (including the phytoplankton and water weed at night). Respiration, as an ecological process, is essentially the reverse of photosynthesis:

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6H_2O + 6CO_2 + \text{heat}.$$

For our purposes, it is sufficient to note that plants produce organic matter that is either used in their respiration or in making their tissues. Plant bodies become food for animals or substrate for organisms of decay. Phytoplankton remove nutrients from the water, while decomposer organisms recycle them.

Nitrifying and denitrifying bacteria have a major influence on ammonia and nitrate concentrations. High concentrations of nitrite result when nitrification stops before nitrite is oxidized to nitrate or because denitrifying bacteria produce nitrite instead of nitrogen gas under some conditions.

Dense phytoplankton blooms can cause shallow thermal stratification. Dead phytoplankton settles into the hypolimnion, causing DO depletion and the accumulation of potentially toxic microbial metabolites. Sudden thermal destratification of water bodies resulting from high wind, heavy rains or other factors can mix hypolimnetic water with surface water, leading to DO depletion and fish kills.

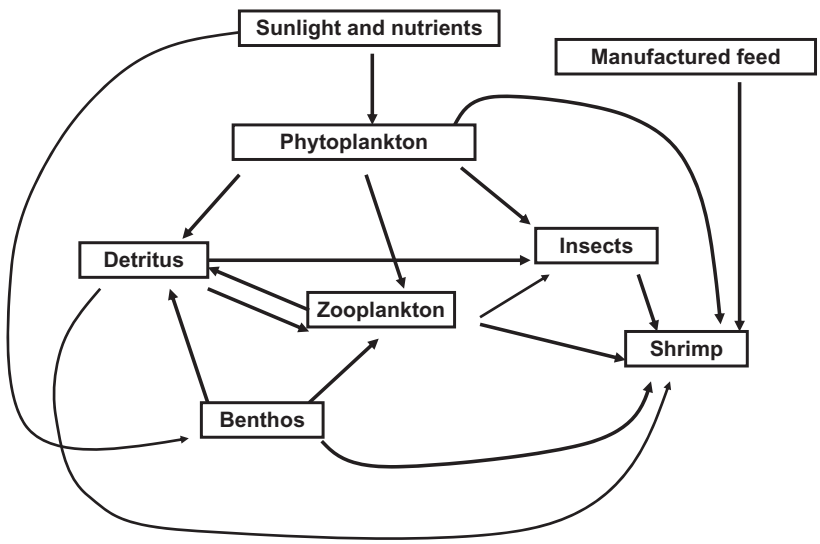


Fig. 3.5 The food web of a shrimp pond.

In the afternoon, DO and pH will be higher in surface water than in deeper water while the opposite occurs with carbon dioxide. In water bodies that do not stratify thermally, oxygen depletion will not usually occur in bottom waters, but sometimes anaerobic zones will still develop at the sediment–water interface.

Phytoplankton profoundly affect pH, and concentrations of DO and carbon dioxide during the 24-hour cycle of light and darkness. In daylight, photosynthesis uses carbon dioxide and releases DO faster than respiration releases carbon dioxide and uses oxygen. This results in an increase in DO concentration and a decrease in carbon dioxide concentration. The decrease in carbon dioxide will cause pH to increase. At night, photosynthesis stops but respiration continues. Thus, DO declines and pH decreases because carbon dioxide concentration increases. The amount of fluctuation of the three variables increases as phytoplankton abundance increases. Depletion of DO may occur at night in water bodies with especially dense phytoplankton blooms (Fig. 3.6). The likelihood of DO depletion increases during cloudy weather, because daytime DO concentration does not rise as high as usual, and night-time concentrations will fall lower than normal.

Dense phytoplankton blooms may suddenly die because of exposure to excessive light or unexplained factors. Decomposition of the dead phytoplankton can result in DO depletion and fish kills.

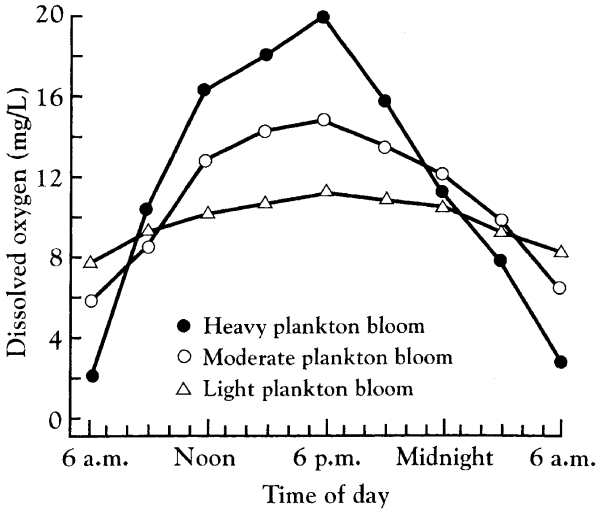


Fig. 3.6 Effect of time of day and plankton density on dissolved oxygen concentration in surface water of aquaculture ponds.

3.2.14 Bottom soils and water quality

In ponds with acidic bottom soils, the overlaying water will be of low alkalinity and poorly buffered. Soils have a great affinity for phosphorus, and they exert control over phosphorus concentration and phytoplankton growth. Organic matter accumulates in pond bottoms, and bacteria degrade this material and recycle the nutrients in it. Bacterial activity uses oxygen faster than it can diffuse or infiltrate into pond bottoms. Below a depth of a few centimetres in oligotrophic water bodies or a few millimetres in eutrophic ones, sediment is anaerobic. This situation is often depicted by plotting redox potential versus depth (Fig. 3.7): the more positive the redox potential, the greater affinity there is for electrons and thus the tendency for an oxidizing environment. In oxygenated water, the redox potential typically is about 560 millivolts (mV). The sediment water interface is usually oxidized and similar in redox potential to overlying water. Redox potential drops rapidly with increasing depth in the sediment.

Organic matter is decomposed by fermentation under anaerobic conditions, but this process only partially degrades organic matter. Certain bacteria use oxygen from inorganic compounds in place of molecular oxygen to decompose organic matter completely to CO_2 and H_2O – denitrifying bacteria are an example. Other bacteria can use oxygen from iron and manganese compounds, sulfate, and carbon dioxide in decomposing organic matter. These processes results in the release of nitrogen gas and nitrite, ferrous iron, manganous manganese, hydrogen sulfide, and methane, respectively. Nitrite and hydrogen sulfide in particular are potentially toxic to aquatic animals. Other than methane and nitrogen gas, reduced substances do not normally diffuse into the water column because the sediment–water interface tends to be aerobic.

Soils can exchange cations with pore water or overlaying water. Equilibrium is established between cations in the water and cations in the sediment. This process has not been studied thoroughly, but it is known that potassium applied to ponds in fertilizer declines to near pre-treatment levels within a few months because of soil uptake (Boyd *et al.*, 2007).

Exchangeable aluminum on sediment can be neutralized by liming material to increase sediment pH. Low pH in soil results from the following reaction:

$$\text{Soil} - \text{Al} \leftrightarrow \text{Al}^{3+} + 3\text{H}_2\text{O} \leftrightarrow \text{Al(OH)}_3 + 3\text{H}^+.$$

Liming material (as illustrated with $CaCO_3$) can be applied to the sediment to neutralize the hydrogen ion:

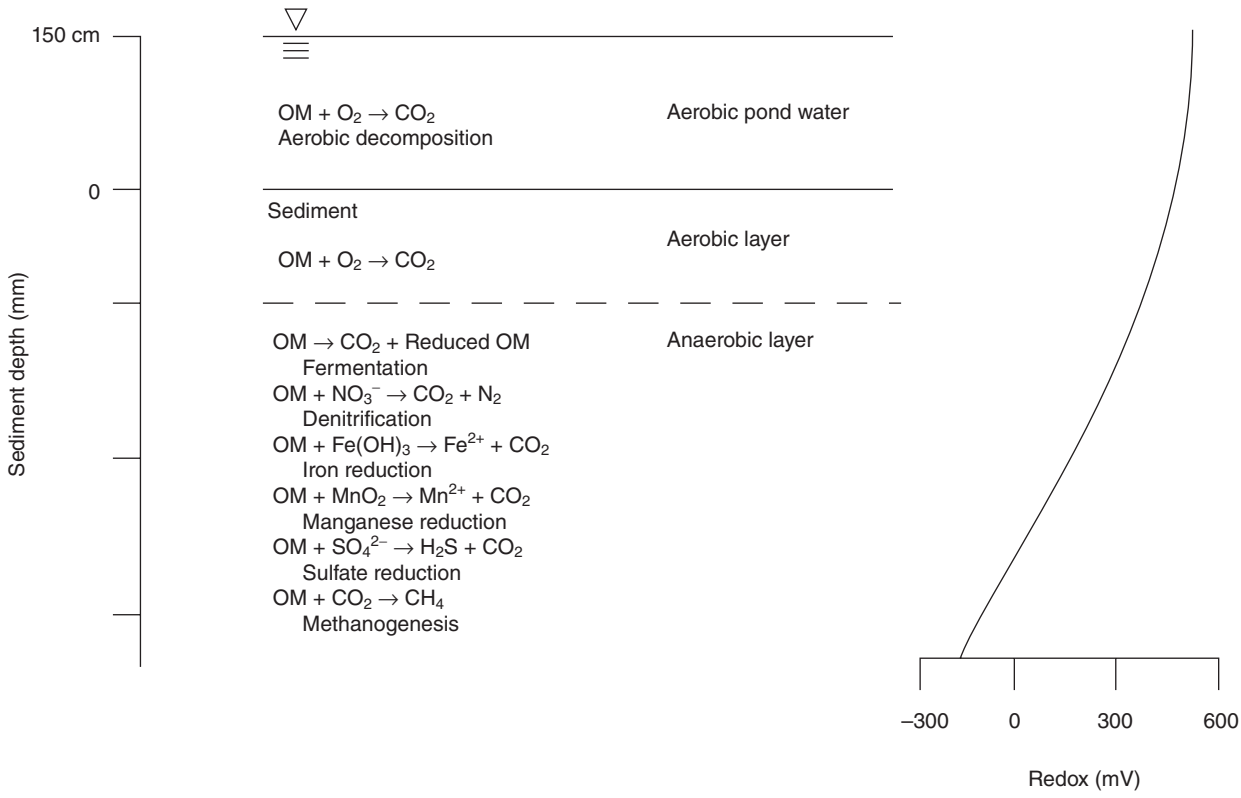


Fig. 3.7 Schematic representation of changes in microbial respiration with decreasing redox potential in aquaculture pond sediment.

$$1\frac{1}{2}CaCO_3 + 3H^+ \rightarrow 1\frac{1}{2}Ca^{2+} + 1\frac{1}{2}CO_2 + 1\frac{1}{2}H_2O.$$

Neutralization of H^+ results in more aluminum ions being released from the soil to hydrolyze and precipitate as aluminum hydroxide with the resulting hydrogen ion continuing to be neutralized by calcium carbonate. Calcium ion from the reaction of $CaCO_3$ and H^+ replaces Al^{3+} on the soil. This causes soil pH to decline. Enough calcium carbonate or other liming agent is applied to neutralize the acidity that can potentially result from exchangeable aluminum. Replacement of exchangeable aluminum with calcium will raise soil pH to near 7.

3.3 EFFECTS OF WATER QUALITY ON CULTURE SPECIES

The effects of these water quality variables tend to follow three patterns (Fig. 3.8). In Fig. 3.8 'Performance' includes factors such as level of feeding, growth, normal behavior, survival, fecundity, and immunity.

3.3.1 Water temperature

Temperature is perhaps the most important water quality variable for it directly or indirectly affects other water quality variables, natural productivity, and culture species. It is seldom possible to alter water temperature in outdoors culture, and cultured species that grow well at the water temperature range of a particular site must be selected.

Increasing temperature increases the rate of physical processes, chemical reactions, and metabolism and growth of organisms. According to van Hoff's law, the rate of chemical reactions will double with a 10 °C increase in temperature. This relationship applies to aquatic animal growth within their range of temperature tolerance. The factor of increase is called the Q_{10}, which is usually about 2 for aquatic animals. Of course, if temperature exceeds optimum, the growth of aquatic animals will decline.

Water temperature ranges for some common aquaculture species are provided in Table 3.5. This table gives the lower critical range, the range over which feeding occurs, the optimum range for growth and the upper critical range.

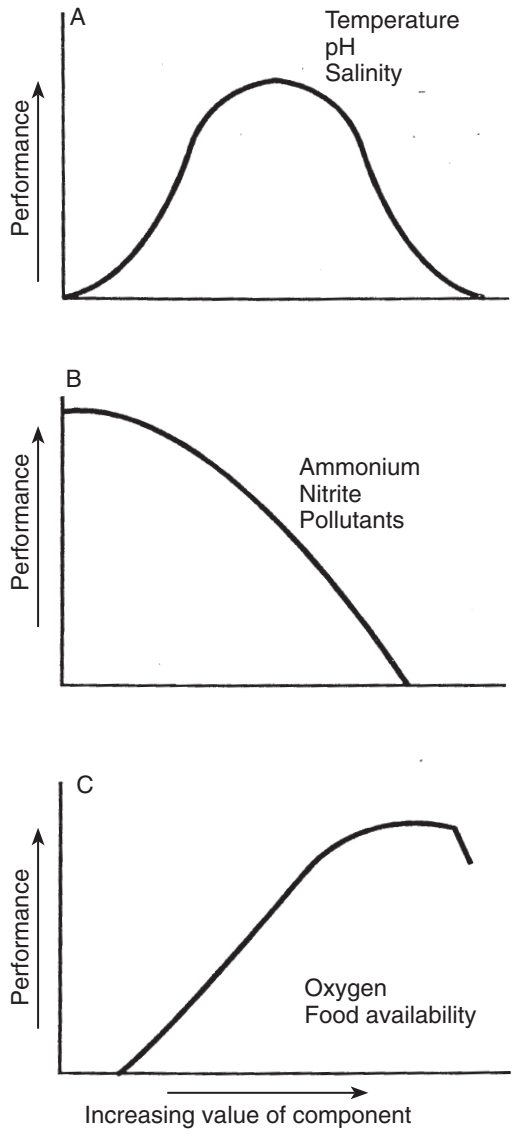


Fig. 3.8 Three curves showing different patterns of the influence of environmental parameters on cultured animals. A. With a distinct optimum level. B. Toxic environmental parameters. C. There is an broad optimum, but an adverse effect of very high levels of the parameter. After Tomasso (1996).

To illustrate interpretation of data in the table, channel catfish may die at water temperatures near 0 °C and 40 °C. They will not eat at temperatures below 15 °C or above 34 °C. The best growth is achieved at temperatures of 25–30 °C.

The relationship between temperature and disease is complex, affecting both host and pathogen. The immune system of aquatic animals functions best within the optimum temperature range for growth (Table 3.5). Thus, epizootics are most common at lower or higher than optimum temperature. Sudden changes in temperature also impair the effectiveness of the immune system.

Water temperature must be monitored during the culture period because it is useful to know if suboptimum growth is the result of water temperature. In some tropical regions, water temperature averages a few degrees lower in the dry season than in the wet season. Nevertheless, producers often fail to recognize the temperature effect and seek other reasons for slower growth in the dry season.

3.3.2 Salinity

Aquaculture species have a range of tolerance for salinity, for they must maintain a suitable salinity of internal fluids through osmoregulation or ion regulation. To understand osmoregulation, consider that a solvent will pass through a membrane from a dilute solution to a more concentrated solution. To visualize this phenomenon consider that the membrane is being bombarded continuously on both sides by molecules of solvent and solute alike. On the side of the membrane facing the dilute solution, more molecules of the solvent will strike the membrane surface than on the side of the membrane facing the concentrated solution because there are more solvent molecules per unit volume in the dilute solution than in the concentrated solution. There will be a net movement of solvent molecules from the dilute solution to the concentrated solution until equilibrium is attained between the two sides.

We can think of the body fluids of aquatic animals as one solution, the water as the other solution, and the part of the animal that separates the two solutions as the membrane. Freshwater fish have body fluids that are more concentrated in ions than the surrounding water; they are hypersaline, or hypertonic, to their environment. Saltwater species have body fluids more dilute than the surrounding water; they are hyposaline or hypotonic to their environment. Osmoregulation in freshwater fish involves the uptake of ions from the environment and restriction of ion loss. The freshwater fish tends to accumulate water because it is hypertonic to the environment, so it must excrete water and retain ions. On the other hand, osmoregulation for the marine fish requires constant intake of water and excretion of ions. Because the marine fish is hypotonic to the environment, it loses water. To replace this water, the fish takes in salt water; but to prevent the accumulation of excess salt, it must excrete salt.

Table 3.5 Critical water temperatures for six representative fish species. Reproduced from Boyd and Tucker (1998), with kind permission from Springer Science+Business Media B.V.

Species	Lower critical[1] range (°C)	Optimum[2,3] range (°C)	Upper critical range (°C)
Oreochromis mossambicus (Java tilapia)	10–14	18–(28–32)–34	36–42
Sciaenops ocellatus (red drum)	8–15	18–(22–28)–30	34–40
Ictalurus punctatus (channel catfish)	0–10	15–(25–30)–34	35–40
Micropterus salmoides (largemouth bass)	0–10	12–(25–30)–32	32–38
Morone saxatilis (striped bass)	0–6	10–(14–24)–28	30–34
Oncorhynchus mykiss (rainbow trout)	0–4	5–(10–16)–20	22–26

[1]Upper and lower critical temperatures describe the ranges over which significant disturbances in metabolism may occur even if the fish are slowly acclimated to those temperatures. The more extreme value in the range is an approximation of the critical thermal maximum or minimum – the temperature at which only brief survival is expected.

[2]The optimum range is the temperature range over which feeding occurs.

[3]Values in parentheses are the ranges for fastest growth.

Invertebrates such as decapod crustaceans and bivalves tend to conform to their environmental salinity more than fish. They tend to be isotonic in seawater, although the ion concentrations of their body fluids differ from seawater. Thus, they must regulate the uptake and loss of particular ions across their body surface. Crustaceans and especially bivalves living in freshwater or low salinity have lower body fluid concentrations than those in seawater. However, they are hypertonic to their environment and must regulate water and ions in the same manner as freshwater fish.

Each species has an optimum salinity range. Outside of this range the animal must expend considerable energy for osmoregulation and ion regulation at the expense of growth and other processes. If salinity deviates too much from optimum, the animal will die because it cannot maintain homeostasis.

Many freshwater fish can live in waters with salinities up to 5–10‰, but they may not reproduce or grow well at salinities above 3 or 4‰. Some species such as tilapia and rainbow trout can tolerate much higher salinity. Marine species are adapted to growth in ocean water and do not grow well at low salinity. Estuarine species are adapted to a wide range of salinity. Most species of cultured marine shrimp survive and grow well at salinities of from 1–2‰ up to 35–40‰. Suboptimum salinity is especially stressful on aquaculture species when temperature also is outside the optimum range.

3.3.3 pH

The optimum pH for growth and health of most freshwater aquatic animals is in the range of 6.5 to 9.0. Acid and alkaline death points are approximately pH 4 and pH 11. Marine fish evolved in the highly buffered seawater that is not subject to wide variation in pH. Consequently, most marine animals cannot tolerate as wide a range of environmental pH as freshwater animals, and the optimum pH is usually between pH 7.5 and 8.5. Some species evolved in estuaries where there is typically large variation in pH in response to variations in river discharge and tidal flow. Brackishwater inhabitants are rather tolerant of extremes of pH.

The gills of aquatic animals are the primary target of elevated, environmental H^+ concentration. The seven major effects of low pH on gill structure and function in freshwater fish in approximate order of occurrence as pH declines from about 6 to 4 follow (Boyd and Tucker, 1998):

1. Inhibition of sodium and chloride uptake.
2. Increased ion permeability and ion efflux.
3. Increased hydrogen ion influx.
4. Increased mucus production.
5. Mucus coagulation and precipitation.
6. Inhibition of gas exchange.
7. Damage to epithelial layers.

Maintaining internal ion balance is the major problem facing animals in low pH environments. Increased mucus production and gill damage at low pH also impair respiratory efficiency. Respiration is further compromised by blood acidosis, which decreases the affinity of hemoglobin for oxygen. Under mild acid stress, animals expend extra metabolic energy for maintenance of gill function at the expense of growth and immune function. Under extreme acid stress, the animal is not capable of maintaining homeostasis and dies.

Acid stress is most common in bodies of water where pH has declined because of human activities. For example, long-term acidification of lakes because of acid precipitation is having disastrous effects on natural fish populations in certain areas of Europe and North America. Low pH in aquaculture systems usually can be controlled through liming.

3.3.4 Dissolved oxygen

Oxygen is needed to oxidize organic matter from food to provide energy for biological activities. Low DO concentration may cause chronic problems of poor appetite, slow growth, and increased susceptibility to disease. It also may result in mortality, with sudden severe DO depletion causing massive mortality.

Most of the oxygen carried in fish blood is associated with hemoglobin in red blood cells, although a small fraction dissolves in the plasma (Boyd and Tucker, 1998). The association of oxygen and hemoglobin is a loose one, and deoxygenated hemoglobin readily accepts oxygen at the gill and easily releases it to the tissues elsewhere in the body. Loading and unloading of hemoglobin with oxygen is governed by oxygen tension. At the gills, the tension of oxygen in water is higher than in the blood, and oxygen is loaded onto hemoglobin. In the tissues, oxygen is used rapidly and tissue fluids have a lower oxygen tension than blood entering the tissues from the arterial system. So, hemoglobin unloads oxygen to the tissue fluids.

Active fish, such as trout, that evolved in high-oxygen environments characteristically have "low-affinity" hemoglobin that easily unloads oxygen at the tissues, an advantage when oxygen is needed during exercise. The disadvantage of low-affinity hemoglobin is that the hemoglobin is not fully loaded with oxygen unless the oxygen tension at the gills is high. Warmwater fish evolved in environments where low DO concentrations were more common; they have "high-affinity" hemoglobin that is capable of effectively loading with oxygen over a wide range of oxygen tensions. This is advantageous to fish that must endure periods of low DO concentration.

Invertebrates do not have hemoglobin in blood. Most species contain another pigment, hemocyanin, which functions in much the same manner as hemoglobin.

Coldwater fish are usually more sensitive to hypoxia than are warmwater fish. Critical DO concentrations that negatively affect growth are lower than 5–6 mg/L for trout and salmon, and 3–4 mg/L for warmwater fish. However, when expressed as oxygen tension, the driving force for diffusion of oxygen across the gill epithelium and for oxygen loading by hemoglobin, the differences in critical oxygen tensions for growth are more limited.

Fish feed best, grow fastest, and are healthiest when DO concentrations are above 5 mg/L. As DO concentrations fall below 5 mg/L, fish compensate for decreased availability of oxygen through behavioral and physiological changes. Ventilation volume increases because less oxygen is available in a given amount of water. Fish minimize extraneous activity to reduce metabolic oxygen demand. Certain physiological responses to low DO concentrations increase the capability for gas exchange at the gills. These adaptations and responses allow warmwater pond fish to survive for days even when DO concentrations are as low as 1–2 mg/L. At some point, compensatory responses are no longer sufficient and oxygen demand of tissues exceeds the amount that can be supplied. At about that point, fish swim to the surface in an attempt to exploit oxygen in the surface film. Eventually, the energy requirement for metabolism in the brain is not met and fish die. Adult fish can live for several hours at DO concentrations as low as 0.3–0.5 mg/L and fingerlings may survive short exposure to lower concentrations. Small fish consume more oxygen per unit weight than large fish because of their higher metabolic rate per unit weight. Small fish are also more effective at using oxygen in surface films than large fish.

Coldwater fish usually die at a slightly higher oxygen concentration than required to kill warmwater fish. For example, rainbow trout may die at DO concentrations of 2.5–3.5 mg/L, and they do not grow well at DO concentration below 5 mg/L. Crustaceans are remarkably similar to fish in their tolerance of low DO concentrations.

Wide daily fluctuations in DO typically occur in intensive aquaculture ponds. In channel catfish ponds, DO concentrations often exceed 15 mg/L in the afternoon but fall below 5 mg/L by dawn. Usually, fish growth decreases if DO concentrations before sunrise drop below 25% saturation. Concentrations of DO above saturation provide no benefit to fish or crustaceans because the oxygen-transporting pigment will fully load with oxygen when water is saturated with DO.

3.3.5 Carbon dioxide

Carbon dioxide concentrations of more than about 60–80 mg/L have a narcotic effect on aquatic animals and higher concentrations may cause death. Lower concentrations may stress fish by interfering with respiration or by causing formation of calcareous deposits in the kidney. High concentrations of dissolved carbon dioxide reduce carbon dioxide excretion at fish gills causing elevated levels of plasma carbon dioxide and uncompensated respiratory acidosis. These conditions decrease the affinity of hemoglobin for oxygen and reduce oxygen uptake by blood at the gills, even if DO concentrations are high. Exposure to high carbon dioxide concentrations reduces respiratory efficiency and decreases the tolerance of animals to low DO concentrations. An upper limit of 20 mg/L dissolved carbon dioxide is often suggested for salmonids. If DO concentrations are above about 5 mg/L, carbon dioxide concentrations of at least 30 mg/L can be tolerated by warmwater species. Nevertheless, fish can sense small differences in carbon dioxide concentrations and try to avoid areas with high concentrations.

Most studies have concentrated on the effects of long-term exposure to specific carbon dioxide concentrations. Such information has application to flow-through culture systems and closed-recirculating culture systems where carbon dioxide concentration is relatively stable over time. In ponds, carbon dioxide concentrations may change by an order of magnitude between night and day. No studies have been made of the effects of short-term exposure of fish or crustaceans to fluctuating carbon dioxide concentrations so it is difficult to assess the importance of carbon dioxide to pond aquaculture.

3.3.6 Gas supersaturation

Water can become supersaturated with gases by several processes: rapid increase in temperature; mixing of air-saturated water masses of different temperatures; expulsion of air into surrounding water during ice formation; air entrainment by falling water; leaks on the suction side of pumps; submerged aerators; photosynthesis (oxygen only); sudden changes in barometric pressure. Gas supersaturation is expressed as the ΔP value which is calculated by subtracting local barometric pressure from total gas pressure in the water or by measuring it directly with a saturometer.

Aquatic animals living in gas-supersaturated waters may develop a condition called gas bubble trauma. Supersaturation is an unstable condition and as gases come out of solution they form bubbles. If the gases in solution diffuse across the gill before coming out of solution, emboli will form in the vascular system and other tissues. Acute gas bubble trauma occurs at high levels of supersaturation, usually at ΔP values of 67–270 kPa or greater. Eggs will float to the surface, and larvae and fry may exhibit hyperinflation of the swim bladder, exophthalmos, cranial swelling, edematous and swollen gill lamellae, hemoperitoneum, gas bubbles in yolk sac, and distention and rupture of the yolk sac membrane. In juvenile and adult fish, the most common symptoms of acute gas bubble trauma are gas bubbles in the blood, bubble formation in gill filaments, on the head, in the mouth and in fin rays. The eyes will also protrude. Mortalities of 50–100% are common in acute gas bubble trauma, and death is caused by vascular emboli that restrict blood flow resulting in tissue anoxia. When aquatic animals are exposed to ΔP values of 33–100 kPa on a continuous basis, chronic gas bubble trauma may develop. Usual symptoms are bubble formation in the gut and buccal cavity, hyperinflation or rupture of the swim bladder, and low level mortality over several days or weeks. Mortality is often related to secondary stress-related infections.

Although animals may potentially develop gas bubble trauma at any time there is a positive ΔP, occurrence of the syndrome is strongly influenced by the depth at which animals spend most of their time. Greater depth and higher hydrostatic pressure mean that a larger DO concentration is necessary for supersaturation. Water depth can also affect the potential for developing gas bubble trauma because rates of photosynthesis and solar heating decrease with depth.

Despite the frequent occurrence of supersaturated conditions in surface waters, gas bubble trauma rarely occurs in aquaculture ponds. Apparently, if supersaturation of surface water reaches harmful levels in ponds, animals will move into deeper waters where ΔP is lower. In most ponds, supersaturation will only threaten eggs or fry restricted to the surface by lack of mobility. In shallow ponds with clear water and abundant submersed vegetation, the entire water volume can become strongly supersaturated and there is no haven for animals.

3.3.7 Ammonia and nitrite

Ammonia nitrogen exists in two forms, NH_3 and NH_4^+, in a pH- and temperature-dependent equilibrium:

$$NH_3 + H_2O \leftrightarrow NH_4^+ + OH^-.$$

As pH rises, un-ionized ammonia increases relative to ammonium. Water temperature also causes an increase in

Table 3.6 Percentage un-ionized ammonia (NH_3) in aqueous solution at different pH values and water temperatures. From Boyd (1990).

	Temperature (°C)								
pH	16	18	20	22	24	26	28	30	32
7.0	0.30	0.34	0.40	0.46	0.52	0.60	0.70	0.81	0.95
7.5	0.96	1.10	1.28	1.46	1.68	1.92	2.22	2.56	3.02
8.0	2.88	3.32	3.83	4.37	4.99	5.71	6.55	7.52	8.77
8.5	8.74	9.98	11.38	12.85	14.46	16.31	18.40	20.70	23.57
9.0	22.87	25.57	28.47	31.37	34.42	37.71	41.23	44.84	49.02
9.5	48.41	52.04	55.66	59.00	62.24	65.50	68.71	71.76	75.00
10.0	74.78	77.46	79.92	82.05	84.00	85.82	87.52	89.05	90.58

the proportion of un-ionized ammonia, but the effect of temperature is less than that of pH. The common analytical procedures for ammonia nitrogen measure total ammonia nitrogen (TAN) which includes both NH_3 and NH_4^+. Percentages of un-ionized ammonia at different temperature and pH values (Table 3.6) may be used to estimate NH_3-N from TAN.

The tolerance of aquatic organisms to ammonia varies with species, physiological condition, and environmental factors. Lethal concentrations to warmwater fish and crustaceans for 24–96 h exposure are between 0.4 and 2.0 mg/L of un-ionized ammonia.

TAN toxicity to fish and other aquatic creatures is attributed primarily to NH_3. As ammonia concentration in water increases, ammonia excretion by aquatic organisms diminishes, and levels of ammonia in blood and other tissues increase. The result is an elevation in blood pH and adverse effects on enzyme-catalyzed reactions and membrane stability. Ammonia increases oxygen consumption by tissues, damages gills, and reduces the ability of blood to transport oxygen. Its toxicity is usually expressed by reduced growth rate instead of mortality. However, disease susceptibility also increases in organisms exposed to sublethal concentrations of ammonia.

Ammonia concentrations in ponds are difficult to evaluate. Because of daily fluctuation in pH, un-ionized ammonia concentrations change continuously. Although afternoon pH may be high in ponds, it seldom remains at its greatest level for more than 1 or 2 hours.

Nitrite-nitrogen, NO_2-N, may accumulate to concentrations of 1–10 mg/L or more in water of aquaculture systems under certain conditions. When nitrite is absorbed by fish, it oxidizes ferrous iron in hemoglobin to ferric iron to form methemoglobin that is not capable of combining with oxygen. Blood containing significant amounts of methemoglobin is brown, so nitrite poisoning is commonly called "brown blood disease." Crustaceans contain hemocyanin, a compound similar to hemoglobin but with copper instead of iron. Reactions of nitrite with hemocyanin are poorly understood, but nitrite can be toxic to crustaceans.

The amount of methemoglobin in channel catfish cultured in ponds varies from 5 to 90% of the total hemoglobin. A slight brown color is apparent when methemoglobin reaches 25 or 30% and a chocolate-brown color is obvious at concentrations of 50% or more.

Some species of fish are able to reduce methemoglobin back to hemoglobin through the action of methemoglobin reductase. When NO_2-N concentrations in the water decline or when fish are transferred to water with low NO_2-N concentration, methemoglobin levels quickly decline. However, it may take fish 2 or 3 weeks to recover completely.

Determination of the highest permissible NO_2-N concentration for pond waters is also difficult, because the toxicity of nitrite is closely related to DO concentration and several other factors. However, pond managers must be concerned when NO_2-N concentrations exceed 2 or 3 mg/L.

The simplest procedure for counteracting nitrite toxicity in fish is to treat water with chloride to reduce the molar ratio of nitrite to chloride. A chloride : nitrite-nitrogen ratio of 1 : 30 prevents negative effects of high NO_2-N concentration on channel catfish (Tucker and Hargreaves, 2004). Catfish farms try to maintain about 100–150 mg/L of chloride in ponds by treatment with common salt (NaCl). Water exchange or replacement can also be effective in reducing NO_2-N concentration.

3.3.8 Hydrogen sulfide

Sulfide is an ionization product of hydrogen sulfide and participates in the following equilibria:

$$H_2S \leftrightarrow HS^- + H^+$$

$$HS^- \leftrightarrow S^{2-} + H^+.$$

The pH regulates the distribution of total sulfide among its forms (H_2S, HS^-, and S^{2-}). Un-ionized hydrogen sulfide (H_2S) is toxic to aquatic organisms; the ionic forms have no appreciable toxicity. Analytical procedures measure total sulfide. The percentages of un-ionized hydrogen sulfide at different pH values at 28 °C are presented in Table 3.7. The percentage of hydrogen sulfide decreases as the pH increases.

Concentrations of 0.01–0.05 mg/L of hydrogen sulfide may be lethal to aquatic organisms. Any detectable concentration of hydrogen sulfide is considered undesirable. The presence of hydrogen sulfide may be recognized without water analysis, for the "rotten-egg" smell of hydrogen sulfide is detectable at very low concentration.

3.3.9 Total alkalinity and total hardness

Total alkalinity results primarily from bicarbonate in waters for aquaculture, and fish have no direct requirement for bicarbonate. There is evidence that crustaceans need bicarbonate when moulting, and 75–100 mg/L is considered sufficient for this purpose. Waters with low total alkalinity are poorly buffered and the availability of carbon dioxide is limited. Waters containing 50 mg/L or more total alkalinity have more stable water quality and are more productive than waters of lower total alkalinity. Waters with total alkalinity more than 200–300 mg/L may have high pH and low availability of phosphorus.

Table 3.7 Percentage un-ionized hydrogen sulfide (H_2S) in aqueous solution at different pH values and water temperatures. From Boyd (1990).

	Temperature (°C)								
pH	16	18	20	22	24	26	28	30	32
5.0	99.3	99.2	99.2	99.1	99.1	99.0	98.9	98.9	98.9
5.5	97.7	97.6	97.4	97.3	97.1	96.9	96.7	96.5	96.3
6.0	93.2	92.8	92.3	92.0	91.4	90.8	90.3	89.7	89.1
6.5	81.2	80.2	79.2	78.1	77.0	75.8	74.6	73.4	72.1
7.0	57.7	56.2	54.6	53.0	51.4	49.7	48.2	46.6	45.0
7.5	30.1	28.9	27.5	26.3	25.0	23.8	22.7	21.6	20.6
8.0	12.0	11.4	10.7	10.1	9.6	9.0	8.5	8.0	7.6
8.5	4.1	3.9	3.7	3.4	3.2	3.0	2.9	2.7	2.5
9.0	1.3	1.3	1.2	1.1	1.0	1.0	0.9	0.9	0.8

Groundwater from some aquifers is both supersaturated with carbon dioxide and high in total alkalinity. When such water is exposed to the air, carbon dioxide is lost, and a portion of the bicarbonate is transformed to carbonate and precipitates as calcium carbonate. This phenomenon is usually not harmful in ponds or other large grow-out units, but precipitation of calcium carbonate on eggs or fry of fish or shrimp in hatcheries may be harmful.

In freshwater aquaculture systems, total hardness concentration will usually be similar to that of total alkalinity. The most common aberration involves certain well waters with hardnesses drastically lower than alkalinities. When used in ponds, such water may develop very high pH in response to photosynthesis. In brackishwater and seawater, total hardness will be many times greater than total alkalinity. This condition does not cause problems with culture species.

Calcium is a major contributor to total hardness in freshwater and low hardness is an indicator of low calcium concentration. In seawater, magnesium contributes much more to hardness than does calcium.

3.4 WATER QUALITY MANAGEMENT

Most water quality problems associated with source water originate from natural phenomena or land use practices on catchments. Common natural limitations are high concentrations of humic substances in waters from woodland watersheds, fluctuations in salinity, and acidic low-alkalinity water from catchments with acidic soils. There are also many problems associated with land use practices. High turbidity in surface water is common where agriculture, logging, mining or other activities have disturbed soils on the catchment area. Water from agricultural areas may be contaminated with pesticides. Pollution from domestic and municipal sources has high concentrations of nutrients and organic matter. Industrial pollution can be a source of heavy metals, potentially toxic inorganic and organic chemicals, organic matter, and acids and bases. Limitations from turbidity and acidity can usually be corrected at aquaculture facilities, but little can be done at the farm level to mitigate other types of pollution in source water.

Turbidity from suspended soil particles can best be corrected by eliminating the source of the turbidity on the catchment. If this cannot be done, a settling basin may be constructed to remove suspended soil particles from water before it enters production units.

3.4.1 Liming of acidic, low-alkalinity water

Ponds built in areas with acidic-sulfate soils may have waters with pH values so low that culture species will not survive or, if they survive, they will not grow well. Such situations may be corrected by heavy liming and management procedures designed to minimize the contact of acid-sulfate soil with the air. However, it is best to avoid using sites with potential acid-sulfate soils for aquaculture.

The more common acidity problem occurs in ponds constructed on soils with a high concentration of exchangeable aluminum. Waters in such ponds have pH of 5.5–6.5 and alkalinity below 20 mg/L. Fish will grow in such waters, but natural productivity of fish food organisms will be low, and waters will be poorly buffered against pH change in response to phytoplankton photosynthesis. Liming materials increase the pH of bottom soils and elevate alkalinity and hardness in water. These changes improve conditions for microbial activity and growth of benthic animals; increase the availability of carbon dioxide, phosphorus, and other nutrients; enhance phytoplankton growth; and improve the survival and growth of the aquacultural crop.

The most common liming materials are agricultural limestone, burnt lime, and hydrated lime. These products are made from limestone, which is a relatively soft rock composed of calcium carbonate ($CaCO_3$ or calcite), calcium magnesium carbonate ($CaCO_3 \cdot MgCO_3$ or dolomite) or a mixture of these carbonates.

Limestone in its natural form is not highly soluble. By pulverizing it into fine particles, the surface area/mass ratio is increased to enhance solubility. In good-quality agricultural limestone, most particles will pass through a 60-mesh sieve (0.15-mm openings). Other materials that have been used for liming include chalk (soft limestone), marl (loose carbonate deposits often mixed with clay), crushed seashells, and wood ash.

Agricultural limestone neutralizes acidity with release of calcium to calcium ions, carbon dioxide, and water. Magnesium carbonate in agricultural limestone reacts with acidity in a similar manner, with the release of magnesium ions.

Reaction of agricultural limestone with carbon dioxide results in dissolution of calcium carbonate and an increase in the calcium and bicarbonate concentrations in solution. Magnesium carbonate reacts in a similar manner, and magnesium and bicarbonate result.

Application of agricultural limestone to water normally increases total alkalinity and hardness by roughly equal amounts. The solubility of calcium carbonate in water in equilibrium with normal atmospheric concentrations of carbon dioxide is about 60 mg/L. In ponds, there is usually more carbon dioxide available from organic matter decomposition and more calcium carbonate is dissolved. Nevertheless, if pond waters contain more than 80–100 mg/L alkalinity, agricultural limestone will not usually dissolve.

Agricultural limestone reacts in soil much the same way it does in water. However, the calcium and magnesium ions, which result from its reaction with hydrogen ions or carbon dioxide, exchange for aluminum and iron ions absorbed on soil colloids. This reduces the acidity of the soil and causes its pH to increase. The pH of soil, however, cannot be increased above 8.3 by application of agricultural limestone.

Burnt lime is made by burning limestone in a furnace at high temperature. Hydrated lime is made by treating burnt lime with water. Both burnt lime and hydrated lime are usually fine white powders. When put into ponds, calcium oxide reacts with water and forms calcium hydroxide which neutralizes acidity and causes pH to rise. Calcium hydroxide also can react with carbon dioxide. Burnt lime and hydrated lime have the same effects as agricultural limestone, but initially cause a much higher pH. Burnt and hydrated limes are not normally applied to pond water during the culture period, to prevent potentially harmful high pH levels. These two materials, however, can be applied to the bottoms of empty ponds at 1000–2000 kg/ha to raise pH and kill pathogens and other unwanted organisms.

The strength of liming materials is reported as a neutralizing value, with the value of pure calcium carbonate established as 100%. Based on this system, the neutralizing values of pure compounds used as liming material are: calcite, 100%; dolomite, 108%; hydrated lime, 135%; burnt lime, 178%. Of course, individual samples of liming materials will vary because of impurities.

Ponds with total alkalinity below 50–60 mg/L must be limed. The liming rate needed to increase soil pH and total alkalinity can be estimated by one of several lime requirement tests which will not be described here (Boyd and Tucker, 1998). An alternative is to measure pond soil pH in a 1 : 1 mixture of dry pulverized pond soil and distilled water. The liming rate can be selected based on bottom soil pH, from Table 3.8. Another approach is to apply agricultural limestone at 1000 kg/ha and check total alkalinity after 3 or 4 weeks. If the alkalinity is still low, more agricultural limestone may be applied.

It is important that agricultural limestone be spread over the entire pond. This can best be done when ponds are empty between crops (Fig. 21.8), but limestone also may

Table 3.8 Lime requirements (kg/ha) based on soil pH and soil texture.

	Soil texture		
Soil pH	Clayey	Loamy	Sandy
<4.5	5000	4000	2500
4.5–5.0	4000	3000	2000
5.1–5.5	3000	2000	1500
5.6–6.0	2000	1500	1000
6.1–6.5	1500	1000	750
6.6–7.0	1000	750	500
7.1–7.5	500	375	250
>7.5	0	0	0



Fig. 3.9 Spreading agricultural limestone over the surface of a full pond.

be spread over the pond surface from a boat (Fig. 3.9). Acidic ponds usually will need to be limed after every crop if they are drained for harvest. Acidic ponds that are not drained for harvest usually need to be limed at 3- to 4-year intervals.

3.4.2 Pond fertilization

Fertilizers are frequently used in pond aquaculture to stimulate phytoplankton productivity and enhance the availability of natural food organisms. Turbidity created by phytoplankton also shades pond bottoms to discourage the growth of underwater aquatic weeds. Ponds may be fertilized with organic manures such as animal excrement, agricultural by-products or grass. They also may be fertilized with chemical fertilizers.

3.4.2.1 Manures

A wide variety of organic materials may be used to fertilize ponds. Application rates must not exceed about 25 kg/ha of dry matter per day in unaerated ponds to avoid dissolved oxygen depression by microbial degradation of the manure. Fish production in manured ponds can be as high as or higher than that achieved with chemical fertilizers. Manures have their maximum benefit in developing countries where small-scale farmers may have manure available from farm livestock and cannot afford chemical fertilizers. There are, however, several disadvantages to manures:

- Manures have a low percentage of nutrients and vary in composition.
- Because of low nutrient quality, large application rates are necessary.
- Large applications can cause dissolved oxygen depletion and accumulation of partially decomposed manure in pond bottoms.
- Manures encourage growth of algal mats.
- Manures may contain antibiotics put into animal feeds and they have high concentrations of trace metals.
- Many consumers do not prefer aquaculture products from manured ponds.

3.4.2.2 Chemical fertilizers

The two most common fertilizers used worldwide in aquaculture are triple superphosphate and urea. Other common fertilizers used in aquaculture are listed in Table 3.9. Most fertilizers are packed in bags and sold as dry granules or prills (spherical pellets), but ammonium polyphosphate and phosphoric acid are liquids.

Nitrogen in fertilizers is present as urea, ammonium or nitrate. Phosphorus occurs as orthophosphate or polyphosphate, while potassium appears in its ionic form. Fertilizers dissolve in water to release their nutrients. Urea begins to hydrolyze at once and is completely transformed to ammonia and carbon dioxide within hours or days. Polyphosphate also quickly hydrolyzes to orthophosphate.

The grade of a fertilizer is usually reported as percentages of nitrogen (N), phosphorus oxide (P_2O_5), and potassium oxide (K_2O). Thus, triple superphosphate usually is a 0-46-0 fertilizer, diammonium phosphate is an 18-48-0 fertilizer, and urea is a 45-0-0 fertilizer. Table 3.9 shows the approximate grades of common commercial fertilizers. Note that some primary fertilizer sources contain one primary nutrient, such as urea or triple superphosphate, while others may contain two primary nutrients such as

Table 3.9 Approximate grades of common commercial fertilizers. From Boyd (1990).

Fertilizer	Percentage composition		
	N	P_2O_5	K_2O
Urea	45	0	0
Calcium nitrate	15	0	0
Sodium nitrate	16	0	0
Ammonium nitrate	33–35	0	0
Ammonium sulfate	20–21	0	0
Superphosphate	0	18–20	0
Triple superphosphate	0	44–54	0
Monoammonium phosphate	11	48	0
Diammonium phosphate	18	48	0
Calcium metaphosphate	0	62–64	0
Potassium nitrate	13	0	44
Potassium sulfate	0	0	50
Potassium chloride (muriate of potash)	0	0	60

diammonium phosphate and potassium phosphate. A mixed fertilizer is made by blending two or more primary fertilizer sources to provide two or three primary nutrients. Mixed fertilizers representing a wide range of grades can be purchased in some countries.

Sometimes, fertilizers will be supplemented with the secondary nutrients, calcium, magnesium, and sulfur. The usual sources of these elements are calcium and magnesium sulfates. Supplements of trace elements, iron, manganese, zinc, copper, boron, and others, may be added to fertilizers.

Fertilizer granules or prills are water soluble but settle to the pond bottom before completely dissolving. Much of the phosphorus may be adsorbed by the bottom soil instead of remaining in the water. This problem can be lessened by using liquid fertilizers.

Liquid fertilizers are denser than water and are diluted 1 : 10 with pond water and splashed over pond surfaces or released into the propeller wash of an outboard motor while a boat is driven over the pond surface. If liquid fertilizers are not available or considered too expensive, granular or prilled fertilizers may be placed in 10 to 20 times their volume of pond water, pre-dissolved by vigorous stirring, and splashed over the water surface.

Typical application rates of N and P_2O_5 are 2–10 kg/ha each per application. Many farmers apply excessive nitrogen. I recommend applications of 2 kg N/ha and 8 kg P_2O_5/ha for freshwater ponds, and 8 kg/ha each for N and P_2O_5 for ponds filled with brackishwater or seawater.

Diammonium phosphate or monoammonium phosphate are good fertilizers for freshwater because they have $N:P_2O_5$ ratios of about 1 : 3 and 1 : 4, respectively. For brackishwater or seawater, a fertilizer with a $N:P_2O_5$ ratio of 1 : 1 is best, and a mixed fertilizer with a 20-20-0 grade is a good choice. In brackishwater ponds for shrimp culture, many farmers want to encourage diatom growth. This can be done by applying nitrogen fertilizers weekly at 2–3 kg N/ha. Nitrate is especially efficient in promoting diatoms, and sodium nitrate can be used as a diatom-promoting fertilizer.

Fertilizer may need to be applied at intervals of 2 to 4 weeks to maintain phytoplankton blooms. However, more frequent applications may be necessary to initiate blooms. In ponds with feed applications, nutrients enter the water from feed wastes, and applications of fertilizers are often unnecessary or only necessary until feeding rates reach 15 or 20 kg/ha/day.

Once blooms have been initiated, fertilizer can be applied according to need as judged from the Secchi disk visibility. A Secchi disk visibility of 25–40 cm is usually considered adequate. As an example, where the normal application rate is 2 kg N/ha and 8 kg P_2O_5/ha, the application rate might be adjusted for Secchi disk (SD) visibility as follows: SD ≤ 25 cm, no N or P_2O_5; SD = 26–30 cm, 0.5 kg N and 2 kg P_2O_5/ha; SD = 31–35 cm, 1 kg N and 4 kg P_2O_5/ha; SD = 36–40 cm, 1.5 kg N and 6 kg P_2O_5/ha; SD > 40 cm, 2 kg N and 8 kg P_2O_5/ha.

Fertilizers containing ammonium or urea are potentially acidic because nitrifying bacteria in water can oxidize ammonia to nitrate and yield hydrogen ion. The hydrogen ion will react in water to decrease total alkalinity and pH. The adverse influence of nitrification on alkalinity and pH can be counteracted by routine applications of agricultural limestone to ponds where alkalinity is naturally low. In brackishwater, seawater, and freshwater with alkalinity above 50 mg/L, acidity from nitrogen fertilizers is not a problem.

3.4.3 Feeding, mechanical aeration, and water circulation in ponds

The production of aquatic organisms in ponds can be greatly increased by application of manufactured feeds. For example, in channel catfish farming, feeding can increase production from 200–300 kg/ha in fertilized ponds to 2500–3000 kg/ha in fed ponds before low dissolved oxygen (DO) concentration develops as a result of

waste from feeding. Mechanical aeration may be applied to ponds and feeding rates can be increased drastically, and production of 8000–10 000 kg/ha is sometimes realized.

3.4.3.1 Feeding and water quality

Only a portion of the feed applied is eaten. Fish quickly ingest feed pellets and, with careful feeding, 90% or more of the feed will be consumed. Shrimp nibble on feed pellets and 20–40% of feed may not be consumed directly. About 80 or 90% of the feed eaten by the culture species is absorbed across the intestine, and the remainder becomes feces.

Absorbed nutrients are used in metabolism and, to a lesser degree, for growth. Water, carbon dioxide, ammonia, phosphate, and other basic compounds are excreted as metabolic wastes (Fig. 3.10). A relatively small amount of the nutrients in the original feed input is captured in biomass of harvested animals. In one example, aquacultured shrimp contain only 12.2% of the carbon, 25.8% of the nitrogen, and 14.2% of the phosphorus applied in feed.

In fish culture, the percentages of feed carbon and nitrogen harvested in fish are similar to those for shrimp. However, unlike shrimp, fish have bones made of calcium phosphate and thus contain more phosphorus than shrimp. The percentage of feed phosphorus removed in fish at harvest is typically around 25–35%.

Wastes from feeding have a tremendous influence on pond water quality. Micro-organisms readily decompose organic matter in uneaten feed and feces. This decay process removes dissolved oxygen from the water and releases carbon dioxide, ammonia, phosphate and other mineral nutrients. The culture species also consumes dissolved oxygen and excretes minerals similar to those generated by decomposition.

Ammonia and phosphate stimulate growth of planktonic and benthic algae. Algal abundance in ponds increases as feed inputs increase. Ponds with dense blooms of planktonic algae typically have high dissolved oxygen concentrations during the day, but lower dissolved oxygen at night can stress or kill the culture species (Fig. 3.6).

When feeding rates exceed 30–40 kg/ha/day, DO concentration often will fall to less than 3 or 4 mg/L at night. Low DO stresses culture species, so mechanical aeration is necessary at higher daily feeding rates (section 3.4.3.2). Dissolved oxygen levels must be monitored, even in ponds with mechanical aeration, to avoid excessive feed input for the amount of aeration applied.

Water exchange sometimes is used to flush plankton and nutrients from ponds and improve water quality. However, this practice can lead to water quality deterioration in receiving water bodies.

Ponds have a large capacity to assimilate wastes resulting from addition of feeds. As mentioned above, bacteria

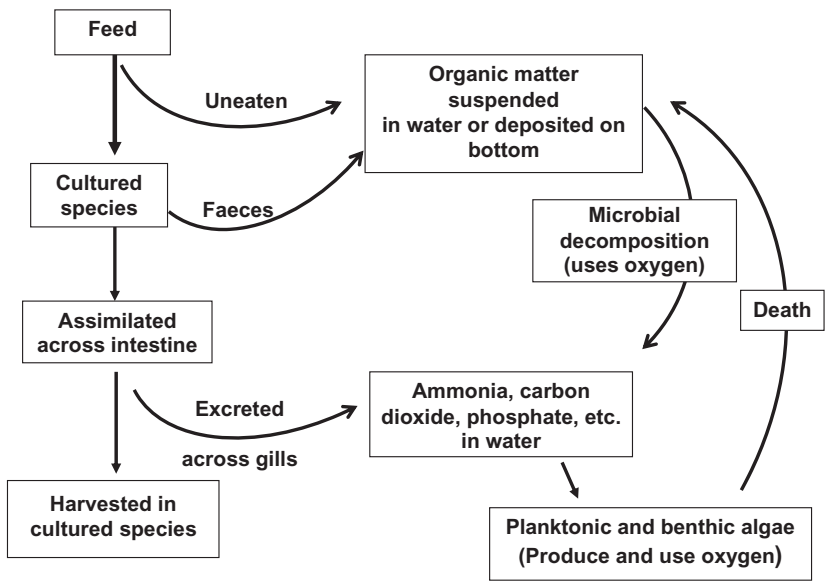


Fig. 3.10 Fate of feed in an aquaculture pond.

Table 3.10 Nitrogen and phosphorus budgets as percentages of the original input of the two elements in feed to catfish ponds. Reproduced from Boyd (2004).

Pathway	Nitrogen	Phosphorus
Harvest in fish	32	3
Ammonia volatilization	13	–
Denitrification	17	–
Sediment accumulation	23	58
Effluent	16	11



Fig. 3.11 Cracks in soil after the pond bottom has dried for 2 weeks.

mineralize organic matter to carbon dioxide, ammonia, and phosphate. Ammonia is lost to the atmosphere by volatilization (diffusion). It also is oxidized to non-toxic nitrate by nitrifying bacteria. Nitrate can be denitrified to nitrogen gas, which diffuses into the atmosphere. Carbon dioxide is converted to organic carbon by photosynthesis or it diffuses from pond waters to the atmosphere. Bacteria can transform carbon dioxide in sediment to methane, which also diffuses to the atmosphere. Sediment usually has a large capacity to fix phosphorus in insoluble iron, aluminum, and calcium phosphates. Some of the organic matter in ponds is relatively resistant to microbial decay and accumulates in sediment as stable organic matter.

The "budgets" for nitrogen and phosphorus in experimental channel catfish ponds at Auburn University are provided in Table 3.10. However, ponds may receive greater inputs of nutrients in feed than can be assimilated quickly and water quality deteriorates. When this happens, the culture species will be adversely affected and feed conversion efficiency will decline.

When ponds are drained for harvest, organic matter and nutrients in the water are discharged. The decomposition of new organic matter in pond bottoms can be hastened by allowing pond bottoms to dry and crack to facilitate aeration (Figs 3.11 and 21.8). By doing this each time a pond is drained, relatively low organic matter concentrations can be maintained in pond bottoms. For example, research ponds at Auburn University are drained and their bottoms dried annually. Some of these ponds are more than 50 years old, but bottom soils contain only 2% organic carbon. Of course, pond bottoms may eventually become saturated with phosphorus, which can lead to high concentrations of dissolved phosphorus and dense blooms of algae.

Feed inputs to ponds must not exceed the capacity of ponds to assimilate the resulting wastes. The most reliable way of determining how well a pond is assimilating wastes from feeding is to monitor early morning DO concentrations. Feeding and mechanical aeration rates must be adjusted to maintain DO concentrations above 3–4 mg/L in the early morning when lowest concentrations occur.

3.4.3.2 Mechanical aeration

Mechanical aeration is widely used in pond aquaculture to prevent low DO concentrations, especially at night; to improve the efficiency of feed use; and increase production of the cultured species.

Mechanical aerators increase the rate at which oxygen from air enters water by providing a greater surface area between air and water to facilitate diffusion. This can be achieved mechanically by two methods. Water may be splashed into air to increase its surface area and accelerate the rate of diffusion. Alternatively, bubbles of air may be released into water to create a large surface area between bubbles and water through which oxygen from the bubbles enters the water.

It is important to note that aerators are most effective when DO concentrations in water are low, for the driving force causing oxygen to enter the water is the difference in pressure between oxygen in air and oxygen in water. Moreover, most aerators transfer oxygen from water to air when water is supersaturated with DO.

In addition to transferring oxygen to water, aerators circulate pond water. Water circulation is beneficial in moving oxygenated water away from aerators to prevent localized saturation and loss of oxygen-transfer efficiency. It also prevents ponds from thermally stratifying. Aerator-induced water currents transport oxygen throughout the

pond for use by the culture species. Circulation creates ripples on the water surface that favor the outward diffusion of ammonia and other toxic gases. It also delivers water containing dissolved oxygen over the pond bottoms to avoid localized pockets of anaerobic sediment.

In intensive ponds, aeration is necessary during daytime to provide circulation even though little or no oxygen transfer results. However, the amount of aeration applied usually must be reduced during daytime to minimize oxygen loss to the air and lessen energy costs. At night, aeration must be increased to prevent low DO concentration.

There are many kinds of mechanical aerators. Paddle wheel aerators (Fig. 3.12) and vertical turbine aerators are two common types of aerator (Fig. 3.13). Diffused-air aeration systems release bubbles of air near pond bottoms to rise through the water column. Another type is the propeller-aspirator-pump aerator, which releases tiny air bubbles into turbulent water created by a rapidly rotating impeller.

The standard aeration efficiency (SAE) is the quantity of oxygen an aerator will transfer per horsepower (hp) in 1 hour. Typical SAE values range from 1 to 2 kg oxygen/hp/h. SAE is measured for 20 °C, 0 mg/L DO, and clear tap water. During operation in a pond containing 3–4 mg/L DO, the actual oxygen transfer rate will be 40–50% of the SAE.

Experience and calculations based on pond oxygen budgets suggest that production can be increased by about 400 kg for each horsepower of aeration applied (1 hp ≈ 0.75 kW). For example, suppose 1500 kg/ha can be produced without mechanical aeration. To increase production by 3500 kg/ha to 5000 kg/ha, the minimum aeration requirement will be about 9 hp/ha (3500 kg/ha ÷ 400 kg/hp). However, probably not less than 1.5 times the minimum, i.e. 14 hp/ha, should be used, to be safe.



Fig. 3.12 A paddlewheel aerator in a shrimp pond in Thailand.

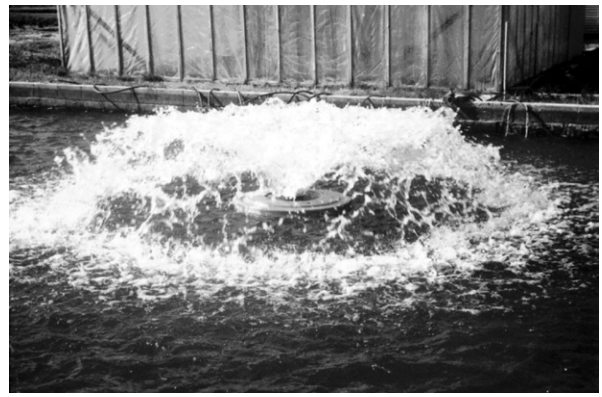


Fig. 3.13 A vertical pump aerator.

Few studies have examined the best ways of positioning aerators in ponds. Fish move to aerated zones, so positioning is less important in fish ponds than in shrimp ponds. In fact, in channel catfish farming, all aerators are often located at one end of the pond. This practice allows the use of a single electrical service panel and greatly reduces the amount of electrical wire between the panel and aerators.

In shrimp ponds, the long-standing practice of positioning aerators to create a circular movement of water is probably as good as any other alternative. To avoid erosion, aerators must not be mounted in water less than 0.75 m deep or near embankments. Water currents also must not impinge on embankments for the same reason. Moreover, water currents from one aerator must not collide with currents from another.

3.4.3.3 Mechanical water circulation

Conserving DO from photosynthesis by blending the surface water with deeper water in the daytime should lessen the amount of mechanical aeration required at night. Several types of mechanical water circulators have been developed for use in aquaculture, but two types have the greatest potential.

The most well-known circulation device is the air-lift pump (Parker and Suttle, 1987). These pumps are con-

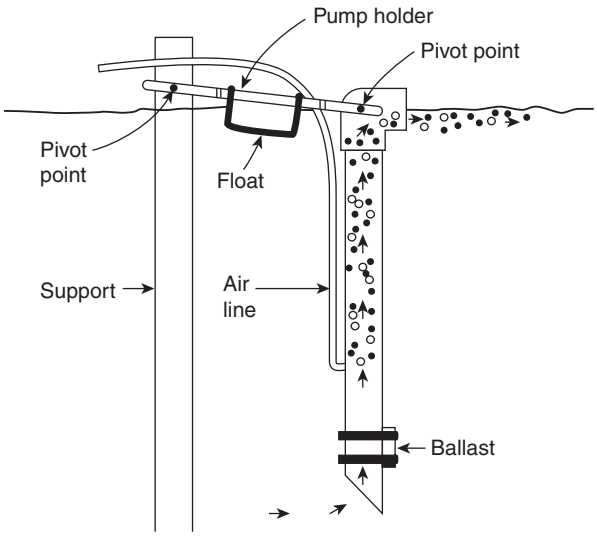


Fig. 3.14 An air-lift pump.

structed of a length of PVC pipe, termed the eductor, and a PVC elbow. Air from a low-pressure, high-volume blower is released through a right-angle hose adaptor into the PVC pipe (Fig. 3.14). Alternatively, air may be released through a diffuser to produce smaller bubbles. Rising air bubbles lift water through the eductor and discharge it at the surface. A pump holder with flotation device is attached between the anchor post and pump to allow the pump to pivot in response to changes in water depth (Fig. 3.14). Ballast must be provided at the bottom of the pump. Many variations in the designs of air-lift devices have been used, and equations have been developed for predicting their discharge. The pumps create water circulation by lifting water from near the bottom and releasing it at the surface. Air-lift pumps also cause some aeration, because oxygen in the rising air bubbles diffuses into the water.

Horizontal, axial flow water circulators are most suitable for use in ponds because they can discharge much more water than air-lifts (Fig. 3.15). A water circulator powered by a 3 hp gear motor that incorporated 76-cm diameter fan blades mounted within a 92-cm diameter, 111-cm long housing was capable of discharging about 60 m^3/min when operated at 90 rpm. Several companies have manufactured modified versions of this design.

Horizontal discharge water circulators are not designed to effect aeration. Nevertheless, they increase turbulence at the water surface, which can slightly increase gas transfer between air and water.



Fig. 3.15 A horizontal, axial-flow water circulator.

Research has not provided convincing evidence of the benefits of water circulators, but energy costs are rising worldwide, and the possibility for using mechanical water circulators to lessen the requirements for mechanical aeration appears worthy of a second round of scientific investigation and farm trials.

3.4.4 Oxygen management in raceways and cages

In flow-through aquaculture systems (Fig. 2.4), rapid water flow prevents the growth of plankton and flushes out wastes (Soderberg, 1995). Nearly all the DO in inflowing water is available to fish. Stocking and feeding rates in trout raceways are established so that DO concentration does not fall below 5 mg/L and stress the fish.

An equation for calculating the carrying capacity of flow-through systems is:

$$\text{Carrying capacity (kg)} = \frac{Q(DO_1 - 5)(7.2)}{\frac{F}{100}}$$

where Q = inflow (m^3/min), DO_1 = DO in inflow (g/m^3), F = feed in % body weight per day.

Suppose that water containing 10 mg/L DO enters a raceway at 10 m^3/min. The water supply has 5 mg/L of available DO or

$$72\,\text{kg DO/day } (5\,\text{g/m}^3 \times 10\,\text{m}^3/\text{min} \times 1440\,\text{min/day} \times 10^{-3}).$$

It usually is possible to apply 5 kg feed for each kilogram of DO, so 360 kg feed can be applied daily. The fish biomass necessary to fully use the available DO at a feeding rate of 3% body weight daily would be 12 000 kg (360 kg feed/day ÷ 0.03).



Fig. 3.16 Cage culture of trout in Bosnia. (Photograph by Melba Reantosa, courtesy of Rohana Subasinghe.)

Aeration also may be used in to increase carrying capacity in flow-through systems. However, in raceways, almost the entire amount of DO supplied by aeration would be available to the fish, for there are few other organisms present to use it.

In cage or seacage culture (Figs 3.16 and 3.17) or culture of bivalve shellfish, the DO concentrations in the water result from natural conditions and usually cannot be influenced by management. Sites for such aquaculture projects should provide high-quality water and good water circulation. Many cage culture operations have experienced serious losses to DO depletion when thermal destratification of eutrophic lakes occurred (Fig. 3.18).

3.4.5 Miscellaneous treatments

3.4.5.1 Control of high pH

Several treatments have been used to lower pH. In some cases, water exchange may be used to flush out phytoplankton and this will often lower pH. Copper sulfate treatment will kill algae, causing a decline in carbon dioxide use in photosynthesis and a resulting drop in pH. This practice, however, can cause low DO concentration when dead algae decay. Organic amendments such as manure and molasses have been applied because carbon dioxide released when these materials decay will depress pH. This treatment is seldom highly successful and it also can cause DO depletion. In emergencies, aluminum sulfate may be applied. Aluminum ion hydrolyzes to release hydrogen ions and lower pH.

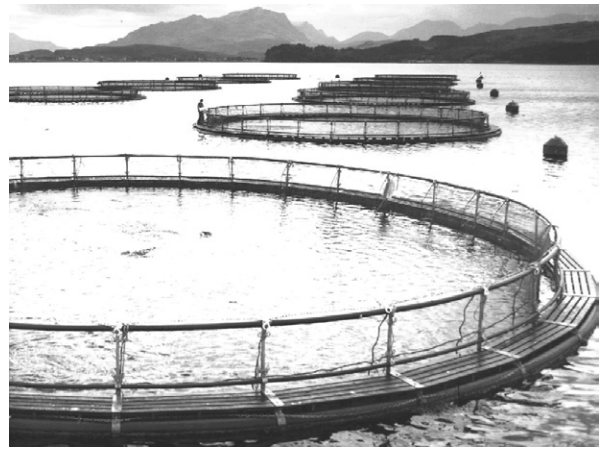


Fig. 3.17 Fish culture seacages. (Note the relative size of the figure on the second cage as an indication of the dimensions of the seacages.)

Waters with low total hardness and high total alkalinity concentration, e.g., 3–10 times more alkalinity than hardness, may have pH values of 10 or more when photosynthesis is rapid. Application of calcium sulfate (gypsum) to increase calcium concentration can be an effective treat-

ment. It usually is recommended that enough calcium sulfate be added to bring total hardness concentration up to that of total alkalinity. The treatment rate for gypsum may be estimated as:

$$CaSO_4 \cdot 2H_2O = 2(\text{total alkalinity} - \text{total hardness}).$$



Fig. 3.18 Dead fish in cages following dissolved oxygen depletion in a lake.

This treatment has a residual effect that varies with the flushing rate of the pond.

3.4.5.2 Controlling phytoplankton with copper sulfate

Phytoplankton, and especially blue-green algae, are highly sensitive to copper. The traditional copper formulation used as an algicide is copper sulfate containing 26% copper. Although copper sulfate readily dissolves in water, it quickly disappears from solution through precipitation and reaction with soils (Fig. 3.19).

The cupric ion is the toxic form of copper to plants, but it is only slightly less toxic to aquatic animals. The concentration considered acceptable for phytoplankton control ranges from 0.10 to 1.0 mg copper per litre of water. The maximum recommended concentration of copper for fish is usually 0.02 mg/L in acidic low-alkalinity water and 0.2 mg/L in water with high alkalinity and pH.

The solubility of cupric ion is greatest at low pH and decreases rapidly with increasing pH. This poses two problems for the use of copper sulfate as an algicide. At low pH, the relatively high concentration of cupric ion favors high toxicity to phytoplankton, but there is risk of harming the culture species. At higher pH, there is less danger in harming the culture species, but it is more difficult to

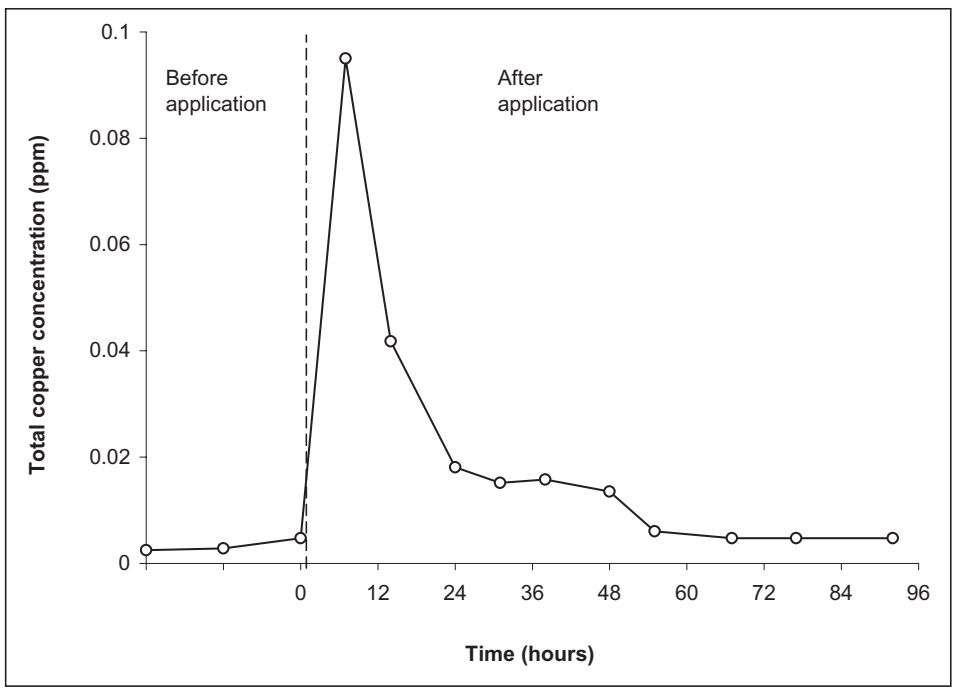


Fig. 3.19 Changes in copper concentration following treatment of pond with copper sulfate.

achieve a great enough cupric ion concentration to control phytoplankton.

Copper ion can be chelated with organic compounds such as triethanol amine or ethylene diaminetetraacetic acid (EDTA). The benefit of treating water with chelated copper products is that the concentration of dissolved copper can be maintained at an elevated level for several days, even in waters with a high pH and alkalinity.

Chelated copper is toxic to phytoplankton, but it is not nearly as toxic as cupric ion to aquatic animals. Thus, chelated copper products for phytoplankton control can be used in acidic, low-alkalinity waters without fear of harming the culture species. Moreover, in high-alkalinity water of greater pH, an effective copper concentration can be achieved more easily by using chelated copper products. The main disadvantage of chelated copper compounds is their high cost, which is usually several times greater than copper sulfate per unit weight of copper.

In high-alkalinity waters with pH of 7.5 or more, relatively large applications of copper sulfate can be made without endangering culture species. In fish culture, the usual copper sulfate dose is 0.01 of the total alkalinity. For example, if total alkalinity is 135 mg/L as calcium carbonate, the copper sulfate dose must be 1.35 mg/L.

In channel catfish farming, copper sulfate treatments are a widely used means of thinning blue-green algae before harvest for the purpose of avoiding off-flavors and rejection of the fish by processing plants. Many catfish ponds in the south-eastern United States have been treated with copper sulfate several times per year for 10 or more years. The copper quickly disappears from the pond water, and copper concentrations return to pre-treatment levels within 72 hours (Fig. 3.19). Copper accumulates in the sediment of ponds, but because of its low solubility at pH 6 or above, contamination of pond waters by copper released from sediment does not occur. Copper sulfate can be applied by several methods. The most common methods are broadcasting crystals over pond surfaces, dissolving the algicide in water for spraying over pond surfaces, and suspending copper sulfate in porous bags in front of aerators or where inflow enters ponds. Copper sulfate can also be used to treat mat-forming algae around pond edges. The usual method is to broadcast copper sulfate crystal over algal mats.

Chelated copper algicides can be mixed with water and sprayed over pond surfaces. Also, some workers mix chelated copper algicides with water and drain the solution from a boat-mounted tank into the turbulence caused by the propeller of an outboard motor while driving the boat over the pond surface.

3.4.5.3 Clearing pond waters of clay turbidity

Muddy ponds often have low-alkalinity, acidic water. The application of agricultural limestone and fertilizer to increase alkalinity, phytoplankton abundance and pH can cause clay particles to settle in some ponds. When liming and fertilization are not effective, flocculating agents may be applied to precipitate suspended clay particles and clear water of turbidity. The most common agents are: organic matter, usually hay; calcium sulfate (gypsum); and aluminum sulfate (alum).

The use of organic matter to treat turbidity is problematic because of the large quantities needed. The treatment rate usually are 2–3 t/ha of hay or 500–1000 kg/ha of animal manure. Such large applications of organic matter can cause depletion of DO and mortality of culture animals.

The principle of gypsum treatment is to increase calcium concentration and encourage the flocculation of clay particles by neutralizing the negative charges on their surfaces. Gypsum must be applied at 300–400 mg/L for best results. Depending upon the hydraulic retention time, calcium concentration will be elevated for months or even years to provide future protection against turbidity from suspended clay particles.

Alum dissolves in water to temporarily increase the concentration of aluminum ions. Aluminum ions are trivalent and many times more effective than divalent calcium ions in flocculating clay particles. In most water, an application of 25–30 mg/L of alum effectively removes clay turbidity within a few hours. Some clays are more difficult to flocculate, so it is advisable to conduct simple tests by establishing different alum concentrations in water samples to determine the effective alum treatment rate for a particular pond.

Alum must be applied on a calm, clear day to prevent wind action or rainfall from disturbing the floc. Particles of floc usually become obvious within 10–15 min after treatment, and the pond clears of turbidity within a few hours.

Because alum forms sulfuric acid in water, it reduces the total alkalinity and lowers pH. Each milligram per litre of alum consumes about 0.5 mg/L of total alkalinity. To assure residual total alkalinity and a safe pH, alum treatment rates must not exceed the total alkalinity in concentration. For example, if the total alkalinity is 25 mg/L, the maximum, safe alum treatment is 25 mg/L.

3.4.5.4 Mineral amendments

Several uses of mineral amendments already have been discussed. In addition, in fish hatcheries and nursery ponds it may be necessary to increase salinity for certain species.

This can be done by applying industrial-grade, mine-run salt which is about 96–98% sodium chloride. In some countries, brine solution (100–250‰ salinity) from coastal seawater evaporation ponds may be applied as an alternative to salt.

Saline surface and groundwaters found in some inland regions are used to culture marine and brackishwater species. These waters often have different proportions of major ions than found in seawater. In particular, low concentrations of potassium and magnesium have been associated with poor survival and growth of some shrimp and fish species. Although the specific requirements of marine and brackishwater species for major ions are not known, they should grow well in waters with ionic proportions similar to those of seawater. The concentrations of ions that would be expected in seawater diluted to any given salinity may be estimated:

$$C_p = S_p \times \frac{34{,}500\,\frac{mg}{L}}{C_{sw}}$$

where C_p = equivalent seawater concentration of the major ion (mg/L); 34 500 mg/L = normal salinity of seawater; C_{sw} = normal concentration of the major cation in seawater (Table 3.1).

Minerals such as fertilizer-grade potassium chloride (muriate of potash), potassium magnesium sulfate (Kmag®), and magnesium sulfate (Epsom salt) have been applied to ponds to correct ionic imbalances.

3.4.5.5 Neutralizing acidity from nitrification in intensive systems

Nitrification is a natural water purification process by which certain bacteria oxidize and transform potentially toxic ammonia to non-toxic nitrate. This process is especially important in intensive aquaculture ponds and water re-use systems, where ammonia can reach concentrations harmful to fish and shrimp. However, nitrification produces acidity that causes pH and total alkalinity to decline.

Fish and shrimp usually eat most of the feed offered to them. A large proportion of the consumed feed is absorbed across the animal's intestines and the rest is expelled as feces. Uneaten feed and feces decompose and release carbon dioxide, ammonia and other inorganic nutrients. Of the nitrogen absorbed across the intestine, more is converted to ammonia and released into the water than is converted into biomass. Nitrification will oxidize ammonia, creating considerable acidity.

Nitrogen inputs to ponds in feeds usually are much higher than fertilizer nitrogen applications. The amount of calcium carbonate required to neutralize the potential acidity of feed can be calculated (Boyd, 2007) using the following equation:

$$\text{Calcium carbonate equivalence of feed (kg)} = \text{Feed input (kg)}(\%\ \text{Crude protein in feed})(0.01285)$$

In a pond producing 5000 kg shrimp per hectare per crop using feed with 35% crude protein and achieving a feed conversion ratio of 2.0, the calcium carbonate equivalence of the feed is 4498 kg/ha. In a water re-use system receiving 1000 kg/day of feed containing 30% crude protein, the calcium carbonate equivalence is 385 kg/ha/day. In intensive systems, especially water re-use systems, nitrification can cause alkalinity and pH to decline rapidly. Frequent use of liming materials may be necessary.

3.4.5.6 Zeolite

Zeolite is a naturally occurring mineral that can also be produced synthetically. It is used in industry for water softening and as medium for molecular sieves. In recent years, there has been interest in possible agricultural and aquacultural applications of zeolite. In aquaculture, zeolite is in filters for removing ammonia from water in tanks that hold and haul fish.

Some shrimp farmers treat ponds with zeolite, thinking that it will remove ammonia from water. In shrimp pond water, concentrations of sodium, potassium, calcium, and magnesium ions are much greater than the ammonium concentration. These cations compete with ammonium for exchange sites on zeolite, and the ability of zeolite to remove ammonium from water is much lower than the total capacity of the mineral to absorb cations.

The author once conducted a laboratory trial to determine the influence of salinity on ammonia removal by zeolite from water. In freshwater, 1 kg zeolite removed 9 g NH_3-N. It would require 1111 kg zeolite to remove 1 mg/L NH_3-N from a 1-ha pond of 1 m depth (10 000 m^3). At a salinity of 4‰, 1 kg zeolite removed 0.12 g NH_3-N (83 000 kg zeolite to remove 1 mg/L NH_3-N from 10 000 m^3 water). At 32‰ salinity, 1 kg zeolite removed only 0.04 g NH_3-N. Zeolite is therefore not a useful treatment to remove ammonia from ponds. The costs of product and labour to apply it at the high rates needed for effective action are prohibitive.

3.4.5.7 Bacterial products

Probiotic use in aquaculture is increasing as producers attempt to improve pond soil and water quality, enhance survival, and improve the growth of culture species.

Probiotic use is common in Asian aquaculture and is spreading to the West. Probiotics include an array of products that are usually of microbial or botanical origin. The ones used as bottom soil and water quality enhancers are primarily cultures of living bacteria, enzyme preparations, or the two combined.

The waste in aquaculture ponds – primarily uneaten feed, feces, and remains of plankton – is ordinary organic matter that readily decomposes. Micro-organisms of decay respond to substrate (nutrient source), and their abundance increases rapidly until substrate is depleted. Microbial numbers then decline rapidly and only resting bodies, such as spores, remain. This pattern of growth is illustrated in Fig. 9.4 with data from microbial cultures, but the same pattern occurs in ponds. The addition of more substrate will again elicit the growth of micro-organisms. This suggests that to increase microbial action, one only needs to add substrate, for the micro-organisms already are present.

Because micro-organisms respond to substrate and micro-organisms capable of assimilating aquacultural wastes are present, it seems unlikely that a paucity of bacteria impairs sediment and water quality. More plausible explanations are that the assimilative processes in ponds have been exceeded by the aquacultural waste load, or environmental conditions deteriorate and limit microbial assimilation.

Enzymes are organic catalysts that can accelerate chemical reactions but are not expended in the process. An example is the acceleration of the conversion of urea to carbon dioxide and ammonia by the enzyme urease. Enzyme preparations used as probiotics usually are extracts from homogenates of yeast cells, and they function extracellularly. Proponents of these products argue that adding enzymes to ponds increases the activity of extracellular enzymes and favors greater rates of waste assimilation by microbes. This may seem to be a reasonable assumption. However, in most ponds, active microbial flora already produce enzymes and the small quantities added in treatments with enzyme preparations are insignificant.

Several replicated pond studies conducted over the past 20 years at Auburn University and Mississippi State University in the United States did not demonstrate improvements in sediment and water quality in ponds treated with bacterial inocula and enzymes. Even at doses several times the treatments suggested for ponds, the amendments did not cause positive changes in water quality in laboratory systems.

A few studies have shown that probiotics can increase fish and shrimp survival. The mechanism by which probiotics enhance survival is puzzling, but it is not related to improvements in water or sediment quality.

3.5 EFFLUENTS

Discharges from extensive aquaculture facilities are low in concentrations of nutrients and organic matter. Thus, they usually are not considered a significant source of pollution to receiving waters. Fertilizers and feeds are applied in semi-intensive and intensive culture, and effluents from these systems have a greater potential for causing water pollution.

Feed is the main source of nutrients in aquaculture discharges. The fate of the nutrients and other wastes depends upon the type of culture system. In static ponds, water remains in ponds for long periods and natural processes degrade organic matter, nutrients are sequestered in sediment, ammonia diffuses into the air, denitrification returns nitrogen to the air, and suspended solids settle out. A portion of the wastes enter natural waters when overflow occurs following rainfall or when ponds are drained for harvest. Nevertheless, static ponds tend to serve as sediment traps, and a relative small percentage of the carbon, nitrogen, and phosphorus applied in feed is discharged. For example, the discharge from channel catfish ponds contained about 3.1% carbon, 28.5% nitrogen, and 7.0% phosphorus applied in feed (Boyd and Tucker, 1998).

In ponds with water exchange, the hydraulic retention time is less, and natural processes have less time to remove suspended solids, organic matter, and nutrients resulting from aquaculture. For example, a typical watershed pond in the south-eastern United States will usually discharge two or three times its volume during the winter and early spring, and discharge little or no water during the rest of the year. Most of the feed is applied during warm months when discharge is minimal. However, in a coastal shrimp pond in Central America, water may be exchanged at a rate of 5–10% of pond volume per day, flushing ponds completely within 10–20 days.

In raceways and other flow-through culture systems, culture units are flushed several to many times per day. In trout raceways, it is not uncommon for raceway units to be flushed three times per hour. It is possible to remove uneaten feed and feces that settle in the ends of raceway units (Fig. 3.20), but suspended solids and nutrients pass through the system into natural waters.

In cage culture, all of the feed ingredients not converted to fish biomass and removed at harvest enter the water body into which cages are installed (section 2.2.6). Some of the solid waste is heavy enough to settle to the bottom

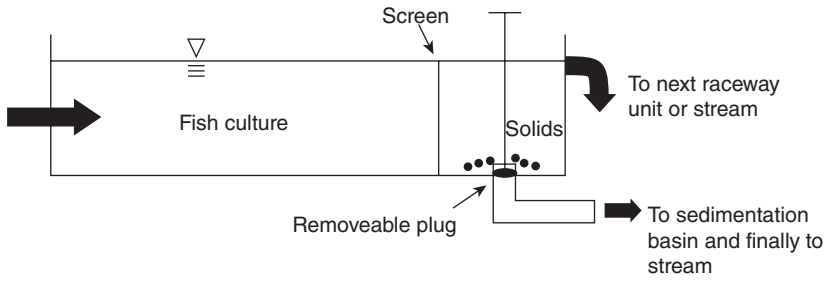


Fig. 3.20 Removal of solids from the lower end of a trout raceway.

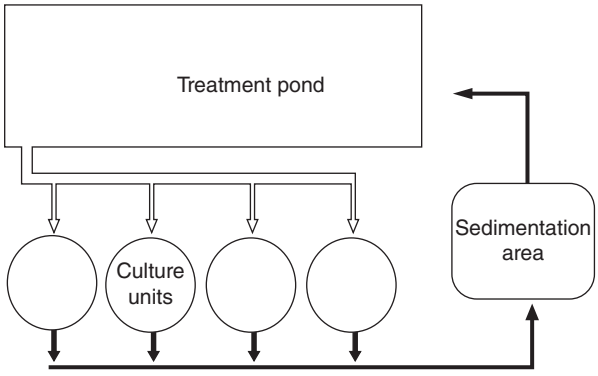


Fig. 3.21 Scheme of water recirculation in an aquaculture system using a pond (reservoir) for waste treatment by natural processes.

beneath the cages while some of the lighter solids remain suspended in the water. Fish excrete ammonia, carbon dioxide, and other wastes that enter the water as soluble nutrients.

There are two types of water re-use systems. Outdoor systems recycle water from grow-out units into treatment ponds and the water is eventually returned to the culture units (Fig. 3.21). These systems will discharge when heavy rainfall occurs. Indoor systems (Fig. 3.22) rely on waste treatment devices such as settling basins, mechanical filters, foam fractionators, biological filters, etc. However, they will discharge to the outside when basins and filters must be cleaned and when freshwater must be added to dilute total dissolved solids (salinity) concentration that increases over time.

In recent years, there has been increasing concern about negative impacts of aquaculture effluents on natural water bodies into which they are discharged. Many governments have developed regulations to limit the volume and improve the quality of aquaculture effluents. Limits for concentrations of selected water quality variables and discharge volume have been established for aquaculture effluents in some nations. Producers comply with these limits in order to discharge effluents. In other nations, best management practices (BMPs) are mandated. These practices usually include methods for reducing discharge and for assuring better conversion of feed nutrients to biomass of culture species (Tucker and Hargreaves, 2008). Many aquaculture associations have developed BMPs for voluntary adoption by their members and growing numbers of consumers in developed countries are seeking aquaculture products resulting from environmentally responsible culture methods. Several organizations such as the Global Aquaculture Alliance and the World Wildlife Fund are developing eco-label certification programs for aquaculture species (Tucker and Hargreaves, 2008). The Food and Agriculture Organization of the United Nations is making international guidelines for eco-label certification of aquaculture products. When completed, these guidelines will serve as a stimulus for greater effort towards aquaculture certification.

3.6 SUMMARY

Water quality is an important consideration in aquaculture. The potential species that may be cultured depends upon the salinity and water temperature range at a particular site. Source water may be of impaired quality because of low pH, high turbidity, or pollution. Turbidity can be controlled by eliminating its source on the watershed or by use of a settling basin to clarify water before use. Sources of pollution usually cannot be controlled by aquaculturists, and highly polluted sites should be avoided.

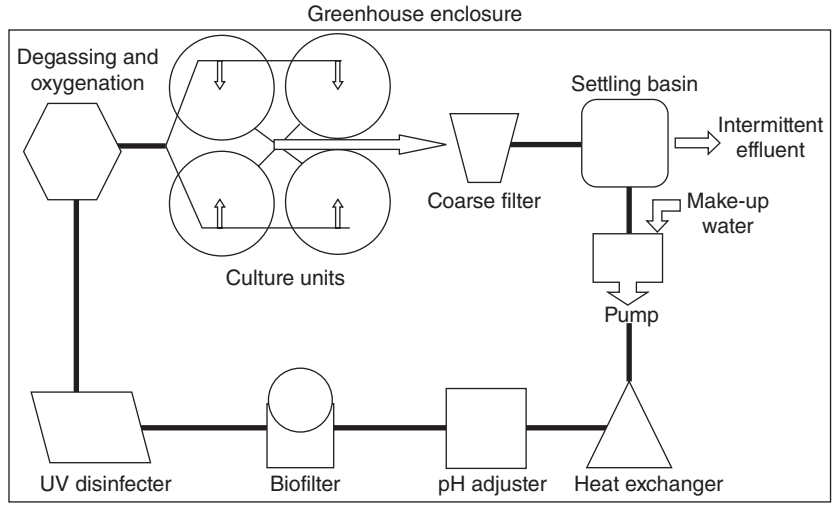


Fig. 3.22 Scheme of a closed recirculating system for indoor aquaculture in a tank room or greenhouse using a series of water treatments to maintain water quality. There is still need to regularly remove some water (intermittent effluent) and replace this with clean water.

Low pH can be corrected by liming, but this usually is done in culture units. Fertilizers are used to increase natural productivity in pond aquaculture, but much greater production of fish and shrimp may be achieved by the application of manufactured feed. Feeding wastes impair water quality, but mechanical aeration can be applied to increase the availability of dissolved oxygen and allow greater production. There is no method of reducing inputs of ammonia nitrogen to ponds aside from lowering fertilization rates or stocking and feeding rates. However, the natural processes of nitrification, uptake by microorganisms, and volatilization usually prevent excessive concentrations of ammonia. Nitrite toxicity may be counteracted in freshwater ponds by application of chloride ion. Copper sulfate often is used to kill blue-green algae that produce odorous compounds responsible for off-flavor in culture species. Turbidity from suspended soil particles in culture ponds can best be removed through treatment with liming materials, calcium sulfate, or aluminum sulfate. Bacterial amendments and zeolite do not improve water quality in ponds.

Effluents from aquaculture ponds can cause pollution of water bodies into which they are discharged. Thus, practices should be implemented at aquaculture facilities to reduce the volume and improve the quality of effluents.

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4
Environmental Aspects

Martin Kumar and Simon Cripps

4.1 PUBLIC IMAGE

Environmentally sustainable technology is in the long-term interests of the aquaculture industry. However, in some regions, especially within developed countries, aquaculture has a poor public image because of its apparently inadequate concern for the environment (New, 1999): 'Aquaculture suffers from the negative public perceptions caused by the relatively few but well publicized cases of severe local environmental degration and social disruption caused by some forms of aquaculture.'

The negative environmental impacts attributed to aquaculture have most often resulted from:

- poor planning;
- inappropriate site selection;
- inappropriate management procedures;
- lack of attention to environmental protection;
- 'goldrush' circumstances where many sought to join lucrative new industries as soon as possible.

This has led to antagonism towards further developments and there are many current examples of heated controversies within communities about new aquaculture developments and their impact.

In the terrestrial environment, many traditional agricultural industries have caused substantial to catastrophic environmental damage. Some level of environmental impact is almost inevitable with each industry because agriculture, by its nature, is not part of the natural environment. These impacts of agriculture are, however, generally accepted by the community because:

- these are long-established primary industries;
- often their deleterious effects on the environment occurred centuries ago and there is no memory of the natural environment;
- these industries are our main source of food.

Modern aquaculture is developing as a latecomer within a world that is very different from that in which much of agriculture development occurred. It is a world where there is considerably greater concern for the environment in many regions. Many established agricultural industries would not exist in their current form, or exist at all, if the environmental criteria being applied to aquaculture developments had been applied to them.

Many fisheries would not exist in their current form or at their current level of harvesting if rigorous environmental criteria were applied. It is inevitable that many fisheries are having significant adverse affects on aquatic environments, having long since reached the maximum level of extracting commercial products. Aquaculture offers an alternative source of seafood that may have less impact on the environment (see sections 1.4 and 1.5).

There is no value, however, in pointing to the injustice of more rigorous environmental criteria being applied to aquaculture. The industry must convince governments and communities by its actions and words that it is a responsible and environmentally sound industry. A poor public image will continue to hinder the development of aquaculture in many countries.

The modern aquaculture industry is maturing and adapting to current values and philosophies, and it is responding

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to environmental concerns. This chapter describes the various interactions between the aquaculture industry and the environment, with emphasis on the means by which adverse impacts can be avoided or reduced.

As the importance of aquaculture grows worldwide, the concept of sustainable aquaculture is increasingly recognized to incorporate environmental, economic and social parameters. Aquaculture development must be advanced in a manner that is environmentally sustainable and that protects the quality of the environment for other users. Reciprocally, it is important for society to provide appropriate environments for aquaculture.

In some countries the functional requirements for governing aquaculture are met by a diversity of legal mechanisms derived from various legal bases, which are mainly generic rather than specific to aquaculture. The institutional frameworks, legislation and management processes reflect agendas other than those specific to aquaculture, and the historical evolution and family of laws of each country (Glenn and White, 2007). With regard to the management of pollution loadings and the sustainability of aquaculture relative to the carrying capacities of host water bodies, the outputs are subject to legal standards and monitoring, while the contributory inputs are assessed and controlled from initial application through to day-to-day farm operations. In many respects, pollution and sustainability are the critical determinants of whether approvals are given.

There are also positive impacts of aquaculture on the environment. Aquaculture can be used as a tool to treat wastewater (Kumar *et al.*, 2000). Water and nutrients can be recycled through an aquaculture operation. When industries must comply with environmental standards that require treatment of effluent, this often constitutes an added operational cost. If, however, the treatment itself produces income, minimises pollution and complies with environmental standards, it not only increases profitability, but also enhances the sustainability of the industry. The 'waste', which provides income by producing a valuable product, effectively becomes a 'resource'. A number of by-products, such as bio-energy (gas and heat), aquaculture products (fish), and aquatic plant and agricultural products, can be produced while organic waste is treated. Thus, this chapter also deals with the positive environmental impacts of aquaculture.

4.2 IMPACTS FROM LAND-BASED AQUACULTURE

In general, environmental effects associated with extensive aquaculture systems are considered to be minimal. As the production and intensity of aquaculture increase, the potential impacts of pollutants from aquaculture also increase. A serious environmental impact of land-based aquaculture (including coastal areas) occurs when untreated effluent is discharged into surrounding bodies of water. Major environmental concerns about pond, tank, raceways and coastal farming include:

- destruction of natural habitats (natural vegetation, such as forests and mangroves);
- eutrophication and sedimentation in natural bodies of water caused by effluent;
- excessive use of resources such as water, foodstuffs and electricity;
- negative effects on native fisheries and biodiversity.

A number of measures are currently used to minimise the negative impacts of land-based aquaculture. Environmentally friendly and economically viable techniques and management measures are being developed to recover nutrients from effluent and re-use wastewater. Although aquaculturists are progressing towards a system in which the discharge of effluent is minimised, the problem of effluent disposal continues to challenge aquaculture and other intensive farming industries.

4.2.1 Effluent

Aquaculture is not the only guilty party as a source of effluent. In fact, in many situations in the developed countries it may only be a relatively minor contributor to effluent input. Its outputs may pale into insignificance compared with industrial, agricultural and municipal outputs into the same water mass. Nevertheless, aquaculture's contribution is specifically reported and this leads to the perception of aquaculture as being a major polluter (New, 1999).

4.2.1.1 Sources of pollutants in aquaculture effluent

The environmental impact of aquaculture from effluent discharge depends strongly on:

- species cultured;
- culture methods;
- stocking density;
- feed composition;
- feeding techniques;
- hydrography of the site.

Nutrient-rich effluent discharged from aquaculture systems can cause reduced DO concentration, raised

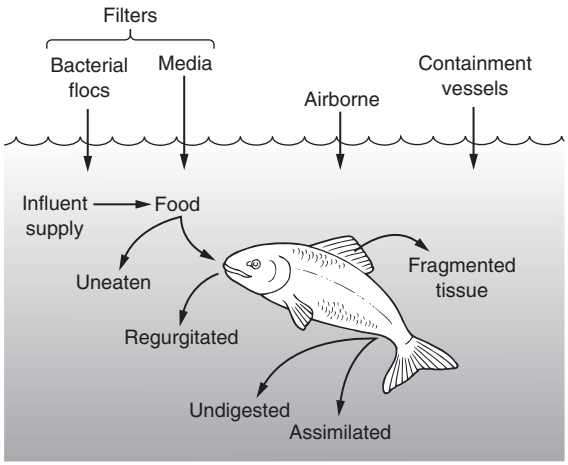


Fig. 4.1 Suspended particle sources in an aquaculture system.

biological oxygen demand (BOD)[1], increased suspended solids (SS) and increased P and N compounds. The concentrations of P and N are factors in the growth of aquatic plants. However, they are also important nutrients for cultured organisms and enter farms in the form of fertiliser or animal feed. The nutrient compounds are associated with suspended solids in effluent water. Thus, the removal of suspended particles from aquaculture effluents is important in reducing its nutrient content.

Nutrients and SS in aquaculture effluent come from a variety of sources (Fig. 4.1). Particles are carried into the farm with the water supply and also produced within the culture system. In flow-through systems, particles consist mainly of uneaten or regurgitated food, faeces and fragmented tissue. In recirculation systems, additional inputs may come from water-conditioning devices, such as filter media escaping from retaining vessels or bacterial flocs breaking away from tertiary filter media (Cripps, 1993).

Depending on the species and culture techniques, up to 85% of the P and 52–95% of the N entering a fish culture system as feed may be lost into the environment through feed wastage, fish excretion, faeces production and respiration (Wu, 1995). Some feed may be lost from the tank or pond outlet or through the bottom of a cage before the animals have eaten it. This loss is increased with over-feeding. Nutrients also pass through the animals and reach the environment as faeces. If feed management is poor, the food conversion ratio (FCR) will be poor, resulting in increased wastage of feed. In high-density cultures, or those in large volumes, it can be difficult to assess the amount of food required. In such cases, it is normal to err on the side of caution and to over-feed to ensure good growth. The FCR has, however, improved, and N and P levels in feeds have decreased as the aquaculture industry has developed (Figs 4.2 and 4.3).

Feed wastage is evident in the farming of shrimp as well as fish. In the Philippines, for example, it has been estimated that 4500 ha of intensive shrimp culture ponds were supplied with 43 200–108 000 t of formulated feeds in 1992 and that ca. 15% of this (6480–16 200 t) was not consumed (Primavera, 1994). Thus, careful feed management is an important aspect in limiting the environmental impacts of aquaculture and an important economic consideration for aquaculturists.

A number of studies have estimated the total N and P discharged into receiving waters from aquaculture of various species. It has been estimated that for each metric ton of channel catfish (*Ictalurus punctatus*) produced, an average of 9.2 kg of N and 0.57 kg of P is discharged (Schwartz and Boyd, 1994). Similarly, for each metric ton of juvenile Atlantic salmon (*Salmo salar* L.) cultured in freshwater, there were environmental loadings of 71 kg of N and 10.9–11 kg of P (Kelly *et al.*, 1996). A recent study conducted in an intensive system for the culture of sea bream (*Sparus aurata*) in earthen ponds estimated the direct consequences of discharge into receiving waters. The study estimated that:

- 9105 kg of total suspended solids (TSS);
- 843 kg of particulate organic matter (POM);
- 235 kg of BOD;
- 36 kg of NH_4^+-N, 5 kg of NO_2^--N, 7 kg of NO_3^--N;
- 3 kg of PO_4^{3-}-P

were discharged into the environment for each 1000 kg (1 t) of fish cultured (Tovar *et al.*, 2000).

A further source of potential pollutants in aquaculture effluents is that from undigested food, which is eaten by the animal but not assimilated and is passed out as faeces. In a formulated diet, 15–20% of the ingested dry matter may not be digestible. Using figures for the salmonid culture industry in Norway as an example, it is possible that ca. 47 000 t of feed were lost to the environment as faeces in Norway in 1989. Advances in feed composition and feeding practices over recent years have decreased that

[1]The amount of oxygen required by aerobic micro-organisms to decompose the organic matter in that water: high levels of organic matter lead to high BOD and potentially low DO levels from the aerobic decomposition.

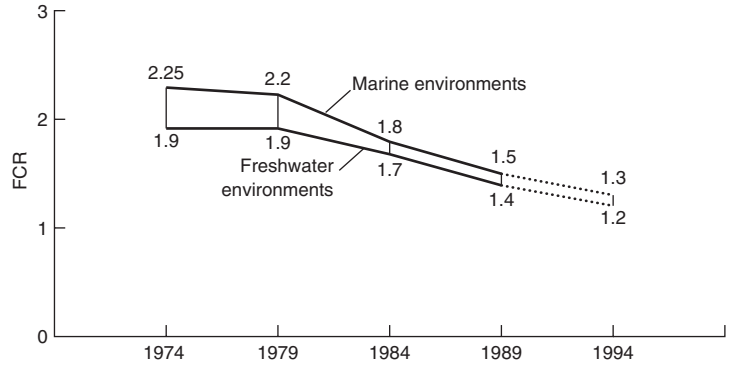


Fig. 4.2 Changes in the food conversion ratio (FCR) in Nordic fish feeds. After Ackefors and Enell (1994).

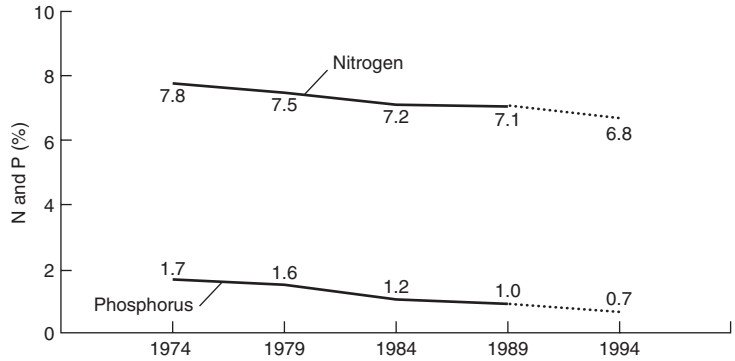


Fig. 4.3 Changes in the nitrogen (N) and phosphorus (P) content in Nordic fish feeds. After Ackefors and Enell (1994).

figure considerably and are partly responsible for the economic success of Norwegian fish farming into the twenty-first century (section 15.5.1).

Similarly, it has been estimated that in intensive shrimp ponds in Asia, only 17% of the food presented is converted to shrimp biomass. About 15% of the feed remains uneaten or is lost through nutrient leaching, 20% is egested as faeces and a large part of the remainder (48%) is lost to the environment as excreted metabolites and moulted shells (Primavera, 1994) (section 3.4.3.1).

Feeds with high nutrient content can also result in losses to the recipient water. Fish utilise protein N for growth, as an energy source and for metabolic functions. It is more cost-effective (excluding flesh quality considerations) and better environmentally if diets contain only sufficient protein to support growth and metabolic requirements so that energy requirements are met from dietary carbohydrates and lipids ('high-energy diets'). These protein- or energy-sparing diets have become popular in recent years and, in conjunction with improved feeding techniques, have resulted in substantial reductions in the wastage of N into the environment.

Table 4.1 shows the concentrations of suspended solids, total phosphorus (TP), total nitrogen (TN) and BOD in effluents from farms in Europe. There is surprisingly close agreement between the studies in the concentrations of all four variables. This is despite the range of geographical locations and variations in farm type and farm management practices between studies. Table 4.2 indicates typical industrial effluent contaminant concentrations for comparison. When placed in the context of other industries and typical municipal wastewater, it can be seen that the

Table 4.1 Reported contaminant concentrations in aquaculture effluent in Europe. Reproduced from Cripps and Kelly (1996) with permission from John Wiley & Sons.

Location	SS (mg/L)	TP (mg/L)	TN (mg/L)	BOD (mg/L)
21 EIFAC farms	9	–	–	5
Typical in Norway	3	0.100	0.5	–
Rogaland, Norway	1.6–14.1	0.08–0.27	0.43–0.70	–
Northern Sweden	[6.9]	0.11	0.70	–
Northern Ireland	–	0.11	0.531	–
Typical range	5–50	0.05–0.26	0.5–5.0	5–20
Finland	–	0.055	–	–
>31 UK farms	11.1	0.082	–	4.00
Typical in Denmark	5–50	0.05–0.15	0.5–4.0	3–20
Typical values	14	0.125	1.4	8

BOD, biological oxygen demand; EIFAC, European Inland Fisheries Advisory Committee; SS, suspended solids; TN, total nitrogen; TP, total phosphorus.

Table 4.2 Reported domestic and industrial wastewater contaminant concentrations versus typical aquaculture values. Reproduced from Cripps and Kelly (1996) with permission from John Wiley & Sons.

Facility	SS (mg/L)	TP (mg/L)	TN (mg/L)	BOD (mg/L)
Aquaculture (typical)	14	0.125	1.4	8
Domestic wastewater				
Weak	350	4	20	110
Medium	720	8	40.5	220
Strong	1200	15.000	85	400
Stormwater runoff	170	0.350	3.5	14
Meat processing	300	–	3	640
Paper pulp mill	–	–	–	1800

BOD, biological oxygen demand; SS, suspended solids; TN, total nitrogen; TP, total phosphorus.

concentrations (as opposed to mass flow) of these four contaminants in aquaculture effluents are relatively low. Separation and concentration of the contaminants from aquaculture wastewater for subsequent disposal are therefore difficult because of their low concentrations.

4.2.1.2 Impacts of effluent on aquatic systems

Eutrophication can be translated from Greek as 'nutrient (food) rich'. Nutrient enrichment in both fresh- and saltwater is a natural process. Nutrients can be carried into low-nutrient (oligotrophic) areas, which then become mesotrophic (intermediate nutrient content) and ultimately become eutrophic when the nutrient level becomes high. This natural process can be greatly accelerated by additions of nutrients from untreated sewage and from discharges from agriculture, aquaculture and other industries (Pillay, 1992; Welch and Lindell, 1992). As the concentration of organic matter increases, so also do the populations of aerobic bacteria, which use oxygen to break down organic matter. The DO content of the water then decreases, resulting in stress, death or exclusion of other aquatic species. The point at which the DO concentration

is at its lowest is usually some distance downstream from the point at which discharge water enters the recipient body of water. Thus, in a lake or river, this 'critical point' may be some distance from the farm. In contrast, settlement from cage culture often occurs below the cages and potentially harmful effects are close to the cultured organisms.

According to Boyd (2001), levels of suspended solids in effluents must be limited for several reasons:

1. The suspended solids may form a plume of discoloured water in the discharge area, and the plume may reduce light penetration and phytoplankton productivity.
2. Restricted light penetration by turbidity affects sea grasses, coral reefs and other sensitive underwater habitats.
3. Sedimentation in shallow water may interfere with local navigation, fishing and other beneficial uses.
4. Excessive sedimentation can stress or kill trees where effluent is directed into mangroves.
5. Sediment accumulation can bury and smother benthic organisms.
6. A high sediment oxygen demand can produce undesirable anaerobic conditions, and toxic metabolites (e.g. ammonium, methane and hydrogen sulphide) may enter the water from anaerobic sediment and harm sensitive aquatic animals.

In addition, large quantities of suspended particles may cause clogging of the respiratory apparatus of aquatic animals and result in poor feed uptake by visual and filter feeders.

The deposition of suspended solids is pronounced in slow-moving or shallow water. In such locations, the majority of benthic species can be excluded, and only those that are opportunistic survive. The correlation between pollution and biomass (or diversity) is so strong that indices have been devised that can be used to assess pollution levels by calculating the biomass, species diversity or presence of sensitive species intolerant to pollution.

An increase in dissolved nutrients can greatly increase primary production, resulting in large concentrations of phytoplankton or algal 'blooms'. If a bloom contains a species of toxic algae, then this can be dangerous for both humans and aquatic animals. Such blooms have occurred in many parts of the world and have affected bivalve aquaculture in particular (section 23.5.3.1); however, it appears that aquaculture has often been the victim of these problems rather than the cause.

4.2.2 Effects of land-based aquaculture on the natural habitat

Land-based aquaculture, which includes aquaculture in the coastal regions, has had well-publicised negative impacts on the natural habitat. Large-scale removal of mangrove forest for marine shrimp culture and pressure on forest resources in mountain areas for freshwater aquaculture have been well documented (Kumar and Hiep, 1995). Again, however, aquaculture is blamed where other activities, such as destructive logging of mangroves for timber and fuel, and clearing for settlements and agriculture, also play their part in habitat deterioration (New, 1999; Walton *et al.*, 2007).

4.2.2.1 Coastline degradation

The large-scale exploitation of coastal regions for aquaculture is a major concern in some developing countries. Environmental issues may, of necessity, take second place to food production or economic development in these countries. In Indonesia and the Philippines, it is estimated that 300 000 ha and 200 000 ha of mangroves, respectively, have been converted into marine shrimp ponds (Fig. 4.4). The Philippines has lost more than 66% of its mangroves since 1920 (Primavera, 1994). Similarly, substantial mangrove losses have occurred in other south-east Asian countries. For example, 102 000 ha of mangroves in Vietnam were cleared for shrimp farming between 1983 and 1987, and approximately 65 200 ha of mangroves were converted into shrimp farms in Thailand between 1961 and 1993



Fig. 4.4 Banks being built for a shrimp farm near a coastal village on Los Negros, Philippines. The natural wetland will be destroyed and the mangroves in the distance lost. (Photograph by Clem Tisdell.)

(Primavera, 1998). Similarly in South America, around 21 600 ha of shrimp ponds in Ecuador have been developed in mangrove areas. The processes of mangrove forest clearance are continuing in these regions. Alternatively, there is also research into 'mangrove-friendly' aquaculture.

This development is a major concern for several reasons. Mangroves are the main factor stabilising many coastal and estuarine regions. Without the extensive root systems of mangroves, the mud flats on which they grow can easily be washed away, particularly during tropical storms. Mangrove clearance also influences the quality of water available to agriculture and may cause changes in stream flow, siltation and coastal sedimentation characteristics. In estuaries where mangroves have been cleared for aquaculture ponds, flooding may result because the water volume retained by the ponds exceeds the carrying capacity of the estuary. Without the stabilising effects of mangroves, the land is gradually eroded despite the building of pond walls.

Mangroves are a major source of coastal productivity, adding huge amounts of organic detritus to coastal ecosystems. Mangroves are also known to be important nursery grounds for a wide range of species and, in particular, species that support coastal fisheries. Removal of mangroves can result in depletion of a wide range of native species and can lead to serious reductions in stocks of fished and non-commercial species. In South America, for example, young shrimp are hand-netted from beaches and grown on in coastal farms. Serious mangrove depletion has resulted in a sharp decline in shrimp stocks, which has affected the viability of the shrimp farms themselves.

Similarly, temperate marshes can be highly productive regions containing vegetation and organic detritus, which are a food source for many organisms. They also prevent coastal erosion, buffer flood damage, act as over-wintering and nesting grounds for birds, and contain many species requiring conservation (Pillay, 1992).

Clearing mangroves and coastal marshes for aquaculture development is not sustainable because of the large-scale environmental and social degradation, and the depletion of fisheries stocks that may result. Despite this, large amounts of aid money are still directed towards this type of development, with only the promise of short-term profits.

Ways of reducing these impacts include the following:

1. Legislation against further facilities in sensitive coastal areas, using zonation plans or by the total prohibition of new developments without independent impact assessment prior to granting aid.
2. Intensification of farming practices.

Sustainable use of mangroves, is summarised by the following guidelines:

- do not alter the hydrology;
- do not alter the tidal flooding and surface circulation patterns;
- do not alter the physical structure, biological activity or chemical properties of the substrate.

Mangrove forests are being re-established in some regions by replanting with propagules, but these tend to be monogeneric plantings, which may lead to forests that are ecologically inadequate compared with normal multispecific forests. A study by Walton *et al.* (2007), however, found that even monogeneric stands are effective in restoring normal populations of a predator such as the mud crab (*Scylla* species), indicating a high level of ecological recovery.

4.2.2.2 Impacts on other resources

In some developing countries, farmers are clearing forest to accommodate agriculture, including aquaculture. Alternatively, some developing nations have recognised the negative environmental impact of such practices and are taking positive steps towards environmental protection for the long-term sustainability of the farming sectors.

Use of grass carp as a major aquaculture species has had a significant negative impact on forests. Grass carp require large amounts of grass or green vegetation as food (40 : 1 FCR). They are cultured predominantly in Vietnam (Fig. 4.5) in small-scale operations where farmers often obtain this foliage from the forest (Fig. 4.6). A large number of farmers looking for green leaves in the forest to feed cultured grass carp in their ponds can have major impacts on forests . Recognising the long-term environmental impacts of undesirable aquaculture practice, the government is taking measures to protect forests and is providing assistance for more sensitive fish culture practices. Also, the government is investigating alternative species composition for better economic return without including grass carp in freshwater polyculture.

Some freshwater aquaculture practices use excessive water. The diversion of streams, the addition of canals and reservoir construction has negatively affected the environment in some areas. Freshwater resources are becoming even more limited and require conservation. Freshwater



Fig. 4.5 Grass carp (*Ctenopharyngodon idella*).



Fig. 4.6 Farmers collect large quantities of fresh grass or green foliage for feeding grass carp in Vietnam.

aquaculture ponds must be operated using appropriate water management techniques, which minimise water exchange.

4.3 IMPACTS OF AQUACULTURE WITHIN LARGE WATER BODIES

The culture of aquatic animals within large water bodies has the potential to cause both onshore and offshore impacts on the surrounding environment. Large water bodies including oceans, rivers and lakes are widely used for aquaculture purposes. Commonly used methods for aquaculture include:

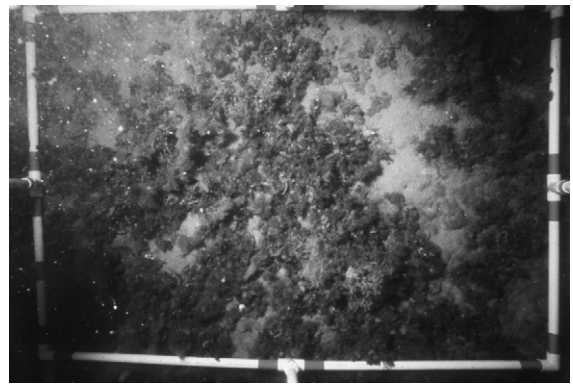


Fig. 4.7 Seafloor beneath a seacage for fish culture showing accumulation of organic matter.

- cage or pen culture for fish (both marine and freshwater fish);
- culture of bivalve molluscs within a few kilometres of the shoreline;
- ranching activity (stock enhancement programmes).

4.3.1 Pollutants from cage culture and their impact on the environment

Dense populations of animals such as farmed fish in cages (section 2.2.6) may be a major source of organic and inorganic compounds in water. Such high densities of animals may produce marked changes in the chemical composition of the water (e.g. DO components and N/P ratio), which may subsequently affect populations of phytoplankton. The main sources of pollution are animal faeces and excretions, and feed waste. Unlike its land-based counterpart, cage culture relies upon natural water movement to deliver water and oxygen to sustain production and remove wastes. The impact of aquaculture on the surrounding biota has been a growing concern because of the rapid expansion of cage culture in the past few decades. Uneaten feed and faeces contribute significantly to the overall solid-waste production from cage systems (Fig. 4.7). The limited potential that exists for treatment of the waste material produced is a key issue in the environmental concerns raised against cage aquaculture.

The effects on the benthic community structure of organic loading originating from fish farms are most pronounced under and in the immediate vicinity of fish cages. They are less so at increasing distances from the farming operation. Accumulation of waste products in farm sediments usually results in dramatic changes in sediment

chemistry and also the macro- and meiobenthic communities (Karakassis *et al.*, 1999). If the sedimentation rates from cages are high, the fauna may disappear completely. The community becomes more diverse and normal with increasing distance from the cages. The benthic fauna play an important role in the supply and mineralisation of organic matter and affect nutrient cycling in the sediment. Nitrogen cycling is faster, and both nitrification and denitrification are enhanced compared with azoic sediments. Various studies have shown that microbiological metabolism in sediments is stimulated by organic wastes from cages. In a shallow marine cage farm in Kolding fjord, Denmark, microbiological metabolism in the sediments was measured at ca. 10 times (525–619 mmol/m^2/day of CO_2) that at a reference site, most of which could be accounted for by sulphate reduction (Holmer and Kristensen, 1992). Studies on the production of macrobenthic populations in the vicinity of cage farms in western Scotland (Pearson and Black, 2001) suggested that the production of infaunal benthos close to the cages is 4–6 times greater than background levels.

Site rotation and 'fallowing' are methods used by cage culture industries to minimise impacts in the vicinity of fish cages (section 15.4.2). The fallowing of sites and rotation of cages has now become recommended practice in many areas where hypertrophic sedimentary conditions are a problem. A study undertaken in western Scotland on the benthic faunal succession and sedimentary conditions during the fallowing periods indicated that communities adjacent to fish cages returned to normal 21–24 months after destocking (Pearson and Black, 2001). The switch from highly enriched to moderately enriched communities took 9 months, followed by a further 9 months to achieve a lightly enriched status.

The rapid expansion of cage culture in the last decade, particularly in the salmon industry, has increased the use of many chemicals which may have direct environmental impacts. Poor water quality and crowded conditions induce stress in caged fish, contribute to impaired growth and predispose them to disease. This in turn necessitates increased use of medicinal solutions. Pesticides are being used to combat sea-lice infestations, and disinfectants help to prevent the spread of viral infections. These substances add to the chemical wastes going into the environment and may have a negative impact on it (Haya *et al.*, 2001).

4.3.2 Cage farming impacts on wild fauna and flora

Cage culture also has direct impacts on wild fish populations by supplying food, either directly or through an increase in algal and zooplankton biomass, and by providing refuge in fish cage structures. Escaped fish may also have ecological impacts. A review undertaken by Phillips *et al.* (1985) of the freshwater sector showed that populations of species including roach and native brown trout have increased as a consequence of rainbow trout cage culture.

Meadows of seagrass, *Posidonia* species, cover vast areas in shallow regions of the Mediterranean. They are regarded as a cornerstone of the littoral ecosystem, providing a wide variety of niches and accounting for the high diversity of these areas. A recent study concluded that, although nutrient input from fish cages resulted in an increase in leaf length, and increases in the biomass of epiphytes and fish life of the region, there was evidence of decreased meadow density and total disappearance beneath cages (Pergent *et al.*, 1999). The study suggested that *Posidonia* species is a useful bioindicator for monitoring fish farm impacts in these environments.

4.3.3 Impact of bivalve culture

Bivalve culture has positive and negative impacts depending on the culture practice. Bivalves filter feed on phytoplankton and other fine organic particles (section 23.2.2). Bivalve culture usually requires the introduction of structures into the water body, from which the bivalves are either supported, protected or suspended. The introduction of such structures has an immediate effect on local hydrography and provides a substratum upon which other epibiota can settle and grow. The introduction of high densities of cultured organisms increases local oxygen demand and elevates the input of organic matter into the immediate environment.

The effects of biodeposits from suspended mussel culture on the local benthic environment have been considered in a number of studies. Heavy sedimentation of faeces and pseudo-faeces beneath mussel farms leads to organic enrichment and thus alters macrofaunal communities (section 23.6.2). Sedimentation rates up to three times higher than at reference sites were recorded in several studies, suggesting that average biodeposits in suspended culture could reach quantities up to 345 kg/m^2/year (Chamberlain *et al.*, 2001). A study on biodeposits conducted in Ireland indicated that, in general, the effect of sedimentation was restricted to a radius of 40 m around the farm. There is increasing awareness of the potential environmental effects of bivalve culture and a number of measures can be undertaken to minimise these impacts (section 23.6.2).

On the positive side, bivalves have the capacity to filter from 1 to 60 L per individual per hour. Since the 1990s, research has demonstrated the ability of oysters and other shellfish to filter the water column and therefore remove the effects of over-nitrification in coastal and estuarine waters. Other research has shown that wetlands restoration can be used as a denitrification tool as well. Both of these strategies use natural processes and have the benefits of not only removing nitrogen and its effects but also can provide benefits such as stimulating the regrowth of valuable eelgrass beds, and revitalising finfish populations and crustacean nurseries. Both have the ultimate effect of providing not only clearer estuarine waters but a potential source of sustainable food stocks.

4.4 GENERAL IMPACTS ON THE ENVIRONMENT

There are general environmental impacts that are applicable to both land-based and water-based aquaculture. National development plans and policies for aquaculture must account for the general and specific impacts of aquaculture, and approval processes must include stringent measures to prevent or minimise such impacts.

4.4.1 Disease transfer

The transfer of disease between farms and the environment has long been a contentious issue. Farmed animals may infect wild animals but, also, indigenous organisms may carry endemic pathogens to and between farms. There is disagreement about which of these two vectors is the more common or important.

In terms of environmental impacts, two main types of pathogen can be identified:

1. Obligate pathogens, which occur only when there is a suitable host as a substrate for growth.
2. Opportunistic pathogens, which are widespread, almost ubiquitous, and cause disease when the resistance of the host is reduced.

Stress on fish resulting from high-density, intensive cultivation is often sufficient to allow opportunistic pathogens to take hold and form a disease reservoir capable of infecting other culture stocks or the surrounding environment (Chapter 10). Alternatively, natural populations may also be under considerable stress as a result of suboptimal conditions, such as a poor food supply or chronic obligate disease. They can then form a disease reservoir, with the potential to disperse over a much larger area than farmed species.

Several vectors for the transfer of diseases between cultured animals and the environment have been determined. Escaped animals have received the highest media profile. Intermediate hosts can be important vectors. Wild fish, for example, may move in and out of floating cage systems. Bivalves are known to contain and concentrate pathogens in addition to various chemicals and heavy metals. Although the presence of pathogens in such intermediate hosts may have originated from culture facilities, the hosts themselves do not necessarily suffer. In the case of bivalves, the organisms can be depurated with clean water to ensure that diseases and contaminants are flushed away. Effluents can both carry pathogens from a culture facility and predispose the surrounding environment to disease outbreaks by chemically stressing indigenous fauna or by improving the environment for the growth of pathogens (e.g. increasing the organic matter).

There is no firm evidence that predators of cultured organisms carry pathogens out of culture facilities, although pathogens are found in faeces and regurgitated food from birds that feed at aquaculture farms. Also, parts of the cultured organism may be lost or discarded after predation, allowing them to decompose away from the farm. The practice of removing dead animals from farms and dumping them at sea is now illegal in most countries. Solid or liquid wastes from processing facilities, such as blood or offal, can also contain high concentrations of pathogens. Animals cultured for restocking purposes may carry disease with them when they are stocked into a fishery.

All sensible measures must be taken to ensure that escapes and predator attacks are reduced. Treatment of wastewater can reduce the incidence of pathogens and prevent the build-up of conditions that predispose an area to disease. Dead animals from the culture population must be removed rapidly and regularly to ensure that pathogens do not build up. They must be disposed of with care, possibly at a land tip or by some form of heat and chemical treatment.

The use of unprocessed whole fish by-catch (‘trash fish’) from the fishing industry as an aquaculture feed has greatly decreased in recent years and is illegal in some countries. Such feed, particularly if ungutted and not fresh, can harbour disease.

The use of prophylactic drugs in intensive culture has decreased in some industries (section 19.2) and increased in others. Although these drugs help to reduce the reservoir of pathogens that could be transferred to the environment, their widespread use has potentially detrimental effects, including the build-up of drug-resistant strains in wild and

cultured animals. Management strategies that are less aggressive and more sustainable are showing great success in reducing disease transfer. Maintaining only one year-class of animals in a region at one time has prevented the transfer of disease throughout the life cycle of the culture animals. In Scotland, neighbouring companies have agreed on a joint strategy in which a region can be left fallow between generations, thus reducing direct disease transfer or transfer via secondary hosts and the sediments. Adequate spacing between farms is also important, so that the carrying capacity (the ability of the environment to accept effluent) is not exceeded, and to prevent direct transfer of pathogens.

The presence of a disease in the environment may not be the result of farming operations. This link is often difficult to prove because so many contributing factors are involved. Also, farm diseases do not always disseminate in the environment if stress levels are low. Microbial activities may well increase in sediments, but they are not necessarily pathogenic.

Diseases in culture organisms, and the transfer of disease to the environment, can be greatly reduced by careful management (Chapter 10). Some of the management practices used to minimise the occurrence and transfer of diseases in aquaculture systems are discussed in more detail in the later chapters of this book, which outline specific culture methods for various species/groups (Chapters 13–25).

4.4.2 Impact on genetics

4.4.2.1 Introduction of exotics

The introduction of exotic species or strains into a new region is not confined to aquaculture, nor is the introduction of animals and plants through natural, accidental or deliberate causes a new phenomenon. For example, terrestrial animals have been deliberately introduced for public health reasons (e.g. pathogen competitors), for reduction of pests (e.g. a new predator) or for improved farming efficiency (e.g. a faster-growing stock).

Within the aquatic environment, natural movement of stocks into a new area or region can occur as a result of climatic events, such as glaciation at high latitudes or river flooding in the tropics. There are examples of accidental introduction from the aquarium fish trade and the discharge of ballast water from shipping, the latter vector being a particularly serious problem in California, Australia and New Zealand. Deliberate introductions include predatory fish brought into a catchment to improve the vitality of a managed stock and also ranching.

Introductions and transfers have been defined as follows:

- Introductions are movements beyond the present geographical range of a species and are intended to insert totally new taxa into the flora and fauna.
- Transfers take place within the present geographical range of a species and are intended to support stressed populations, enhance genetic characteristics or re-establish a species that has failed locally.

The escape of some species of cultured animals is not covered well within the above definition. At many, if not the majority of, locations, cultured animals are grown within their natural range; however, the cultured stock may be a different strain from local wild individuals of the same species. Escapes may then be classified as threatening introductions rather than transfers. Despite precautions, escapes from farms are common. The breaching of containment vessels, ponds, cages, tanks, etc. during storms or floods is common. Predators such as birds and marine mammals take and then drop live prey. Damage to pond walls by burrowing mammals and to seacages by seals occurs. Operational losses may occur during grading, transferring between vessels and harvesting. The potential for losses because of poaching and vandalism must also be considered.

The scale of this problem varies with location. In some lakes in which cages have been broken, the majority of a class of animals, e.g. salmonid fishes, may be of cultured origin. An example of this is the presence of rainbow trout (*Oncorhynchus mykiss*) in some Scottish freshwater lochs. At more open marine locations in which few escape incidents have occurred, there may be insignificant introduced populations. Mills (1989) estimated that the number of escaped salmonids in Britain was 15% of the farmed production at that time. The World Wildlife Fund (WWF, 2001) concluded that in Norway alone at least half a million farmed salmon escape from aquaculture facilities annually, so that 30–50% of coastal catches may be farmed fish.

Escaped animals may have the following impacts:

- interbreeding with wild stock, i.e. genotype modification;
- competition for space and food – niche competition;
- transfer of disease;
- changes in the local ecology by changing trophic interaction;
- habitat modification.

Although precautions can be taken, escapes are bound to occur. Both the frequency and magnitude of such losses must however be minimised, and in many countries this is becoming a legal requirement. The following procedures will help to reduce losses:

- regular inspection and maintenance of pond walls, weir gates and farm effluent gratings;
- secondary back-up containment facilities;
- predator deterrence: nets, scarers, etc.;
- underwater predator netting around net cages;
- site surveys, prior to farm construction, covering flood frequency, height above water table, etc.;
- adequate security or circumspect location to avoid or deter poaching and vandalism;
- good public relations to reduce vandalism;
- all in-farm animal handling operations to be conducted within confined areas.

The introduction of non-native species into a new region must be considered with great caution. Once in a new location, the ecology of the species may alter, or unforeseen consequences, such as unpredicted interspecific competition, may occur. For example, introduced brown trout are thought to be a major factor in the decline of several species of freshwater fish in south-eastern Australia. The major contributing factors are thought to include competition for habitat, food resources and predation.

Exotic species have been introduced to facilitate the development of aquaculture in a number of cases. For example, a number of species of shrimp, including *Marsupenaeus japonicus*, *Litopenaeus vannamei*, *L. styirostris* and *Fenneropenaeus chinensis*, have been introduced into the Philippines since the 1960s. The effects of these introductions on endemic species and the local environment are unknown.

4.4.2.2 Genetic interaction

There is increasing concern about the interbreeding of wild and cultured stock (WWF, 2001). Controversy concerns not only the effects, but also the extent to which this occurs. 'Genetic pollution' can be associated with the introduction of new species into an area or the escape of organisms from a culture facility. Rather than just outcompeting the native species, or transferring diseases, escaped animals may interbreed with wild stocks. The mode and scale of possible genetic interaction from introductions and escapees has been described in the previous section.

Cultured species, in which the life cycle has been closed in captivity, have been bred to be suitable for farming. Such adaptations, e.g. reduced stress in high stocking density situations, may not necessarily be suitable for success in the wild. There is some evidence to suggest that hatchery-reared salmonids do not compete well in the wild with corresponding native stocks. By implication then, interbreeding between wild and cultured stocks may produce less fit individuals. The following list indicates other possible impacts of interbreeding:

1. Loss of genetic diversity by interbreeding of wild stocks with relatively uniform culture stocks (Crozier, 1993).
2. Loss of distinct stock integrity. Some native strains, although not necessarily suitable for culture directly, may form a store of a particular genotype, e.g. resistance to a particular disease, or fast growth in warm, low-oxygen conditions. By back-crossing these genes, the genotype can be incorporated into a culture or a threatened species. Interbreeding with these distinct stocks can dilute such characteristics and result in loss of the strain.
3. Cross-bred hybrids can be sterile or less fertile, so the native stock becomes less viable and may ultimately be lost.

A particular problem exists when animals are cultured as part of a restocking programme in which individuals will eventually mature and may reproduce with existing members of a wild population. As mentioned above, giant clams are a good example of animals that were cultured for this purpose and which were transferred between Pacific nations. Because of concern for the potential genetic implications of these transfers, guidelines were drawn up that outlined preferred practices for hatchery production of spat and for translocation of spat (Lucas, 1994).

4.4.3 Chemical additions

A wide range of chemicals are currently used in the aquaculture industry. These have been summarised by Beveridge *et al.* (1993) as follows:

- chemotherapeutants, e.g. parasiticides, antimicrobials;
- vaccines;
- hormones, e.g. growth stimulants, sex control;
- food additives, e.g. vitamins, pigments;
- anaesthetics;
- disinfectants;
- water treatment compounds, e.g. pH regulators, herbicides, pesticides and piscicides;

- antifoulants (originally tin and copper compounds);
- construction additives, e.g. stabilisers, pigments.

4.4.3.1 Antibiotics

Compared with the public health or general veterinary fields, relatively few chemo-therapeutants are suitable or available for use with aquaculture species for human consumption. This is because of problems of strain resistance and because few pharmaceutical companies consider aquaculture to be a sufficiently large market to target, primarily because of licensing costs.

Pressure from environmentalists also limits marketing. The main classes of therapeutants are antibiotics, nitrofurans, sulphonamides, acriflavine, copper sulphate/potassium permanganate stains, formalin/formaldehyde, oxolinic acid, iodine and di-*n*-butyl tin oxide.

The use of antibiotics in aquaculture is widespread. Although the timing, quantity, concentration and type are carefully controlled in developed countries, they have been subject to misuse, especially in Asian shrimp culture. Antibiotics can be added to the feed to control bacterial diseases or used prophylactically to prevent the outbreak of diseases (e.g. section 16.9.1.4). Considerable quantities can be used.

Most antibiotics are associated with particulate matter and leave the farm on particles in the effluent. They will therefore have a large impact on sediments. The period of activity or persistence of antibiotics in the environment varies with environmental conditions, such as temperature and substrate, and according to the particular antibiotic used.

The major environmental impacts of antibiotics are on non-target species, including humans, and on the resistance of the target organisms. For example, resistant strains of *Vibrio* bacteria have developed in shrimp ponds in south-east Asia as a result of the widespread prophylactic use of antibiotics (Pillay, 1992). The main impacts on the environment have yet to be definitively quantified, but possible effects may include:

- resistance of the target organisms to antibiotics, leading to the occurrence of more virulent strains;
- antibiotic resistance of non-target pathogens of other species, resulting in the development of increased disease incidence in non-culture species;
- reduction in general microbial action within the sediments, leading to rapid degradation of site quality;
- persistence within the cultured organism, which can result in allergic reactions in humans if the organisms are consumed too soon after antibiotic treatment.

4.4.3.2 Pesticides

Some chemical treatments for sea-lice (*Lepeophtheirus salmonis*) contain dichlorvos, an organophosphate. Concentrated dichlorvos is a highly toxic neurotoxin that is dangerous when inhaled, absorbed across the skin or ingested. Treatments are stressful to the cultured fish, and toxic to various molluscs and crustaceans (Jackson and Costello, 1991). Its half-life in seawater is 4–7 days and its breakdown to harmless products reduces its capacity to bioaccumulate; however, because of the serious negative impacts, alternatives are now being widely used.

The use of herbicides, pesticides and piscicides was reviewed by Pillay (1992). For example, teaseed cake, derris powder and rotenone are commonly used to remove pests from aquaculture ponds. The toxicity of these compounds appears to last for 2–4 days, although 20 mg/L rotenone concentrations can remain toxic to juvenile fish in tropical shrimp ponds for 8–12 days.

4.4.3.3 Steroids

Steroid hormones are used for inducing maturation and spawning, and for sex reversal. These chemicals are used commercially only for broodstock and not for marketed animals (sections 7.7 and 18.5.7).

4.4.3.4 Pigments

Cultured animals may not have access to naturally occurring pigments, so any carotenoids required must be added to the diet (e.g. in salmonid culture). Although 88% of the pigments may not be retained by the cultured organism, they oxidise rapidly in the environment. There are no data to indicate a significant environmental impact from carotenoids.

4.4.3.5 Antifouling

A good flow of water is required through floating net cages at all times. They must therefore be kept clean from fouling organisms such as seaweeds and bivalve molluscs. Similarly, pipes and pumps in land-based systems must be kept clean. It is now common practice to impregnate the netting with some form of antifoulant, either at the factory or on site. The most commonly used antifoulants have been either organotin, e.g. tributyl tin oxide (TBT) or copper-based compounds. These compounds are highly toxic to bivalves, and there is growing evidence that some fish species may also be affected. Research is continuing to find less toxic biocide-free antifoulants and a water-curing epoxy-based coating has been developed (de Nys and Ison, 2008). Again, suitable management practices can reduce the amount of antifoulant required and therefore

the effect on the environment. Cleaning nets from seacages on land, after drying, is labour intensive, but it reduces the chemical requirement for antifouling (section 15.4.5).

4.4.4 Social aspects

4.4.4.1 Socio-economic impacts

The development of aquaculture has a number of advantageous impacts, particularly in rural areas. These include employment, the stabilisation of rural populations, improved economy, improved infrastructure (communications, roads, etc.) and, most importantly, in developing countries, the production of a fresh supply of protein-rich food (food security) (section 26.3.2). Even in industrialised countries, a fresh, local supply of quality fish or shellfish is desirable. However, these developments may result in social problems in some cases, depending on the region:

- displacement of traditional local industries such as capture fisheries;
- reduction in employment because of intensive and relatively skilled aquaculture activities;
- depletion in the seed required for capture fisheries (e.g. shrimp postlarvae);
- conversion, and therefore degradation, of a traditional multi-use subsistence habitat into a monoculture facility (Pollnac, 1992);
- changes in traditional water use and consumption;
- increased farm intensity, which may lead to increases in income differential and social stratification;
- overexploitation of the carrying capacity of a region, reducing the possibility of other uses.

4.4.4.2 Aesthetics

The location of farms in areas of outstanding natural beauty may be a problem (Figs 4.8 and 4.9). Aquaculture farms are often situated in remote areas with little other development. Very often these areas are also considered to be in need of protection. A conflict of interest then arises between those who require jobs and income in remote communities and those who wish to keep the area unspoilt. This kind of conflict is not limited to aquaculture developments.

There are ways of minimising the visual impact of aquaculture facilities in scenic and recreational regions. If possible, buildings should be kept low lying or placed away from the shoreline, and their colour must blend in with the surroundings. Tanks and cages must similarly be unobtrusive. Brightly coloured buoys for floatation purposes are particularly noticeable and must be avoided, although navigation buoys must of course remain visible. Equipment not actually in use, such as nets, tanks, forklift trucks, etc., must not be abandoned or stored in obvious sites.



Fig. 4.8 Co-existence of oyster farms and yachting anchorage in the Rivière d'Etel, France. (Photograph by Peter Gugerell.)



Fig. 4.9 Long-lines for mussel culture in a loch in northern UK.

4.4.4.3 Noise

The noise from aquaculture facilities may be a problem. In the worst cases, constant disturbance and noise from farm boat motors and vehicles can scare off shy animals and annoy local residents. Compressed air devices that give off explosive noises at regular intervals to scare off nuisance birds will be irritating to neighbours. Similarly, automatic feeding devices that fire food into tanks or cages on a regular basis, perhaps every 5 minutes, throughout the day can be irritating. Although such noise would scarcely register on a sound meter, the annoyance value is similar to that of a dripping tap at night. Pumps may run continuously. Paddle wheels, used especially at night to sustain good DO levels in ponds, may be a problem. There is inevitably noise associated with aquaculture, as with agricultural activity. The level will obviously depend on the kind of operation. Chosing a site that is an appropriate distance from residential areas can be an important consideration.

4.4.4.4 Other social impacts

The following list summarises some of the main social issues associated with aquaculture developments (Gowen, 1991):

- competition for space with other users;
- traditional fishing;
- navigation;
- anchorages and marinas for recreation boating;
- different forms of aquaculture, and between aquaculture and other industries (e.g. wood pulp);
- amenity, recreation and tourism;
- visual impact and loss of wilderness aspects of the countryside;
- restrictions of access to land, foreshore and inshore areas, which may affect outdoor activities (water sports and harvesting of shellfish for non-commercial purposes);
- reduction in the amenity value of freshwater for recreational fishing;
- reduction in property values.

Many of these conflicts can be resolved at the planning stage by adequate discussion and legislation. The fact that many such conflicts are not resolved amicably indicates that in many cases planning consent procedures and legislation are inadequate. It is in the interest of all parties to ensure that well-defined impact assessment and planning regulations exist and are implemented and that protected areas are not compromised. This will help ensure that environmental impacts are adequately considered and developments are appropriate.

4.4.5 Public health issues

Aquaculture operations can have several impacts directly on humans, in their roles as the operators of aquaculture facilities and consumers of aquaculture products.

4.4.5.1 Safety at work

Aquaculture is an industry that is frequently located both onshore and in deep water, contains heavy and electrical equipment in proximity to water, and has to operate in all weather. This leads to a variety of potential hazards:

- electrical and mechanical hazards, e.g. electrocution;
- hazardous chemicals, e.g. organochlorine sea-lice treatments;
- boating accidents;
- drowning;
- muscular injuries;
- diving accidents and practices, e.g. multiple ‘bounce’ dives for the removal of fish mortalities from cages.

4.4.5.2 Product safety

In addition to obvious public health considerations, it is particularly important for the aquaculture industry, which frequently markets its products as clean and healthy, that contamination of products is minimised. Legal regulations also usually require some level of security. Aquaculture facilities sited inland or inshore are particularly susceptible to product contamination. This can range from slight tainting to greater levels of contamination by bioaccumu-

lating or biomagnifying substances. Serious contamination can have an important impact on consumers. Substances that can cause serious contamination of aquaculture products include heavy metals, such as mercury; petroleum compounds from oil spills; microbes, such as those resulting from sewage (particularly important in the bivalve mollusc industry); organochlorines entering the farm as contamination or as pesticide treatment; and algal toxins resulting from algal blooms, such as 'red tides'. Careful site selection can play an important part in reducing contamination from particular sources. Good management will monitor the prevailing ambient conditions, and so specific pollution incidents may be avoided, e.g. closure of pond intakes during toxic algal blooms. The controlled purification of bivalves in clean water (depuration) for a period prior to marketing is often a legal requirement and greatly reduces the potential threat to human health. In areas where a serious pollution incident has occurred, an exclusion zone, in which culture animals cannot be sold for consumption, may be required to safeguard not just the consumer but also the reputation of the product and the local industry. This occurred following oil contamination of a small number of salmon farms in Scotland.

4.4.6 Miscellaneous impacts

4.4.6.1 Capture fisheries

There are several other possible impacts from aquaculture that have not been described above. At present their incidence is infrequent, their effect is small or current social thinking considers them to be unimportant.

The relationship between aquaculture and capture fisheries is important to consider. For example, the collection of seed from the wild (such as young shrimp and juvenile fish) for aquaculture could detrimentally influence capture fisheries. Alternatively, production of juveniles that have been cultured through their early life stages (during which mortality in the wild is high), for release to supplement natural stocks, may have a beneficial impact on capture fisheries (section 1.7). Cultured animals can also be used to enhance or replace overfished wild stocks, augment sport-fishing stocks and replace populations depleted through industrial developments, such as the damming of rivers used by migrating fish.

As capture fisheries decline and aquaculture expands, competition is likely to increase between the two in terms of product price and quality. This may ultimately result in a shift in labour, first on a local basis and then nationally. A benefit of this is that natural stocks may have a better chance of recovery, although market forces will probably ensure that further stock protection legislation is still required.

4.4.6.2 Animal welfare

Another consideration of the impact of aquaculture activity is the welfare of the cultured species itself. Although animal welfare has received little consideration in the past, concern is increasing. The welfare of animals can be summarised by 'five freedoms':

1. Freedom from thirst, hunger or malnutrition.
2. Freedom from thermal or physical discomfort.
3. Freedom from pain, injury or disease.
4. Freedom from fear and distress.
5. Freedom to express a normal pattern of behaviour.

There is currently a lack of knowledge about the impact of culture on aquatic vertebrates, and squid and octopus. The welfare of the animals at various stages in the culture system may require further attention if public opinion requires it. Factors to consider include water quality, stocking density, culture environment (e.g. tank or net dimensions), physical handling, disease control, harvesting, transport and slaughter. Care must be taken not to attribute human feelings and preferences to the culture species, but clearly, animal welfare must be considered and openly discussed.

4.5 IMPACT ASSESSMENT

4.5.1 Environmental impact assessment

An *environmental impact assessment* (EIA) is the gathering of information required to estimate the possible environmental impacts resulting from a development. The results of the study are presented in an *environmental impact statement* (EIS). The EIA is usually conducted by the developer. Regulating authorities normally inspect the EIA protocol and the result is the EIS. These authorities ensure the correct monitoring of the predicted impacts during and after development. The EIS can also be made available to other relevant government authorities, financiers and the local community. The main stages of the EIA, conducted with the aid of specialists, are:

- scoping and impact identification;
- baseline studies;
- prediction and evaluation;
- identification and mitigation measures;
- assessment or comparison of alternatives;
- impact monitoring/auditing (from Pillay, 1992).

Assessments must be restricted to the most serious and likely impacts to focus efforts most efficiently. The EIS must include the following information, described in detail by O'Sullivan (1992):

- a description of the proposed development;
- the data necessary to identify and assess the main effects which the development is likely to have on the environment;
- a description of the measures envisaged in order to mitigate these effects;
- a non-technical summary.

EIA guidelines vary with local legislation and the nature of the development. Protocols have been presented by a number of workers. One such method is shown in Fig. 4.10.

There are issues in ensuring the aquaculture farmer is following the regulatory measures, although most of the countries follow a monitoring procedure for ensuring compliance.

Effective monitoring provides essential research data for many processes, including identification of impacts, development of methods for future monitoring and the validation of decision-making tools used by environmental managers. Monitoring is also used in validating model programs for general use and in a post-operational capacity to confirm models generated for a particular site. The latter is important as any prediction should be tested and management decisions based on the original prediction modified in light of the results (Telfer and Beveridge, 2001).

4.5.2 Environmental quality objectives

In many countries, *environmental quality objectives* (EQOs) are established so that the environment can be managed in such a way that these objectives would be achieved.

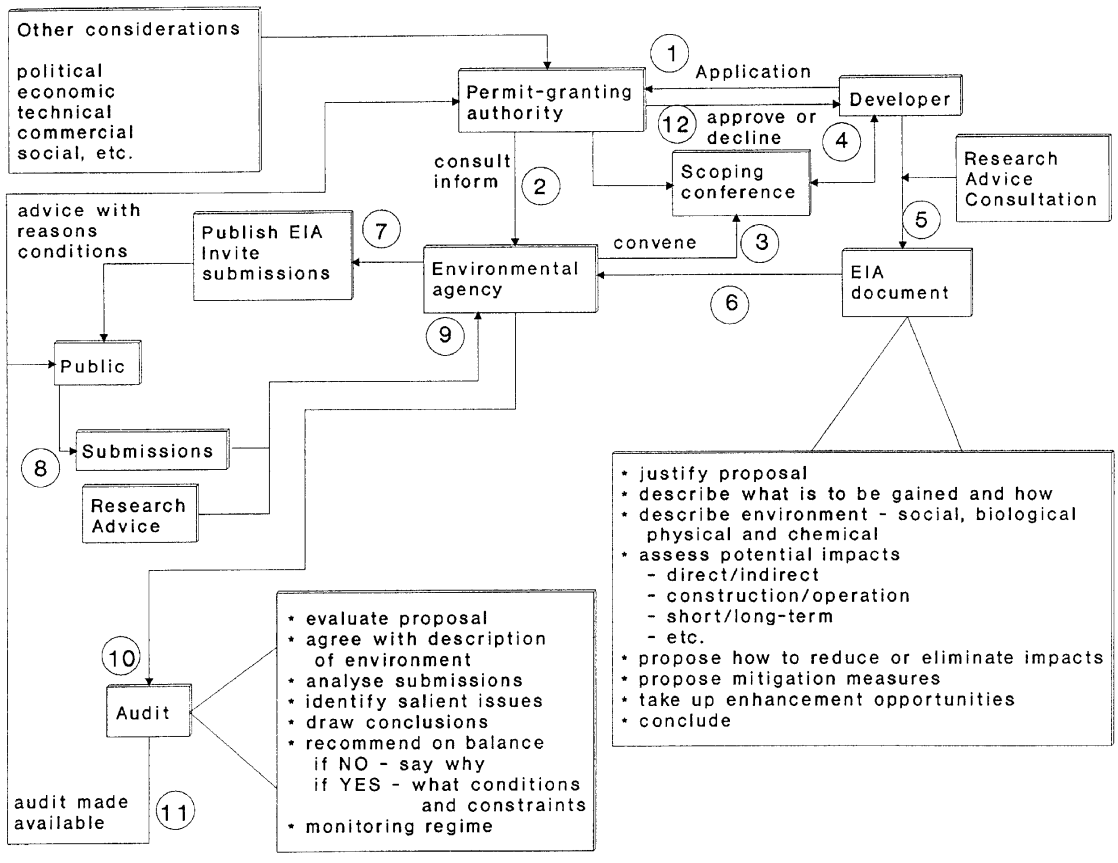


Fig. 4.10 Example of an environmental impact procedure. Based on GESAMP (1991) with permission.

Environmental quality standards (EQSs) are then set for specific variables in order that the objectives are attained. Therefore, quality standards are now implicit within any process of regulation, enforcement and quality control. EQSs are considered to be important to protect the consumer, the environment and also the 'product' (Fernandes *et al.*, 2001). The environmental quality objectives should be defined for coastal waters and standards should then be set to address those objectives. They will require a degree of flexibility to take account of geographic differences. Examples could include: safeguarding water quality; conserving aquatic ecosystems; protecting consumers; consideration for other end users. Quality standards may be set internationally or they may be locally derived from available data (e.g. sediment quality) to provide operational guidelines. Inherent within the EQO/EQS (EQS = *environmental quality standard*) approach is the concept of a mixing zone (or AZE = *allowable zone of effect*) where it is accepted that standards may not apply or be less stringent. This approach requires a definition of the extent of this zone and the criteria to be achieved within it. Monitoring approaches should also be informed by the following: species cultured (e.g. finfish or shellfish), biomass, methodology, technology, location (e.g. open sea, coastal, enclosed bay, semi-enclosed sea, fjord, estuarine, land-based), type of feed and chemicals used. Specific sampling variables to be measured should be determined by the set EQOs and EQSs, the type of operation and the nature of the environment. The extent and frequency of sampling should be determined by the scale of any impacts (Telfer and Beveridge, 2001). The results of monitoring surveys should provide the basis for remedial action, if required. A non-site-specific regional survey should be conducted periodically to assess the wider impacts of aquaculture operations. This should be the responsibility of the regulatory authorities and could be integrated into national monitoring programmes.

4.6 INTEGRATED WASTEWATER TREATMENT AND AQUACULTURE

Effluents are a major issue for aquaculture industries (section 3.5). Some governments are demanding the implementation of best management practices (BMPs) or aquaculturists involved in the same industry may implement BMPs for their region.

Aquaculture can have positive impacts on the environment. Integrated farming has significantly enhanced agricultural production and sustainability in many parts of the world, including parts of Asia, where integrated aquaculture has been practised for many centuries (sections 2.3.6 and 14.5.2). An underlying process in integrated farming is recovering resources such as nutrients and water for re-use. This improves the sustainability of the system and minimises environmental pollution. Integrated farming and wastewater treatment embrace a diverse set of technologies that link fish culture to terrestrial farming.

It has increasingly been recognised that organic waste including sewage is not necessarily a pollutant but a nutrient resource that can be recycled through integrating farming practices. Extensions of the traditional practices of recycling domestic sewage through agriculture, horticulture and aquaculture, being basically biological processes, have been in vogue in several countries. The sewage-fed fish culture of Munich, Germany, and 'bheries' in Calcutta, India, are world famous. The emphasis in these practices has been on the recovery of nutrients from the wastewaters. Taking a cue from these practices and deriving information from the new databases in different disciplines of wastewater management, aquaculture is recognised as an important tool in many developing countries and is being adapted as a standardised technology for treatment of domestic sewage. Waste-fed aquaculture is a proven nutrient-recycling technology that is practised successfully in many countries. Properly designed and managed sewage-fed fish ponds offer a viable low-cost wastewater treatment cum usage opportunity. Several studies have clearly demonstrated that fish production of 5–7 t/ha/year is achievable in tropical climates where year-round growth occurs.

4.6.1 Waste-fed aquaculture in India

The concept of using aquaculture as a tool for wastewater treatment has been evaluated through a systematic research programme carried out over a period of 5 years by the Central Institute of Freshwater Aquaculture, Bhubaneswar, India. In collaboration with the Public Health Engineering Department, Government of Orissa, the Indian Aquaculture Sewage Treatment Plant (ASTP), comprising duckweed and fish culture, was designed. Its field facility has been developed under a project on 'Aquaculture as a tool for utilisation and treatment of domestic sewage'. The ASTP comprises a set of duckweed ponds where algae and duckweeds are utilised in the removal of the nutrients and the reduction of BODs and CODs (chemical oxygen demands). These are complemented by fish ponds and marketing (holding) ponds (CIFA, 1998). The system can receive primary-treated sewage after the removal of solids. The intake BOD levels for the ASTP are in the range of 100–150 mg/L and, consequently, it may be necessary to incorporate an anaerobic unit in which the organic load and

BOD levels are very high. Duckweed culture, before the fish ponds, aids in the removal of excessive nutrient concentration and residues. The waste contains BOD_5 levels of about 100 mg/L. After treatment in the system with a total retention period of 5 days, the final effluent BOD_5 is brought down to 18–22 mg/L (section 3.2.5). Fish ponds are stocked with five carp species: catla (*Catla catla*), rohu (*Labeo rohita*), mrigal (*Cirrhinus migrila*), silver carp (*Hypophthalmichthys molitrix*) and common carp (*Cyprinus carpio*). The production levels recorded from the fish ponds are in the ranges of 3–4 t/ha/year.

4.6.2 Vietnam

Wastewater has been used in aquaculture and agriculture in areas near Hanoi for many decades. Tuan and Trac (1990) described the three systems involving waste-fed fish culture that are used:

1. Fish culture.
2. Fish–rice rotation.
3. Fish–rice–vegetable rotation.

The average net yield from fish culture is about 2.1 t/ha/year. Increased gross yield has also been achieved (4–7 t/ha/year) by controlling the sewage flow and thereby adjusting the N/P ratio along with the organic load. Tilapia (*Oreochromis mossambicus* and *O. niloticus*), silver carp and common carp are the main species utilised in the waste-fed culture system in Hanoi.

Luu and Kumar (2000) presented the national status of sewage-fed aquaculture systems in Vietnam. In medium-sized towns and cities, where the sewage output is relatively high and the catchment area is suitably designed for aquaculture, fish culture is a common practice. In larger cities, where sewage is available around the year, the intermediate catchment areas are always used for aquaculture. Aquaculture is also widely practised in most of the sewage lakes. In Hanoi, there is a daily discharge of 320 000 m^3 of sewage that flows by gravity to the flood plains of Thanh Tri district, where it is used and treated by the agricultural–aquacultural systems. Other lakes, such as Truc Bach and West Lake, Bay Mau Lake, are simple catchment areas for domestic sewage and are used for aquaculture.

The sewage lakes or ponds are usually stocked with fingerlings of Chinese major carp (silver and grass), Indian major carp (rohu and mrigal), tilapia and common carp. In the intermediate catchment lakes, where the water level is less exchangeable, algal blooms develop quickly, sometimes resulting in sudden planktonic collapse and DO depletion to critically low limits. Experience shows that in such lakes the percentage of fish species such as silver carp and tilapia, which feed on phytoplankton, algae and detritus, can be increased to 50–60%. On the other hand, in sewage lakes and ponds where water is periodically pumped to balance the nitrogen content, the fish species thriving predominantly on detritus, zooplankton and zoobenthos can be stocked at a higher ratio. Stocking density depends on the quality of sewage. However, the commonly followed stocking density is up to four fingerlings per m^2 within the size range 30–70 g. With this stocking density, fish productivity of these ponds/lakes reaches 5–7 t/ha/year without other inputs, such as feed, fertilisers and chemicals.

4.6.3 China

According to Zhang (1990), the use of municipal wastewater in China has developed rapidly since the 1950s. In 1985 the total area of wastewater-fed aquaculture involving more than 30 sites was 8000 ha, with a total fish production of 30 000 t. In the 1970s, people in China, especially environmentalists, started to review the positive and negative sides of waste-fed aquaculture. After the review, the government continued its development of waste-fed aquaculture. Most of the waste-fed aquaculture in China is located near cities, and it contributes a large part of commercial fish products to city markets. Silver carp and bighead carp are the main species stocked, along with common carp (*Cyprinus carpio*) and crucian carp (*Carassius auratus*). The stocking density is usually 15 000 per hectare of 20- to 30-g fingerlings. In general, fish yields from wastewater-fed ponds are 2–4 times higher than those from ordinary fish farms. The production ranges from 1.5 to 11 t/ha/year. In China, polyculture systems, using Chinese major carp species with common carp, are common. The efficient usage of food resources coupled with year-round growing conditions (temperature) allows the systems to obtain high production rates. The major problem in waste-fed aquaculture in China is that some industrial toxic waste is mixed with municipal sewage. A great deal of effort has gone into separating industrial pollutants from wastewater.

4.6.4 Germany

Waste-fed aquaculture dates back more than a century in Germany. According to Prein (1990), two groups of systems can be distinguished:

1. Polishing.
2. Wastewater-fed fish ponds.

The former systems receive well-treated effluent from wastewater purification systems and are subdivided into:

- ponds that receive drainage effluents from sewage fields;
- ponds that receive effluents from other biological treatment systems.

The latter systems are designed to purify raw wastewaters that have only been mechanically pre-treated. Net freshwater fish production from waste-fed aquaculture averages 500 kg/ha per 7 months (860 kg/ha/year), with loading rates equivalent to 2000 persons/ha/year. Prein (1990) recorded over 90 installations across the country, ranging from small, single ponds, receiving wastewater amounts equivalent to the treatment of only a few dozen people, up to large systems, e.g. Munich, with 233 ha designed to treat the wastewater from 500 000 people and to produce a gross fish yield of 100–150 t/year. The main fish species used are common carp (*Cyprinus carpio*) and tench (*Tinca tinca*). The German system was designed to operate in temperate latitudes with low stocking densities. Thus, the fish yields are lower than those of tropical waste-fed aquaculture systems where year-round optimum growth is possible because of tropical temperature conditions.

4.6.5 Hungary

In Hungary, the first experimental sewage-fed fish culture trial was carried out in the city of Fonyod on a total water surface area of 211 ha. The technological package developed in Hungary was the culmination of a 5-year research project plus 5 years of monitoring a commercial operation system. The sewage-fed fish pond technology in Hungary can receive, process, utilise and purify domestic sewage and produce 12–20 kg/ha/day. It applies basic principles of complete grazing pressure on both planktonic and benthic communities by implementing polyculture methods using silver carp and common carp, which are able to utilise the planktonic and benthic fish food resources completely (Olah, 1990) (Fig. 2.10).

The results from the Hungarian farm indicate that nutrient levels in the sewage are substantially reduced, e.g. ammonia from 48–50 mg/L to 0.3–0.5 mg/L, total N from 50–55 mg/L to 2–3 mg/L and total P from 10–12 mg/L to 0.70–1 mg/L. At the same time, the DO level has been increased to 8.3 mg/L.

4.7 INTEGRATED RESOURCE MANAGEMENT

4.7.1 Integrated farming

Modern integrated farming is an approach to farming that incorporates the concept of integrated resource management (IRM). This approach integrates the management of additional enterprises, particularly aquaculture, with those of the existing farming system and with their natural resource systems, so that opportunities for rehabilitation and synergism can be exploited (section 2.3.6). IRM systems with aquaculture as a major or minor component differ from traditional farming systems. The basic concept is to use aquaculture as a tool for recycling wastewater and nutrients without creating adverse environmental impact. In other words, IRM can complement and improve the overall recycling and efficient use of many types of farm and other wastewater in a sustainable way (Fig. 4.11).

In the system shown in Fig. 4.11, animal wastes are collected in a reception pit and then fed semi-continuously into an aerobic reactor, which is aerated through a membrane diffuser. Microbial populations in the reactor degrade organic material to CO_2 and microbial biomass. The aerobic process significantly reduces the organic content (BOD and COD) of the slurry, and transforms N and P. The slurry is separated into solid and liquid phases, with the solid phase subsequently composted. The liquid phase is diluted and passed into shallow microalgal ponds, which are mixed slowly using paddle-wheels. Prolific algal growth occurs, with nutrient removal via biotic and abiotic processes. Effluent from the ponds is fed into fishponds to produce zooplankton and aquatic plants on which the fish feed. The final effluent from fishponds is passed to constructed wetland, from which there may be recycling, and is also used for irrigation.

Overcoming constraints of enhancing food and economic security while minimising environmental impact is a prime concern of many developing nations in south-east Asia. Vietnamese rural families are required to make better use of the available land, particularly within the highland region. About 80% of the total land area of Vietnam is mountainous with depleted forest. The Vietnamese government is trying to address this problem through promoting an integrated farming model. The integrated farming system (section 2.3.6) practised in Vietnam has provided a significant boost to the income levels of farmers in lowland areas. Research is being carried out to maximise the production and sustainability of integrated farming in the mountain region.

4.7.2 Modern research focus and approach

The fundamental attitude to the way waste research is carried out has been drastically changed. Research focus has been shifted from mono-disciplinary to multi-disciplinary collaborative research to exploit 'waste' nutrients. This is where great opportunity lies – to view them instead as an immense

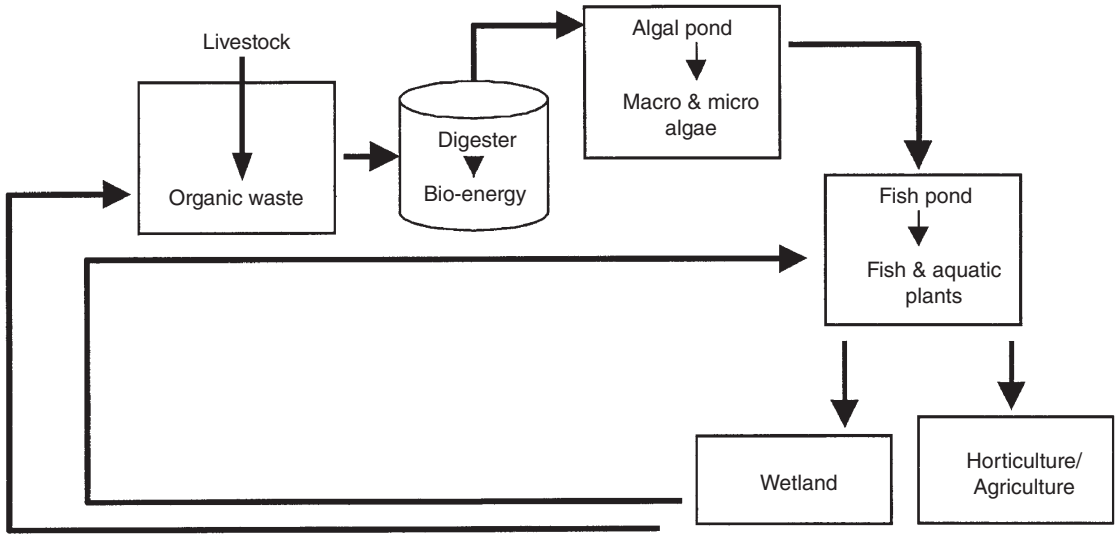


Fig. 4.11 Integrated wastewater treatment model at Urrbrae, South Australia.

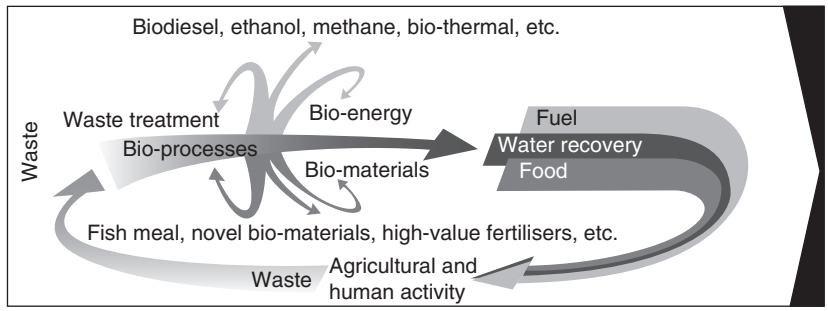


Fig. 4.12 Integrated biosystem research concept.

and unexploited resource which can, with ingenuity, persistence and smart technology, be reclaimed and new industries developed. The collaborative structure provides the ideal platform for undertaking such research.

Water resource and organic pollution are the two key issues, which will limit aquaculture industrial growth and sustainability. The overarching principle of the current IBS research is to add value to waste streams, solid or liquid, derived from either agriculture or urban environments. The added value may be from the production of energy and biofuels, biomaterials or fertilizers and recover clean water (Fig. 4.12). While the added-value product provides the economic incentive for the process, the initial waste feedstock must be stabilised. This requires that the risk to both the environment and human health be minimised. The risk may be due to the presence of toxic compounds or pathogenic micro-organisms initially present within the waste stream, which are unaffected by the process, or similar contaminants produced during the added-value processing. In either case ecological and environmental risk will need to be assessed, managed to acceptable levels and communicated to the various stakeholders. Integrated risk management is focused to address the legitimate concerns and maintain high safety standards.

4.8 CONCLUSIONS

Aquaculture has a poor public image in some regions. However, some of the negative impacts of aquaculture cannot be regarded as serious in comparison with other industries, including many forms of agriculture.

Nevertheless, impacts such as nutrient discharges and coastal degradation are very serious. For the sake of the future expansion of the aquaculture industry, it is important to minimise environmental impacts and minimise negative public perception of the industry. Attention also needs to be paid to those impacts that the public (i.e. consumers) consider to be important, even if scientific evidence suggests otherwise. It is important to note that by incorporating aquaculture into waste treatment, the incentive to reclaim nutrients from wastewater, release clean effluent and simultaneously produce fish has proved successful in many parts of the world.

Serious impacts are often an indication of bad management, wrong site location or poor planning, which will also be detrimental to the culture economics. In such an ecologically based industry, minimal impacts go hand in hand with good business. Aquaculturists have to work with and within the environment to succeed.

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5
Desert Aquaculture

Inland: Sagiv Kolkovski, Yitzhak Simon and Gideon Hulata
Coastal: Sagiv Kolkovski and Nasser Ayaril

5.1 INTRODUCTION

The term 'desert aquaculture' refers to aquaculture production of fish, shellfish and aquatic plants in arid areas or in areas where water supply is restricted. It sounds paradoxical, given the obvious lack of suitable surface waters for such a purpose in these areas. However, during the past two decades, the commercial 'desert aquaculture' sector has been thriving in several countries around the world, utilising various sources of water including fresh and brackish groundwater resources and pumped seawater.

Desert aquaculture offers many advantages in areas that may be otherwise unproductive, and where:

- large quantities of brackish groundwater that can only partially be used for agriculture are available;
- there is clean and unpolluted water (seawater or from other sources) due to few or no existing industries;
- there is a warm ambient climate;
- geothermal bores can maintain high temperatures in the winter through greenhouse use;
- the dry climate allows water cooling in summer;
- there is inexpensive land;
- geographic isolation provides a natural quarantine;
- there are minimal ecological risks;
- year-round production can be achieved.

These advantages have attracted large investment over recent years into production of a diversity of aquatic organisms, including:

- fish species such as barramundi, common carp, mullet, striped bass, tilapia (*Oreochromis* species and hybrids) and ornamental fish;
- crustacean species such as redclaw crayfish, brackish and marine shrimp, e.g. *Litopenaeus vannamei, Fenneropenaeus indicus* and *Penaeus monodon*, and brine shrimp (*Artemia* sp.);
- microalgae such as *Dunaliella salina.*

Different approaches have been developed to utilise different water sources in similar arid areas including:

- use of groundwater;
- rainwater reservoirs functioning as irrigation reservoirs and 'mega' fishponds;
- intensive recirculating systems;
- large-scale agri-aqua water usage;
- large-scale land-based pond systems.

Although over the years and in various countries around the world there have been many attempts to develop systems and solutions for desert aquaculture, only a few have been found to be commercially viable. This chapter looks at some of these solutions in Israel and Saudi Arabia, but the experiences in these places are applicable to other countries with similar water and weather conditions in at least some parts of their geography. These countries or regions include the interiors of Central and North Africa (with projects currently being developed in Algeria,

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Morocco and Libya), eastern Asia, North America and Australia, and most of the countries in the Middle East. The developments are also applicable to countries with limited or no coastline, and to heavily populated countries with limited land areas available for aquaculture. Although the chapter discusses 'Desert Aquaculture', the systems and methods described in it may be applied to any area where aquaculture depends on limited water resources from terrestrial sources.

5.2 THE ISRAELI EXPERIENCE

Israel is located in a semi-arid zone, with distinct winter (wet) and summer (dry) seasons, and a low annual rainfall of around 500 mm/year. The only large inland water body is the Lake of Galilee, which mainly supplies freshwater for human consumption. Moreover, in the central-north areas of Israel, where the majority of the rainfall occurs, the hilly and mountainous land cannot naturally hold water. In spite of the obvious climatic constraints and overall shortage of water, both agriculture and aquaculture are highly developed in Israel (Mires, 2000).

To deal with these impediments, different solutions and methods to maximise water use have been developed. These solutions include:

1. Reservoirs to store rainwater during the wet season. Israeli agriculture is now largely intensive and depends on irrigation from these reservoirs during the dry summer. Recently, it has become common to use irrigation reservoirs for fish culture in integrated farming systems (section 2.3.6). These integrated agriculture-aquaculture systems use the water twice:
   (a) within an aquaculture production system;
   (b) subsequently to supply irrigated agriculture systems.

   This system, now a few decades old, was a significant step in the intensification of inland fish culture in Israel (Hepher, 1985; Sarig, 1988).
2. Large-scale recirculating systems, in which water from outdoor fish ponds, raceways and tanks is passed into sediment ponds to remove the solids. The water is then passed to an adjacent water reservoir, and good-quality water is then returned from the reservoir to the fish rearing systems.
3. Highly intensive recirculating systems that incorporate water filtration systems, such as drum filters, biological filters, protein skimmers and oxygen injection systems. Highly intense systems may support up to 50 kg of fish per m^3 of water. Culture is intensive, as the stock is entirely dependent on a comprehensive artificial diet and there is acute management of water parameters (section 2.4.4). These systems are usually compact, take up a relatively small area and are extremely efficient with water usage.
4. Greenhouse technology was adopted from desert agriculture and includes environmental control, i.e. humidity, temperature, light and radiation. These conditions are important in arid areas, which have large temperature changes between day and night and summer and winter.

5.3 REGIONAL VARIATION IN ISRAEL

Israel can be divided into two climatic regions:

1. The southern arid/semi-arid areas have very low annual precipitation (<100 mm) and consist of the Negev Desert and the Arava Valley. These areas are part of the Surian-African Break. The Arava Valley sits between two mountain systems: the Jordan from the east and the Negev from the west. It starts at the southern end of the Dead Sea, some 400 m below sea level and finishes at the Gulf of Eilat (an extension of the Red Sea). The Negev Desert lies west of the Arava Valley and occupies most of the southern part of Israel.
2. The central-north of the country has a relatively high annual rainfall (>600 mm). This region is the most populated area of Israel and the competition for resources is intense. These areas include the flats near the Mediterranean Sea, the hilly area towards the east that borders the Lake of Galilee, and the Galilee and Golan mountains in the north.

The two areas differ in their sources of water, its use, and the types of integrated aquaculture systems employed. In the southern, more arid region, water is pumped year-round from geothermal bores. It is used for highly intensive aquaculture in a very highly integrated system of water management from the bore to the end user. In the central-north region, irrigation reservoirs storing winter rainfall also operate as large fish ponds with semi-intensive culture (Mires, 2000). As noted above, while this central-north region has moderate rainfall compared to the southern region, it potentially suffers from water shortages throughout much of the year due to its topography.

5.4 AQUACULTURE IN GEOTHERMAL WATER

Arid aquaculture in southern Israel began in 1979 with the discovery of locally available geothermal water (at 60 °C)

near Moshav Faran, a settlement in the Arava Valley (20°22′N, 35°09′E). The idea of using hot groundwater for highly intensive aquaculture, to achieve maximum growth throughout the year, has subsequently been developed commercially. Combined greenhouse heating of microalgae cultures (*Spirulina* and *Dunalliela* species) and fish ponds has also been successfully trialled, but did not prove economically viable.

For both ecological and economic reasons (the cost of 1 m^3 or 1000 L of brackishwater in Israel is about US$0.05), the design of integrated aquaculture projects in arid regions with agriculture areas as end users is critical. Unlike the central-north areas of Israel, integrated aquaculture in the southern, more arid, areas is based on highly intensive systems with very tight water budgets. Water loss is minimal and is predominantly due to evaporation. However, even when there is no need for heating during the summer, most of the fish farms have water exchange of at least 10% per day to maintain water quality. A small fish farm of 2000 m^3 will therefore use about 200 m^3 of water per day, which in turn will irrigate about 4 ha of crops in the desert summer. In winter, when a large amount of water is needed to supply the heat energy to the fish ponds in the aquaculture system, there is a need to find a solution for all the output water or effluent (Fig. 5.1).

There are two options for transferring heat energy to the fish ponds in these production systems:

1. A closed system using heat exchangers. When using a closed system, the geothermal water is used for heating the fish pond via a heat exchanger.

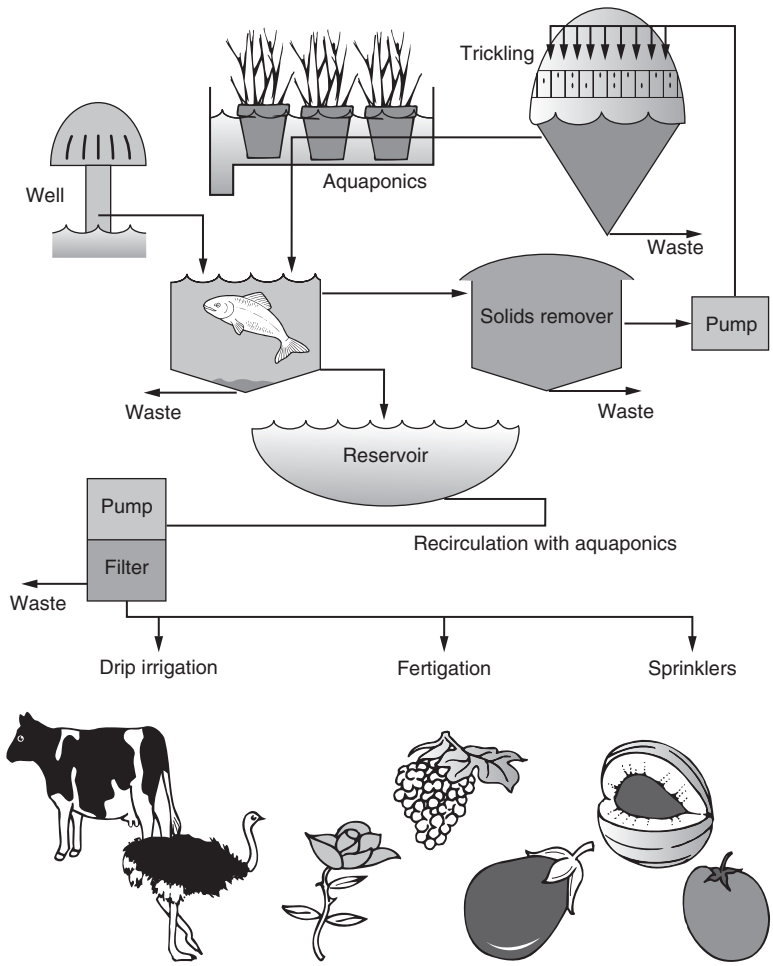


Fig. 5.1 Schematic description of a form of desert aquaculture (see text for description of processes).

2. Direct supply of water to the fish pond. When a direct supply of geothermal water to the fish pond is used for heating, the water is also used to flush organic matter from the pond and contributes overall to the water quality of the pond. Accordingly, the outlet water is loaded with suspended solids, micro-organisms, algae and plankton due to the high nutrient loading in the intensive rearing system (Zoran *et al.*, 1994; Milstein *et al.*, 1995).

When the end use of the effluent is drip irrigation, the water needs to be filtered or otherwise treated prior to being distributed under pressure through the dripping system. Usually, a small reservoir (0.1–1 ha surface area) is attached to the fish farm for this purpose. This reservoir, together with water treatment facilities, is used to provide a buffer between the agriculture project (e.g. greenhouse or open field) and the aquaculture system. Fish are also reared in this reservoir, but at relatively low biomass per unit volume or area. The water treatment facilities typically include high-pressure pumps, a chlorine injection system (or other form of disinfection) and an automatic filtration system. Secondary filtration is undertaken at each irrigation head to ensure good water quality for final reticulation and to prevent drippers from clogging with particulate waste matter.

The bore water salinity is crucial for any agricultural crop, with 0–5‰ salinity being an acceptable concentration in most cases in Israel. However, most of the geothermal water available in Israel is considered too saline (8–12‰), especially if increased salinity occurs in fish ponds due to evaporation. Rearing sensitive crops is not feasible at these higher salinities, although other crops, e.g. watermelons, alfalfa and tomatoes, are highly successful. 'Desert sweet tomatoes', a brand name for a very sweet variety of tomato that was developed in Israel and is produced in saline groundwater, has been extremely successful in both local and European markets. Salinities up to 8‰ can be used to produce a variety of crops, such as date palms, olives, certain citrus varieties and varieties of green vegetables.

Of five model pilot-scale farms established during the 1980s and 1990s, two have been expanded to full commercial scale of 200–400 t/year aquaculture production. These farms were built from modular units of $8 \times 300\,m^3$ capacity ponds inside a greenhouse (Fig. 5.2). The ponds are connected to a water treatment unit that includes a settlement pond (100–200 m^3 capacity) together with an 'activated suspension' method (bio floc technology, BFT) for nitrification (conversion of nitrogen as ammonium and organic residues) into protein by bacteria (Avnimelech *et al.*, 1989, 1994; Avnimelech, 1998).



Fig. 5.2 Interior of greenhouse containing large tanks for intensive fish culture in Israeli arid Negev.

Several different types of water use systems have been developed for these fish ponds:

1. Stand-alone pond systems that include the ponds, settlement pond and water treatment system. These systems function primarily as aquaculture systems with only small daily amounts of water discharge.
2. Pond systems that are connected to an irrigation reservoir that enables water exchange (Fig. 5.3). A water treatment system may be included for treating the pond effluent before it returns to the reservoir. Some of the irrigation reservoirs are also stocked with fish that can maintain the water quality, i.e. by controlling the algae and other aquatic organisms. This method (Laventer, 1987) involves a range of fish species that are usually stocked in these reservoirs to control the aquatic environment. These are usually a variety of carp species that feed at different levels of the food chain. For example, silver carp filter unicellular algae, bighead carp feed on zooplankton, grass carp eliminate vegetation and black carp control aquatic mollusc populations (Fig. 2.10). The fish are not considered an environmental threat (due to wild spawning) since they can be stocked as sterile triploids. In Israel there are centres and hatcheries that specialise in producing these species (for example, Gan Shmuel and Hama'apil fish breeding centres).

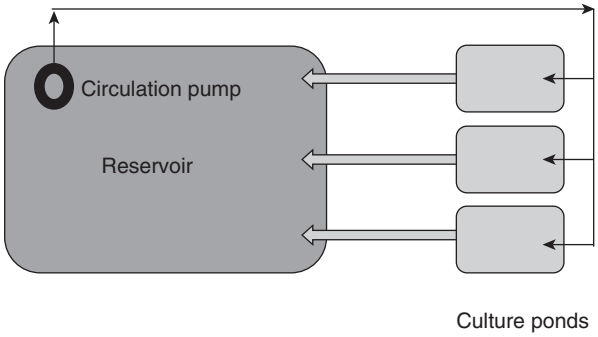


Fig. 5.3 Scheme of an open-pond intensive fish farm with a reservoir serving as a biofilter. Reproduced from Mires (2000) with permission of John Wiley & Sons.

Intensive culture units also accompany dual-purpose irrigation/fish culture reservoirs, which serve as a biological filter and water source. One example is the facility of Kibbutz Neve Etan fish farm (32°30′N, 35°32′E) that includes $8 \times 200\,m^3$ circular concrete ponds (Fig. 5.4). Water is pumped from the fish ponds to a settlement pond for solids removal and then pumped to the main irrigation reservoir. In most cases



Fig. 5.4 Open-pond intensive fish farm in the Bet Shean Valley, which is adjacent to a reservoir serving as a biofilter. (The reservoir is to the left, outside the field of view.)

the reservoir is very large with a capacity of several million m^3 of water. The effluent water from the fish farm is 'diluted' in the reservoir, which acts as a biological filter, so the water that is pumped back into the pond systems is relatively clean. Since these reservoirs are used for a dual propose, i.e. agriculture and aquaculture, synchronisation between fish and crop production needs to be managed so that at the end of the summer, when the reservoir is nearly empty or very low, the fish will be harvested. See section 5.5 for more on the operation of dual-purpose reservoirs.

In the earlier-built reservoirs, i.e. those not specifically designed for fish culture, with depth of over 5 m, stratification of water may occur during the rearing season and can cause mortalities. This happens when the summer-time temperature of the upper water layer reaches or exceeds 30 °C while the temperature of the water 2–3 m below the surface is only 20–22 °C (section 3.2.8) Organic materials and nitrogenous compounds accumulating in the bottom layer lead to anoxic conditions near the bottom. Afternoon winds, which are very common in Israel's northern valleys during summer, may stir up water layers in a stratified reservoir, whereby the noxious bottom layer is brought to the surface and severely affects the welfare and survival of fish due to collapse of algal populations, reduction in DO levels and hydrogen sulphide poisoning. Different methods were developed to break up or prevent the stratification, mainly using aeration (Milstein *et al.*, 2000, 2001; Milstein and Zoran, 2001; Zoran and Milstein, 1998).

3. Overwintering tilapia ponds are employing biofloc technology with minimal exchange of water (up to 5% per day) during daily sludge removal. The carrying capacity of these ponds ranges between 10 and 20 kg/m^3. Water quality can be controlled by adding starch as an energy source for the nitrifying bacteria. To be efficient, starch application should start as soon as nitrite levels begin to rise.

5.5 WATER-LIMITED AQUACULTURE

With water limitation throughout much of the year and lack of natural water containments, the development of aquaculture in the central-northern region of Israel also required specific systems to be developed where dual-purpose reservoirs are used for irrigation and fish culture.

5.5.1 The annual cycle

Fish are reared in the reservoirs over the irrigation period of spring to autumn (April to November). Fish harvesting is carried out in autumn, when the reservoirs are nearly empty and awaiting to be refilled over winter. Reservoirs begin to fill during late autumn with a combination of ground (spring) water and early winter rains. When the water column reaches a height of 1 m (normally around October) the reservoir can be stocked with carp and mullet fingerlings that can survive the low winter temperature. These fish reach market size within a year. The reservoir continues to fill until January or early spring, depending on its geographical location and holding capacity. Common reservoirs are at least 8 m deep, and their water capacity ranges from 500 000 to over 1 000 000 m^3. In the early spring, when water temperature reaches 22–23 °C, overwintered tilapia fingerlings are added to the reservoir. In late spring or early summer (May to June), air temperature in the valleys in northern Israel climbs to over 30 °C, making irrigation of field crops a necessity. The stored reservoir waters are pumped out for crop irrigation and the water level in the reservoir drops gradually while the fish continue to grow. By September/October, when only 1–2 m depth of water remains, the fish farmers progressively harvest the fish and prepare for final draining of the reservoir and harvesting of the remaining fish.

In the 1960s, the first reservoirs were constructed solely for irrigation in the Harod Valley (32 °33′N, 35 °22′E) to serve the needs of the kibbutz settlements in that region. Five reservoirs were constructed, with a total surface area of 90 ha. These rather shallow reservoirs collected and stored brackish ground (spring) water, flowing year round, for use during the dry summer. The reservoirs were subsequently deepened to increase their storage capacity for winter rains. The farmers of these communal settlements who used to rear their fish in shallow earthen ponds, then decided to also use the reservoirs for fish culture. In a few years it became apparent that rearing fish in such reservoirs was logical and profitable, although professional and technological expertise were lacking.

This secondary use of water for fish culture, by introducing aquaculture into irrigation reservoirs, improved the efficiency of water usage and reduced the cost of water needed for fish culture in conventional earthen ponds. However, the main drawback was harvesting the fish from these reservoirs, since the engineers planning their construction did not consider such activities in their initial design. This led to dramatic technological developments during the late 1970s, when many new reservoirs were constructed and were specifically planned for dual-purpose use, i.e. they were equipped for efficient harvesting of the fish (Fig. 5.5). This development, in turn, changed the emphasis on water usage in this region. In the newly

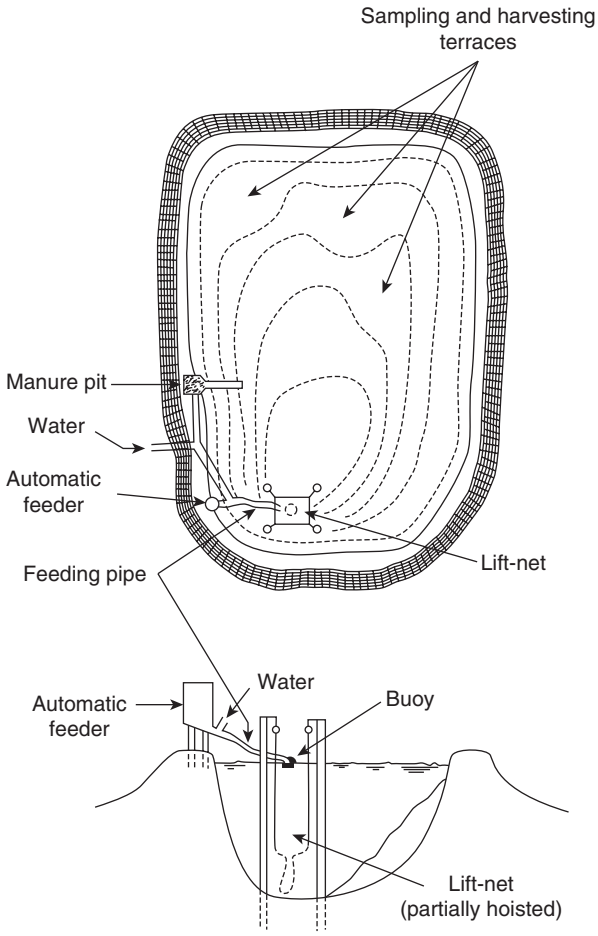


Fig. 5.5 Fish culture reservoir and installations. Schematic view from Hepher (1985), by courtesy of Springer.

constructed reservoirs, fish culture became the primary activity and crop irrigation a 'by-product'. In 2008, almost all fish farms in the central-northern region of Israel operated such reservoirs (Fig. 5.6), which have proved to be an efficient and profitable tool for fish culture.

In Israel, both in conventional earthen fish ponds and in reservoirs, freshwater fish are typically cultured in a polyculture system, stocked with different species of fish (Hepher, 1985). Most reservoirs are stocked with:

- 80% common carp and tilapia (at various % combinations);
- 20% accompanying species, such as grey mullet, grass carp, red drum and the silver carp × bighead carp hybrids.

Only about 30% of reservoirs are used for monoculture of either carp or tilapia. The main advantages of the monoculture system relate to easier harvesting operations and storage of recovered fish.

Juvenile fish to be stocked into reservoirs are initially reared to an appropriate size in conventional fish ponds that are shallow and earthen. None of the target species can grow from a 1 g juvenile (at first stocking) to market size from spring to autumn (ca. 8 months), which is the usual operational period of a reservoir. Thus, the farm must have additional rearing ponds in order to efficiently operate a reservoir for fish culture. These ponds will also be used at harvest time to hold fish until they are marketed (all through the year), since a farm cannot market the whole harvest of a reservoir at once. Thus, the ponds are operated all year round: rearing juvenile fish in the spring and summer, and holding fish for market or for stocking the reservoirs (in the following spring) during the winter. The stored fish are fed maintenance rations during winter and this increases operating costs.

Rearing tilapia in reservoirs, either in monoculture or polyculture, poses a real challenge – biological as well as economical. Being tropical fish in origin, winter water temperatures in Israel preclude their stocking during winter (January to April, when temperatures are below 15 °C). Thus, the annual production cycle is geared to stocking tilapia, previously nursed to weight of at least 50 g and overwintered, in the spring so that they can reach market size before temperatures drop down in the fall. These tilapia are already sexually mature when stocked into the reservoir, and capable of reproducing if both males and females are stocked. This, in turn, would lead to a population explosion, competition for resources, resulting in large amounts of unwanted fish filling the reservoir. Stocking of all-male or nearly all-male (>95% males) seed will practically eliminate the problem (sections 7.7.3 and 16.5.1). Adding predators, such as *Dicentrarchus labrax* or red drum (*Sciaenops ocellatus*), can further reduce the numbers of tilapia fingerlings surviving in the reservoir.

Fish production in the reservoirs has intensified during the last decade, and average production rose from 0.5 kg/m^3 to 1.5–2.0 kg/m^3. This increase was the result of intensification (increasing stocking density and feeding rates) that was enabled by increasing aeration inputs (from one to 4–5 aeration devices per 0.1 ha; Fig. 5.7) and improving feeding quality (nutritional content as well as physical integrity of floating pellets produced by extrusion). This was also accompanied by improving harvesting technology and infrastructure on fish farms.



Fig. 5.6 Aerial view of the Einan reservoir, located in the Hula Valley, northern Israel, having a surface area of 45 ha and a mean depth of 15 m when full. (Photograph by courtesy of David Jansen.)



Fig. 5.7 A battery of aeration devices in a fish culture reservoir in Maoz Haim, Bet Shean Valley, Israel. (Photograph by courtesy of David Jansen.)

5.5.2 Limitations and constraints

The developments described above and the economic superiority of reservoir-based fish culture over other systems (section 5.5.3), are not without problems and limitations. Growing fish in large, deep reservoirs is a major challenge and a high-risk operation. It involves a day-to-day effort to cope with unforeseen biological and technological difficulties, where the ability of the farm manager to respond to unexpected events is limited due to the large volume of water and biomass of fish at hand.

The price of water for agricultural use is continually increasing. Fish farmers are forever seeking ways to make more efficient use of water, in order to lower the production cost of fish. Dual-purpose reservoirs, recirculation of water on farms and water re-use for irrigation constitute a remarkable effort toward achieving this goal. However, the high evaporation rate during the hot summers and the recent dry winters have led to increased salination of impounded water in some northern regions in Israel, to the extent that the water can hardly be used for irrigation of traditional crops or even for growing common carp. Thus, some previously dual-purpose reservoirs are currently used solely for culture of more salt-tolerant fish species (e.g. tilapia and marine species), affecting their economy. Another important issue is the environmental protection regulations that are becoming increasingly strict. Close cooperation is required between farm operators and environmental protection officers to coordinate the release of large amounts of water when draining reservoirs in a way that will benefit, rather than harm, the natural environment in the region.

5.5.3 Economics of reservoirs vs. conventional fish ponds

Detailed examples of production figures for dual-purpose reservoirs are presented in Hepher (1985) and Sarig (1988). Production inputs and associated capital depreciation costs per unit weight of fish output in such reservoirs compare favourably with those in conventional earthen fish ponds (Table 5.1).

To compare water use in particular, production of 1 kg of tilapia requires:

- 7.5 m^3 of water in conventional earthen ponds with semi-intensive culture (medium biomass);
- 4.5 m^3 in concrete ponds with intensive culture;
- 4.0 m^3 when cultured extensively in reservoirs;
- 1.5 m^3 in an industrial culture system that is indoors and highly intensive.

Production costs of the major species are rather similar for the two culture systems (stand-alone and dual-purpose reservoir); with those in dual-purpose reservoirs being slightly lower (Table 5.2). Most of this difference is due to the different feed conversion ratios (FCR) obtained in the two culture systems and the cost of water. Current farm-gate prices (to the farmer) are (1 ILS is approximately US$0.29):

- 10–12 ILS/kg for carp;
- 11–13 ILS/kg for tilapia;
- 6 ILS/kg for silver carp (and its hybrid);
- 12–17 ILS/kg for mullet and red drum.

A detailed breakdown of production costs of fish in dual-purpose reservoirs is shown in Table 5.3.

Table 5.2 Comparative costs of rearing carp and tilapia to commercial size in reservoirs and conventional earthen ponds.

Reservoirs	Conventional earthen ponds
8 ILS/kg of carp	9 ILS/kg of carp
10 ILS/kg of tilapia	11 ILS/kg of tilapia

1 ILS ≈ US$0.29.

Table 5.1 Production inputs and associated capital depreciation costs for producing 1000 kg of fish in ponds and reservoirs in Israel.

Item	Dual-purpose reservoirs	Earthen ponds	Comments
Water	–	50 000 m^3/ha	Reservoir water price is charged to irrigated field crop
Feed	1300 kg	2200 kg	Ponds are used for holding fish during winter, so more feed is required overall
Labour	5 days	6 days	
Seed	4000/ha	5000/ha	
Energy	5000 kW	6000 kW	
Depreciation	2000 ILS	2500 ILS	

1 ILS ≈ US$0.29.

Table 5.3 Itemised costs of producing 1 kg of fish in dual-purpose reservoirs. Price and cost figures in ILS/kg.

Item	Unit	Quantity	Unit price	Cost
Water	m^3	3	0.15	0.45
Feed	kg	1.3	2.2	2.90
Fingerlings (50 g)	number	4	0. 5	2.0
Energy (pumping, aeration)	kWh	5	0.4	2.0
Maintenance, machinery		1	0.3	0.30
Marketing		1	1.0	1.00
Labour, management	days/t	0.003	400.0	1.20
Depreciation		1	1.44	1.44
TOTAL				12.30

1 ILS ≈ US$0.29.

5.6 INDOOR AQUACULTURE FACILITIES

As in many places around the world, indoor aquaculture facilities have developed rapidly during the past decade to accommodate the need for an appropriate environment for recirculating aquaculture systems (RAS). Indoor RAS unitsare also gaining popularity in non-desert, water-limited regions and a few RAS units have been established as a private initiative on kibbutz fish farms (e.g. Sde Eliyahu in the Bet Shean Valley, and Ein Hamifratz in the northern coastal plain).

Understanding that water limitation for fish culture will become even more acute, the Department of Fisheries of the Israeli Ministry of Agriculture has given its support to the design, construction and evaluation of five commercial pilot RAS units, in order to test alternatives for production of fish with minimal water usage. Each pilot project is different (in terms of design and water treatment system) to enable comparison and identification of the optimal design.

Each indoor facility consists of the following components:

- climate-controlled building;
- hatchery, nursery and production tanks;
- aeration and liquid oxygen systems;
- water treatment unit (the heart of the RAS), including solid removal, biological filter;
- gas removal and water distribution system;
- feeding system;
- control and alarm systems;
- harvesting, sorting and dispatch of produce to the market.

The fish reared in the various pilot systems are tilapia, hybrid striped bass and gilthead seabream (the latter in saline water). A computerised simulation model has been developed and implemented for design and management of such RAS (Halachmi, 2006, 2007; Halachmi *et al.*, 2005). While the evaluation is still in progress, preliminary results suggest a remarkable saving in water used for fish production. However, the high construction and operation costs can only be covered by selling fish at high prices. With the current market prices of fish in Israel, the economic viability of all the tested systems is questionable. Calculations show that profitability can be reached when the price of the fish at the farmgate is at least 14 ILS, and annual production from a 0.2 ha facility exceeds 500 t.

Greenhouses are also used for overwintering tilapia in temperate climate zones. After harvesting from earthen ponds or reservoirs during autumn, tilapia need to be overwintered until marketing during the winter or restocking for grow-out the next spring. Greenhouses are used to ensure that water temperatures will not drop below 16 °C for a 24-h period. Fish farms in Israel have invested in constructing such greenhouses, in which water exchange is up to 5%. Use of greenhouse-covered ponds improves the well-being and survival of tilapia during overwintering.

5.7 DESERT COASTAL AQUACULTURE TECHNOLOGY – THE SAUDI ARABIAN EXPERIENCE

5.7.1 Introduction

During the past decade, a new aquaculture industry has developed in an area that previously had very little, if any, aquaculture development (or any other industry, for that matter) (Al-Balla, 2007). The area, one of the harshest environments in the world with less than 25 mm annual



Fig. 5.8 A chain of mountains forms a backdrop to the operations of the National Prawn Company (NPC) at Al-Gallah valley, Saudi Arabia. (Photograph courtesy of NPC.)

rainfall, is located in Saudi Arabia on the east coast of the Red Sea (Fig. 5.8). Initially the concept and the technology were developed and adopted from Asian, South American and other countries by the National Prawn Company (NPC).

The high profitability of prawn farming around the world led to uncontrolled shrimp farming on a large scale, which resulted in deterioration of environment, spread of diseases and the industry's decline. Thailand and Ecuador are just two examples of the many countries that suffered from this issue. Unlike traditional tropical shrimp farming in ponds of brackishwater or low salinity, desert coastal aquaculture has the advantage of culturing shrimps in highly saline seawater, reducing the pathogens, while the intense sunlight available throughout the year makes it possible to oxidise the pond bottom after harvesting (Fig. 5.9).

Although isolated and with air temperatures reaching up to 50 °C, the Saudi Arabian desert and coastline provide stable conditions for this type of aquaculture.

Other advantages include:

- availability of large unutilised areas of suitable land;
- seawater of the highest quality (there is no industry or human activity for hundreds of kilometres around);
- a remote location, reducing the chances of disease transfer.

With a coastline of about 2300 km along the Red Sea and Arabian Gulf alone, there is vast potential for future development of desert coastal aquaculture.

NPC was developed as a vertically integrated company with self-supply of postlarvae (PLs) through a breeding programme, feed mills and processing plants for the final products. This large (currently, the largest in the world) development enables the company to control and integrate every step of the development, preventing mistakes and environmental damage caused by disorganised and scattered ponds with no environmental planning, as was the case with some marine shrimp farming in south-east Asia and Central America.

5.7.2 Marine shrimp species

Several shrimp species were tested for their suitability for the high salinity of the Red Sea (38–40‰), including *Penaeus semisulcatus*, *P. monodon* and *Fenneropenaeus indicus.* Early farming operations were with the giant tiger prawn, *P. monodon*. However, physiological experiments identified *F. indicus* as the most suitable species to



Fig. 5.9 Dry pond, National Prawn Company, Saudi Arabia. (Photograph courtesy of NPC.)



Fig. 5.10 Cultured and harvested *Fenneropenaeus indicus.* (Photograph courtesy of NPC.)

withstand the highly saline waters. As a result, this is the species currently grown in the region (Fig. 5.10).

A breeding programme was initiated to overcome the shortage of *F. indicus* broodstock in the Red Sea and today it is the central part of the operation, with the 25th generation of domesticated *P. indicus* and the 5th generation of intensively selected shrimp being available in the stocks of the National Prawn[1] Company (NPC). In 2008, NPC produced and stocked 663 million PLs. Average production of PLs from the hatchery is 600 million with an annual capacity of 1.2 billion.

5.7.3 Grow-out

5.7.3.1 Ponds

Experience gained during the pilot stage of the project led to commercial operations in 1996, with construction of 100 × 1 ha ponds near Al-Lith, about 200 km south of Jeddah, on the Red Sea coast (20 °17′N, 40 °02′E). These pilot ponds continue to be part of NPC operations and are used for commercial production of marine shrimp. The average annual production from these 100 ponds was 350, 701, 643 and 580 t in each of the years 2003 to 2006, respectively.

Based on this commercial stage, a large-scale operation has been developed in two phases:

[1] NPC uses the term 'prawn' for what is called 'marine shrimp' or 'shrimp' in this book. The terminology for these penaeid crustaceans varies according to country and species.



Fig. 5.11 Excavator building dyke between ponds. (Photograph courtesy of NPC.)

- Phase I had 11 farms totalling almost 2800 ha, with annual production of 11 500 t).
- Phase II has 15 farms totalling almost 3500 ha, which are expected to produce 17 500 t annually, and it is already under way with the first set of ponds being stocked by the end of 2009.

The ponds were constructed by excavation of the sandy soil and creating dykes (Fig. 5.11). Dykes are the sloping walls that separate ponds and provide a broad surface for the service vehicles to travel along. Seepage in sandy soil can be a severe problem for ponds. There are some areas of the NPC farms where this is a problem, in which case clay soil is trucked from other areas and mixed in a 80/20 ratio with sand to create a less permeable surface layer. In other areas there is clay below the top soil. In general, the pond substrates are compacted to reduce seepage (Figs 5.12a and 5.12b). Pond preparation involves ploughing and liming the pond substrate between crops of shrimp (Fig. 5.13), as is common to much pond aquaculture (section 21.4.4).

Stocking density is 15–18 shrimp per m^2 in 10 ha (100 000 m^2) ponds and 25 shrimp per m^2 in aerated 1 ha ponds. The shrimp are harvested when their average body weight reaches 22–25 g.

5.7.3.2 Intake pumping station

The intake pump station on the shore of the Red Sea for Phase I was designed to cater for a flow of 90 m^3/s at a

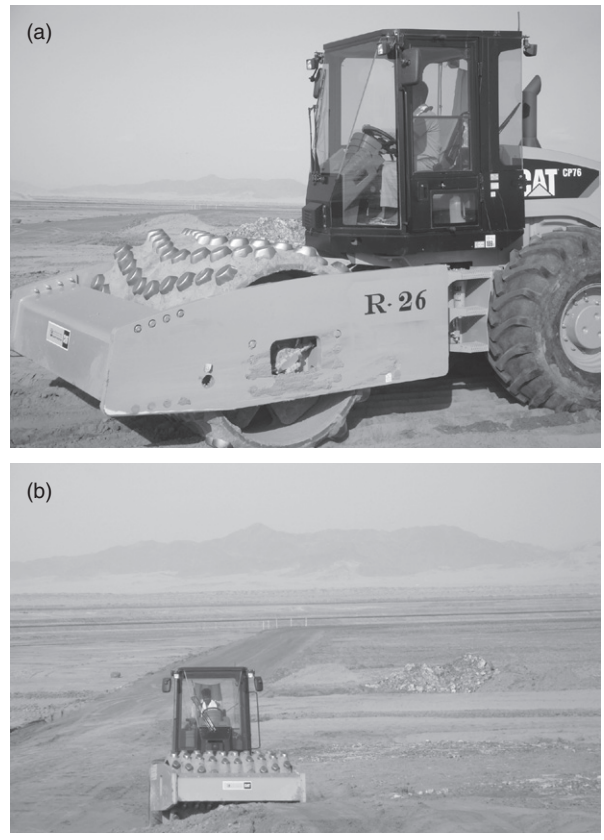


Fig. 5.12 (a) Compactor. (b) Compactor working on pond substrate. (Photographs courtesy of NPC.)



Fig. 5.13 Tractor with equipment to break up top soil. (Photograph courtesy of NPC.)



Fig. 5.14 Culvert at Farm 5 opening into the main drain canal (running parallel to the mountain range). (Photograph courtesy of NPC.)

static head of 2.5 m. The station is equipped with 12 submersible, vertical pumps. The intakes are provided with screens to avoid the inflow of contaminants and debris. The seawater flows through a 35 km intake canal to the grow-out ponds. Another pump house will be built for Phase II.

5.7.3.3 Drain pumping station

The outflow water pump station is designed for a flow at the rate of 90 m^3/s (similar to the intake) at a static head of 1.1 m (Fig. 5.14). The station is equipped with 17 submersible horizontal pumps (mixer type) facilitating low consumption of power. The discharge column of 1800 mm is installed with flap valves to protect against backflow of seawater. The effluents from the ponds are passed through a series of effluent treatment ponds, equivalent in surface area to 10–25% of the pond area of each farm, prior to discharge through a 38-km long wastewater canal.

This project has made a significant contribution to the development of this arid region, with almost 2000 employees from around the world, who are accommodated in the township area. Over 200 km of sealed and unsealed roads have been constructed on the project site. With a predicted annual production of 30 000 t and vertical integration of every aspect of production, the magnitude of the project is significant and can serve as an excellent example of coastal desert aquaculture that is very large, sustainable and environmentally friendly.

5.8 BRINE SHRIMP (*ARTEMIA* SP.) PRODUCTION IN WESTERN AUSTRALIA

The culturing of *Artemia* sp. in saline ponds as part of salt works or stand-alone ponds in coastal arid areas is not new. During the past three decades, small-scale harvesting of *Artemia* biomass and cysts from these types of pond has taken place in many areas around the world (Dhert *et al.*, 1992; Zmora and Shpigel, 2006). In most cases, production of *Artemia* involves shallow ponds with seawater being pumped or diverted to them to compensate for evaporation. The hypersaline (usually > 150‰) environment is ideal for *Artemia*, because no predatory species can live in this environment. In most cases, the ponds are fertilised to create algae blooms as a food source for the *Artemia* (Sorgeloos *et al.*, 1986; Teresita *et al.*, 2003). This method of *Artemia* culture usually results in low yields per pond area or volume. One of the problems is managing and balancing the algae production and *Artemia* growth, since *Artemia* can bloom quite rapidly, feeding on the algae, and then the culture may collapse due to a shortage of food. Supplementing *Artemia* ponds with other food sources,

such as soy, rice bran and other grains or agriculture products, has been trialled and on a small scale can provide alternative feed sources.

Artemia culture in a closed environment, such as tanks, has also been trialled (Dhont and Van Stappen, 2003; Zmora and Shpigel, 2006). Although these small-scale experiments (3000-L tanks) produced tens of kg of *Artemia* (Zmora and Shpigel, 2006), there has been no further development towards large-scale, commercial *Artemia* production in tanks anywhere in the world.

Recently, a new production unit was commissioned in Australia for large-scale production of *Artemia* biomass and future production of cysts. The *Artemia* culture system is an adjunct to the production of *Dunaliella salina* microalgae for the extraction of β-carotene (section 13.3.2) in a hypersaline pond system located at Hutt Lagoon, Port Gregory, Western Australia (Fig. 5.15). It is an elongate lake that sits in a dune swale adjacent to the coast. The lake is about 14 km in length, with its long axis oriented in a northwest direction, parallel with the coast. It is about 2 km wide and its area is about 2500 ha. Although not a completely arid area, the lagoon is hypersaline and the technology which has been developed could be implemented in many coastal areas around the world.

Initial investigations into growing *Artemia* in shallow ponds demonstrated a bio-security risk of windborne cysts being transferred to infect adjoining algae ponds. Therefore, a different approach was taken and tank production units were developed.

The *Artemia* grow-out system is based on 32 000-L polyethylene (LDPE) storage tanks (40 currently installed with plans to increase to 200 tanks). The tanks are grouped into production modules of ten, with access walkway/work platforms between paired modules (Fig. 5.16). Each tank is plumbed for flow-through water and aeration. Each module is connected to a central harvester that has the ability to filter and separate the *Artemia* to different sizes. The modules are flow-through systems with water being pumped from the hypersaline lagoon and mixed with normal seawater to reach the optimal salinity. Both water sources are pre-filtered to prevent any debris entering the culture.

The *Artemia* are fed on both live *Dunaliella salina* sourced directly from the production ponds and supplementary levels of concentrated algae.

Innovative filtration systems have been developed to cope with the massive exchange of water through the tanks. These systems remove the sticky *Artemia* faecal pellets and other contaminants such as moults, without harming the live *Artemia*.

Special attention was given to harvesting methods and bio-security, in order to prevent any *Artemia* or cysts returning to the algae production ponds where they could cause catastrophic damage to production of algae.

The scheme's commercial viability is based on the link between the two production systems, algae and *Artemia*, and the shared facilities, power, etc. Moreover, the algae biomass left after β-carotene extraction, usually considered a waste product, is an ideal food source for the *Artemia*.

5.9 SPECIES FOR WATER-LIMITED AQUACULTURE

It is not a simple matter to give a list of species that are suitable for use in aquaculture systems in conditions of water shortage. This is for several reasons:

1. It is difficult to characterise these culture systems. As described in this chapter, there are many different systems from low-biomass reservoirs to high-biomass intensive systems at almost any possible salinity. They all work on the principle of 'smart usage' of the water available.
2. A decision on the 'best' cultured fish or shellfish species should take into account many local and non-biological factors, such as market value and volume, cost of all the operation segments (e.g. labour, land and electricity), distance to market, timing and mode of marketing (live, fresh chilled or frozen).

In addition to the general considerations for choosing the best species in any aquaculture project (section 2.8), specific biological and other attributes are required for a species to be suitable for water-limited aquaculture. In general, aquaculture species reared in water-limited aquaculture systems need to be more stress-tolerant than species reared in flow-through systems or seacages. The former are more likely to have broader tolerances of adverse water quality variables associated with crowding: low DO, low pH, high N compounds and high suspended solids. Depending on the environment or ability to economically control these variables, the species may be required to tolerate substantial temperature and salinity variations.

Various fish species are currently reared around the world in water-limited systems, including recirculating systems, pond systems and others. Probably the most common fish species for water-limited (fresh- or brackishwater) systems are tilapia. They may be reared intensively or semi-intensively in polyculture systems with other fish

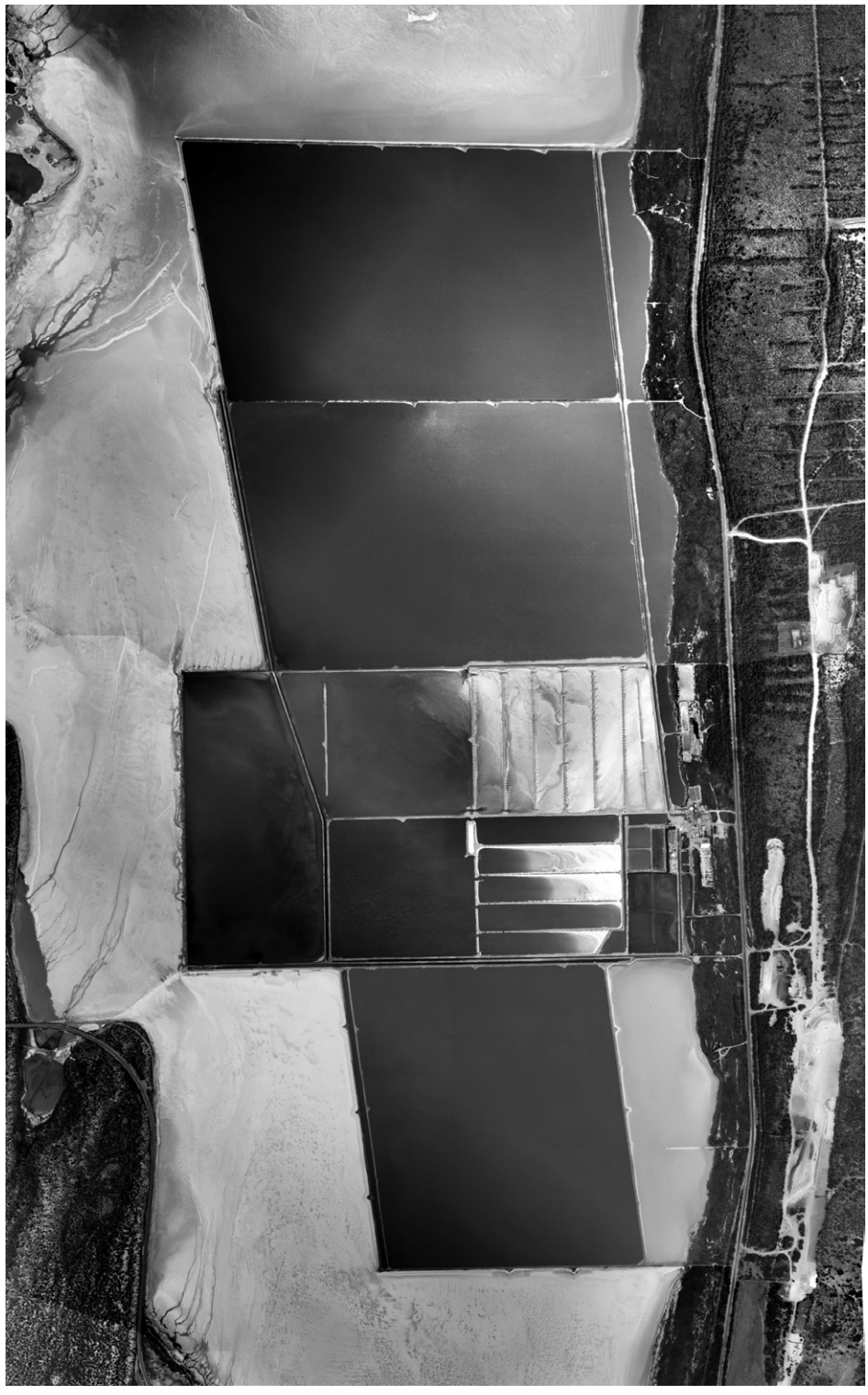


Fig. 5.15 Aerial photo of *Dunaliella salina* ponds, Hutt Lagoon, Port Gregory, Western Australia. The ocean is on the right of the photograph. (Photograph courtesy of Cognis Australia Pty Ltd.)



Fig. 5.16 *Artemia* production system, Port Gregory, Western Australia. The rectangular ponds in the background are culturing *Dunaliella salina*. The *Artemia* culturing system is a series of covered units in the foreground of the ponds.

species, such as carp and mullet. In many countries, tilapia are considered to be the 'best' candidate for water-limited systems. They are relatively stress-tolerant and can be reared in a range of salinities, from freshwater to almost seawater salinity (sections 16.3.1 and 16.8.8). Tilapias can be reared in very high densities of up to 50 kg/m^3. The rearing period is relatively short (6 months to market size), and in many countries there are very good market values and demand. Other fish species suitable for water-limited aquaculture systems include barramundi, hybrid striped bass, carp and mullet.

Arid coastal aquaculture has seen considerable development and investment in the past few years. Although water availability is not a problem in this situation, hypersaline conditions are likely to be problematic. Minimal relative humidity and often high temperatures lead to high evaporation from the expanses of seawater and create hypersalinity. Thus, species used for arid coastal aquaculture must tolerate and, even more than tolerate, thrive in high salinities. *F. indicus* in Saudi Arabia is an example of such a species.

This form of aquaculture offers real opportunities to utilise arid land for which there is no other use. Marine shrimp, fish and algae (such as *Dunalliela salina* grown for its β-carotene content at high salinity) are currently being produced in several arid coastal regions around the world. Usually, these projects need high capital investment due to their location and scale of production.

5.10 CONCLUSIONS AND FUTURE DIRECTIONS

During the past few years, restrictions on the use of water for agriculture and aquaculture have been aggravated by prevailing drought conditions in Israel, Australia and other agricultural countries. This has focused attention on the development of 'water smart' farming and aquaculture systems. Therefore, more productive use of saline groundwater in desert areas is increasingly important. Very high-density and high-yield systems that can maximise the use of the water are being developed by:

- recirculation through water treatment systems;
- recirculation through large reservoirs that can also be used for irrigation.

Various integrated agri-aquaculture systems are also being tested. Accordingly, Israel is currently investing in the development of salt-tolerant crops that are suitable as the end water users after first use by aquaculture. Integrated

aquaculture systems with these crops, including water-efficient pond/reservoir and recirculating/greenhouse systems, are being further developed and improved. Other priorities for the aquaculture industry in Israel are:

- evaluation of new aquaculture species that can suit these systems and which have local and export market potential;
- importation of exotic plants from Africa for evaluation;
- genetic selection of current species for higher salinity tolerance.

Many countries face the problem of increasing salination where soil becomes increasingly saline due to salty groundwater rising, thus rendering soil unsuitable for agriculture. For example, Western Australia has already lost 30% of its agricultural land due to rising groundwater. This problem needs to be addressed by finding salt-resistant crops and, in the longer term, by rehabilitation of the soil. In Australia, during the past few years, attempts have been made to find solutions for the use of saline water for aquaculture (Allan 2008).

The smart use of water is sometimes needed where it is impossible to dump vast amounts of saline water into the environment. In this situation, systems with full or partial recirculation are the best solution. This is also the case whenever water quality is a problem and there is a need for water treatment prior to use to address problems such as high iron and low pH.

A different approach to utilising arid land is evident in desert coastal aquaculture. Desert coastal areas are underutilised and completely uninhabited in many countries around the world (e.g. the Sahara desert in Morocco; North African countries such as Algeria and Libya; Persian Gulf countries, Oman, Yemen and many other countries with vast desert coastal areas). This type of aquaculture needs large investment and therefore is suitable for high-value and large-volume aquaculture, as demonstrated in Saudi Arabia.

Finally, even after finding the right aquaculture species for the local conditions and markets, and the optimal rearing system, the most important aspect is the aquaculturist behind the system. Trained and knowledgeable personnel are the key to any successful aquaculture venture.

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6
Reproduction, Life Cycles and Growth

John S. Lucas and Paul C. Southgate

6.1 INTRODUCTION

The three major groups of aquaculture animals will be considered in this chapter: fish, bivalve molluscs and decapod crustaceans (the last two are the major shellfish groups). The morphologies of the species in these three groups are extremely different (see invertebrate and vertebrate textbooks), as are their reproductive systems, life cycles and patterns of growth. However, although the details and requirements of the various life stages of aquaculture species vary enormously, there are some general patterns.

6.2 REPRODUCTIVE PHYSIOLOGY

6.2.1 Fish

The sexes in cultured fish are separate and their paired gonads are located dorsolaterally in the body cavity. Reproductive activity is confined to a particular season of the year. Reproduction is usually triggered by environmental cues, such as increase in day length or water temperature (in temperate and tropical species), or changes in salinity or turbidity (in tropical species). These cues trigger hormonal changes within the animal brought about by stimulation of the pituitary gland.

The pituitary gland contains three major hormones concerned with reproduction:

1. Gonadotrophin-release hormone (GnRH) controls the release of gonadotrophin from the pituitary.
2. Gonadotrophin-release-inhibitory factors (GnRIF, primarily dopamine), inhibit the release of gonadotrophin from the pituitary.
3. Gonadotrophins (GtHs) regulate the release of gonadal steroid from the gonad. Gonadotrophins are composed of GtH I (follicle-stimulating hormone, FSH) and GtH II (luteinising hormone, LH).

The major male and female gonadal steroids are 11α-ketotestosterone and 17β-oestradiol, respectively. They control the major aspects of reproduction such as reproductive behaviour, oocyte maturation, spermatogenesis and ovulation. These steroids also have a negative-feedback influence on GtH production from the pituitary. This hormonal system is shown in Fig. 6.1. Although low levels of GtH are found in the blood throughout the year, final maturation of gametes and ovulation is brought about by a surge in GtH levels in response to final environmental cues.

Knowledge of this system allows aquaculturists to control reproduction in captivity and obtain spawnings of high-quality eggs when required. Control of reproduction also allows hatchery managers to plan for maximum food production for larvae and juveniles when needed. In captivity, however, the final environmental cue for reproduction is often lacking, in which case eggs do not undergo final maturation and fish do not ovulate or spawn because of the lack of a surge in GtH levels. This problem is usually overcome in one of two ways:

1. Environmental manipulation. The environmental cues necessary for gamete maturation and spawning are provided. This method requires precise knowledge of the factors governing reproduction in a particular species.

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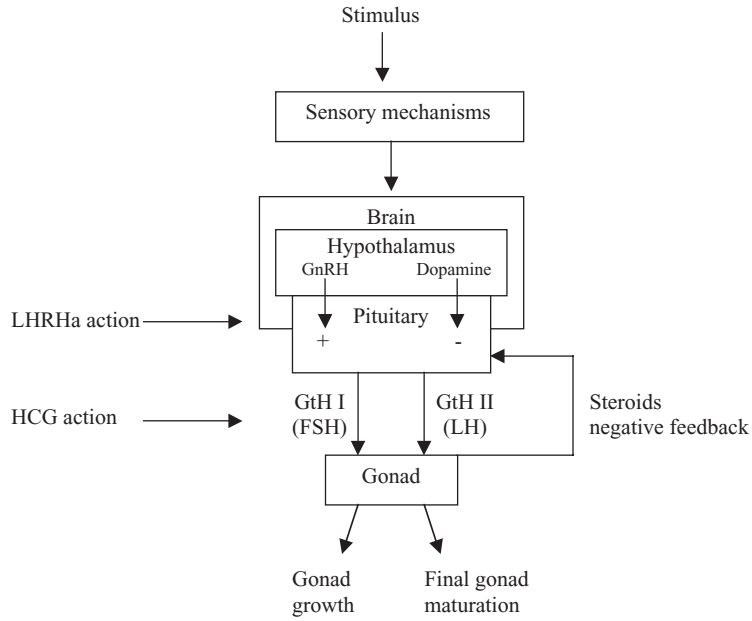


Fig. 6.1 The reproductive endocrine physiology of fish showing the sites of action of LHRHa and HCG used to artificially induce maturation.

Table 6.1 The actions of human chorionic gonadotrophin (HCG) and luteinising hormone-releasing hormone analogue (LHRHa) in influencing reproduction in fish.

Hormone	Action
HCG	Acts directly on the gonad to induce the release of gonadal steroids (sex hormones)
LHRHa	Acts on the pituitary gland to stimulate the release of gonadotrophins (GtH)

Important cues include water temperature, salinity, photoperiod and food availability.

2. Hormonal manipulation. The final GtH surge is artificially attained by injection of appropriate hormones into the fish. Although crude pituitary extract can be used, human chorionic gonadotrophin (HCG) and luteinising hormone-releasing hormone analogue (LHRHa) are more commonly used.

The actions of these hormones are detailed in Table 6.1 and their points of action within the maturation sequence of fish are shown in Fig. 6.1.

Successful spawning induction depends on a number of factors, which are outlined in the following sections.

6.2.1.1 Stage of maturity of brood fish

Artificial surges in GtH will be an ineffective spawning inducer unless female oocytes have previously reached a certain stage of development. To determine this, a small sample of developing eggs is removed from the female and observed microscopically. Cannulation is used, in which a fine plastic tube is passed up through the oviduct to the ovary. Gentle suction by the mouth at the other end of the tube provides a small sample of eggs for inspection. Eggs must possess yolk globules and be of a certain size. The required size can only be determined experimentally for each species but, as a rule of thumb, they should be at least half the diameter of eggs at spawning. Ripe males should exude milt (sperm) from the genital opening when pressure is applied to the abdomen.

6.2.1.2 The correct hormone to use and hormone dosage

HCG and LHRHa are the preferred hormones for spawning induction in fish; both are available commercially and available in purified form.

1. LHRHa is more effective in bringing about oocyte maturation. It is usually used at a dosage of 10–50 µg/kg fish.
2. HCG is usually used at dosages of 250–2000 IU (international units of activity) per kg of fish.

Initially, a mid-range dose is used, and the optimum dose is determined by trial and error. If the dose is too low, it will fail to induce a spawning. Too high a dose will cause final oocyte maturation to occur too rapidly and will result in poor egg quality. A very low dose will be effective if the oocytes are very mature (determined by cannulation).

6.2.1.3 Method of hormone administration

Hormones are administered either intramuscularly (IM) or intraperitoneally (IP) (into the body cavity). IM injections have the disadvantage of hormone loss when the needle is withdrawn; however, minimising the injection volume can reduce this. IP injections avoid the problem of hormone loss, but can result in damage to internal organs or injection into the intestine, where the hormone will be ineffective.

Hormones can be administered by injection of liquid or as a pellet containing the hormone. Usually, the hormone is mixed with cholesterol and compressed to form a pellet, which is injected into the muscle. The advantage of using pellets is that the release of the hormone into the blood system occurs more evenly and does not result in a sudden increase in hormone levels as does liquid injection. Cellulose can also be incorporated into the pellet to help regulate the rate of hormone release: the more cellulose, the slower the rate of release.

6.2.1.4 Timing of hormone administration

Hormonal induction of spawning is more successful if the hormone is administered so that spawning occurs at a time of day when it would occur naturally. To achieve this, knowledge of the time of natural spawnings and the length of time taken between hormone administration and ovulation ('latent period') is required. For example, barramundi spawn naturally after dark and have a latent period of ca. 36 h. Therefore, the hormone is administered at around 07.00 h to induce a spawning after dark the following night.

As detailed above, GnRIF inhibits the release of GtH within the pituitary (Fig. 6.1). GnRIF is actually dopamine, and dopamine antagonists such as pimozide and domperidone can block its inhibitory action. Thus, spawning may be facilitated by administering a dopamine antagonist to brood fish. The use of a combination of GnRF analogue (e.g. LHRHa) and a dopamine antagonist (e.g. domperidone) to induce ovulation and spawning of cultured fish is known as the 'Linpe method'. A mix of LHRHa (stimulating GtH) and pimozide (inhibiting GnRIF) is available commercially and spawning kits are available for the major groups of cultured fish such as carps and salmonids.

6.2.2 Decapod crustaceans

The sexes in cultured decapod crustaceans are separate, and the paired gonads are located dorsally and laterally to the gut. Their reproduction and gonad maturation are hormonally regulated. Synthesis and release of crustacean reproductive hormones occur in response to both exogenous and endogenous factors and, in the wild, reproduction is closely related to seasonal cues. These seasonal cues are similar to those for fish, e.g. photoperiod, water temperature and food availability.

Reproduction in decapod crustaceans is controlled by hormones released from the sinus gland and associated centres (X-organ and Y-organ) within the eyestalks. These hormones are gonad-inhibiting hormone (GIH), moult-inhibiting hormone (MIH) and several other hormones. A commonly used technique to induce reproductive maturation in shrimp is eyestalk ablation. This removes the eye, together with the eyestalk and its source of hormones. Eyestalk ablation is usually unilateral (applied to one eye only) and is achieved by:

- cutting off the eyestalk;
- cauterising, crushing or ligaturing the eyestalk.

In females, ablation results in an increase in total ovarian mass, owing to the acceleration of primary vitellogenesis and the onset of secondary vitellogenesis. In males, ablation induces spermatogenesis, enlargement of the vas deferens and hypersecretion in the androgenic gland. Eyestalk ablation also removes the source of MIH and other compounds that control moulting (ecdysis). Reproduction in decapod crustaceans is often characterised by a pre-copulation/pre-spawning moult.

Gonad maturation after ablation can be very rapid in marine shrimp, and females can develop full ovaries within 3–4 days. At mating, males insert a spermatophore (a large bundle of non-motile spermatozoa) into the thelycum (receptacle for the spermatophore) on the ventral surface of a female shrimp. Fertilisation occurs externally upon ovulation and passage of the oocytes through the



Fig. 6.2 Blue swimmer crabs (*Portunus pelagicus*) mating. The male crab is on top of the female, which is on its back, so their ventral surfaces are in contact. The female is soft post-moult and the male has forced its abdomen (visible as white edges) under the female's abdomen to transfer sperm (as spermatophores) to her genital apertures. (Photograph by courtesy of Ria Tan.)

gonadophore. The eggs are shed by the female shrimp into the water column. Spermatophores may remain implanted through several ovarian maturation cycles and may fertilise as many as six spawnings within one moult cycle.

Copulation in decapod crustaceans typically involves a soft-shelled female that has just moulted and a hard-shelled male (Fig. 6.2). As decapod crustaceans moult regularly (section 6.4.2), it means that fertilisation can be reliably obtained by keeping female and male broodstock together in appropriate conditions. This is used for hatchery production in freshwater prawns, freshwater crayfish, crabs, lobsters and some marine shrimp species. In the groups other than marine shrimp, the fertilised eggs are not shed into the water column, but are retained on the female's abdominal appendages (pleopods) until the larvae or juveniles hatch (Fig. 22.12). Females brooding eggs on their pleopods are known as 'berried' or ovigerous females.

6.2.3 Bivalves

Most species of cultured bivalve have separate sexes, but the total situation within the group is more complex than in fish and decapod crustaceans. Some cultured bivalves are hermaphrodites, e.g. some scallops, with eggs and sperm produced simultaneously. Other bivalves change sex during development. Usually these are protandrous, with younger individuals being male and older individuals being female. Some bivalves may undergo more than one change (often annual) in functional sexuality and are said to show rhythmical consecutive hermaphroditism (e.g. *Ostrea* species). Although the causes of sex change in bivalves are poorly understood, gene-activated components that respond to environmental factors, sex-determining genes and food supply have all been suggested.

As in fish and decapod crustaceans, gamete maturation in bivalves is influenced by a number of exogenous factors, including water temperature, food availability, light intensity and lunar periodicity. It is also influenced by endogenous factors including hormones, genetic factors and levels of endogenous energy reserves. Gonad maturation in bivalves relies on both direct food intake and utilisation of endogenous energy reserves. Water temperature is perhaps the major influence on gonad maturation in bivalves. However, the relationship between increased water temperature and increased natural phytoplankton production (food availability) is also very important.

Major spawning cues for bivalves include a change in water temperature, a change in salinity, lunar periodicity and chemicals (pheromones) associated with water-borne gametes from other individuals. With the exception of lunar periodicity, these are also commonly used spawning cues in bivalve hatcheries (see section 6.3.2). Gametes are generally liberated into the surrounding water where they are fertilised. In some species (e.g. *Ostrea edulis*), fertilised eggs are retained in the mantle cavity, where they develop into swimming larvae before being released.

6.3 LIFE CYCLES

At breeding, mature adults shed their gametes (eggs and sperm) freely into the water or the male impregnates the female. Fertilisation results in a zygote and subsequently an embryo, which develops within the egg. Embryonic development occurs over a period of hours or days, depending on the species and temperature. In a generalised life cycle for a species with planktonic larvae, the embryo emerges from the egg as a swimming larva that develops into a juvenile over a period of days, weeks or even, in some species, months. This may be a progressive process, as in shrimp and most fish; or the larva may abruptly settle out of the plankton onto an appropriate substrate and metamorphose into a juvenile, as in bivalves and other benthic invertebrates. The juvenile then grows progressively over a period of months or years until it reaches sexual maturity and the life cycle is completed (Fig. 6.3).

From the viewpoint of aquaculture, these life cycles can be divided into the following sequence of stages:

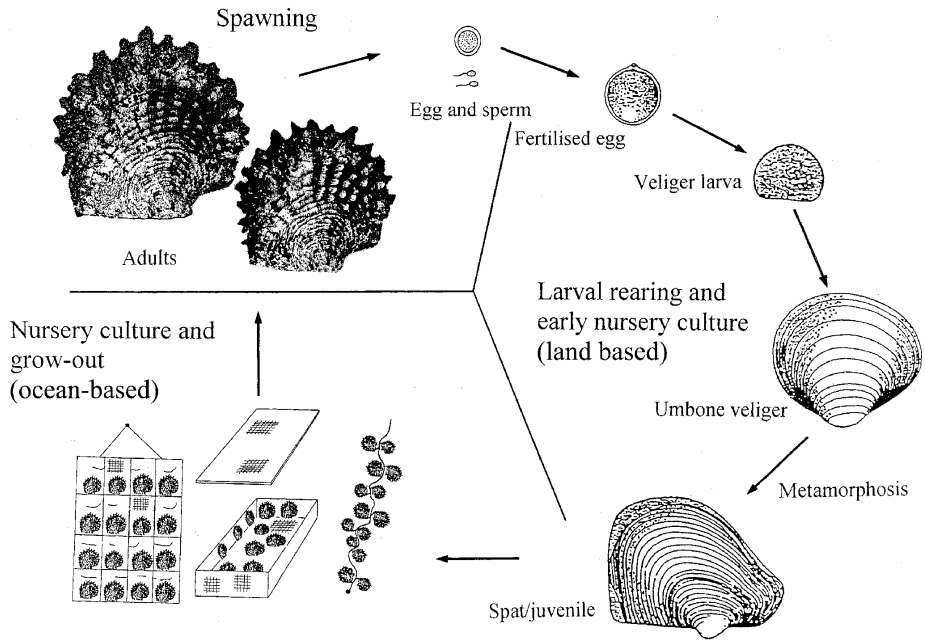


Fig. 6.3 Generalised life cycle of a pearl oyster (*Pinctada* species) showing the phases of culture.

1. Broodstock conditioning to produce ripe adults for spawning.
2. Spawning, either naturally or induced (the latter being more common in aquaculture).
3. Egg fertilisation.
4. Larval rearing.
5. Postlarval and juvenile rearing.
6. Grow-out rearing to commercial size.

These phases of aquaculture are outlined in the following sections.

In intensive aquaculture, many of these phases in the life cycle of a species require different culture techniques. For example, intensive fish culture uses different culture methods for larvae, juveniles and grow-out stock. In extensive aquaculture, however, there may be very little change in culture techniques throughout all phases of the life cycle, e.g. extensive culture of tilapia and freshwater crayfish.

6.3.1 Broodstock selection and conditioning

This involves selecting appropriate animals to serve as sources of gametes. It may involve selecting stock with particular genetic traits (section 7.4). In many cases the broodstock are simply obtained from the field. Most aquatic animals become sexually mature and reproduce during the warmer months of the year in response to increasing photoperiod, food availability and rising water temperature. Knowing the major cues for gonad maturation, conditions in an aquaculture hatchery can be manipulated to bring on sexual maturity outside natural reproductive seasons. This is known as broodstock conditioning, and it allows hatchery production on a year-round basis.

6.3.2 Spawning

Methods of inducing spawning in individual species and groups are described in detail in other chapters. However, it is appropriate to summarise these methods in five categories:

1. Mild stress, such as abrupt temperature increases, e.g. with bivalves.
2. Manipulating the body's reproductive hormones by injection or implantation of reproductive hormones into the body in fish or by eyestalk ablation in shrimps.
3. Transferring gametes from a container where animals have spawned to one where they have not and thus allowing the pheromones associated with gametes to trigger responses from the recipients, e.g. bivalves.

4. Gonad stripping, where the broodstock are known to have ripe gametes. The body is appropriately massaged to expel gametes, e.g. fish (Fig. 15.3), or gonads are removed from a dead animal and lacerated to release gametes, e.g. bivalves.
5. Injecting a neurotransmitter substance directly into the gonad of a ripe animal, presumably to cause gonad contractions, e.g. some bivalves.

It is evident that the greatest range of induced spawning techniques is used with bivalves (section 23.4.2). To an extent this reflects the fact that they are more readily induced to spawn than fish or shrimp.

6.3.3 Egg fertilisation

Where the broodstock sexes are segregated, fertilisation involves mixing an appropriate amount of sperm suspension with a suspension of eggs. The objective is to run a course between low fertilisation rates, as a result of insufficient sperm, and polyspermy, where eggs are penetrated by a number of sperm (Stephano and Gould, 1988). When an egg is penetrated by a sperm, its surface often changes to prevent further sperm penetration. If, however, sperm are very abundant, other penetrations may occur before the barrier is established, and the egg then contains two or more haploid nuclei from sperm. These result in a polyploid ($2 + n$) zygote and the subsequent embryo develops abnormally.

6.3.4 Larval rearing

Embryonic development is usually a brief process, from a few hours' to several days' duration. It requires the developing embryos to be cleaned of any debris and chemicals from spawning induction and fertilisation. The embryos need to be maintained in finely filtered (e.g. 0.2–2 μm) water to reduce levels of bacteria, which may potentially invade the surfaces of the eggs. Developing embryos must also be provided with adequate levels of dissolved oxygen.

The larval stages of most fish, crustaceans and bivalves are planktotrophic, i.e. dependent on exogenous food supplies for much of their development. When larvae first hatch, however, they usually have sufficient energy reserves in the yolk to support development for a day or more before they need to feed. These reserves were present in the egg and were passed on from the mother. Fish larvae, for example, do not develop a functional gut until some time after hatching: they depend on endogenous reserves until they can support themselves by feeding on appropriate foods in their environment (Fig. 6.4). The embryos and early larval stages of most aquatic animals utilise lipids and proteins from yolk reserves to fuel development to this point. It has been estimated, for example, that embryonic development in some bivalves utilises about 70% of the lipid reserves of the egg (Gallager and Mann, 1986). Bivalve larvae must again rely on endogenous energy reserves at the end of larval development, during metamorphosis, when they temporarily lose the ability to feed. Unlike fish and crustacean larvae, which retain the ability to feed during the transition from larva to juvenile, bivalve larvae must accumulate substantial energy reserves during larval development. In some species (e.g. abalone and trochus), the eggs contain sufficient nutrient reserves to support larval development through to the juvenile phase without the larvae having to

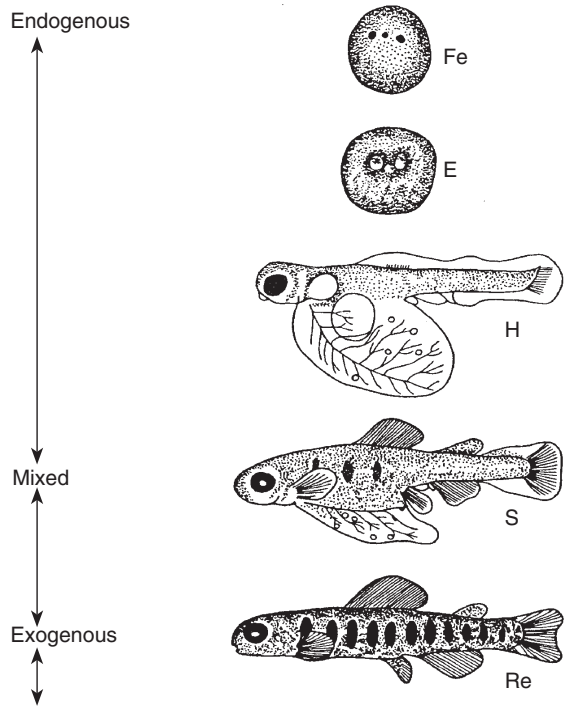


Fig. 6.4 Diagram showing early development in salmonids. E, eye development of embryo inside the egg; Fe, fertilised egg; H, hatching with large yolk sac; S, larva beginning to seek external food; Re, completion of yolk sac resorption and total reliance on exogenous nutrition. Reproduced from Kamler (1992), modified from Raciborski (1987) with permission from the Polish Academy of Sciences.

feed. This lecithotrophic development is usually relatively brief and associated with relatively large and yolky eggs.

The life cycles of freshwater animals often differ from this general pattern in that planktonic larval stages are omitted or much abbreviated. This is known as direct development. Freshwater species with direct development typically produce larger and yolkier eggs than related marine animals. These large eggs support a comparatively longer embryonic period to result in the hatching of juveniles or near-juveniles. The eggs are usually protected during this long embryonic development and the adult may carry them, e.g. on the female crayfish's pleopods or in the mouths of some fish, or there may be 'nests' and patterns of reproductive behaviour to protect broods of eggs. (Egg-protecting behaviour also occurs in some freshwater and marine fish that have normal larval stages.)

Planktotrophic larval development is often the most demanding phase of the life cycle for aquaculture, and it can last from several days to many weeks. The larvae are physically and physiologically fragile. They require well-controlled environmental parameters, such as dissolved oxygen (DO), pH, nitrogen waste levels, bacterial populations, levels of organic and inorganic particles, and temperature. They are often reared in large tanks to buffer these environmental parameters; but tank culture also has hazards, such as contamination and pathogenic bacterial blooms.

As the larvae switch from relying on their endogenous yolk reserves to feeding, providing appropriate food becomes a major aspect of larval culture. There are two main forms of food:

1. Microalgae for the filter-feeding larvae of molluscs and shrimps.
2. Rotifers and brine shrimp nauplii for the larvae of fish and older larvae of shrimp (Chapter 9).

Larval density is important in hatchery culture. The advantage of high larval density is that more postlarvae can be produced per unit volume of culture tank. However, if larval density is too high, it may compromise water quality and affect larval growth and survival. The culture tank water is usually changed at regular intervals to maintain water quality. The density of larvae is reduced as they grow, despite inevitable attrition that reduces numbers. If one larva per mL of culture water completes development, this corresponds to one million larvae from 1000 L of culture water. Aquaculture hatcheries use larval rearing tanks up to 20 000 L and produce tens of millions of postlarvae per rearing.

6.3.5 Postlarval and juvenile rearing

It is necessary to provide appropriate substrates in the culture system to induce bivalve larvae to settle. The larvae are selective because, at settlement and metamorphosis, they attach and make a habitat commitment for the remainder of their lives. For fish and shrimp larvae there is no abrupt metamorphosis. There is, however, a progressive change in behaviour of shrimp postlarvae as they become more substrate oriented. The same pattern occurs in benthic fish.

Marine and brackish water crabs, such as the mudcrab, are another example of an abrupt metamorphosis from planktonic larval stages to the first benthic juvenile stage. In this case it is via a transitional megalopa stage.

The early part of this period is often a particularly difficult one for rearing fish, as the postmetamorphic juveniles must be progressively weaned off live feeds on to artificial diets. Standard procedures have been developed for the major culture species (e.g. section 9.5.1), but this is often one of the particular points of difficulty in developing the culture process for a new fish species.

Postmetamorphic juveniles of fish, decapod crustaceans and bivalves are very small, physiologically fragile and vulnerable to predation. Over a period of days to months or more than a year, they are grown in protected conditions, before they are put out into the grow-out environment. This may involve a period of culture in tanks at the hatchery and an onshore nursery, followed by a period of protected culture in the field. Care is required to minimise mortality when transferring juveniles from the relative comfort of the hatchery (e.g. adequate feed and filtered water, which may be heated above ambient temperature) to field-based nursery conditions with cooler water, fluctuating food supply, predators, disease and fouling.

6.3.6 Grow-out rearing

Grow-out rearing is the final phase, during which the juveniles are put out into the adult environment and reared until being harvested. They are not treated in the same manner throughout this phase; factors such as food pellet size, pond size and mesh sizes of protective or enclosing nets are varied as they grow.

6.3.7 Other considerations

In some cases, the whole culture process from spawning broodstock to final marketing takes place on one farm. In other cases, the cultured organisms may be sold several times during the culture process. Broodstock may be captured by fishing or reared on a specialist farm and sold to the hatchery. Late larval stages or early juveniles are often

sold from a specialist hatchery to nursery growers. Juveniles ready to be grown out may be sold from a specialist nursery to grow-out farms. This is because these different phases of culture require particular expertise and facilities. Most typically, the culture process involves a hatchery or hatchery/nursery selling juveniles to farmers, because the hatchery aspect is most technically demanding and because one hatchery can supply many farms. In some Asian countries, such as Taiwan, this process is taken further. There is a series of specialised facilities with expert technical staff who culture specific early-development stages of fish. During development, the fish may be sold on to other facilities a number of times. Each facility may culture equivalent stages of a number of species.

The above describes general methods for culturing stock from gametes to harvesting. In many cases, however, only the later part of the life cycle is cultured on a particular farm and the difficult early stages are omitted. Many aquaculture industries rely on natural recruitment of juveniles, thereby omitting the more technically demanding phases of aquaculture outlined in sections 6.3.1, 6.3.2, 6.3.3 and 6.3.4. Relying on natural recruitment is possible by knowing the times and localities of this recruitment and other biological information about the recruits, such as settlement substrate. It may be possible to stock coastal ponds with recruits, e.g. juvenile fish, shrimps or crabs, by filling the ponds at high tides when juveniles are abundant in the adjacent inshore waters. Alternatively, the recruits may be netted from coastal water when they are abundant and then stocked into ponds. In the case of bivalve larvae, it is a matter of providing appropriate substrates, e.g. wooden battens for oyster larvae or raked particulate sediment for cockle or clam larvae, to attract late-stage larvae to settle from the water column. In freshwater ponds, the adults may be allowed to mate and care for their eggs, and then the juveniles are removed at an appropriate stage. There may still be several commercial stages in this process, whereby juveniles from the field, obtained from privately owned settlement substrates or by fishing, are sold to farmers for grow-out.

Natural recruitment is often the start of an extensive or semi-intensive culture process, i.e. where the stocking rate is low to moderate and capital is limited. It is the basis of many major aquaculture industries, but there are the inherent problems, as in fisheries, that natural recruitment levels vary from year to year, and are not completely predictable in time. Fishing pressure on the wild adult stock may reduce its reproductive output and hence natural recruitment. Another disadvantage of relying on natural recruitment is that there is no opportunity for stock improvement. It is impossible to select for desirable traits for aquaculture.

6.4 GROWTH

6.4.1 Size vs. age

There are two patterns of growth occurring in the developing organism:

1. Absolute growth rate, e.g. rate of increase in length or mass per unit time, is quite insignificant initially, when the animal is very small (Fig. 6.5). Then, as the animal

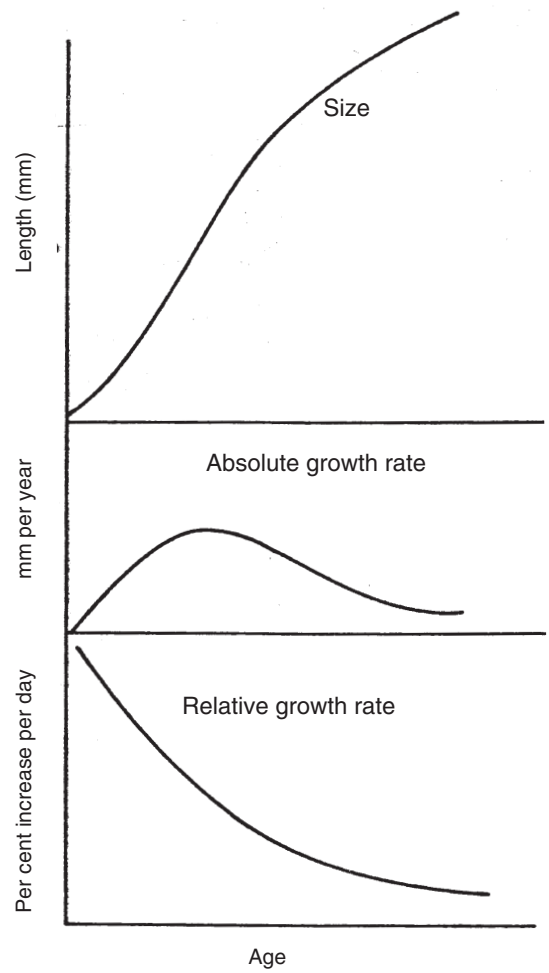


Fig. 6.5 Generalised curves of size, absolute growth rate and relative growth rate vs. age. Reprinted from Malouf and Bricelj (1989) with permission from Elsevier Science.

grows, its capacity to feed and assimilate food progressively increases. The absolute growth rate increases and the size vs. age curve becomes steeper. This is known as the exponential phase of growth.

2. Relative growth rate, e.g. growth increment per unit body mass per unit time, is very rapid during the early phases of an animal's life cycle (Fig. 6.5). That is, growth relative to size is very rapid. It may be 200% and more during the first week or so of development and remain at a high level during the early months.

The size vs. age relationship reflects the changing pattern of absolute growth, producing a sinusoidal curve (Fig. 6.5). There is an upper inflection in the size vs. age curve, and the upper curve typically slopes gently towards a final theoretical size, L_∞, which it would reach if the animal lived indefinitely.

Some complex logistic equations, such as the von Bertalanffy growth function (VBGF), are used to describe the relationship between size and age. One form of the VBGF is:

$$L_t = L_\infty(1 - b\mathrm{e}^{-Kt})^{-1}$$

where L_t is size at time t; L_∞ is maximum size; b and K are growth coefficients; b is related to the ratio of maximum size to initial size; and K is the growth coefficient, often used in comparisons of growth rates between populations and species.

However, over short periods of measurement (months), when the animals are in the exponential (rapid) phase of growth, a relative growth coefficient can be obtained from the following equation:

$$k = \frac{(L_2 - \ln L_1)}{(t_2 - t_1)}$$

where L_1 and L_2 are sizes at initial time, t_1, and final time, t_2, respectively. This growth coefficient is simple to calculate and is widely used in comparative studies.

6.4.2 Growth in decapod crustaceans

Growth in decapod crustaceans basically follows the pattern outlined in section 6.4.1. The pattern of growth is, however, complicated by moulting, whereby the animal sheds its old exoskeleton (Fig. 6.6) and expands rapidly during a short period before a new exoskeleton hardens. Thus, growth appears to occur in a series of abrupt steps rather than as a continuous process (Fig. 6.7). The details of the moult cycle are outlined in Table 6.2.



Fig. 6.6 The empty shell of a female mitten crab, *Eriocheir sinensis*, discarded after it has completed moulting. (Photograph by Christian Fischer.)

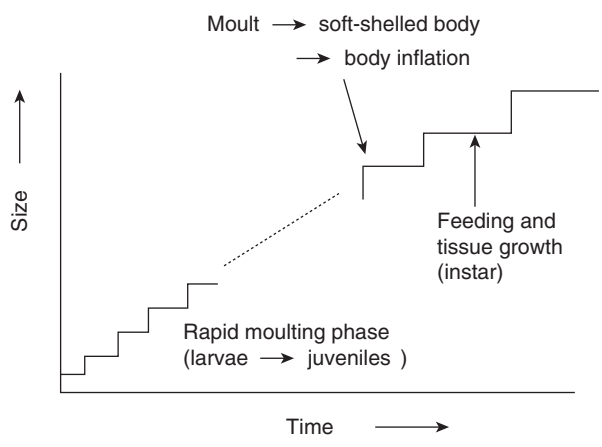


Fig. 6.7 Staircase-like pattern of growth of decapod crustaceans with regular moults to expand in size by 'inflation' alternating with hard-shelled periods (instars) during which the animal feeds and grows tissue.

The decapod crustacean's life is divided into a series of intermoult periods or instars, which may be numbered consecutively for convenience. These are punctuated by moults (or ecdyses) and the animal continues to moult, unless it goes into a final terminal instar when it ceases to moult (terminal anecdysis). The moults are more frequent earlier in life and the size increments at moults are rela-

Table 6.2 Events of a moult cycle in a decapod crustacean, from one inter-moult period (instar X) to the next (instar X + 1).

Phase	Duration	External body size	Organic tissue mass	Behaviour	Shell
Intermoult (instar X)	Days–weeks	Fixed	Increasing	Feeding; locomotion	Hard
Pre-moult	Hours–several days	Little change	Decreasing to increase organic content of body fluids	Ceasing feeding; may seek cover	Shell decalcifying
Moult	Minutes–hours	Rapid increase	Low	Rapidly shedding old shell; no feeding; may be concealed	Old shell discarded; new soft shell
Post-moult	Hours–days	Little change	Recommences increasing	Recommences feeding	Hardening
Intermoult (instar X + 1)	Days–weeks (months[1])	Fixed	Increasing	Feeding, locomotion	Hard

[1]Last instars of some large species, e.g. marine lobsters, mudcrabs.

tively larger, but absolutely smaller. The result is a size vs. age relationship that fundamentally looks like the relationship in Fig. 6.5, but with a stepwise pattern instead of a smooth curve (Fig. 6.7).

The processes of:

- moulting to expand body dimensions; and
- growth, in terms of adding organic tissue and energy content,

are completely independent (Table 6.2). The decapod crustacean feeds and builds up organic tissue during the intermoult period. This is the critical period of growth, although there is no change in external dimensions: body size is constrained by the exoskeleton. The animal does not feed during the periods immediately before, during and after moulting. Its shell is too soft for feeding and it is vulnerable. This is the period of non-growth, although it is the period when the body expands rapidly by absorbing water.

6.4.3 Energetics of growth

The growth rate depends on the extent to which net energy intake exceeds metabolic rate, and to what extent energy is diverted from growth to reproduction in mature animals (section 8.3). Energy committed to gametogenesis is a major component of the body's resources in many aquaculture animals. Hence gametogenesis is at least part of the cause for growth tapering off with age.

The energy for growth may be expressed as a summation of input and outputs.

$$G = I - M - E - F - R$$

where G is the growth rate; I is the food ingestion and absorption rate; M is the metabolic rate; E is the excretion rate of waste molecules from metabolism; F is faeces production; R is gametogenesis; and $[I - (E + F)]$ is the net rate of energy intake, i.e. food intake – (excreted wastes and faeces). The equation can be reorganised:

$$G = [I - (E + F)] - M - R$$

or

$$G = [I - (E + F)] - M \text{ during immaturity.}$$

6.4.4 Measuring growth

6.4.4.1 General

A very important aspect of growth is to measure it accurately in the organisms being cultured. This is a means of predicting when they will be ready for harvest. Evidence of suboptimum growth rates draws attention to factors such as health, adequacy of feeds and nutrition, environmental quality, stock source, etc., that may be adversely influencing performance.

The change in size of a substantial representative sample of the cultured organisms must be measured to determine

the growth rate. For example, an appropriate sample from each pond on a shrimp farm may be at least 100 individuals. The size of aquaculture organisms may be measured in various ways, but different methods are more appropriate for seaweeds, fish, bivalves and crustaceans. The four main measurements of size used in aquaculture are listed below:

1. A linear dimension measured with callipers or a ruler, according to size. The advantages of this method are that it is quick and easy, and the animal is not unduly harmed. The disadvantages are that it cannot be used for flexible organisms (e.g. seaweeds) and it does not give an indication of the condition (fat/thin) of the animal.
2. Wet weight of the whole living organism, weighed on scales. The advantage of this method is that it is quick and easy, and the animal is not unduly harmed.
3. Dry weight. The dead organism, or a representative part of it, is dried in an oven at ca. 50 °C until all the water has evaporated. The advantages of this method are that it gives a tissue weight value that is not complicated by water content and the value is relatively quickly and easily obtained. With any assessment of animal tissue growth, whether it is fish, bivalves or crustaceans, dry tissue weight is the most accurate. In times of physiological or nutritional stress, the water content of animal tissues may increase, leading to an increase in wet tissue weight and giving a false impression of tissue growth.
4. Ash-free dry weight (AFDW). Once the dry weight of an organism (or part thereof) is determined, it is heated in a furnace at ca. 500 °C for ca. 24 h. All organic matter is burnt off in the furnace, leaving only inorganic ash. The weight of ash is then subtracted from the dry weight. This gives the AFDW, which was the dry weight of organic matter in the animal. The particular advantage of AFDW determinations is that it is more accurate for animals with very large inorganic components, such as shells (e.g. bivalves), for which it is difficult to estimate organic tissue content because it is relatively small. An alternative to AFDW is to use condition index (section 23.2.5).

Determining dry weight and AFDW requires specialised equipment such as a drying oven, 500 °C furnace and a sensitive balance. As such, linear measurement and wet weight determination are used most routinely as means of assessing size or growth of aquaculture organisms.

Frequently it is sufficient for the aquaculturist to observe measured growth, but in some circumstances there may be a need to calculate growth rates, e.g. for comparisons of growth rate through the seasons or between batches. The growth rate may be calculated on the assumption of linear growth, i.e. change in size over period of measurement. However, as illustrated in Fig. 6.5, growth is not linear. The growth coefficient provided in section 6.4.1 can be used as a more accurate value for comparisons of relative growth rate, provided the animals are in the appropriate phase of growth.

6.4.4.2 Fish

Fish length has long been used as an assessment of size and growth. Two measurements are typically used:

1. Standard length (SL). SL is measured from the tip of the snout to the base of the caudal fin. In fish larvae, however, SL is from the tip of the snout to the tip of the notochord.
2. Total length (TL). TL is measured from the tip of the snout to the tip of the caudal fin. However, measuring TL may be inaccurate if there is damage to the caudal fin, and it is difficult to measure accurately in larvae and juveniles.

As noted previously, length takes no account of fatness or condition of a fish and may therefore be a poor guide to tissue growth. Consequently, wet weight is also frequently used for size and growth assessment, usually in combination with length. Although wet weight is most commonly used as a measure of condition in fish, there are advantages of using dry weight for fish (section 6.4.4.1).

6.4.4.3 Bivalves

Bivalves are usually assessed in terms of shell dimensions, e.g. shell length (SL), shell height (SH) and shell width (SW). If only one dimension is used, as is often the case, it is the largest dimension of the shell. This is not the same in all bivalves, e.g. the largest shell dimension is SH in oysters and SW in clams.

The major problem with shell dimensions as a measure of growth is that they take no account of tissue growth. The bivalve shell tends to grow, regardless of the condition of the animal. It is not unusual for the shell to increase in dimensions at an abnormally slow rate while tissue mass declines. This can happen during periods of inadequate phytoplankton abundance or in response to adverse environmental conditions or disease. Clearly, shell increments in these circumstances do not represent growth. They show only apparent growth. This is where AFDW determina-

tions would indicate the true situation with regard to the state of the tissue.

There is also a simpler method for determining the relative amount of tissue in relation to the shell. This is called the condition index (CI) and the method for determining it is described in section 23.2.5. The CI is used for table oysters and is more concerned with gonad condition and how 'fat' the oysters are than with tissue growth *per se*.

6.4.4.4 Decapod crustaceans

Decapod crustaceans tend to be measured by a linear measurement of their carapace. Crabs, with a carapace that is broader than it is long, have their maximum carapace width (CW) measured. Other decapod crustaceans, marine shrimp, freshwater prawns, freshwater crayfish, etc., with carapaces that are longer than they are broad, have their carapace lengths (CL) measured. The last group of crustaceans have a well-developed tail (abdomen). This tends to flex vigorously when they are held, hindering total length measurements, which are not usually used.

Because of the moult cycle, the water content of decapod crustacean tissues is very variable and wet weight measurements of individuals are dubious. Wet weight measurements of large groups of individuals are more reliable as they accommodate the range of moult conditions in the group. The total wet weight is then divided by the number of individuals. This technique has the advantage of being more rapid than taking individual carapace measurements, but it only gives a mean size.

Frequency of moulting is a fair indication of relative growth rate in a cultured population of decapod crustaceans but it is difficult to assess. It is no substitute for size measurements.

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7
Genetics

Rex Dunham

7.1 INTRODUCTION

Genetic intervention has been used to enhance animal and plant agriculture production for centuries and has intensified during the last two centuries. These techniques are now being applied to aquatic organisms to improve production traits. Aquaculture genetics has tremendous potential for enhancing aquaculture production. Modern research on aquaculture genetics began sporadically 80 years ago and became commonplace in the 1970s. During the last three decades, research in this area has steadily grown, and now research on traditional selective breeding, genetic biotechnology, transgenics, and genomics is quite active.

Knibb *et al.* (1998) considered that in their genetics most marine fish production remains equivalent to the use of undomesticated wild ancestral cattle, chicken, etc. in ancient terrestrial agriculture. This may be true for marine fish, many molluscs and crustaceans, and new aquaculture species in general. But after 40–50 years of genetics and breeding research, coupled with genetic biotechnology, this is no longer true for established species such as carps, catfish, salmonids, tilapias and oysters. The best available genotypes may have performance levels of up to 10-fold that of poor performing wild genotypes, and the rate of progress and genetic gain certainly rivals that for terrestrial livestock. Regardless of the species and the genetic gain made as of today, much greater genetic progress can continue to be accomplished.

Effective programs have goals and plans, and this is also true for genetic enhancement programs. Goals are the important traits of economic importance that we want to improve, and the extent to which we consider it feasible to improve these traits. Genetic enhancement programs are then the plans that we use to accomplish these goals and objectives. The primary purpose of this chapter is to review these genetic enhancement programs to show how effective they have been with the focus on production traits of foodfish and shellfish.

7.2 BASIC GENETICS

7.2.1 Gene action

There are two basic types of gene action, dominant/recessive and additive. In the case of dominance, only one copy of the dominant allele (*A*) in a diploid organism is needed for expression of the associated trait. In the case of the recessive allele (*a*), two copies of the allele are needed for the recessive phenotype to be observed. In a completely dominant system (Table 7.1a), a large unit of phenotypic change occurs when going from the homozygous recessive genotype (*aa*) to the heterozygous genotype (*Aa*) and no unit of change when going to the homozygous dominant genotype (*AA*). For additive gene action, the alleles, of course, act in an additive fashion with equal units of change when comparing the different genotypes ($aa \rightarrow Aa \rightarrow AA$) (Table 7.1b) or, like mathematical addition, the stronger allele will make a greater contribution to the phenotype (Table 7.1c). The alleles at one locus can affect the expression of alleles at a second locus. This type of gene interaction is termed epistasis.

7.2.2 Qualitative traits

Qualitative traits are phenotypes that are expressed in an all-or-none fashion. For example, albino or normal colora-

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Table 7.1 Basic types of gene expression. (a) Complete dominance. (b) Incomplete dominance. (c) Overdominance. (d) Co-dominance (additive gene expression).

	Genotypes	Phenotypes	Unit of phenotypic change between genotypes
(a) Complete dominance	*bb*	red	large
	Bb	black	none
	BB	black	
(b) Incomplete dominance	*bb*	white	large
	Bb	dark grey	small
	BB	black	
(c) Over-dominance	*ss*	malaria prone	large
	Ss	healthy (best)	large
	SS	sickle cell (sick)	
(d) Co-dominance	*rr*	white	large equal
	Rr	pink	units of change
	RR	red	

tion is usually a result of gene expression from a single or only a few loci. Coloration and deformities are examples of qualitative traits.

Qualitative traits such as changes in color, finnage, scale pattern or deformities can be desirable or detrimental in aquaculture. Obviously, qualitative traits are important and the primary basis for the ornamental aquaculture industry. Deformities can be valuable in the ornamental trade, but are usually undesirable in the foodfish industries. If these qualitative traits are a result of dominant gene action, they can be easily eliminated as all homozygous dominant and heterozygous (carrier individuals) phenotypes are obvious, and those individuals can be immediately selected against, resulting in a population with none of the dominant detrimental allele. An example of this is the saddleback mutation in tilapia (Tave *et al.*, 2003). On the other hand, it is extremely difficult to eliminate a deleterious recessive allele from a population as heterozygous carriers cannot be identified by simple visual observation (Table 7.1a). If the trait was of high economic importance or damage, the heterozygous carriers could be eliminated, by mating them with individuals of known genotype and examining the phenotypic ratios in the progeny, i.e., progeny testing. Since fish are highly fecund and produce large numbers of progeny, progeny testing could eliminate the deleterious allele from the population in a single generation.

When deformities are observed, fish culturists often assume that the deformities have a genetic basis and that they are likely to be increasing because of inbreeding in the population. However, these assumptions are usually false. Most deformities observed are usually environmentally induced and are often related to low egg quality or poor water quality in the hatchery.

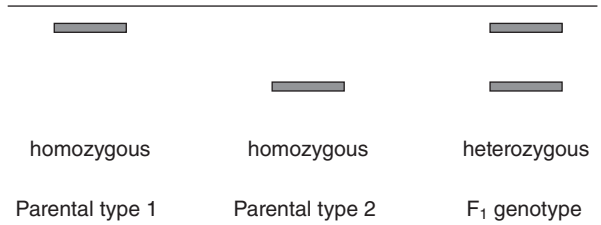


Fig. 7.1 Co-dominance: both alleles are expressed equally. DNA or protein banding patterns illustrate the concept.

There are different types of dominance (Table 7.1), as discussed earlier. In the case of complete dominance, the trait is fully expressed in the heterozygous and homozygous dominant genotypes. Albinism (in contrast to normal coloration) and saddleback are examples of complete dominance in fish.

The heterozygous genotype allows a major, but not complete, unit of change in the phenotype in incomplete dominance. The homozygous dominant genotype is necessary to make the complete, maximum shift in the phenotype. In the case of overdominance, the phenotype of the heterozygous genotype is outside that of the two homozygous genotypes (Table 7.1c). The phenotype associated with each allele is observed in the case of co-dominance (Fig. 7.1).



Fig. 7.2 Mirror scale pattern in common carp. Genes for scale pattern in common carp have epistatic interaction, pleiotropy, and semi-lethality.

- *Epistasis* is the interaction of genes at different loci. Alleles at one locus can affect the expression of alleles at another locus. Epistasis is the basis of some color types in fish. For instance, it is the explanation for some red and black color variants in tilapia and scale pattern in the common carp is also influenced by epistatic gene action (Fig. 7.2).
- *Pleiotropy* is when one gene affects more than one trait. The alleles and loci that affect scale pattern in common carp are an excellent example of this phenomenon. These genes not only affect scale pattern, but also growth, survival, other meristic traits, tolerance of low oxygen, hemoglobin and hematocrit , and ability to generate fins. Some mutations can also have semi-lethal or lethal effects, resulting in reduced viability or death. Again, certain alleles affecting scale pattern in common carp have semi-lethal or lethal effects and this applies to the alleles responsible for saddleback in tilapia.
- *Heterosis* is the increased function of any biological quality in hybrid offspring ('hybrid vigour').

7.2.3 Phenotypic variation

Individual phenotypes, the appearance, characteristics and performance of individuals, are a result of three main components:

1. The environment.
2. The genotype.
3. The interaction between the genotype and the environment.

Thus phenotypic variation (V_p) is a result of genetic variation (V_g), environmental variation (V_e) and variation due to the genotype–environment interaction (V_{ge}):

$$V_p = V_g + V_e + V_{ge}$$

Genetic variation in populations has several components as well:

- additive genetic variation;
- dominance genetic variation;
- variation due to epistasis interaction;
- variation due to maternal heterosis.

The type of genetic variation has a bearing on the success of the genetic enhancement program being attempted. For selection to be successful, a trait must have significant heritability and the ratio of additive genetic variation to total phenotypic variation must be high. Dominance, epistasis, and overdominance are the genetic basis of heterosis; the relative performance of crossbreeds and hybrids compared to their parents. Thus, significant dominance-related variation must exist for crossbreeding and hybridization programs to be successful.

7.3 DOMESTICATION AND STRAIN EVALUATION

Use of established, best-performing domestic strains is the first step in a genetic improvement program, and the mechanism to make the most rapid initial progress in genetic improvement. Domestic strains of fish usually have better performance in aquaculture settings than wild strains of fish. Strain variation is also important, since strain affects other genetic enhancement approaches, such as intraspecific crossbreeding, interspecific hybridization, sex control, and genetic engineering.

When wild fish are moved to aquaculture or hatchery environments, they are exposed to a new set of selective pressures that will change gene frequencies. Thus an organism better suited for the aquaculture environment

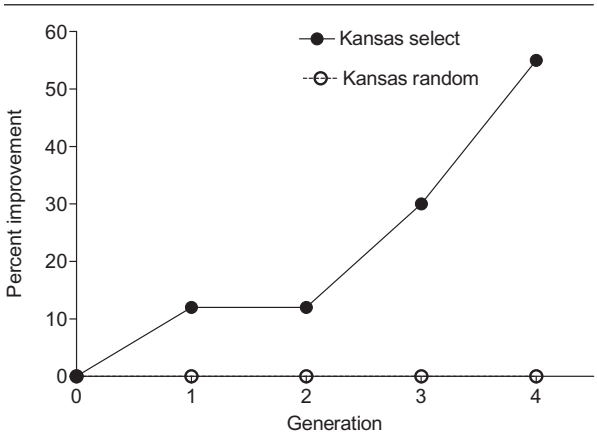


Fig. 7.3 Percent improvement for Kansas select channel catfish compared to Kansas random channel catfish after first, second, third, and fourth generations of selection for increased body weight.

begins to develop. This process, termed domestication, occurs even without directed selection by the fish culturist. Domestication effects can be observed in some fish within as few as one to two generations after removal from the natural environment (Dunham, 1996).

In channel catfish (*Ictalurus punctatus*) an increased growth rate of 3–6% per generation was observed due to domestication selection (Fig. 7.3), and the oldest domesticated strain of channel catfish (98 years), the Kansas strain, has the fastest growth rate of all strains of channel catfish.

Domesticated common carp in Hungary exhibited better growth and resistance to *Aeromonas hydrophila* than wild strains.

Although most domesticated strains usually perform better in the aquaculture environment than wild strains, there have been exceptions, such as wild Nile tilapia, *Oreochromis niloticus*, and rohu, *Labeo rohita*, which appeared to grow better in the aquaculture environment (Dunham *et al.*, 2001). However, the explanation for this anomaly appears to be related to a lack of maintenance of genetic quality and genetic degradation in the domesticated strains compared to these wild fish. Poor performance of some domestic tilapia is related to poor founding (parental) lines, random genetic drift, inbreeding, and introgression with slower growing species, such as *O. mossambicus*, and slower-growing strains such as Nile tilapia from Ghana (Table 7.2).

Domestication of farmed shrimp was relatively slow compared to that of finfish because of:

Table 7.2 Strain variation in Nile tilapia. Comparison of Egypt, Ivory Coast and Ghana strains for some traits that are important for aquaculture.

Trait	Best performing strains
Growth	Egypt, Ivory Coast
Reproduction	Ghana
Cold tolerance	Egypt
Seinability[1]	Egypt, Ghana

[1]Vulnerability to be seined in harvesting.

- the use of wild broodstock and postlarvae;
- a lack of understanding of shrimp reproductive biology for domestication of the species;
- endemic disease challenges;
- laws restricting movements of shrimp and disease-free certification;
- the relatively recent nature of shrimp aquaculture (Dunham *et al.*, 2001).

As is the case with fish, domesticated shrimp are more cost-effective than wild strains for aquaculture application, but the reproductive performance of domesticated *Penaeus monodon* is similar to wild broodstock.

Strains of fish exhibit large amounts of variability for many different traits, Channel catfish and rainbow trout strains differ in growth, disease resistance, body conformation, dressed carcass percentage, vulnerability to angling and seining, age of maturity, time of spawning, fecundity, and egg size. Okamoto *et al.* (1993) reported that an infectious pancreatic necrosis virus (IPNV)-resistant strain of rainbow trout showed 4.3% mortality compared with 96.1% in a highly sensitive strain. Other strains of fish in some marine species vary for upper temperature tolerance, loss of swimming equilibrium, and disassociated caudal fin.

7.4 SELECTION

7.4.1 Selective breeding

Research on selection in fish for relevant aquaculture traits began in the 1920s (Embody and Hayford, 1925), but very little selection was conducted prior to 1970. Unfortunately, during this time period, several potentially high-impact experiments did not include adequate genetic controls to prove genetic gain. From 1970 to the present, research on selection and traditional selective breeding has continued

Table 7.3 Examples of improvements from selective breeding in aquacultured species.

Species	Parameter	Number of generations of selection	% improvement
Rainbow trout	Body weight	6	30
Coho salmon	Growth rate	10	50
Atlantic salmon	Growth rate	1	7
Channel catfish	Growth rate	4	55
Brook trout	Resistance to bacterial furunculosis	3	2–69 (survival)
Rainbow trout	Resistance to *Flavobacterium psychrophilum*	1	32
Common carp Vietnamese	Body weight	6	5% per generation
GIFT Nile tilapia	Body weight	8–14	13
Bivalve molluscs	Growth rate	1	8–9
White-leg marine shrimp	Growth rate	1	4.4
White-leg marine shrimp	Resistance to Taura virus	1	12.4% survival

to grow rapidly (Dunham, 1996) despite the excitement about and increased funding in the area of molecular genetics and genomics. In general, the response to selection for growth rate in aquatic species is very good compared to that with terrestrial farm animals and these programs have been highly successful (Table 7.3). Fish and shellfish often have higher genetic variance compared to farmed land animals, and Gjedrem (1997) indicates that the genetic variation for growth rate is 7–10% in farmed terrestrial animals and 20–35% in fish and shellfish. Fecundity is also higher in aquaculture species compared to warm-blooded agricultural animals, allowing for higher selection intensity for aquaculture production improvement, and a few hundred heritability estimates have been obtained for several traits of cultured fish and shellfish (Tave, 1993).

Selection for increased body weight has a high probability of success in the vast majority of aquatic organisms and in the vast majority of strains within a species. Six generations of selection increased body weight by 30% in rainbow trout, an increase of 7% was achieved within a single generation in Atlantic salmon, and an increased growth rate of 50% was achieved with ten generations in coho salmon. Body weight was improved in channel catfish, by 12–20% with one to two generations of genetic selection, and the best line grew twice as fast as typical nonselected strains. After three generations, the growth rate of channel catfish in ponds was improved by 20–30% and this was further increased to 55% after four generations of selection in a Kansas strain of channel catfish (Fig. 7.3).

Selection for body weight has also been successful in marine species such as the gilthead seabream (*Sparus auratus*). Selection improved growth of the marine shrimp, *Marsupenaeus japonicus*, with improvement in the growth, survival and total yields obtained in two selected lines (10–15% increase in mean yields).

Selection is an effective genetic enhancement program to improve growth rates in bivalves. One generation of mass selection for growth rate in Pacific oysters increased growth by 8%, and mass selection of adult oysters gave a strong response to selection for growth rate in *C. virginica*. In other experiments, a 10–20% gain in growth rate of oysters was achieved after one generation of selection. A genetic gain of 9% increased growth rate in Sydney rock oysters (*Saccostrea commercialis*) was achieved in a generation, and in the Chilean oyster (*Ostrea chilensis*). A 9% per generation of selection for growth rate has been estimated for the hard-shell clam or quahaug (*Mercenaria mercenaria*). Rate of genetic improvement in these bivalves appears to be similar to that of finfish.

Selection has been effective for improving disease resistance, but not as consistently as selection for body weight. Strain variation for selection response is more prevalent for disease resistance than for body weight, and often no selection response is found for some strains, while others will exhibit significant enhancement of disease resistance as a result of selection. In the case of salmonids, selection for disease resistance has been particularly successful (Embody and Hayford, 1925). Three generations of selection for resistance to endemic bacterial furunculosis in brook trout (*Salvelinus fontinalis*) improved survival

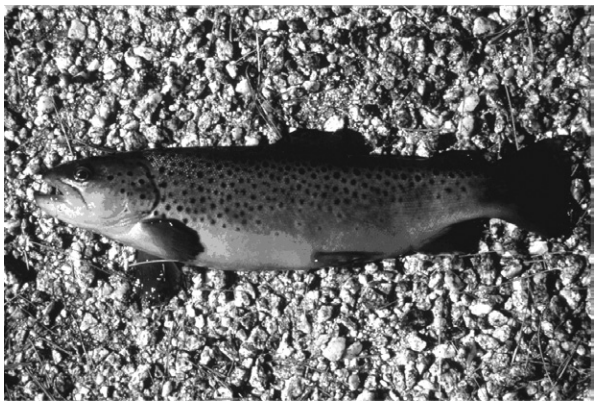


Fig. 7.4 The brown trout, *Salmo trutta.*

from 2% to 69%. Resistance to furunculosis in brown trout and brook trout has been improved via selection (Fig. 7.4). One generation of selection increased resistance to *Flavobacterium psychrophilum* (bacterial cold water disease) in rainbow trout by an absolute 32% and a relative 105%.

Selection for increased disease resistance and survival has also been successful in crustaceans. A response for one generation of selection of 4.4% for growth rate and 12.4% for survival was obtained in the marine shrimp, *Litopenaeus vannamei*, when exposed to Taura syndrome virus. More dramatically, resistance to Taura syndrome virus had an absolute 30% improvement and a relative improvement of 100% with two generations of selection in *L. vannamei*. Additionally, growth and pond survival were improved by 5–6% per generation.

Heritability, additive genetic variation, and selection response can vary among strains for body weight, and tilapia and common carp are a couple of the more prominent examples of this phenomenon. Body weight of common carp initially appeared unresponsive to selection as five generations of selection for increased body weight resulted in no genetic gain, and five generations of family selection resulted in modest gains of about 5–10%. However, in a Czechoslovakian strain of common carp heritabilities for body weight were estimated at 0.15–0.49. Vietnamese common carp had a heritability of 0.3 for growth rate, and six generations of selection increased body weight by 5% per generation.

Mass selection has improved body weight in *Oreochromis mossambicus*, red tilapia, Nile tilapia, *O. shiranus* and *O. aureus* (Dunham *et al.*, 2001). However, selection for increased body weight in red tilapia has been variable. Even greater variability to the selection response has been observed in Nile tilapia, from no response in some strains, 1–7% gain per generation in others, and as much as 11% per generation in the Philippines for GIFT strain. The lack of response in some strains may reflect a narrow genetic base in the founder stock or sole use of mass selection in cases where additive genetic variation was low. Some selection programs for Nile tilapia were moderately successful, for example, 14% body weight increase over two generations for a synthetic Egyptian strain. Selection for increased growth in GIFT Nile tilapia was much more productive, with 77–123% growth improvement. During the first two generations of selection, responses were similar to selection in Nile tilapia grown in low-input environments as were found in the first two generations in the GIFT population, which was selected in a variety of environments. The 11% genetic gain per generation in GIFT tilapia was better than that obtained in most other species of fish, which typically average 5–7% per generation, as demonstrated for salmonids following approximately ten generations of selection. However, other exceptional examples exist such as the channel catfish, which had an increased body weight of 14% per generation over four generations, and the 13–14% increase per generation observed in some cases for salmon.

Response to selection can differ depending on the direction of selection. Body weight of common carp in Israel was not improved over five generations, but could be decreased in the same strain selected for small body size. Virtually identical results for Nile tilapia have also been reported. In general, it is easier to select to make traits smaller rather than larger, which, of course, would rarely have aquaculture significance. There are exceptions to the observations above, as common carp in the Czech Republic responded to selection for increased body weight, but not for decreased body weight.

Body conformation can be dramatically changed via selection. Heritability for body depth is quite high in common carp. Recently, a significant heritability was found for deformities in the Atlantic cod, *Gadus morhua* (Praebel *et al.*, 2009). The implication is that deformity rate could be reduced through selection.

Reproductive traits tend to have high selection responses. Spawning date can be shifted in coho salmon, *Oncorhynchus kisutch*, by about 14 days with four generations of selection.

Long-term selection appears feasible in fish. Ponzoni *et al.* (2009) evaluated the 8th to 14th generations of GIFT Nile tilapia selected for increased body weight. The

Table 7.4 Examples of correlated responses to selection.

Species	Trait selected	Correlated traits		
		Positive	No correlation	Negative
Channel catfish	Increased body weight	Dressout %;[1] feed consumption; FCE[2]	Body composition; seinability[3]	Tolerance of low DO
Atlantic salmon	Growth rate	Feed consumption; FCE		
European whitefish	Growth rate	FCE		
Rainbow trout	Muscle lipid content		Dressout %; fillet %	
Rainbow trout	Bacterial cold water disease resistance		Body weight; thermal growth coefficients	

[1](Body weight without head, viscera and skin) × 100/total body weight.
[2]Food conversion efficiency.
[3]Vulnerability to be seined in harvesting.

response per generation was 13% even after this length of time.

7.4.2 Correlated responses to selection and indirect selection

When selection is conducted upon one trait, positive, negative or no correlated responses to selection can occur for other traits depending upon the nature of genetic correlations among traits (Table 7.4). Additionally, in mass selection programs there is the potential for decrease in performance in some traits, long-term, because of the accumulation of inbreeding. Although selection for body weight has generally been associated with positively correlated responses such as increased survival and disease resistance, in some cases long-term selection results in decreased bacterial resistance either due to changes in genetic correlations or due to inbreeding depression. Increased fecundity, fry survival and disease resistance were correlated to selection for increased body weight in channel catfish after one generation of selection for body weight. Three and four more generations of selection resulted in increased dressout percentage[1], decreased tolerance of low oxygen and no change in body composition or seinability. Progeny from select channel catfish had greater feed consumption, more efficient feed conversion and greater disease resistance than controls.

Atlantic salmon experience a positive correlated response in feed conversion when selected for growth rate. Wild salmon had a 17% higher intake of energy and protein per kg growth compared with fish from the 4th generation selected for growth rate. The wild fish had 8% lower retention of both energy and protein. There are strong genetic correlations between growth rate and feed conversion in European whitefish, *Coregonus lavaretus*, and channel catfish, and the nature of the heritabilities and genetic correlations indicate that indirect selection for feed conversion by selecting for growth rate would more effectively improve feed conversion than direct selection for feed conversion efficiency.

The relationship between body weight and carcass traits is not consistent from one species to another. The nature of the heritabilities and genetic correlations among body weight, visceral fat, muscular fat, muscular moisture, and muscular ash in gilthead seabream, *Sparus auratus*, would allow development of a selection index to improve growth, fat content, texture, and carcass yield simultaneously. Dressout and fillet percentage also had positive heritabilities in gilthead seabream. However, body weight and fillet percentage had a negative genetic correlation, indicating that it might be difficult to simultaneously select for both traits. In the case of sea bass, *Dicentrarchus labrax*, body weight, visceral percentage, visceral fat percentage, fillet percentage, and head weight percentage all had significant heritability. Body weight had a positive correlation with each of these traits except a negative correlation to head weight percentage, indicating selection for increased body weight would also increase fillet perentage, but result in fish with a higher visceral fat percentage. Body weight, percent fat, relative head length, relative body height, relative body width, percent processed body, and fillet yields had moderate to high heritabilities in common carp in the Czech Republic. Body weight was highly correlated with

[1](Body weight without head, viscera and skin) × 100/total body weight.

fat percentage. Relative head length had strong negative correlation with fat percentage, dressout percentage, and fillet percentage. Thus, indirect selection for reduced relative head length should increase fillet percentage and dressout percentage, but also increase fat percentage. Selection for increased body weight would also result in a fattier common carp.

Muscle lipid content in rainbow trout responded to bidirectional selection. Selection for muscle lipid content did not impact dressout percentage or fillet percentage.

A variety of genetic relationships exist among growth and survival traits. Selecting for resistance to bacterial cold water disease resistance in rainbow trout did not affect body weight or thermal growth coefficients. Resistance to furunculosis, infectious salmon anemia, and infectious pancreatic necrosis all had relatively high heritabilities in Atlantic salmon. Additionally, the genetic correlations among these traits were all zero, which should allow simultaneous selection for all three traits without any negative correlated responses to selection.

Heritability for upper thermal tolerance is significant in rainbow trout. Genetic correlation between this trait and body weight was essentially zero, thus no correlated responses to selection would be expected when selecting for either of these traits on the corresponding trait. Melanin deposits of Atlantic salmon were negatively correlated with pericarditis, and pericarditis was not correlated with body weight; however, pericardial fat was correlated with body weight. Shell closing strength has a high heritability in Japanese pearl oysters, *Pinctada fucata*. This trait is correlated with high summer survival as the ability to close the shell tightly appears to be a major survival trait.

7.5 INBREEDING AND MAINTENANCE OF GENETIC QUALITY

Even if genetic enhancement is not a goal, loss of genetic quality and avoiding reduction in performance will always be a goal. It is as important to prevent production losses due to inbreeding as it is to increase production from genetic enhancement. This is especially true for species with high fecundity, such as carp, where few broodstock are necessary to meet demands for fry and broodstock replacement. The detrimental effects of inbreeding are well documented and can result in decreases of 30% or greater in growth production, survival, and reproduction (Dunham, 1996) once the inbreeding coefficients reach 0.125 and 0.250 (levels of the same alleles from a common ancestor). For many traits, as the inbreeding increases, the extent of the inbreeding depression also increases. Inbreeding depression is easily correctible. When an inbred individual is mated to an unrelated individual, the inbreeding coefficient of the progeny returns to 0.0, the effects of inbreeding on the performance of the progeny are also eliminated, and inbreeding depression is also zero. This has been demonstrated in channel catfish. In most aquaculture businesses inbreeding will not become a problem because broodstock populations are relatively large. If 50 breeding pairs are randomly mated per generation, the accumulated inbreeding should not result in inbreeding depression for 25–50 generations. Formulas for calculating inbreeding and determining the impact of random genetic drift are thoroughly discussed in Tave (1993).

7.6 CROSSBREEDING AND HYBRIDIZATION

7.6.1 Intraspecific crossbreeding

The opposite of inbreeding, the mating of related individuals, is crossbreeding, the mating of unrelated individuals. Intraspecific crossbreeding (crossing of different strains, breeds or races) has the potential to increase growth rate and other traits, but heterosis (differences between offspring and parents) may not be obtained in every case. However, intraspecific crossbreeding is a relatively effective genetic enhancement program to improve growth rate, and tends to be highly effective for improving survival-related traits and reproductive performance.

Approximately 55% and 22% of channel catfish and rainbow trout crossbreeds evaluated, respectively, elicited improved growth rate (Dunham, 1996; Dunham and Smitherman, 1983). Chum salmon crossbreeds, however, had no heterotic increases in growth rate (Dunham, 1996). Common carp crossbreeds generally express low levels of heterosis, and only about 5% of the crossed carp that were evaluated had enhanced growth rates. However, those that exhibited positive heterosis are quite important and are the basis for carp aquaculture in Israel, Vietnam, China, and Hungary.

In the case of channel catfish, reciprocal crosses did not perform the same and there appeared to be a maternal effect on combining ability (Dunham *et al.*, 2001).

The crossing of common carp lines in Hungary (Bakos and Gorda, 1995) demonstrates the frequency of success in long-term crossbreeding for this species. During a 35-year period, more than 140 crosses were tested. Three were chosen for culture, based on an approximate 20% improvement in growth rate (and other qualitative features), compared to parent and control carp lines. Approximately 80% of common carp production in Hungary is generated from these Szarvas crossbreeds (Dunham *et al.*, 2001). In Israel, the crossbreeding of the common carp strain,

DOR-70, and the Croatian line, Nawice, resulted in fast growth, and was widely utilized on Israeli farms. The Czech Republic also utilizes improved growing crossbreeds, South Bohemian × Northern mirror carp and Hungarian 15 × Northern mirror. In Vietnam, crossbreeding of eight local varieties of common carp, along with Hungary, Ukraine, Indonesia, and Czech strains resulted in significant heterosis in the F1 progeny. The Vietnamese × Hungarian common carp crossbreed was particularly popular, due to fast growth and high survival rates under different production conditions. Double crosses among Vietnamese, Hungarian, and Indonesian strains have subsequently been used for carp selection and crossbreeding throughout Vietnam because farmers had difficulty maintaining pure parental lines for the crossbreeding.

Heterosis for growth rate, body shape, fillet yield, and visceral body fat percentage has been observed in Nile tilapia. In the case of the silver barb, *Barbodes gonionotus*, 23–35% higher growth rate was found in crossbreeds than the parent strains. Crossbreeds of different strains of European catfish, *Silurus glanis*, have outstanding adaptability under warmwater holding conditions and mixed diet feeding regimes. Crossbreeding can also improve performance in crustaceans and resulted in heterosis for growth rate, but not survival, in Chinese shrimp (*Fenneropenaeus chinensis*) (Tian *et al.*, 2006).

Crossbreeding often improves survival traits. Strains of cold-resistant carp, Ropsha carp, for cold zones in northern Russia have been developed by crossing local carp and Siberian wild carp from the River Amur. Wild strains of common carp are less susceptible to koi herpes virus/carp interstitial nephritis and gill necrosis virus, whereas domestic strains tend to be vulnerable (Shapira *et al.*, 2005). Two domestic strains, two domestic × wild crossbreeds, and one domestic × domestic crossbreed were compared for viral resistance. In the laboratory, the most resistant genotype was one of the domestic × wild crossbreeds and one of the pure strains was the least resistant. The remaining genetic groups were intermediate in viral resistance. When the challenges were repeated in ponds, the results were the same except the other domestic × wild crossbreed had excellent resistance in ponds, although its performance had been intermediate in the laboratory.

Crossbreeding of the walking catfish, *Clarias macrocephalus*, improves resistance to *Aeromonas hydrophila* infections. Crossbreeding improved phagocytosis activity in African catfish, *Clarias gariepinus*, but did not enhance body weight, total length, the specific immune response to *A. hydrophila*, phagocytic index nor male reproductive performance. In the case of channel catfish, reciprocal crosses did not perform the same and there appeared to be a maternal effect on combining ability (Dunham *et al.*, 2001).

Table 7.5 Number of channel catfish from domestic (D) by domestic (D × D) crosses and domestic × wild (W) (D × W) crosses showing positive, negative or no heterosis (hybrid vigour) for growth rate.

	Heterosis		
Cross	Positive	None	Negative
D × D	4	0	1
D × W	2	3	1

Domestication has a strong influence on the success of crossbreeding programs. Domestic × domestic crosses are more likely to result in heterosis than wild × domestic and wild × wild crosses for several traits. Domestic × domestic channel catfish (Table 7.5) and rainbow trout were more likely to exhibit heterotic growth rates than domestic × wild crossbreeds.

Again, crossbreeding does not always result in genetic improvement. No heterosis was observed for reciprocal crossbreeds between a domestic and wild strain of chinook salmon, *Oncorhynchus tshawytscha*, for growth survival, saltwater growth, saltwater tolerance, stress response and recovery, and fecundity. Crossbreeds between wild and domestic Atlantic salmon were intermediate in performance for body weight, condition factor, and sexual maturation (Glover *et al.*, 2009). When wild strains of European sea bass, *Dicentrarchus labrax*, were crossed, large strain differences were obtained for survival, growth, shape, sex ratio, muscular fat content, visceral yield, and spinal deformities, but not fillet yield (Vandeputte *et al.*, 2008). There was no heterosis among these wild strains and no genotype–environment interactions. This helps illustrate the fact that heterosis is less likely to be obtained from wild strains than for domestic strains.

Apparently, domestication also affects the success of crossbreeding in crustaceans as well. When two wild strains of giant freshwater prawn, *Macrobrachium rosenbergii*, were crossed with a domestic strain (Bangkok), large additive strain effects were observed for growth, but no heterosis. Wild strains were involved and little heterosis observed. The domestic strain was the fastest growing of the strains. The most rapidly growing prawns were some of the crossbreeds. Reciprocal crossbreeds did not have the same performance.

One practice is to develop inbred lines to use in crossbreeding programs to obtain heterosis. The existing data indicates that it is unlikely that true genetic gain will be obtained using this strategy. The crossbreeding of inbred lines of Pacific oysters, *Crassostrea gigas*, often resulted in heterosis for growth and survival. Additionally, crossing of inbred lines of Pacific oysters reduces summer mortality. Similarly, the crossing of two inbred lines of cockle, *Fulvia mutica*, resulted in increased shell length and whole body weight, but intermediate survival. It is likely that this is not true genetic enhancement. Bondari and Dunham (1987) demonstrated that inbreeding reduces growth in channel catfish. Crosses of the inbred lines did indeed grow faster than the parental inbred lines. However, the performance of the crossbreeds only negated the inbreeding depression and was equivalent to that of the original population, so no true genetic gain was obtained as performance has returned to the original baseline. Production of gynogenetic female lines and gynogenetic sex-reversed inbred male lines from common carp with the best combining ability was an important part of the Hungarian crossbreeding programs. A higher heterosis was expected from crossing inbred lines, but the growth rate of F1 crossbreeds was only 10% higher than controls.

One small potential impediment to crossbreeding programs is seed production. Strain mating incompatibilities can occur and impede fry output in channel catfish and Nile tilapia and this appears to be more strongly influenced by the female than the male.

7.6.2 Interspecific hybridization

In principle and in genetic basis, interspecific hybridization is similar to intraspecific crossbreeding. This has been a popular breeding program as over the years fisheries biologists have repeatedly tried to combine the best traits of more than one species, mostly with little success (Argue and Dunham, 1999). Interspecific hybridization rarely results in heterosis. However, interspecific hybridization has resulted in fish with increased growth rate (Fig. 7.5), manipulated sex ratios, sterile animals, improved flesh quality, increased disease resistance, improved tolerance of environmental extremes, and other altered traits (Dunham *et al.*, 2001).

Although interspecific hybridization rarely results in an F1 suitable for aquaculture application, there are a few important exceptions to this rule. The channel catfish female × blue catfish, *Ictalurus furcatus*, male is the only hybrid of nearly 50 North American catfish hybrids examined that exhibits superiority for growth rate, growth uniformity, disease resistance, tolerance of low oxygen levels, dressage percentage, and harvestability. This is by far the best genotype for ictalurid farming. Although they do not show heterosis for such a broad spectrum of traits, crosses of the silver carp (*Hypophthalmichthys molitrix*) and bighead carp (*Aristichthys nobilis*), black crappie (*Pomoxis*

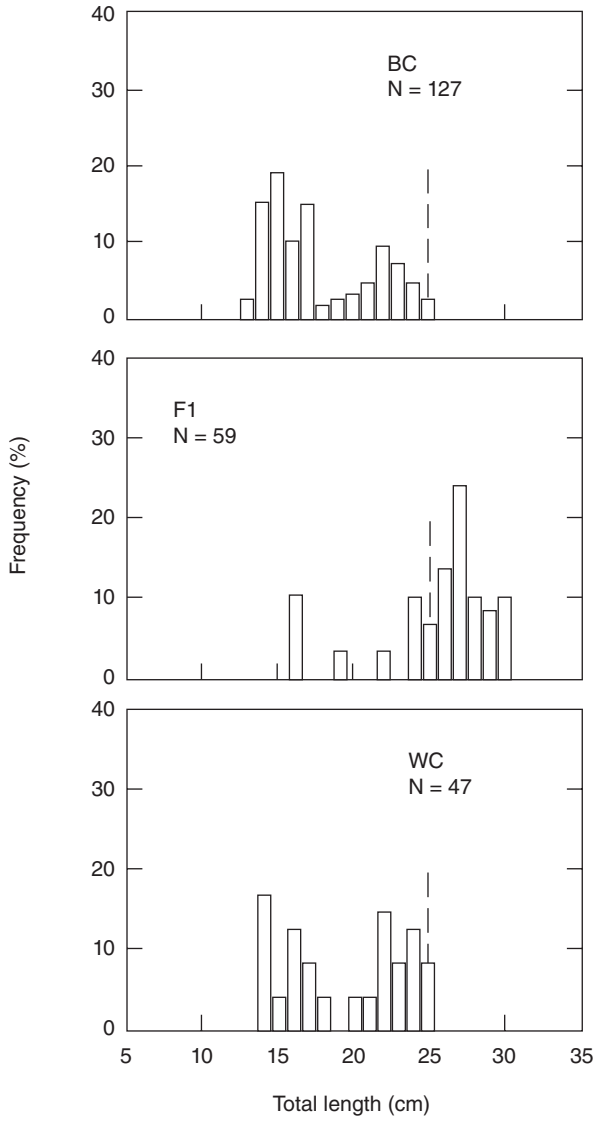


Fig. 7.5 Length-frequency distribution of age1+ (1989 class) black croppies (*Pomoxis nigromaculata*) and white croppies (*P. annularis*) collected in autumn (fall) 1990, showing the increased growth rate of hybrids compared with the parent species. The dotted lines indicate the minimum size for fishing. Reproduced with permission from Taylor & Francis.

nigromaculatus) and *P. annularis* and African catfish hybrids (*Clarias gariepinus, Heterobranchus longifilis*, and *H. bisorsalis*) all exhibit faster growth than parent species. In marine fish, the family Sparidae, hybrids of *P. major* and common dentex, *Dentex dentex*, also grow faster than parental genotypes.

In the case of shellfish, various hybrids between the Thai oysters (*Crassostrea belcheri, C. lugubris*, and *Saccostrea cucullata*) were compared, but no heterosis was observed. However, heterotic pearl production has been achieved in China using interspecific hybridization (Li *et al.*, 2009). The hybrid between the freshwater pearl mussels, *Hyriopsis schlegel* ♀ and *H. cumingii* ♂, increased pearl size by 23%, pearl output by 32%, and the frequency of large pearls by 3.7 times.

Heterosis for a single trait is not necessarily essential for an F1 hybrid or cross to have increased value compared to the parents. The composite performance may make the F1 the culture genotype of choice. The "sunshine" bass between white bass, *Morone chrysops*, and striped bass, *M. saxatilis*, grows faster, with better overall culture characteristics for growth, good osmoregulation, high thermal tolerance, resistance to stress and certain diseases, high survival under intense culture, ability to use soy bean protein in feed, handling tolerance, and angling vulnerability than either parent species. Other examples of crosses that have resulted in improved overall performance in experimental aquaculture conditions include:

- common carp with rohu;
- mrigal (*Cirrhinus cirrhosus*) and catla (*Catla catla*);
- tambaqui (*Colossoma macropomum*) and *Piaractus brachypoma* and *P. mesopotamicus*;
- green sunfish (*Lepomis cyanellus*) crossed with bluegill (*L. macrochirus*);
- gilthead seabream (*Sparus aurata*) with red seabream (*Pagrus major*).

One of the best examples of commercial application of interspecific hybridization is the walking catfish. The primary catfish cultured in Thailand is the hybrid between African (*Clarias gariepinus*) and Thai (*C. macrocephalus*) catfish. Although it does not grow as fast as pure African catfish, it grows faster than the Thai walking catfish and its yellow flesh is still acceptable to Thai consumers, in contrast to the red flesh of the African walking catfish. Another example of a good "compromise" hybrid is the rohu (*Labeo rohita*) × catla (*Catla catla*) hybrid which grows almost as fast as pure catla, but has the small head of the rohu considered desirable in Indian aquaculture. *Catla catla* × *Labeo fimbriatus* (fringe-lipped peninsula carp) hybrids have the small heads of *L. fimbriatus*, plus the deep body and growth rate of catla.

Another potential benefit of interspecific hybridization is that some species combinations result in progeny with skewed sex ratios or monosex progeny. Monosex populations of fish are desirable when growth differences between the sexes, sex-specific products such as caviar are wanted, reproduction needs to be controlled or when other exploitable sexual dimorphism exists. Hybridization in tilapias or centrarchids often results in near monosex hybrids. Hybridization between the Nile tilapia and the blue tilapia, *Oreochromis aureus*, results in predominantly male offspring. Tilapia matings, which produce mainly male offspring, include Nile tilapia × *O. urolepis honorum* or *O. macrochir*, and *O. mossambicus* × *O. urolepis honorum*. Conversely, the hybrid between striped bass and yellow bass (*M. mississipiensis*) produced 100% female individuals.

Theoretically, the production of sterile hybrids can reduce unwanted reproduction or improve growth rate by energy diversion from gametogenesis or reduction in sexual behavior. Karyotype analysis is believed to be a general predictor of potential hybrid fertility. Hybrids of Indian major carps are generally fertile because they share similar chromosome numbers ($2N = 50$). However, when they are mated with common carp ($2N = 102$), the hybrids have what is equivalent to a $3N$ chromosome number and they are sterile (Reddy *et al.*, 1990). A natural triploidy also occurs when crossing between grass carp, *Ctenopharyngodon idellus*, and bighead carp. Grass carp are commonly utilized for aquatic macrophyte control in the US, but there is concern about their establishment in the natural environment, resulting in potential impact on desirable vegetation in the ecosystem. The grass carp × bighead carp is not a viable option for weed control as although this triploid hybrid has reduced fertility, some progeny maintain diploidy and could be fertile. An exception to the chromosome number-fertility rule includes some crosses of sturgeon species with different chromosome numbers that produce fertile F1 offspring.

Hybridization is a good program to improve disease resistance in fish, as is the case for coho salmon (*Oncorhynchus kisutch*) hybrids, which are considered resistant to several salmonid viruses. However, overall viability was poor. Viability increased when hybridization was followed with triploidization. In some cases, the salmon hybrids exhibit the outstanding viral resistance but very poor growth. Triploid Pacific salmon hybrids sometimes show earlier seawater acclimation.

Similarly, increased tolerance of various environmental factors may also be inherited by F1 hybrids when one parent species has a wide or specific physiological tolerance. Several tilapia hybrids display enhanced salinity tolerance. Florida red-strain hybrids (*O. mossambicus* × *O. urolepis hornorum*) can reproduce in salinities as high as 19‰, which is not necessarily a good trait when considering the potential environmental impact.

Just as was the case for intraspecific reciprocal F1 crossbreeds, reciprocal F1 interspecific hybrids usually show different phenotypes and performance. Reciprocal hybrids of *O. niloticus* (N) × *O. mossambicus* (M) demonstrate different salinity tolerances. Genetic maternal effects were evident as the hybrid with the *O. niloticus* mother had a higher survival rate after salinity challenges at 20‰ than pure *O. niloticus*, but lower survival rates than those of the reciprocal hybrid. At 30‰ salinity, a direct transfer killed all tilapia with *O. niloticus* maternal ancestry. Growth rates of N × M hybrids were comparable to those of Nile tilapia, while those of the M × N hybrids and *O. mossambicus* were comparable, but lower, than the first two groups, an additional example of maternal genetic effects.

Backcrosses, MN × N, also showed the highest salinity tolerance (comparable to that of *O. mossambicus*), but no significant differences in salinity tolerances were found in the remaining backcrosses (N × NM, NM × N, N × MN) or pure *O. niloticus*, thus some type of maternal effect from the maternal nuclear genome, cytoplasm or mitochondrial genome continued to be transmitted to the backcross generation. Carcass yield of the backcross hybrids, however, tended to be higher than those of the parent species. Interspecific backcrossing has also been used to successfully introgress genes for cold tolerance and color among closely related tilapia (Table 7.6).

Hybridization among marine species, and among marine and freshwater spawning species, has not shown much promise for developing improved fish for aquaculture application. Reciprocal hybrids between *Sparus auratus* and *Pagrus major* developed vestigial gonads at 2–3 years and were sterile, and no growth or survival superiority was observed compared to the parent species until sexual maturity. Hybridization between European sea bass (*Dicentrarchus labrax*) females and striped bass (*Morone saxatilis*) resulted in viable fry. Only triploid fry survived to 6 months of age and at 8 months, the survivors showed poor growth compared to diploid *D. labrax.* Such hybrids would only be of commercial value where reproductive confinement is needed for ecological reasons and a highly desirable flesh quality was obtained.

Table 7.6 Cumulative mortality from cold exposure for *Oreochromis aureus* (AA), red backcross *aureus* (AR), *Oreochromis niloticus* (NN), and red backcross *niloticus* (RN) illustrating the correlated performance of the red backcross and its associated backcross parent species.

		Cumulative mortality (%)			
Time	Genotypes	AA	AR	NN	RN
1		0	0	7	17
2		0	0	60	80
3		7	10	100	100
4		27	37	100	100
5		100	100	100	100

7.7 CHROMOSOMAL TECHNIQUES

7.7.1 Gynogenesis, androgenesis, and cloning

Gynogenesis and androgenesis are techniques to produce rapid inbreeding and cloned populations. Gynogenetic individuals ("gynogens") produced during meiosis ("meitoic gynogens") are by definition "inbred," since all genetic information is maternal. "Mitotic gynogens" are not homozygous, since crossovers and recombination during oogenesis produce different gene combinations on the chromosomes of the ovum nucleus and nucleus of the second polar body, which is expelled during meiosis. The rate of inbreeding through gynogenesis is roughly equivalent to one generation of full-sib mating. Meiotic gynogens are totally homozygous, with identical genes on each pair of chromosomes. They are more likely to die during embryonic development due to the higher frequency of deleterious genotypes found in 100% homozygous individuals.

Androgenesis, or all-male inheritance, is more difficult to accomplish than gynogenesis, since diploidy can only be induced in androgens at first cell division, a difficult time to manipulate the embryo. Also androgens are totally homozygous, so a large percentage with deleterious genotypes probably die.

Gynogenesis and androgenesis can be used to elucidate sex-determining factors in fish. If the male is the homogametic sex when androgens are produced, the androgens will be 100% ZZ (all male). If the male is the heterogametic sex, XX and YY androgens will be produced, resulting in both sexes.

Fully inbred clonal lines have been produced in zebrafish, ayu, common carp, Nile tilapia, and rainbow trout using both gynogenesis and androgenesis. Technology has not yet been shown to directly target and clone an individual fish. However, two successive generations of mitotic gynogenesis or androgenesis results in a clonal, although randomly generated, population. These individuals within the clonal population should have identical genotypes throughout their entire genome. Since they will be homozygous for sex-determining genes, sex reversal must be used to perpetuate these populations. The performance of individuals within such clones is highly variable. Individuals with extreme homozygosity apparently lose the ability to respond to environmental variables in a consistent, stable manner, and even micro-environmental differences affect performance among individuals. As genetic variation decreases, environmentally induced variation increases, and at a more rapid rate than in heterozygous populations.

7.7.2 Polyploidy

In normal development the diploid egg nucleus undergoes a mitotic division after a sperm penetrates through the outer membrane to fertilize the egg. One of the two 2*n* nuclei resulting from this mitosis is extruded from the egg as the first polar body. The 2*n* nucleus of the egg then undergoes a meiotic division and one of the resulting haploid (*n*) nuclei is extruded as the second polar body. The egg now contains two haploid nuclei: one from the egg and one from the sperm. These fuse to produce a diploid nucleus in a zygote, which then undergoes an initial division into two cells as the first step of embryonic development.

Polyploids, gynogens, and androgens are produced by disrupting the above processes at various stages through shocks (Fig. 7.6). Various chemical, temperature, and pressure shocks are used shortly after fertilization to produce triploidy and shortly before first cell division to produce tetraploidy (Fig. 7.6). The timing of the disruption is criti-

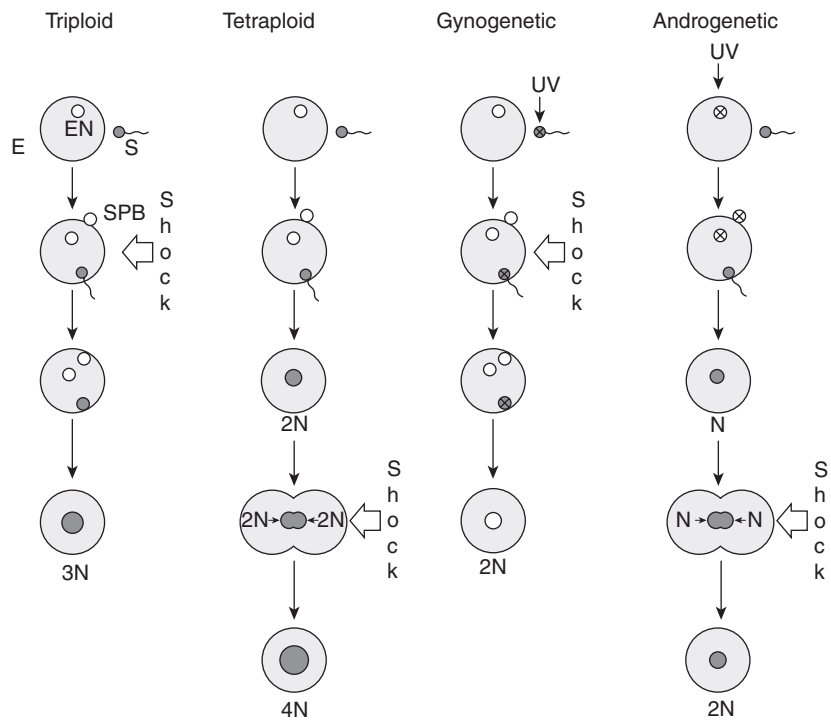


Fig. 7.6 Stages of egg nucleus development when shocks are applied to produce triploids, tetraploids, gynogenetics, and androgenetics. E = egg. EN = egg nucleus. S = spermatozoan. SPB = second polar body. UV = lethal ultraviolet radiation. X = genetic material destroyed by UV irradiation. *N*, 2*N*, 3*N*, and 4*N* = ploidy levels. Redrawn from a figure originally published by Douglas Tave (1990). Chromosomal manipulation. *Aquaculture Magazine*, **16**(1), 62–65. Reproduced with permission from the World Aquaculture Society.



Fig. 7.7 Hydrostatic pressure chamber for inducing polyploidy. It is important to utilize a design that allows the bleeding off of all air to prevent the chamber from exploding and becoming a projectile.

cal and, together with the most effective shock, varies according to the species (Fig. 7.7).

Polyploidy was thoroughly evaluated in fish and shellfish, especially during the period 1970–2000 (Dunham, 2004). Triploid evaluation usually emphasizes the traits of growth, sterility, and flesh quality. Triploid organisms are generally sterile. Females produce less sex hormones and, although triploid males may develop secondary sexual characteristics and exhibit spawning behavior, they are generally unable to reproduce. Triploidy can also be used to restore viability to nonviable interspecific hybrids.

Usually, triploidy will not improve growth rates in finfish until after sexual maturation, which is beyond market size for most species. However, there are exceptions: channel catfish triploids grown in tanks were larger than diploids at about 9 months of age (90 g), which is shortly after the first emergence of sexual dimorphism in body weight. This is not advantageous commercially as triploid channel catfish and triploid catfish hybrids did not grow as rapidly as diploids in commercial environments, such as earthen ponds, and had decreased tolerance of low dissolved oxygen. Triploid salmonid hybrids show similar or slower growth than diploid hybrids, but again may grow faster than controls once they reach maturity. Triploid chinook salmon, *Oncorhynchus tshawytscha*, were less aggressive during feeding than diploid fish, but grew at the same rate as diploids.

In the case of common carp, most 1-year-old triploids had undeveloped gonads and were sterile. The triploids grew slower than their diploid siblings under all conditions investigated. The potential for culture of triploid common carp appears questionable; however, results from India indicate that triploid common carp had a higher dressout percentage than diploid controls, at least partially compensating for the slower growth.

Triploid performance can be influenced by strain and family effects. Diploid Arctic charr, *Salvelinus alpinus*, grew faster than triploids. However, both ploidy level and family affected growth, and family predicted the performance of triploids. Triploids from fast-growing families grew more rapidly than diploids from slow-growing families.

Triploidy can have adverse effects on low oxygen tolerance. Triploid channel catfish and triploid catfish hybrids had decreased tolerance of low dissolved oxygen.

Triploidy generally results in the prolongation of good flesh quality. The flesh of triploid rainbow trout females was superior to that of diploid females because postmaturation changes were prevented. Combining the monosex breeding and triploidy can produce fish with both superior growth rate and flesh quality. The triploid channel catfish had 6% greater carcass yield at 3 years of age, which was well past the time of sexual maturity and market size. However, carcass percentages and resistance to hemorrhagic septicemia (caused by *Aeromonas hydrophila*) were not different between the triploid or diploid Thai walking catfish.

Triploidy can be very beneficial when applied to shellfish culture (Fig. 7.8). Triploid induction in oysters, such as *Crassostrea gigas*, increases their size and flesh quality (Dunham, 2004). Triploid induction in *Saccostrea cucullata* increased the flesh content of the oyster relative to diploid siblings (Kesarcodi-Watson *et al.*, 2001). Triploid oysters do not produce large gonads, increasing marketability and flesh quality. This technique may or may not result in complete genetic sterilization in oysters, as some triploids are able to reverse a portion of their cells back to the diploid state, creating potentially fertile mosaics (Dunham, 2004). Growth performance of sibling triploid and diploid oysters was correlated, but not their ability to reproduce. In regard to summer mortality, performance of triploid Pacific oysters was much more erratic than that of diploids.

Benefits of triploidy are not as straightforward in other species of shellfish. Growth and survival were not different for the blacklip abalone, up to 30 months of age; however, the triploids had a more elongated shell and greater foot muscles than diploids. Triploids had higher feed consumption than diploids, but diploids had superior feed

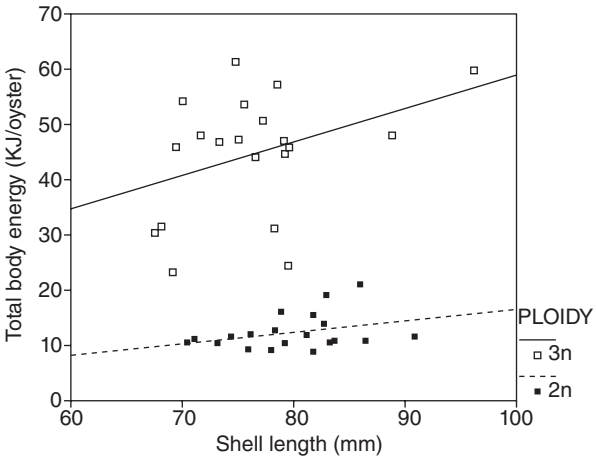


Fig. 7.8 Relationship between total body energy, which to a large extent is soft body tissue, and shell length in adult and triploid oysters of *Saccostrea cucullata*. Each data point is a value for a single oyster. (Reproduced from Kesarcodi-Watson *et al.* (2001) with permission of Elsevier.)

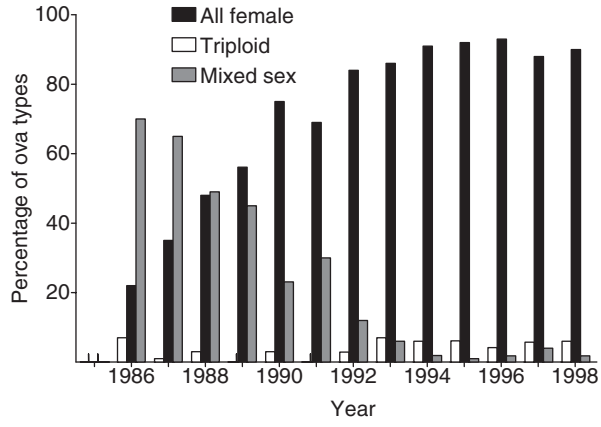


Fig. 7.9 Percentage of all-female, triploid, and mixed-sex rainbow trout, *Oncorhynchus mykiss*, utilized in and from 1986 to 1998, illustrating the increasing and almost exclusive adoption of the all-female production technology. (Figure by David Penman.)

conversion efficiency. Greenlip abalone, *Haliotis laevigata*, had heavy mortality compared to diploids in several life stages. Diploids also grew faster than triploids, although the triploid abalone yielded up to 30% greater meat weight compared to same-length diploid abalone during the spring/summer maturation periods at 36 and 48 months. Diploid abalone produced equivalent meat weights to triploid abalone between the maturation periods. Fatty-acid composition of the meat was the same for triploids and diploids.

In the majority of examples of various species of shellfish, triploidy was beneficial (Dunham, 2004).

Triploidy is the only technique that can guarantee that marine shrimp populations are skewed towards the faster-growing sex, the female. Triploidy is also used to prevent the theft of elite stocks/germplasm. In the case of *Fenneropenaeus chinensis*, triploids had a reduced number of hemocytes. This may be a key for explaining the trend of reduced tolerance of low oxygen in finfish, as well as having implications for crustaceans. The triploid shrimp grew faster during sexual maturation, but not before this time.

Polyploidy is not commercially feasible for all species because the reproductive biology of some species places limitations in artificial propagation technology needed for triploid induction. For instance, mouth brooding of many tilapia, low numbers of eggs per batch, and asynchronous spawning prevents or would greatly impede commercial production of triploid tilapia.

Tetraploidy is extremely difficult to accomplish in finfish. Most tetraploid individuals die as embryos. In the rare cases where a few tetraploids hatched, they were weak, slow growing, had low survival, but were fertile. Tetraploids are viable for shellfish. In this case, they are a valuable tool for crossing with diploids to make triploid populations.

In summary, triploidy is usually not effective for increasing growth rate, but is very effective for sterilization and increasing flesh quality. However, triploidy can be effective for increasing growth past sexual maturation and, in general, is effective for increasing size and growth in shellfish.

7.7.3 Sex reversal and breeding

A variety of strategies and schemes utilizing sex reversal and breeding, progeny testing, gynogenesis, and androgenesis can lead to the development of predominantly, or completely, male or female populations (genetically and phenotypically) and populations with unique sex chromosome combinations. The goals of this strategy are to take advantage of sexually dimorphic characteristics such as growth and flesh quality, control reproduction or prevent establishment of exotic species. All-female populations have been successfully developed for salmonids (Fig. 7.9),

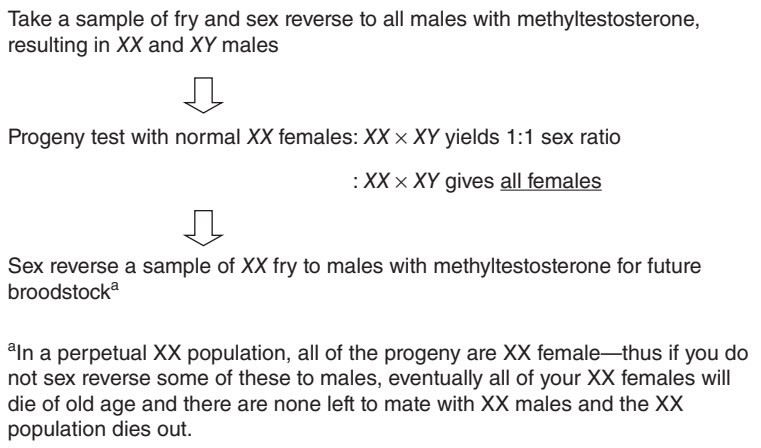


Fig. 7.10 Scheme for producing all-female XX populations of fish.

cyprinids, and tilapia using the scheme presented in Fig. 7.10. Populations of YY males have been established for Nile tilapia on a commercial scale and on an experimental scale for channel catfish (Dunham, 1996), and the procedure is illustrated in Fig. 7.11. Genetic production of monosex populations has the advantage of reduced hormone use compared to direct sex reversal using hormones, which of course has environmental and regulatory implications.

Sex-determining mechanisms were reviewed by Devlin and Nagahama (2002), Dunham *et al.* (2001), and Dunham (2004). While many commercially cultured families exhibit the usual XX/XY sex determination mechanism (carps, salmonids), where XX are females and XY are males, others may be sequential hermaphrodites (changing sex as they mature), such as gilthead seabream and groupers, or have temperature-controlled sex determination in addition to an XX/XY mechanism, such as in Nile tilapia and hirame. Different mechanisms may also be found in closely related species:

- The Nile tilapia has the XX/XY system with the female being homogametic, XX, and the male XY.
- The blue tilapia has a WZ/ZZ system with the male being homogametic, ZZ, and the female, WZ.

Similar differences in closely related species are likely to exist for centrarchids, ictalurids, and perhaps others. Additionally, sex determination has polygenic influences in some species.

Sex ratios of Nile tilapia at 36 °C become a quantitative trait. Three generations of selection for maleness resulted in 93% male progeny, whereas selection for femaleness resulted in a sex ration of 1 : 1. Heritability was high for maleness. The response to selection for femaleness was the result of a lower heritability coupled with maternal effects.

Sex reversal and breeding has allowed production of YY channel catfish males that can be mated to normal XX females to produce all-male XY progeny. Males that are XY can be turned into phenotypic females by use of sex hormones and can then be used as breeders. The sex ratio of progeny from the mating of XY female and XY male channel catfish was 2.8 males/1 female, indicating that most, if not all, the YY individuals are viable. All-male progeny are beneficial for catfish culture, since they grow 10–30% faster than females. YY males are also viable in salmonids, Nile tilapia, goldfish, and channel catfish. The channel catfish YY system has stalled, however, because YY females have severe reproductive problems, and large-scale progeny testing is not economically feasible to identify YY males. A combination of sex reversal and breeding to produce all-female XX rainbow trout is now the basis for stocking most of the culture industry in the United Kingdom, as is the case for the chinook salmon industry in Canada. All-female populations are desirable, in this case, because males undergo maturation at a small size and have poorer flesh quality. Monosex chinook (*O. tshchawytscha*) and coho crossed with chinook have also been produced.

YY male Nile tilapia were as viable and fertile as XY males, and capable of siring 96% male offspring. YY genotypes can be feminized to mass-produce YY males with YY × YY matings, thus eliminating the need for

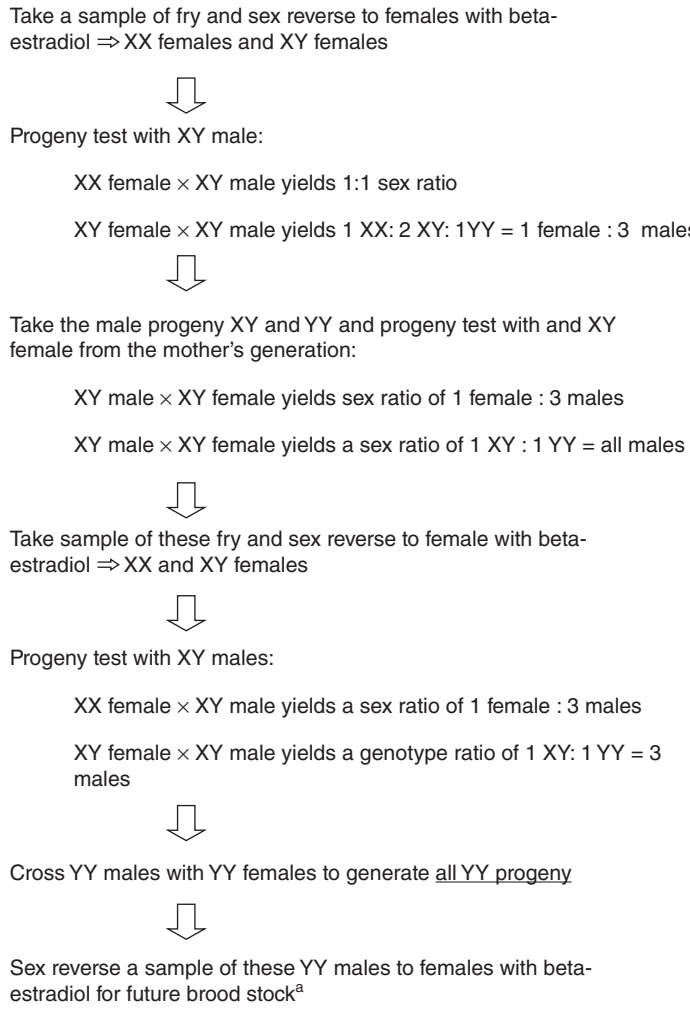


Fig. 7.11 Scheme for producing all YY male populations of fish.

time-consuming progeny testing to discriminate XY and YY male genotypes. This has enabled the production of YY males and then all-male progeny, XY, after crossing with normal XX females. These normal all-males derived from the YY males are sold commercially as "genetically male tilapia" [GMT®] to distinguish them from sex-reversed male tilapia. The YY male technology provides an effective solution to culture problems with early sexual maturation, unwanted reproduction, and overpopulation.

Sex ratios vary widely between spawnings of Nile tilapia, but at the population level, they maintain a normal distribution of around 1 : 1 males to females. Sex ratios vary among strains of Nile tilapia and greater heterogeneity was found in the sex ratios of families collected from a mix of strains, some of which were introgressed with *O. mossambicus*. YY males crossed with XX females produce 95–100% males, and Scott *et al.* (1989) observed no females from the mating of 285 progeny of a single YY

male crossed to ten separate females, indicating the potential to select for lines that can produce 100% males. In fact, three generations of gynogenetic *O. niloticus* have been produced, and males from this line were used for mating with gynogenetic *O. niloticus* females, resulting in consistent production of 100% males.

YY-GMT® technology has commercial application, since YY Nile tilapia, unlike channel catfish, can be sex reversed to produce functional females. The progeny of the YY-GMT® males increase yields by up to 58% compared to mixed-sex tilapia of the same strain as well as greater yields than sex-reversed male tilapia. In addition, YY-GMT® fish have more uniform harvest size, greater survival, and better food conversion ratios. Although the development process is time-consuming and labour-intensive, once developed the production of monosex males can be maintained through occasional feminization of YY genotypes and existing hatchery systems without any special facilities or labour requirements. YY male technology has been widely disseminated in the Philippines since 1995, Thailand since 1997 and, to a lesser extent, in a number of other countries including Vietnam, China, Fiji, and the United States (Dunham *et al.*, 2001). In the Philippines and Thailand, broodstock are distributed from breeding centers to accredited hatcheries to maintain quality control and proprietary control. The availability of further improved GMT®, along with increasing resistance to use of hormones in aquaculture, should allow this technology to impact tilapia culture on a global scale.

In Israel, all-female common carp populations have been established using sex-reversed XX gynogenetic females crossed to males, and using these XX males for breeding. All-female offspring were released to commercial farms and resulted in 10–15% yield improvement over existing commercial stocks. Gynogenesis and sex reversal have also successfully induced *Morone* spp. to produce monosex populations to avoid limitations on introductions to areas where this species is exotic.

Monosex female Java barb are another example of sex manipulation and progeny testing being adapted to a commercial scale over a relatively short period (8 years) in Thailand and Bangladesh. Gynogenetic Java barb were all female and were hormonally masculinised. Most of the breeding of the resultant neomales produced all, or nearly all, female progeny. These gave greater yields in pond culture than mixed-sex batches and, perhaps surprisingly, had higher survival rates than the mixed-sex fish. Monosex female fingerlings from neomale broodstock are now supplied on a commercial scale in Thailand.

7.8 MOLECULAR AND GENOMIC TECHNIQUES

7.8.1 Gene transfer/genetic engineering

7.8.1.1 Early deployment

Transgenic fish have been developed that have improved growth, color, disease resistance, survival in cold and body composition, and that can produce pharmaceutical proteins. Transgenes elicit pleiotropic effects, some positive and a few negative, but most of the negative effects appear to lower fitness traits, which is positive for biological containment. Transgenic fish appear to pose little environmental risk, but this research is not fully conclusive. Transgenic zebrafish with altered coloration have been commercialized and growth hormone gene transgenic salmon, carp, and tilapia are near commercialization. To expedite commercialization and minimize environmental risk, transgenic sterilization needs to be developed. When transgenic fish research was initiated, a large percentage of the work was actually conducted on commercial aquaculture species, but an increasing amount of research is conducted with model species. Potential positive impact of transgenic fish appears likely in many arenas.

Norman Maclean and S. Talawar of Southampton University, UK, were the first researchers to inject cloned genes (Fig. 7.12) into fish (rainbow trout) eggs (Maclean

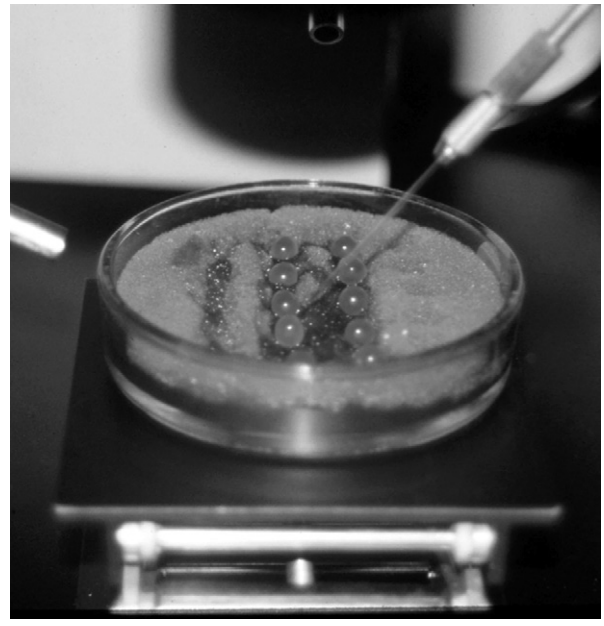


Fig. 7.12 Microinjection of DNA into salmon eggs. (Photograph by courtesy of Robert Devlin.)

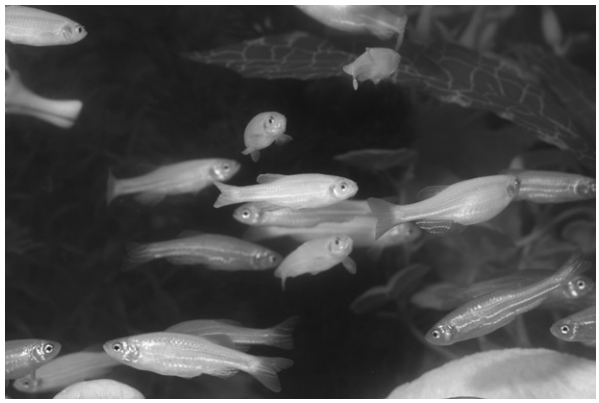


Fig. 7.13 GloFish. (Photograph from www.glofish.com)

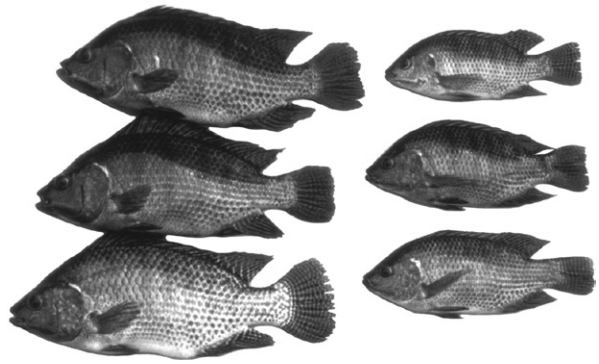


Fig. 7.14 Growth-hormone-transgenic Nile tilapia, illustrating a 2- to 4-fold body-weight enhancement compared with non-transgenic controls. (Photograph by courtesy of Norman Maclean.)

and Talawar, 1984). This was followed by Zuoyan Zhu at the Institute of Hydrobiology in China who reported production of a transgenic fish (Zhu, *et al.*, 1985). Twenty-four years later, transgenic fish application sits not only on the cutting edge, but on the regulatory edge of making its first and major impact. The first application has actually been in the ornamental fish industry rather than in general aquaculture. GloFish (Fig. 7.13), a transgenic zebrafish, *Danio rerio*, containing fluorescent protein, genes GFP, YFP, and RFP, is now commercialized. These fish were actually an output of experiments to develop transgenic fish for environmental monitoring, but were an obvious choice to create a marketing niche in the ornamental fish trade. Commercialization of transgenic fish was thought to have taken place in some countries such as China and Cuba; however, no official documents are available to confirm this, and government representatives indicate that approvals are still pending.

The greatest amount of work has focused on transfer of growth hormone (GH) genes. Due to the lack of available fish gene sequences, transgenic fish research in the mid-1980s employed existing mammalian GH gene constructs, and promoters from viral, bacterial, and mammalian sources. In the early 1990s most GH research then switched to using fish GH constructs. Growth (size and rate) enhancement has ranged from 0% up to a surprising 300% under some conditions (Fig. 7.14). All fish GH constructs introduced into salmonids elevated circulating GH levels by 40-fold in some cases, and induce 5- to 11-fold increases in weight after one year of growth. Precocious smoltification (physiological adaptation from freshwater to seawater) also occurs.

Transgenic alteration of the nutritional characteristics of fish could be beneficial for consumers, and it is now possible to directly alter body composition via transgenesis. Zebrafish transfected with *B-actin*-salmon desaturase genes had enhanced levels of omega-3 fatty acids, docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), in their flesh.

7.8.1.2 Bioreactors

Transgenic mammals such as cows, goats, sheep, and rabbits have been used as biological factories to produce pharmaceutical compounds and biomedical proteins such as clotting factors and blood thinners. Such technology is especially important in the modern world since human extracted products have the potential to be contaminated with HIV, hepatitis viruses, and other human pathogens. These products can also be quite expensive. Transgenically produced biomedical compounds should be safe from human pathogens, should eventually be less expensive, and more widely available.

Fish have potential advantages as bioreactors compared to mammals. These advantages include a short generation interval, low cost of maintenance of the animals, easy maintenance, large numbers of individuals, high density culture, and mammalian viruses and prions are not found in fish. Several examples are now available demonstrating the potential of fish as bioreactors for medical products as well as compounds that can be used in fish spawning:

- CMV-human coagulation factor VII was produced in transgenic zebrafish, African walking catfish, and Nile

Table 7.7 Enhanced resistance to bacterial disease demonstrated by transgenic channel catfish containing cecropin genes in a natural epizootic and an artificial tank challenge.

Transgene	Disease challenge	Survival %		
		Environment	Transgenic	Control
Preprocecropin B	*Flavobacterium columnare*	Pond	100	27
Cecropin B	*Edwardsiella ictauri*	Tank	41	15

tilapia eggs. Clotting activity was detected, indicating proper post-translational modifications. Proteins could be collected in eggs, serum or possibly different proteins in different tissues for other types of genes.

- Transgenic Nile tilapia secreted human insulin in Brockmann Bodies. Islet tissue was used for xenotransplantation and successfully transferred to diabetic nude mice, reversing the effects of diabetes.
- Single chain goldfish luteinizing hormone (LH) gene was injected into rainbow trout eggs. At 4 days of age goldfish LH was isolated from the eggs and the recombinant LH injected into goldfish. Testosterone levels were elevated in male goldfish after the injections, proving biological activity.

7.8.1.3 Disease resistance

Transgenic fish with enhanced disease resistance would increase profitability, production, efficiency, and the welfare of the cultured fish. Genetic gain is also possible through traditional selective breeding, but it appears that the rate of genetic improvement and the consistency of genetic improvement may be greater with the transgenic approach for disease resistance. Selective breeding may also have the drawback that the disease organisms may well respond to selective forces as well, negating some of the selection response in the fish.

Expression of viral coat protein genes or antisense of viral early genes has improved viral resistance in rainbow trout. Shrimp have been genetically engineered with antisense Taura syndrome virus-coat protein gene, resulting in a doubling of the resistance to this disease.

Resistance to bacterial disease may be easier to genetically engineer than for diseases caused by other classification of pathogens, and is more well-studied. Bacterial disease resistance may be improved up to 3- to 4-fold through gene transfer. One approach that has been utilized is the transfer of antibacterial peptide genes.

Insertion of the lytic peptide, cecropin B construct driven by the CMV promoter, enhanced resistance to bacterial diseases such as columnaris and enteric septicemia of catfish 2- to 4-fold in channel catfish, *Ictalurus punctatus* (Table 7.7). A greater percentage (100%) of transgenic individuals containing preprocecropin B construct survived than non-transgenic controls (27.3%) during an epizootic of *Flavobacterium columnare* in an earthen pond. In this case, the transgene appears to have imparted complete resistance.

Also, a greater percentage (40.7%) of transgenic individuals containing cecropin B construct survived than non-transgenic controls (14.8%) when challenged with *Edwardsiella ictaluri*, causative agent of enteric septicemia of catfish, in tanks. There were no pleiotropic effects, and growth rate of the transgenic and non-transgenic siblings was not different.

Transfer of cecropin genes to medaka resulted in increased resistance to *Pseudomonas fluorescens* and *Vibrio anguillarum* which killed about 40% of the control fish in both cases, but only 0–10% of the F2 transgenic fish were killed by *P. fluorescens* and about 10–30% killed by *V. anguillarum*. Family variation was observed, and family variation can be extreme for transgenic fish potentially because of differences in genetic background, variable insertion sites, copy number, epistasis, and other factors. This necessitates the coupling of selection with gene transfer to obtain maximum genetic gain from the gene transfer.

Grass carp, *Ctenopharyngodon idellus*, have been transfected with carp *B* actin-human lactoferrin gene resulting in P1 individuals that were more resistant to *Aeromonas*, and exhibited enhanced phagocytosis and more viral resistance than controls. Japanese flounder keratin promoter linked to both the hen egg white (*HEW*) lyoszyme gene and green fluorescence protein (*GFP*) gene, and transferred to zebrafish resulted in F2 transgenic zebrafish with lytic activity of protein extracts from the liver 1.75 times higher than in the wild type zebrafish. In a challenge experiment, 65% of the F2 transgenic fish survived an infection of *Flavobacterium columnare* and 60% survived

an infection of *Edwardsiella tarda*, whereas 100% of the control fish were killed by both pathogens.

7.8.1.4 Pleiotropic effects

To date, two types of transgenic processes have been successful for improving disease resistance: blocking viruses with antisense and overexpressing antibacterial compounds from distant taxa. This is the extent of reported research using transgenesis to directly improve disease resistance. However, transfer of other genes can indirectly affect disease resistance through pleiotropy both in a positive and negative way. If the pleiotropic effects are known, they could be intentionally manipulated for genetic gain in a manner analogous to indirect selection.

There have been attempts to improve cold resistance in fish. Early research involved the transfer of the antifreeze protein gene of the winter flounder. The primary purpose of this research was to produce salmon that could be farmed under Arctic conditions, but expression levels obtained have been inadequate for increasing cold tolerance of salmon. However, preliminary results with goldfish show some promise for increasing survival within the normal cold temperature range.

The insertion of a transgene, a single gene, can affect more than one trait through pleiotropic effects. If the breeder is fortunate, these pleiotropic effects will impact other traits in a positive way. Transfer of growth hormone genes has been documented to affect body composition, body shape, feed conversion efficiency, disease resistance, reproduction, tolerance of low oxygen concentrations, carcass yield, swimming ability, and even predator avoidance.

Increased growth rate in transgenic individuals may reflect increased food consumption, feed conversion efficiency, or both. Fast-growing common carp containing the rtGH gene were found to have a higher feed conversion efficiency than controls. Additional transgenic common carp families demonstrated increased, decreased, or unchanged food consumption, but had improved feed conversion efficiencies. rtGH transgenic common carp had more protein, less fat, and less moisture than non-transgenic full-siblings (about a 10% change). Growth hormone promotes synthesis of protein over fat, thus the protein/lipid ratio is higher in transgenic fish with elevated growth hormone.

Increased protein levels in the muscle of transgenic common carp also increased levels of amino acids; however, amino acid ratios and fatty acid ratios are virtually identical in non-transgenic and transgenic common carp. Fecundity or precocious sexual development appear to be unaffected by insertion of rtGH in common carp; however, transgenic male tilapia show decreased sperm production. Body shape of common carp is also changed by insertion of *rtGH* genes. Transgenic individuals have relatively larger heads and deeper and wider bodies and caudal areas compared to controls. These morphological changes do not affect condition factor, but do improve the dressing percentage.

Endocrine stimulation can lead to excessive, deleterious deposition of cartilage in some GH transgenic salmon, analogous to mammalian acromegaly. The effect can be severe enough to impair feeding and respiration, reducing growth and viability. Thus the fish with the greatest growth enhancement are those that were only moderately stimulated, and hyper-levels of expression can be detrimental.

Survival is an important commercial trait, and the insertion of the *rtGH* gene altered the survival of common carp. F2 progeny inheriting this transgene had higher survival than controls when exposed to a series of stressors and pathogens such as low oxygen, anchor worms, *Lernia, Aeromonas*, and dropsy. GH transgenic common carp had higher lysozyme activity in the serum compared to age-matched non-transgenic control fish. The serum bactericidal activity in the transgenics was 20% higher than in the controls. Values for leukocrit and phagocytic percent of macrophages in head kidney were higher in transgenics than controls, but the phagocytic indices and relative spleen weights in the transgenics and the controls were not different. GH transgene expression apparently not only stimulated growth, but also the non-specific immune functions of common carp.

Conversely, GH transgenic salmon were more sensitive to *Vibrio* compared to controls, so GH gene transfer does not always confer increased disease resistance. Survival among GH salmon families was sometimes improved, sometimes decreased, and sometimes unchanged relative to controls. These differences in salmon could be related to alterations in expression for a myriad of disease-related genes in relation to the altered growth hormone expression. Growth hormone appears to have pleiotropic effects and causes a cascade of events in a large number of biochemical pathways. Heme oxygenase, acyl-coA binding protein, NADH dehydrogenase, mannose binding lectin-associated serine protease, hemopexin-like protein, leukocyte-derived chemotaxin-2 (*LECT2*), and many other genes had enhanced expression in hepatic tissue of immature transgenic salmon, while complement C3-1, *lectin*, *rabin*, alcohol dehydrogenase, Tc1-like transposase, and *pentraxin* genes had decreased expression compared to non-transgenic controls. Gene expression pattern changed

when transgenic salmon approached maturation with hemopexin-like protein, heme-oxygenase, inter alpha-trypsin inhibitor, *LECT2*, GTP cyclohydrolase I feedback regulatory protein (GFRP), and *bikunin* having enhanced expression and lectin, apolipoprotein, and pentraxin exhibiting depressed expression. *Lectin* was found to be highly suppressed in all F2 and immature F3 salmon. Serum lysozyme activity of the innate immunity system was decreased in both generations of GH transgenic fish. GH transgenic amago salmon had altered hepatic gene expression relating to iron metabolism and innate immunity.

GH gene affects respiration, which in turn could have a multitude of intertwined relationships with and effects on growth, low oxygen tolerance, disease resistance, stamina, and predator avoidance. When subjected to low dissolved oxygen, 0.4 ppm, mean absolute survival was the same for transgenic rtGH and control common carp. However, when mean survival time was calculated, the transgenic individuals had longer mean survival time than the non-transgenic full-siblings. Ventilation rate could also be a possible explanation for the slightly better tolerance of low oxygen exhibited by the transgenic common carp. Transgenic channel catfish with the same rtGH construct as the common carp had a lower ventilation rate when subjected to low dissolved oxygen compared to controls.

Pleiotropy of GH gene for oxygen tolerance characteristics varies from one species to another. GH tilapia have a 58% higher metabolism than controls, compensate for oxygen consumption, and have the same maximum swim speed as non-transgenics. GH tilapia tolerate hypoxia equally as well as controls despite higher demand for oxygen.

GH transgenic salmon have an increased need for dissolved oxygen; however, after 4 days of starvation, GH individuals had the same oxygen uptake as controls. After feeding, GH transgenics had 40–70% increased oxygen demand even when controls consumed equivalent amounts of feed. Adult transgenics had higher oxygen demand, poorer swimming ability, and longer recovery time compared to ocean ranched salmon.

GH transgenesis can have significant metabolic costs. Cardiac function was enhanced by GH transgenesis, but universal upregulation of cardiorespiratory physiology in post-smolt (adult) GH transgenic salmon did not occur. Differences in arterial oxygen transport such as cardiac output and blood oxygen carrying capacity are important for aerobic capacity; however, diffusion-limited processes may be bottlenecks that would need to be enhanced to achieve substantial improvements in metabolic and swimming performance. These diffusion-related limiting factors associated with gill function and morphology may explain differences in results from one study to another.

GH gene transfer alters respiration and metabolism in many ways, which could affect the ability to overcome diseases either in an enhanced or detrimental manner. The results from common carp indicate that GH transfer could be used as an indirect method to transgenically enhance disease resistance. The salmon situation may be different because of their different life history, the fact that they are coldwater rather than warmwater fish, and their GH enhancement is much more dramatic. The extent of pleiotropic effects is likely to be a product of the magnitude of the change in the primary target trait and the associated expression strength of the transgene.

7.8.2 Genomics and QTL mapping

Aquaculture genomics has generated an explosion of information during the past 15 years. Framework linkage maps have been constructed with large numbers of markers; in particular, type I markers of known genes have been generated for a number of aquaculture species. Normalized cDNA libraries for EST analysis and functional analysis have been constructed. Functional genomics has advanced rapidly and the knowledge of gene expression responsible for growth, disease resistance, and response to cold temperature and other traits has been greatly expanded for aquaculture species, utilizing tools such as EST analysis and microarrays. Radiation hybrid panels in tilapia and analysis of BAC libraries in catfish have greatly advanced the area of physical mapping of fish genomes. For some aquaculture species such as catfish and salmon, the majority of the genes have been isolated and cloned. Catfish have about 30 000 genes of which currently around 50% are of known function. Sequencing of fish genomes is well advanced for some aquaculture species and is nearing completion in some cases. This progress in the past 15 years is quite remarkable.

In aquaculture species, initial efforts have begun in QTL mapping. QTL markers for growth, feed conversion efficiency, tolerance of bacterial disease, spawning time, embryonic developmental rates, and cold tolerance have been identified in channel catfish, rainbow trout and tilapia. Putative linked markers to the traits of feed conversion efficiency and growth rate have been identified for channel catfish (Dunham, 2004). In trout and salmon, a candidate DNA marker linked to infectious hematopoietic necrosis (IHN) disease resistance has also been identified and for IPN disease in Atlantic salmon. A single IPN QTL found on LG1 accounts for most of the variation in IPN resistance and a highly resistant line can be developed by

selecting this marker. QTL maps have been developed that have multiple markers for bacterial disease resistance in Japanese flounder as well as for body weight, total length, and a variety of body conformation traits in channel catfish and body weight in Asian seabass, *Lates calcarifer*. A single nucleotide polymorphism (SNP for *RuvB-like protein* in giant tiger shrimp is associated with fast growth rate.

7.8.3 Marker-assisted selection

Marker-assisted selection programs have been successfully evaluated in various animal and plant systems. Much theoretical research has been conducted which indicates that marker-assisted selection has the potential to greatly accelerate genetic improvement in breeding programs. Initial experiments with corn, tomatoes, barley, pigs, and dairy cattle have all given positive results indicating that the utilization of DNA and protein markers has the potential to accelerate genetic improvement in various crops or terrestrial animals.

However, marker-assisted selection is not always the most efficient or cost-effective method. These initial experiments indicate that, when heritability for a trait is high, marker-assisted selection does not provide any faster rate of genetic gain than traditional selection. However, when heritability is low, the rate of genetic gain obtained from marker-assisted selection can be substantially higher than that for traditional selection. Theoretically, new schemes based on whole genome selection may enhance rate of genetic gain even for traits with high heritability.

Marker-assisted selection has not been broadly applied in fish. However, marker-assisted selection has led to the development of a line of lymphocystis disease-resistant Japanese flounder, *Paralichthys olivaceus*. These fish were widely applied on farms and demonstrated high levels of disease resistance and survival. Marker-assisted selection may be a mechanism to improve the efficiency of monosex male production in Nile tilapia. A microsatellite marker has been found on linkage group 23 that is associated with sex determination.

7.8.4 Combining genetic enhancement programs

The best genotypes for aquaculture applications in the future will be developed by using a combination of traditional selective breeding, the new biotechnologies, and molecular/genomic approaches. Initial experiments indicate good potential for this combined approach, with examples using mass selection and crossbreeding, genetic engineering and selection, genetic engineering and crossbreeding, and sex reversal and polyploidy all working more effectively in combination than alone to improve traits such as growth, sterility, and flesh quality.

7.8.5 Genotype-environment interactions

The best genotype for one set of environmental circumstances is not necessarily the best genotype for a second set of environmental circumstances. Genotype–environment interactions occur either when the value of the genotypes change in rank or the relative value of two genotypes substantially change in relation to each other (Dunham, 2004) (Figs 7.15a and 7.15b). Genetically improved animals that work well in a research environment may not necessarily be the best performers under commercial conditions. In general, genotype–environment interactions increase for aquacultured animals with

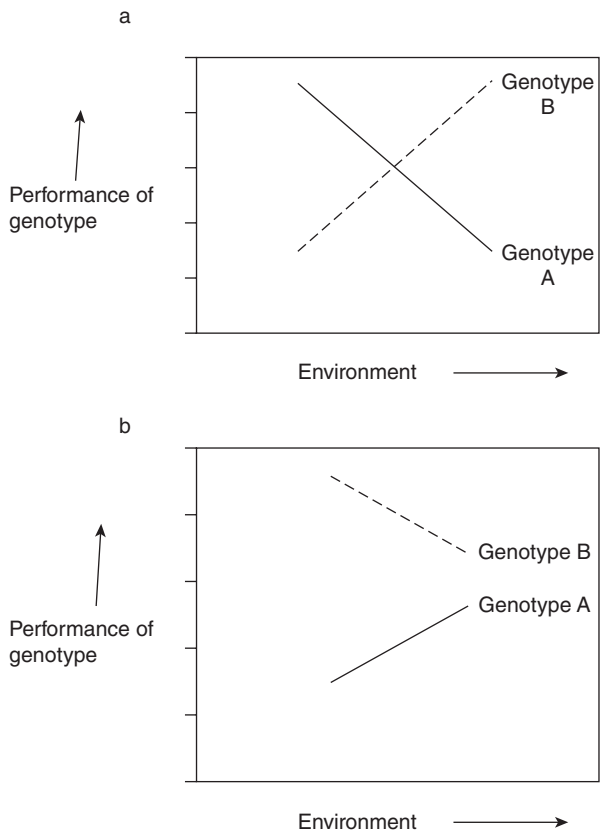


Fig. 7.15 **a**. Genotype–environment interaction occurs when the rank of genotypes changes. **b**. Genotype–environment interaction: rank of genotypes does not change, but the magnitude of the performance difference changes.

increasing genetic distance and increasing environmental differences, especially associated with species such as carp or tilapia that can be cultured simply and low on the food chain or with complete artificial feeds.

Heritability for body weight was higher for the marine shrimp, *Litopenaeus vannamei*, when grown at high densities compared to low densities. Thus, choice of the environment and the subsequent genotype–environment interaction could have impact on the success of selection programs.

Genotype–environment interactions occur in F2 transgenic zebrafish harboring Japanese flounder keratin promoter-hen egg white (HEW) lyoszyme transgene when challenged with either *Flavobacterium columnare* or *Edwardsiella tarda*, under varying challenge conditions. Genotype–environment (nutrition) interactions were also observed when European seabass were fed on fish meal vs. plant-based diets. No genotype–environment interactions were observed for total body weight, foot color, and epipodium pattern in families of greenlip abalone, *Haliotis laevigata*, grown in varying flow rates.

7.8.6 Xenogenesis

Xenogenesis is defined as an organism comprised of elements typically foreign to its species, or a method of reproduction in which successive generations differ from each other. This technology has recently been accomplished in fish by Japanese scientists, and has many potential genetic applications. This procedure is a powerful tool for genomics research, gene knockout applications, cloning, and reviving extinct lines and species.

Xenogenesis is a new biotechnological tool that allows one to interspecifically transfer embryonic stem (ES) cells. Totipotent spermatogonia from the testes of diploid rainbow trout were intraperitoneally transplanted into newly hatched sterile triploid masu salmon, *Oncorhynchus masou*, resulting in xenogenic individuals. Upon maturity, these triploid salmon produced only donor-derived, pure, rainbow trout offspring.

Testes contain six major types of cells: primordial germ cells, spermatogonia A, committed spermatogonia B, spermatids, mature sperm cells, and somatic cells. An isolated germ cell within a spermatocyst is a type A spermatogonium, and these cells possess stem cell potential. These cells divide during spermatogenesis producing isogenic germ cells committed to meiosis. Spermatogonia A from fish were stem-cell-like, and when transplanted into developing rainbow trout, *Oncorhynchus mykiss*, embryos, the embryos produced sperm or eggs derived from the transplanted cells. Similar results were previously found for *Drosophila* male germ-line stem cells, which can regenerate by spermatogonial dedifferentiation.

Both of these cell types, PGCs and spermatogonia A, have been transplanted from a donor species to a related host species with the recipient species producing sperm and eggs (originating from testicular PGCs or SSCs) of the target species. This procedure can be successful, utilizing both cryopreserved or fresh donor cells, opening the possibility of many potential applications. In the case of the salmonid xenogens, testicular development was normal. A 30–70% success rate was achieved among injected host embryos. The xenogenic individuals did have reduced fecundity (possible age effects), but the F1 offspring were normal in genotype, performance, and appearance.

Alternative methods also have the potential to allow xenogenesis and autogenesis via PGC transplantation. A single PGC from pearl danio, *Danio albolineatus*, was transplanted into the blastula of a zebrafish whose native PGC production had been knocked out by an antisense morpholinos oligonucleotide against *dead end.* The donated PGC formed a single testis that produced pearl danio sperm. Xenogenic pearl danio males were sex-reversed to femaleness and mated with untreated males to produce normal, fertile pearl danio offspring. Similarly, the zebrafish host was able to develop goldfish, *Carassius auratus*, and loach, *Misgurnus anguillicaudatus*, testis that produced donor sperm from the injection of a single donor PGC (Zhu *et al.*, 1986). Normally, a few dozen PGCs are needed to form gonads containing germ cells. This study showed that one PGC and perhaps a single SSC are capable of producing a single testis. The next step is to determine whether xenogenesis can be applied on a large scale.

7.9 FUTURE DEVELOPMENTS

Genetic improvement of aquaculture species is an ongoing process. As current demands increase and wild stocks are overexploited, more management tools will be required to increase aquaculture production. Genetic enhancement is an increasingly important component of the management and, if used properly, has strong potential to enhance aquaculture production, efficiency, and sustainability. Although considered old-fashioned, the benefits of traditional selective breeding are far from being completely tapped. Since commercialization of transgenic fish has not yet been approved, the potential impact of this technology has not been fulfilled (Fig. 7.16). Genomic information that has been generated has not yet been utilized for genetic enhancement to any appreciable extent. For genomics to make an impact on genetic improvement of aquaculture species, there will need to be a marriage with quantitative



Fig. 7.16 The high-security research facility for genomics research at Auburn University.

genetics for development of selection indices, breeding schemes, and the generation of the phenotypic information necessary to make genomic data usable for genetic enhancement. A greater number of aquaculture students and young scientists need training in both quantitative and molecular genetics. Little effort has been made in this area and it will be a temporary stumbling block to take full advantage of the genetic information currently being generated throughout the world.

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8
Nutrition

Sena De Silva, Giovanni Turchini and David Francis

8.1 INTRODUCTION

Nutrition is the process by which an animal obtains its energy needs for body metabolism and the basic chemical building blocks, such as amino acids, fatty acids, vitamins and minerals, which are all required for growth and well-being. These needs are met via the ingestion of suitable food materials, which are then subjected to processes of digestion and subsequent absorption of what is needed. The absorbed food substances enter the bloodstream and are transported to organs, tissues and individual cells. In farmed animals in general, the nutritional needs of the stock may be provided in the form of feeds, either completely nutritionally wholesome, where animals rely solely on the provided feed, or partially wholesome, where the remainder of the requirements are obtained through the food produced naturally within the farming system.

Compared to terrestrially farmed animals, aquaculture has developed quite recently as a major food production sector. Aquaculture currently accounts for more than 50% of all foodfish consumed, with the expectation that it will reach 60–65% by 2020, further emphasising its importance as a major food production sector. The science of aquaculture nutrition is relatively new and the major strides in improving the efficiency, sustainability and cost of artificial feeds have yet to be fully realised. The situation is further exacerbated by the fact that, while in terrestrial animal husbandry the number of species concerned is less than ten, in aquaculture it is well over 250, each with its own specific nutritional requirements.

Aquaculture is rather unique among farming systems in that not all cultured species are provided with feeds; for example, the great bulk of molluscs and, of course, seaweeds do not rely on externally supplied feed for growth. For the other two major cultured commodity groups, i.e. crustaceans and fish, the nutrition provided externally to the stock varies according to the culture practices. The broad categorisation of aquaculture practices into: (1) extensive, (2) semi-intensive and (3) intensive (section 2.3) is made on the basis of a number of parameters and inputs (Fig. 8.1). Of these, the most important parameters from a nutritional viewpoint are:

1. Extensive, where the cultured stock is entirely dependent on the food organisms naturally produced in the system, such as, for example, phytoplankton and zooplankton in a pond.
2. Semi-intensive, where the cultured stock obtains a proportion of the nutrition from the food organisms naturally produced and the rest is provided by the farmer.
3. Intensive, where the cultured stock is entirely dependent on externally provided food for its nutrition.

Thus aquaculture nutrition is not straightforward or simple. It is difficult, if not impossible, to deal with all the facets. A number of possibilities exist within a continuum between the extremes of extensive and intensive culture.

The most commonly adopted approach to the subject of aquaculture nutrition is to address the nutrient requirements and related diet formulation aspects of major cultured species. The other option is to deal with diet formulation in less detail and place more emphasis on nutrition-related issues that confront the growing aquaculture sector, particularly in relation to resource utilisation and hence its overall long-term sustainability. In view of

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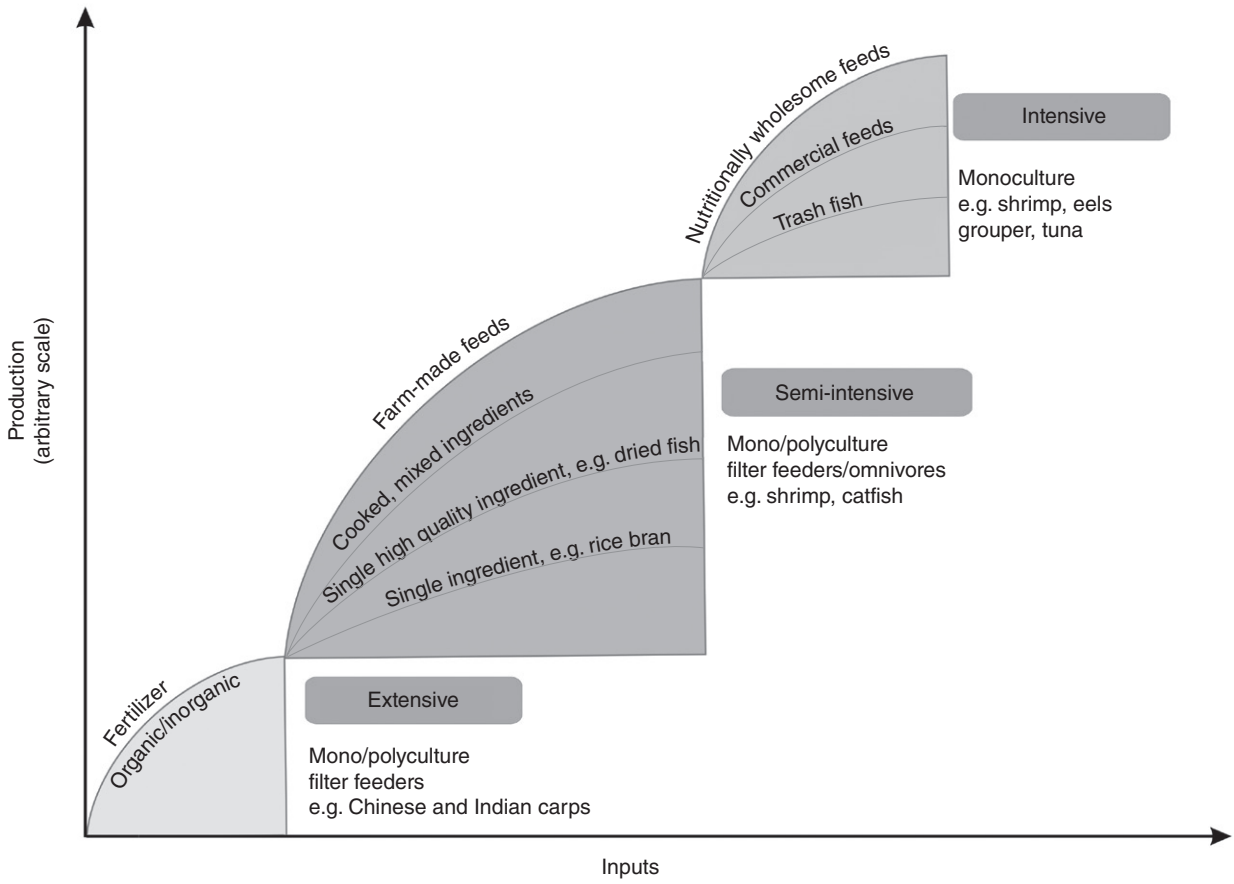


Fig. 8.1 A schematic representation of the effectiveness of fertilisers and different feed types on aquaculture production (from De Silva and Hasan, 2007).

the fact that the nutritional requirements of major cultured species have been relatively exhaustively dealt with (D'Abramo *et al.*, 1997; De Silva and Anderson, 1995; Halver and Hardy, 2002; Lovell, 1998; NRC, 1993; Webster and Lim, 2002b), this chapter focuses more on nutrition issues related to resource usage and related aspects. Furthermore, the main emphasis will be on fish aquaculture, still the most abundant cultured and studied group, with only specific reference to other aquatic animals where significant and noteworthy differences from fish nutrition are known and documented.

8.2 FEED INTAKE, DIGESTION AND NUTRIENT ABSORPTION

Diets for aquatic animals (aquafeed) need to be palatable and easily ingested, as all waste organic material supplied to the aquatic system can impact on water quality and represent a net cost for the farmer. Ingested feeds pass through the fish's digestive system where the various macro-nutrients (e.g. proteins) present in the feed are broken down into basic units that are absorbable (e.g. amino acids) through enzymatic actions. Different species, adapted to different environments and trophic levels, have different digestive systems, which are based on a mouth, oesophagus, stomach, pyloric ceca and an intestine commonly subdivided into the midgut, hindgut and rectum. Some species, particularly those that feed on plant matter (i.e. carps and other cyprinids), have no stomach and are characterised by a slightly different digestive physiology. Briefly, in the different compartments of the digestive system, specific digestive fluids and enzymes are secreted. Hydrochloric acid (HCl) and bicarbonate (HCO_3^-) are

produced and secreted to varying degrees along the digestive system to modify the pH and consequently modulate the activity of different digestive enzymes.

Dietary proteins are broken down in stages to polypeptides, peptides and then amino acids by several different proteases, including pepsin, trypsin and chymotrypsin, which are able to act on peptides and break bonds between specific amino acids. Amylase, chitinase and other various carbohydrate-digesting enzymes are responsible for the digestion of carbohydrates, while lipases, esterases and bile salts are required for the emulsification and subsequent digestion of lipids. Products of digestion, mostly comprising basic molecules such as amino acids, fatty acids and glucose, are then absorbed into the bloodstream by passive diffusion or active transport. More detailed information on digestion and absorption in fish is available in references such as De Silva and Anderson (1995) and Halver and Hardy (2002).

8.3 NUTRITIONAL REQUIREMENTS

Knowledge of the nutritional requirements of a cultured organism is paramount to providing it with a wholesome diet that meets all of its nutritional needs, particularly if the culture practice is intensive, such as in the case of carnivorous fish culture, e.g. salmonids. Each cultured species has its own nutritional requirements that can differ as much as between the requirements of a dog and a cow. That said, the broad requirements, measured in terms of the energy, protein, lipid and carbohydrate content (often referred to as the gross requirements), bear a certain degree of relationship to the natural feeding habits of the organism. For example, carnivorous fish require feeds containing a higher amount of protein when compared to feeds for omnivorous fish species (Table 8.1).

It needs, however, to be stressed that the organism does not have a specific net requirement for protein and lipid *per se*, but it has a specific requirement for the amino acids and fatty acids that are constituents of the proteins and the lipids provided in the diet. Moreover, assuming a hypothetical protein requirement, this is largely affected by the other components of the diet, in particular the protein to energy ratio, which needs to be balanced for optimal growth and well-being of the cultured organism. It is in the above context that the following sections will attempt

Table 8.1 Protein to energy ratios in food for optimum growth of selected cultured fish species. Compiled from Froese and Pauly (2008), Lovell (1998) and Webster and Lim (2002b).

Common name	Scientific name	Trophic[1] level	Digestible protein (DP; %)	Digestible energy (DE; kJ/g)	DP/DE (mg/kJ)
Nile tilapia	*Oreochromis niloticus*	2.2	30.0	12.1	24.7
Silver perch	*Bidyanus bidyanus*	2.9	25–29	13.0–17.0	17.1–19.2
Common carp	*Cyprinus carpio*	3.0	31.5	12.1	26.0
Channel catfish	*Ictalurus punctatus*	3.1	28.8	12.8	22.4
European seabass	*Dicentrarchus labrax*	3.8	43–52	21.0	20.5–24.8
Red drum	*Sciaenops ocellatus*	4.3	31.5	13.4	23.5
Asian sea bass (barramundi)	*Lates calcarifer*	4.3	42.0	15.5	26.7
Arctic char	*Salvelinus alpinus*	4.3	36.0	15.5	23.2
Rainbow trout	*Oncorhynchus mykiss*	4.4	42.0	17.2	24.5
Atlantic salmon	*Salmo salar*	4.4			
	fingerling		40–44	17.4–19.1	23.0
	smoltification		40–44	20.0–22.0	20.0
	grower < 2.5 kg		32–36	16.8–18.9	19.0
	grower > 2.5 kg		32–36	19.4–21.8	16.5

[1]Level in food web: herbivores = 2, predators = 3, carnivores and top predators = 4 or 5 (species are not strictly at one level, hence decimal points).

to clarify briefly the needs of nutrient categories and groups and their interactions.

8.3.1 Energy requirements

Fish, like all animals, require dietary sources of energy for basal metabolism and for growth, reproduction and health. Energy is defined as the ability to do work. Energy is not a chemical compound or a nutrient, but it is an entity that is present in the chemical bonds that hold together the molecules of the nutrients. Energy can be measured only when it is converted from one form to another and therefore the energy content of a feed, a nutrient or a raw material is commonly determined by completely oxidising (burning) all the present compounds to basic molecules such as carbon dioxide, water and other gases. The heat released during this process is measured and the amount of energy (commonly referred to as the gross energy) contained in the product is determined. The basic unit of energy is the joule (J).

$$1\,\mathrm{J} = 1\,\mathrm{kg/m^2/s^2}$$

1 J = the work done by a force of one newton acting on an object to move it through a distance of 1 metre in the direction the force is applied.
1 J = the kinetic energy of a 2-kilogram mass moving at a velocity of 1 metre per second.

This is a relatively small unit and in nutrition it is commonly more convenient to use a multiplier such as the kilojoule (kJ = 10^3 J) or the megajoule (MJ = 10^6 J). The calorie (cal) has been used abundantly in the past (1 cal = 4.184 J); however, the International System of Units (SI) defined the joule as the energy unit and its utilisation is therefore recommended. It has been officially adopted by all world nations except Liberia, Myanmar and the USA.

Various compounds, characterised by different molecules and therefore different types and numbers of chemical bonds, therefore contain different quantities of energy. The three macro-nutrients, namely protein, lipid and carbohydrate, contain approximately 19, 36 and 15 kJ/g, respectively. Consequently, various raw materials containing different proportions of the above three macro-nutrients also contain variable amounts of energy.

Not all gross energy present in a feed (and an ingredient) is directly available to the fish, and the science of the study of the partitioning of dietary energy is commonly referred to as bioenergetics. A comprehensive review on fish bioenergetics is available in Bureau *et al.* (2002). Briefly, to quantify the actual amount of dietary energy available to the organism (net energy), there are several fractions of the original gross energy (GE) contained in the feed that are lost during the processes of ingestion, adsorption and metabolism, and these need to be subtracted to obtain the net energy balance. The first step is to consider the intake energy (IE), which equates to the gross energy in the feed consumed by an animal. The gross energy contained in the faeces (FE), as no food is completely digestible, needs to be subtracted from the gross energy content in the feed, and the resultant is referred to as the digestible energy (DE). Further energy loss that needs to be accounted for includes the energy content of gill and urine excretions, and that remaining is commonly referred to as metabolisable energy (ME). The energy lost as heat increment (HiE) for the processes of digestion and adsorption, product formation, waste formation and excretion also need to be considered and subtracted from the energy balance obtaining, in this way, the net energy (NE). The net energy is the fraction of the gross energy actually available to the animal. However, not all of this energy is then directly available for growth and/or reproduction as further energetic costs of the organism need to be satisfied first. In particular, the net energy is utilised by animals for basal metabolism, voluntary activity and thermal regulation. The remaining fraction, referred to as the recovered energy (RE) is the amount of energy contained in the food which is available for growth, fat deposition and reproduction (Bureau *et al.*, 2002; De Silva and Anderson, 1995; NRC, 1993).

$$\mathrm{IE} - \text{Ingested energy in food}$$

$$\mathrm{DE\ (digestible\ energy)} = \mathrm{IE} - \mathrm{FE\ (faecal\ energy)}$$

$$\mathrm{ME\ (metabolisable\ energy)} = \mathrm{DE} - (\text{excreted energy, e.g. } \mathrm{NH_3})$$

$$\mathrm{NE\ (net\ energy)} = \mathrm{ME} - \mathrm{HiE\ (heat\ increment)}$$

$$\mathrm{RE\ (recovered\ energy)} = \mathrm{NE} - (\text{basal metabolism, activity, thermal regulation})$$

$$\mathrm{RE} \rightarrow \text{growth, fat deposition, reproduction}$$

The above provides a description of the general partitioning of energy, which is neither constant nor static and is affected by a variety of environmental and metabolic processes. As such, the same animal subjected to different environmental conditions or different physiological stages will utilise energy differently. Briefly, the factors affecting the basal metabolic rate are (De Silva and Anderson, 1995):

1. Body size and metabolic rate per unit size. 1000 trout of 1 g will require significantly more energy than 1 trout of 1 kg of body weight.
2. Oxygen availability. The higher the oxygen level, the higher the metabolic rate and therefore the higher the energetic cost of maintenance.
3. Temperature. As fish are poikilotherms, the higher the water temperature the higher will be the basal metabolism and associated energy costs.
4. Water salinity. As osmoregulation is a major energy-consuming process in aquatic vertebrates, it is clear that species living in salinities that are isosmotic with their internal fluids spend less energy in osmoregulation than animals living in hypertonic or hypotonic environments, i.e. marine or freshwater, respectively.
5. Stress. Increased stress levels, which can be induced by a variety of factors ranging from water quality to husbandry conditions and external disturbances, results in increased basal metabolic rate.
6. Physiological cycles. All animals display cycling of their physiological processes resulting in the consequential modification of their basal metabolic rate. Some of these cycles are known and documented (i.e. the seasonality of reproduction), while others are more subtle and poorly understood, as is the case for modifications pertaining to circadian and lunar cycles.

Other factors also need to be considered in the overall energetic balance and in particular factors affecting the non-basal metabolic rate, such as digestion, reproduction and gonad growth, and energy costs for locomotion.

As described above, energy is contained within three macro-nutrients – protein, carbohydrate and lipid – with a considerable interaction between these macro-nutrient components. In general, the aquatic environment is rich in proteins and lipids, and scarce in carbohydrates; and aquatic animals, during evolution, adapted to this nutritional background. Consequently, proteins are the preferred energy source, followed by lipids, while carbohydrates are poorly stored and utilised by aquatic animals. This is likely to be the main difference between aquatic and terrestrial animals, as the latter commonly derive the greatest proportion of their energy requirements from dietary carbohydrates. This is why fish meal and fish oil (the two basic raw materials derived from the reduction process of wild-caught pelagic fish), being optimal sources of protein and lipid, with a relatively low carbohydrate content, are the two most commonly used and preferred raw materials for aquafeed production. This is particularly so for intensively cultured species, which have to be provided with a nutritionally wholesome feed/diet.

Energy concentration is the first nutritional consideration in diet formulation. In fact, maintenance of life process takes priority over growth or other functions and, therefore, energy can be considered as the first basic nutritional requirement of any animal. However, in consideration that protein is likely to be the most expensive component of a diet, derived in most instances from expensive fish meal, and that protein and energy must be kept balanced in a nourishing diet, protein is often a priority in diet formulation (section 9.10.1). In consideration of the poor nutritional value of carbohydrates for aquatic animals and the very high energy content of lipids, diets are usually formulated based on an optimal protein to lipid ratio, underpinning the fulfilment of energy requirements and the protein to energy balance ratio. Different species have different requirements; however, it is possible to consider that common cultured fish have a digestible protein (DP) requirement of 30–45%, a digestible energy (DE) requirement of 12–22 kJ/g and an optimal DP/DE ratio of 17–25 mg/kJ. Table 8.1 shows the digestible protein (DP), digestible energy (DE) and DP/DE ratio for optimal growth of various farmed species, grouped by trophic level, and for Atlantic salmon subdivided according to fish size. The highest DP/DE ratio is in common carp diet (26 mg/kJ) while the lowest is in an Atlantic salmon grower diet (16.5 mg/kJ), clearly suggesting how evolution and adaptation to trophic level has modified nutrient requirements of fish species. This translates into diets extremely rich in energy for the exacting Atlantic salmon and relatively poor in digestible energy for the more frugal common carp. Hence, feeds formulated for different species will have very different inclusion levels of the two raw materials, fish meal and fish oil. The salmon industry consequently utilises a greater percentage of the global fish meal and fish oil production compared to the cyprinid industry in proportion to their contribution to the global foodfish availability.

By comparing diets for cultured fish and terrestrial livestock, it appears that fish have a higher protein requirement, as aquafeeds contain significantly higher protein levels. In reality, the actual protein requirement of fish and terrestrial animals is similar. The difference between them is in significantly lower energy requirements for aquatic animals and this is reflected as a relatively higher requirement for dietary protein. The lower energy requirements of aquatic animals in comparison to terrestrial animals are fundamentally due to lower energetic costs.

- Fish are poikilothermic and hence do not spend energy on regulating body temperature.
- Fish are capable of excreting the base metabolic product of nitrogenous metabolism as its first product, ammonia, by simple diffusion into the aquatic medium. On the other hand, terrestrial animals have to convert this base excretory product into either urea or uric acid, which is energy demanding.
- Fish save energy in locomotion compared to terrestrial husbanded animals, as they are buoyed by the medium and hence require less energy to move.

8.3.2 Protein and amino acid requirements

Proteins are the major organic compounds found in fish tissue, accounting for up to 65–75% of the total fish mass on a dry-weight basis. Proteins are comprised of up to 22 α-amino acids, which are the building blocks of proteins, held in chains by peptide bonds. Amino acids consist of atoms of carbon, hydrogen, oxygen and nitrogen, although some amino acids and, hence, proteins also contain sulphur, phosphorus and iron. Amino acids contain both amine and carboxyl functional groups. Nitrogen accounts for about 16% by weight in the majority of proteins and therefore the crude protein (CP) content of a feed or raw material can be obtained by determining the total nitrogen content and then multiplying this value by a factor of 6.25 ($1/16 \times 100$).

The amino-acid content of proteins differs markedly and each protein is defined by a unique sequence of amino acids that is commonly referred to as the primary structure. Amino-acid proportions are characteristic for each protein. Therefore, theoretically, the ideal protein source for an organism is a protein containing the same amino-acid content and amino-acid proportions of the organism itself. Some protein sources, particularly plant proteins, are moderately to largely deficient in one or more amino acids that are present in fish protein. Fish meal, on the other hand, is the best protein source for farmed fish, containing all the required amino acids in the desired proportions. If fish meal is replaced by an alternative protein source, this alternative will very seldom contain all the required amino acids and in the right proportions. Therefore, various alternative protein sources need to be carefully blended and balanced, often with the inclusion of synthetic amino acids, to make up for the suboptimal amino-acid profiles of alternative sources.

The protein requirements of a great variety of farmed aquatic species have now been determined. However, etymologically it is not correct to use the term 'requirement' when referring to protein, as animals have no specific requirement for protein. They have specific dietary requirements for some essential amino acids, which constitute protein. Nevertheless, it is commonly accepted that 'protein requirement' refers to the minimum amount of high-quality protein needed to meet amino-acid requirements and, hence, to achieve maximum growth. The essential amino acids consist of ten amino acids that all vertebrates require in their diet, natural and or artificial, as animals are incapable of synthesising these (Table 8.2).

Table 8.2 Essential and non-essential amino acids in fish together with commonly used abbreviations.

Essential amino acids			Non-essential amino acid		
Isoleucine	Ile	I	Alanine	Ala	A
Arginine	Arg	R	Asparagine	Asn	N
Histidine	His	H	Aspartic acid	Asp	D
Leucine	Leu	L	Cysteine	Cys	C
Lysine	Lys	K	Glutamic acid	Glu	E
Methionine	Met	M	Glutamine	Gln	Q
Phenylalanine	Phe	F	Glycine	Gly	G
Threonine	Thr	T	Proline	Pro	P
Tryptophan	Trp	W	Serine	Ser	S
Valine	Val	V	Tyrosine	Tyr	Y

A detailed review of the protein and amino-acid nutrition of farmed fish can be found in Wilson (2002). According to the accepted definition of 'protein requirement', the estimated protein requirement of the juvenile stages of a variety of farmed fish species is depicted in Fig. 8.2. In the figure, species have been grouped in conformity to their trophic level, and it is possible to perceive that the higher the trophic level the higher the protein requirement. However, this must be considered as a simple indicative general rule as there are a few exceptions: for example, the grass carp (*Ctenopharygodon idella*) and the milkfish (*Chanos chanos*). Both have a very low trophic level (2.0) but a relatively high protein requirement (40–42%), while the rainbow trout (*Oncorhynchus mykiss*), at the other end of the trophic chain (level 4.4), has an estimated protein requirement of about 40% (Froese and Pauly, 2008; Wilson, 2002). Like fish, most crustaceans have relatively high protein requirements, ranging from 30% to 60% of the dry diet (D'Abramo *et al.*, 1997).

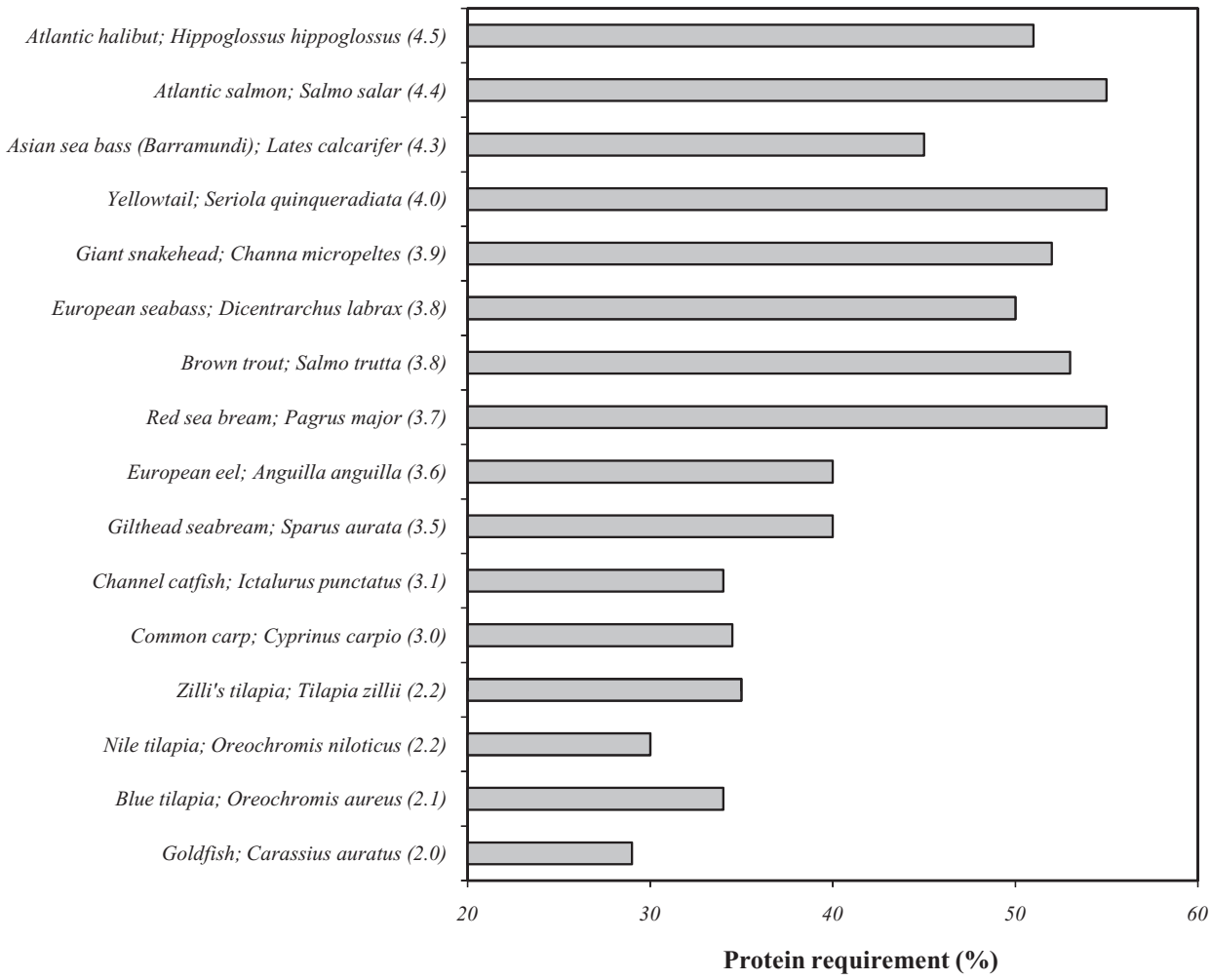


Fig. 8.2 The estimated protein requirement of juvenile fish. Species are sorted according to trophic level (see footnote to Table 8.1). Compiled from Froese and Pauly (2008) and Wilson (2002).

The commonly accepted definition of 'essential nutrients' is that:

- they are required for normal functioning of the organism as they play fundamental roles in the metabolism; and
- their *ex novo* biosynthesis is not possible, and therefore they must be obtained from a dietary source.

As such, essential amino acids (EAA) need to be provided within the diet and particularly within the dietary protein.

The quantitative essential amino-acid requirements of several farmed aquatic species are known (Wilson, 2002) and Table 8.3 shows the specific requirements of juveniles of several species (NRC, 1993), as examples of commonly cultured aquatic species from different trophic levels.

The qualitative amino acid requirements are constant across all studied species. As only 10 essential amino acids (EAA) are required (Table 8.3), it is evident that the quantitative requirements of essential amino acid can vary across species, but only marginally. An examination of the trends for different amino acids within each species reveals relative consistency in terms of quantitative requirements. Phenylalanine, lysine and arginine are the EAA required in the greatest quantities (1.9, 1.7 and 1.6 % of diet, respectively, on average), followed by leucine, threonine,

Table 8.3 Essential amino-acid requirements of juvenile farmed fish species. Requirements are expressed as a percentage of diet. The percentages of essential and non-essential amino acids constituting the dietary protein are also shown. Compiled after NRC (1993).

	Common carp	Channel catfish	Japanese eel	Nile tilapia	Chum salmon
Arginine	1.60	1.00	1.70	1.18	2.60
Histidine	0.80	0.40	0.80	0.48	0.70
Isoleucine	0.90	0.60	1.50	0.87	1.00
Leucine	1.30	0.80	2.00	0.95	1.50
Lysine	2.20	1.20	2.00	1.43	1.90
Methionine	1.20	0.60	1.20	0.75	1.20
Phenylalanine	2.50	1.20	2.20	1.05	2.50
Threonine	1.50	0.50	1.50	1.05	1.20
Tryptophan	0.30	0.12	0.40	0.28	0.30
Valine	1.40	0.71	1.50	0.78	1.20
Total essential amino acid	13.7	7.1	14.8	8.8	14.1
Protein in diet	38.5	24.0	38.0	28.0	40.0
Essential amino acid (% of dietary protein)	*35.6*	*29.7*	*38.9*	*31.5*	*35.3*
Non-essential amino acid (% of dietary protein)	*64.4*	*70.3*	*61.1*	*68.5*	*64.8*

valine, methionine and isoleucine (varying from 1.3 to 1.0 % of diet, on average) and the two least required, in pure quantitative terms, are histidine and tryptophan (0.6 and 0.3 % of diet, respectively, on average). It is important to understand the concept of limiting amino acids. With a feed containing the optimal amount of a hypothetical protein containing each of the required essential amino acids in the required proportions for that fish species, the fish will be able to manifest optimal growth patterns, which may be defined as 100%. The same feed, with the same quantity of protein, but this time with the protein containing only half the required amount of one of the essential amino acids, would achieve a growth of only 50%. This is because in this instance that specific amino acid is a limiting factor, restricting growth by its lack of availability. All other essential amino acids will be catabolised and not deposited; and in affect be wasted.

Table 8.3 shows that, while the requirement for specific amino acids is very small, this is not the case for protein. Summing up all the required essential amino acids of channel catfish gives a value of 7.1%. However, the protein requirement for the species is much greater. This is because proteins are important not only to provide essential amino acids but also as an energy source and for protein turnover. Consequently, the general rule is that ca. 30% of the dietary protein is necessary to fulfil the essential amino-acid requirements and the remaining 70% is required for providing available energy and nitrogen and amino acid back-bones for protein turnover.

8.3.3 Lipid and fatty-acid requirements

The term lipid encompasses a variety of different chemical compounds which are characterised by being hydrophobic and soluble in organic solvents. Lipids are ubiquitous in cells and play a variety of essential functions in live organisms, ranging from being a preferential source of available energy, to being structural components of biomembranes, precursors to eicosanoids, hormones and vitamin D, and also acting as enzyme co-factors and carriers of fat-soluble vitamins (Sargent *et al.*, 2002; Bell and Koppe, 2010). Consequently, from a nutritional point of view, lipids are fundamental components in any diet for the promotion of growth and health, reproduction and other bodily functions. Lipids in aquafeed are provided with the inclusion of oils or fats, and the most commonly used is fish oil, which lately is increasingly being partially replaced by terrestrial vegetable oils, such as canola, soybean and palm oil, or by-products from the rendering industry, such as beef tallow and poultry by-product fat (Turchini *et al.*, 2010).

Lipids are commonly classified into two major classes: simple and complex lipids, or according to their polarity, into neutral lipids and polar lipids, respectively. The simple lipid class includes acylglycerols, wax esters, sterols and free fatty acids, and the complex lipid class includes glycerophospholipids (commonly referred to as phospholipids). With the exception of free cholesterol, all lipids contain one or more fatty-acid molecules, which can be considered as the basic component of lipids, in a similar fashion to what amino acids represent for protein. Triacylglycerols are the most common form of acylglycerols and the major component of neutral lipids. They consist of a glycerol backbone to which three fatty-acid molecules are esterified (joined). Within a polar lipid, phosphoglycerides are the simplest and most abundant. Phosphoglycerides are structurally similar to triacylglycerol; however, the fatty acid esterified at position *sn*-3 is substituted by a phosphate group, accounting for the higher polarity of this molecule.

Fatty acids are molecules that consist of carbon chains of variable length (usually an even number from 12 to 22), with a methyl end (CH_3) on one side of the chain and a carboxyl end (COOH) at the other. As well as their specific names and abbreviations, there are two commonly used fatty-acid formulae, both including the number of carbons (chain length), the number of double bonds (degree of unsaturation) and the position of the double bonds along the carbon chain. The '*n*' or 'ω' annotated formula refers to the position of the first double bond from the methyl end of the molecule, while the 'Δ' annotated formula refers to all the double bonds and their positions, counting the carbons from the carboxyl end of the chain. While the Δ annotated formula is more chemically informative, as it describes exactly the position of each double bond, the *n* or ω annotated formula is more commonly used as it makes the grouping of fatty acids according to their potential metabolic pathways easier, and can be considered more informative from a biological viewpoint. As such, the latter will be used in the following sections. The *n* or ω annotated fatty-acid formula is composed of three numbers:

1. The first number indicates the number of carbons in the chain.
2. The second number specifies the number of double bonds (unsaturations).
3. The third number denotes the position of the first double bond from the methyl end of the molecule.

Thus, palmitic acid is described by the formula 16:0, indicating a fatty acid with a chain of 16 atoms of carbon and no double bonds; oleic acid is described by the formula 18:1*n*-9, indicating a fatty acid with a chain of 18 atoms of carbon, one double bond at the ninth carbon from the methyl end; and arachidonic acid is described by the formula 20:4*n*-6, indicating a fatty acid with a chain of 20 atoms of carbon, four double bonds and the first double bond is at the sixth carbon from the methyl end.

Fatty acids can be grouped in three major classes according to their degree of unsaturation:

1. Saturated fatty acids (SFA; all fatty acids with no double bonds, e.g. 14:0, 16:0 and 18:0).
2. Monounsaturated fatty acids (MUFA; all fatty acids with one double bond, e.g. 16:1*n*-7, 18:1*n*-9 and 20:1*n*-11).
3. Polyunsaturated fatty acids (PUFA; all fatty acids with two or more double bonds, e.g. 18:3*n*-3, 20:4*n*-6 and 22:6*n*-3).

Furthermore, considering their biological peculiarities, further subclasses are increasingly used, such as the highly unsaturated fatty acids (HUFA, also referred to as long-chain polyunsaturated fatty acids, LC-PUFA), which include all polyunsaturated fatty acids with a chain length longer than 20 and three or more double bonds, and the omega-3 (ω-3, *n*-3) and omega-6 (ω-6, *n*-6) classes, which group all polyunsaturated fatty acids with the first double bond at the third or sixth carbon, respectively. The principal fatty acids and fatty-acid classes are shown in Table 8.4.

Lipids contain the highest amount of energy per unit weight (39.5 kJ/g), which is roughly double that of proteins and carbohydrates. Accordingly, a major role of lipids in nutrition is their catabolism via mitochondrial or peroxisomal beta-oxidation to produce metabolic energy in the form of ATP. Due to adaptations to the aquatic environment, which is particularly rich in lipids and poor in carbohydrates, fish metabolism has evolved to preferentially utilise lipids, and specifically fatty acids, as the favoured dietary source of energy. Consequently, lipid sources are the most effective raw materials for optimal balancing of protein and energy in aquafeeds. Dietary lipids, beside their fundamental role as an energy source, are important to supply indispensible nutrients, particularly 'essential fatty acids' or EFA (c.f. essential amino acids) (Sargent *et al.*, 2002; Bell and Koppe, 2010).

Within this context, the definition of an essential fatty acid is quite complex. Fatty acids can be considerably modified by animal metabolism: they can, for example, be produced *ex novo*, they can be elongated or shortened and

Table 8.4 Formulae, names and common abbreviations of principal fatty acids and fatty acid classes.

Formula	Name	Abbreviation	Class	Subclasses
14:0	Myristic acid	–	SFA	–
16:0	Palmitic acid	–	SFA	–
18:0	Stearidonic acid	–	SFA	–
18:1*n*-9	Oleic acid	OA	MUFA	*n*-9
18:2*n*-6	Linoleic acid	LA	PUFA	*n*-6
18:3*n*-6	γ-linolenic acid	–	PUFA	*n*-6
20:4*n*-6	Arachidonic acid	AA	PUFA	*n*-6/HUFA
18:3*n*-3	α-linolenic acid	ALA	PUFA	*n*-3
20:5*n*-3	Eicosapentaenoic acid	EPA	PUFA	*n*-3/HUFA
22:6*n*-3	Docosahexaenoic acid	DHA	PUFA	*n*-3/HUFA

HUFA, highly unsaturated fatty acids; MUFA, fatty acids with one double bond; PUFA, fatty acids with two or more double bonds; SF, saturated fatty acids.

they can also be desaturated (a double bond can be inserted along the carbon chain). However, fish, as with all vertebrates, are not able to add a double bond at the third or sixth atom of carbon of the fatty acid chain and consequently are not able to produce their own *n*-3 and *n*-6 fatty acids, which are required for some essential roles by every cell. The two two bioactive fatty acid groups that are required for cell metabolism are *n*-3 HUFA and *n*-6 HUFA, in particular, eicosapentaenoic acid (EPA, 20:5*n*-3) and arachidonic acid (ARA, 20:4*n*-6), which occur in fish oil. The majority of aquatic species are able to produce EPA and ARA starting from the two basic shorter and less-unsaturated precursors, α-linolenic acid (ALA, 18:3*n*-3) and linoleic acid (LA, 18:2*n*-6), respectively. ALA and LA are produced by plants and algae, but they cannot be biosynthesised *ex novo* by fish or crustaceans, and therefore they should be correctly termed as essential fatty acids.

Interestingly, ALA and LA can be found in abundance in several terrestrial oils. Some aquatic species, however, particularly marine carnivorous fish and many crustaceans, have lost the capability to elongate and desaturate ALA and LA to produce EPA. This is likely to be a result of the adaptation to an environment (food) rich in EPA and ARA. Consequently, these species exhibit a direct requirement for these two HUFAs, which, in this instance, can be considered essential fatty acids. Thus, different species have different essential fatty acid requirements as shown in Table 8.5, with species grouped according to environment (from freshwater to marine) and, within each environment, according to the trophic level (Turchini *et al.*, 2009). Table 8.5 shows that there is a change in species-specific requirements from C_{18} *n*-6 and *n*-3 PUFA towards *n*-3 HUFA with increasing salinity and, within the same environment, moving progressively to increasing trophic levels. Thus, the higher the trophic level the higher the requirements for *n*-3 HUFA and, at the same time, the higher the salinity the higher the requirements for *n*-3 HUFA. This explains why and how the need for the inclusion of fish oil (the only widely available source of *n*-3 HUFA) in aquafeed is highly variable in relation to different species (Turchini *et al.*, 2010).

No other lipids, apart from the EFA discussed above, have been shown to be required by fish. However, crustacean lipid metabolism is different. A peculiarity of crustaceans is that they are unable to biosynthesise sterols *ex novo* (D'Abramo *et al.*, 1997; Teshima, 1997). These sterols (e.g. cholesterol) are required for normal functioning of cells, tissues and organs. Cholesterol, a constituent of cells and biomembranes, is also a metabolic precursor of several hormones, including steroids and moulting hormones. As such, sterols are essential in crustacean nutrition, while they are not for fish or other vertebrates that are able to biosynthesise cholesterol and other sterols *ex novo*. The optimum levels of dietary cholesterol for various crustacean species range from 0.12% to 2.0% and, within a species, may vary according to size and physiological conditions. Similarly, it seems that crustacean lipid metabolism has another peculiarity when compared to that of fish. This relates to an apparent dietary requirement for phospholipids. Some dietary phospholipids are indeed indispensable for survival of larvae and juveniles and for optimal growth in several studied crustaceans species.

Table 8.5 Essential fatty acid (EFA) requirements of fish species, sorted according to trophic level and environment. Compiled after Turchini *et al.* (2010).

					EFA requirements (% dry diet)			
Common name	Scientific name	Trophic level	Environment	Distribution	18:2*n*-6	18:3*n*-3	*n*-3 HUFA	*n*-6 HUFA
Grass carp	*Ctenopharyngodon idella*	2.0	Fw	Temperate	1.0	0.5		
Common carp	*Cyprinus carpio*	3.0	Fw	Subtropical	1.0	0.5–1.0		
Channel catfish	*Ictalurus punctatus*	3.1	Fw	Subtropical	0.5	1.0–2.0	0.5–0.75	
Tilapia	*Tilapia zilli*	2.2	Fw, Bw	Tropical	1.0			1.0
Nile tilapia	*Oreochromis niloticus*	2.2	Fw, Bw	Tropical	0.5			
Milkfish	*Chanos chanos*	2.0	Fw, Bw, Mar	Tropical	1.0	0.5	1.0	
Chum salmon	*Oncorhynchus keta*	3.5	Fw, Bw, Mar	Temperate	1.0	1.0		
Whitefish	*Coregonus lavaretus*	3.5	Fw, Bw, Mar	Temperate			0.5–1.0	
Cherry salmon	*Oncorhynchus m. masou*	3.6	Fw, Bw, Mar	Temperate		1.0	1.0	
Japanese eel	*Anguilla japonica*	3.7	Fw, Bw, Mar	Subtropical	0.5	0.5		
European sea bass	*Dicentrarchus labrax*	3.8	Fw, Bw, Mar	Subtropical			1.0	
Coho salmon	*Oncorhynchus kisutch*	4.2	Fw, Bw, Mar	Temperate	1.0	1.0		
Arctic char	*Salvelinus alpinus*	4.3	Fw, Bw, Mar	Temperate		1.0–2.0		
Barramundi	*Lates calcarifer*	4.3	Fw, Bw, Mar	Tropical			1.0	
Rainbow trout	*Oncorhynchus mykiss*	4.4	Fw, Bw, Mar	Temperate	0.8–1.6	0.7–1.0	0.2–1.0	
Atlantic salmon	*Salmo salar*	4.4	Fw, Bw, Mar	Temperate		0.5–1.0	0.5–1.0	
Turbot	*Psetta maxima*	3.1	Bw, Mar	Temperate			0.6–1.3	0.3
Gilthead sea bream	*Sparus aurata*	3.5	Bw, Mar	Subtropical			0.5–1.9	
Striped jack	*Pseudocaranx dentex*	3.9	Bw, Mar	Tropical			1.7	
Red drum	*Sciaenops ocellatus*	4.3	Bw, Mar	Subtropical			0.5–1.0	
Yellowtail flounder	*Pleuronectes ferrugineus*	3.4	Mar	Temperate			2.5	
Red sea bream	*Pagrus major*	3.7	Mar	Subtropical			0.5–1.0	
Korean rockfish	*Sebastes schlegeli*	3.7	Mar	Temperate			1.0	

Bw, brackishwater; Fw, freshwater; Mar, marine.

These phospholipid requirements can commonly and easily be met by dietary soybean lecithin. The phosphatidylcholine fraction, in particular, seems to be more effective for growth maximisation (Teshima, 1997). It is notable that fish oil is commonly a richer source of cholesterol and phospholipids when compared to other readily available alternative lipid sources.

Once the requirements for essential lipids have been fulfilled, there is no specified minimal or maximal optimal dietary lipid requirement. As previously described, for different species, there is an optimal protein to energy ratio and lipids are the dietary component that plays a major role in determining the energy content of a diet.

In the past two decades, due to the technological advances in aquafeed manufacturing, including implementation of extrusion techniques (section 9.10.2.2), the lipid content of diets has increased significantly. This has resulted in the formulation and realisation of so-called high-energy diets (HED), which could also be named high-lipid diets, considering that dietary energy is fundamentally provided by lipids. HED are able to maximise growth, especially in salmonids, made possible through the protein-sparing effect. This allows dietary protein to be converted into muscle protein more efficiently and results in economic benefits by reducing the need for inclusion of expensive protein sources into aquafeed formulation, and in environmental benefits by reducing nitrogen wastage and release into farm effluents.

High-lipid diets seem to be an easily implemented tool to maximise growth, reduce feed costs and reduce farming production times. However, not all species respond in the same way to an increase in dietary lipids, and the other side of the coin is that fish fed with HED often show excessive adiposity and increased fat content in the fillet. It is well documented that there is a direct and strong relationship between dietary lipid level and fat deposition in the body. Increased fatness of cultured products is currently one of the core issues of anti-farming campaigns in which wild-caught fish are portrayed as a better option to farmed product. Apart from the environmental and ethical issues, some nutritional aspects deserve to be mentioned. An excessive fat deposition in the perivisceral area is extremely detrimental as it represents a net waste and a reduction in yield. On the other hand, an increase in fat content in fish fillet can also be considered as a positive effect. While a fatter fillet can have different sensorial characteristics compared to a leaner fillet (and possible consumer preferences need to be assessed), from a nutritional viewpoint, the fat from fish is highly nourishing and sought after by consumers. In consideration of the recent dietary recommendations relative to the health benefits of adequate intake of *n*-3 HUFA, fatter fish can be considered as a merit of farmed products compared to wild-caught fish. In fact, while health-promoting *n*-3 HUFA levels (percentage of *n*-3 HUFA over the total fatty acids) are generally higher in wild-caught fish compared to farmed products, when expressed as mg of fatty acid per 100 g of fish flesh, farmed fish usually contain significantly more *n*-3 HUFA than wild counterparts. As such, a single portion of farmed fish can easily fulfil the weekly recommended intake of *n*-3 HUFA, while the same-sized portion of a wild fish will possibly fulfil only the recommended daily intake.

If, however, fish oil is abundantly replaced in aquafeed formulation, the resultant fatty acid composition of farmed fish will be significantly affected (Turchini *et al.*, 2010).

8.3.4 Vitamins

Vitamins are a group of organic compounds generally supplied in aquafeeds in trace quantities for the promotion of growth and reproduction and the maintenance of health and function. An extensive review of vitamin requirements in fish nutrition is given in Halver (2002). They exist as parts of complex compounds and act as co-factors in a host of metabolic functions. Vitamins are classified as fat soluble – containing hydrogen, carbon and oxygen (vitamins A, D, E and K) or water soluble – containing hydrogen, carbon, nitrogen and oxygen (B complex vitamins, vitamin C and choline). Each of these is responsible for specific bodily functions. Fat-soluble vitamins are absorbed in the digestive tract concurrently with lipid molecules and can be stored within body fat reserves if provided in excess. Water-soluble vitamins on the other hand are rapidly utilised following absorption, or broken down and excreted if in excess of metabolic requirements.

Vitamin deficiency, known as avitaminosis, can result in increased vulnerability to infection, depressed growth and nutrition-related diseases (Fig. 8.3). Alternatively, excess amounts of dietary vitamins, particularly fat-soluble vitamins, can lead to high storage levels and result in the manifestation of toxic symptoms, known as hypervitaminosis.

The specific vitamin requirements of individual fish species are largely unknown and can vary considerably between species. Ascertaining the true vitamin requirements can prove difficult considering that many vitamins are produced in the gut by micro-organisms. Therefore, published data pertaining to species such as carp and catfish (for warmwater fish species) and rainbow trout (for coldwater species) are generally used in feed formulations.

However, in this situation the possibility exists to over- or under-formulate dietary vitamin levels.

Vitamins are generally unstable within aquafeeds and therefore the level at which they are included in a diet formulation is not necessarily the level available to the fish. This is the result of vitamin denaturing during processing and storage, generally dependent on several variables (processing temperature, oxygen content, light, moisture and pH; section 9.10.3). The stability of vitamins is very variable, ranging from relatively stable (niacin and biotin) to unstable (vitamin C and thiamin). An example of a practical vitamin mix formulation is shown in Table 8.6. It also important that in semi-intensive aquaculture, in the tropics in particular, the cultured stock obtain most of the vitamin requirements through ingestion of natural food organisms produced in the system.

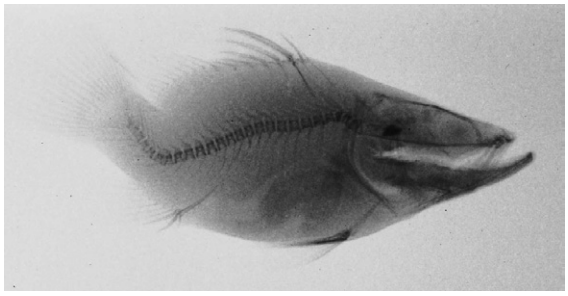


Fig. 8.3 A radiograph of a juvenile barramundi, *Lates calcarifer*, showing deformity resulting from dietary vitamin deficiency.

8.3.5 Mineral requirements

Minerals are inorganic elements required by fish for skeletal formation, enzyme system activation, osmotic maintenance (section 3.2) and acid–base equilibrium regulation. The quantity at which a mineral is required for normal metabolism dictates its level of classification: macro or trace. Macro-minerals are required in high concentrations (g/kg) and include calcium (Ca), phosphorus (P), magnesium (Mg), sodium (Na), potassium (K), chlorine (Cl, in the form of chloride) and sulphur (S). Alternatively, trace minerals are required at much lower concentrations (mg/kg) and include aluminium (Al), chromium (Cr), cobalt (Co), copper (Cu), fluorine (F), iodine (I), iron (Fe), manganese (Mn), molybdenum (Mo), nickel (Ni), selenium (Se), silicon (Si), tin (Sn), vanadium (V) and zinc (Zn). Fish are unique and greatly advantaged in comparison to terrestrial animals, given their ability to directly absorb some minerals from the aquatic medium across their gills and skin. In particular, the marine environment delivers an abundance of minerals, with the exception of P and I, while species cultured in freshwater, which contains fewer available minerals, require a more complete dietary mineral supplementation.

While the cultured animals have requirements for trace elements, there are circumstances where the levels of trace

Table 8.6 Example of water soluble vitamins and mineral mix in the test diet H-440. Reproduced from Halver (2002), with permission from Elsevier.

Complete test diet	g	Vitamin mix	mg	Mineral mix	mg
Vitamin-free casein	38	Thiamine hydrochloride	5	USP XII Salt Mixture No. 2	100 g
Gelatin	12	Riboflavin	20	$AlCl_3$	15
Corn oil	6	Pyridoxine hydrochloride	5	$ZnSO_4$	300
Cod liver oil	3	Choline chloride	500	CuCl	10
White dextrin	28	Nicotinic acid	75	$MnSO_4$	80
α-Cellulose mixture	9	Calcium pantothenate	50	KI	15
				Na_2SeO_3	4
α-Cellulose	8	Inositol	200	$CoCl_2$	100
Vitamin mix	1	Biotin	0.5		
		Folic acid	1.5		
Mineral mix	4	L-Ascorbic acid	100		
Water	200	Vitamin B_{12}	0.01		
Total diet as fed	300	Menadione (K)	4		
		α-Tocopherol acetate (E)	40		

elements (e.g. Cu) are detrimental to the point of being lethal.

In consideration of the ability of aquatic animals to take up minerals from their environment, establishing dietary mineral requirements is complicated by the need to obtain and maintain a water source free of the mineral under investigation. In addition, dietary determinations are made difficult by formulation of mineral-deficient test diets and issues pertaining to tissue mineral stores. The specific function and requirements for minerals are therefore generally poorly understood and incomplete, resulting in dietary formulations based on generalisations made from studies of other species.

Mineral deficiencies in fish can manifest in a multitude of nutrition-related diseases. Briefly and broadly, the most prevalent symptoms of mineral deficiency in fish are a reduction in feeding and growth performance, followed by skeletal malformations and haemorrhaging to the skin and fins. A comprehensive detailed account of mineral deficiency symptoms can be obtained in Lall (2002), and an example of practical mineral mix formulation is shown in Table 8.6.

8.3.6 Carbohydrates and other feed components

Although carbohydrates are considered one of the three main macro-nutrients for fish and crustaceans, carbohydrates play only a minor role and no specific requirement for these compounds has been demonstrated (NRC, 1993; Shiau, 1997; Webster and Lim, 2002a). The nutritional value of carbohydrates varies among fish. In general, warmwater freshwater species can use dietary carbohydrates more efficiently than coldwater and marine species.

Fish can survive, grow and display normal reproductive physiology if maintained on a carbohydrate-free diet. However, carbohydrates are relatively cheap in terms of aquafeed formulation and are a source of available energy. If carbohydrates are not provided within the diet, other compounds (mainly the expensive proteins and lipids) are utilised for energy production and for the synthesis of various biologically important compounds that are normally derived from carbohydrates. Thus, in aquafeed formulation it is important to provide the appropriate concentration of carbohydrates to spare other dietary components (NRC, 1993). Further to this, carbohydrates have very important physical roles in aquafeed: acting as binders, constituting the basic pellet structure and being essential for holding feed pellets together in dry and semi-moist diets (Hardy and Barrows, 2002).

Apart from proteins, lipids, carbohydrates, vitamins and minerals, other minor components are or can be present in aquafeed (De Silva and Anderson, 1995; NRC, 1993). Water is normally added to diets, is present as a natural constituent of raw materials or is an element absorbed from the atmosphere. Finished dry diets (steam pelleted or extruded) commonly contain 5–10% of moisture that is derived from the atmosphere and this low concentration of water greatly increases product shelf-life and eases storage and handling. Fibre, such as cellulose and lignin, is commonly present in many plant-derived raw materials. Fibre is an indigestible carbohydrate and therefore does not play an important role in fish nutrition. However, fibre provides physical bulk to the feed and improves gastric evacuation. A minimum amount of fibre in feed is beneficial, but if it exceeds 8% it can significantly decrease growth and feed efficiency. Vegetable protein sources that are used to replace fish meal in aquafeed formulations usually contain high amounts of fibre, thus contributing to difficulties in using such raw materials to improve the fish-in fish-out balance of aquaculture practices. On the other hand, alternative protein sources derived from the by-products of the terrestrial farmed animal industry often contain an excessive amount of ash. Similar to fibre, excessive amounts of ash in aquafeed formulations can decrease nutrient digestibility, overall growth and feed efficiency.

Other potential non-nutrient components of aquafeeds include pigments, binders, feeding stimulants, enzymes, hormones, antibiotics, antifungal agents and antioxidants (section 9.10.5, Table 9.14).

8.4 TYPES OF FEED

Fish diet formulation and related manufacturing practices have evolved considerably in the last few decades, as previously reviewed in detail (Hardy and Barrows, 2002). The types of feed used in aquaculture, as expected, are as diverse as the aquaculture practices. The feed types range from single ingredients to a mix of ingredients, cooked or raw, to moist and dry formulated feeds, and essentially reflect the type of practice. The former types of feed are mostly used in semi-intensive aquaculture of omnivorous fish species such as carp. These types of feed, including single ingredients, a mix of ingredients, cooked or raw, are generally referred to as supplementary feeds as these augment the food produced within the culture system and contribute to the nutrition of the stock (Fig. 8.4). The dynamics of such feeds and the utilisation efficiencies differ markedly between feeds the culture systems (De Silva, 1993; New *et al.*, 1993).

Within the group of formulated feeds, it is possible to distinguish different categories based on their moisture content and whether manufactured on farm or by

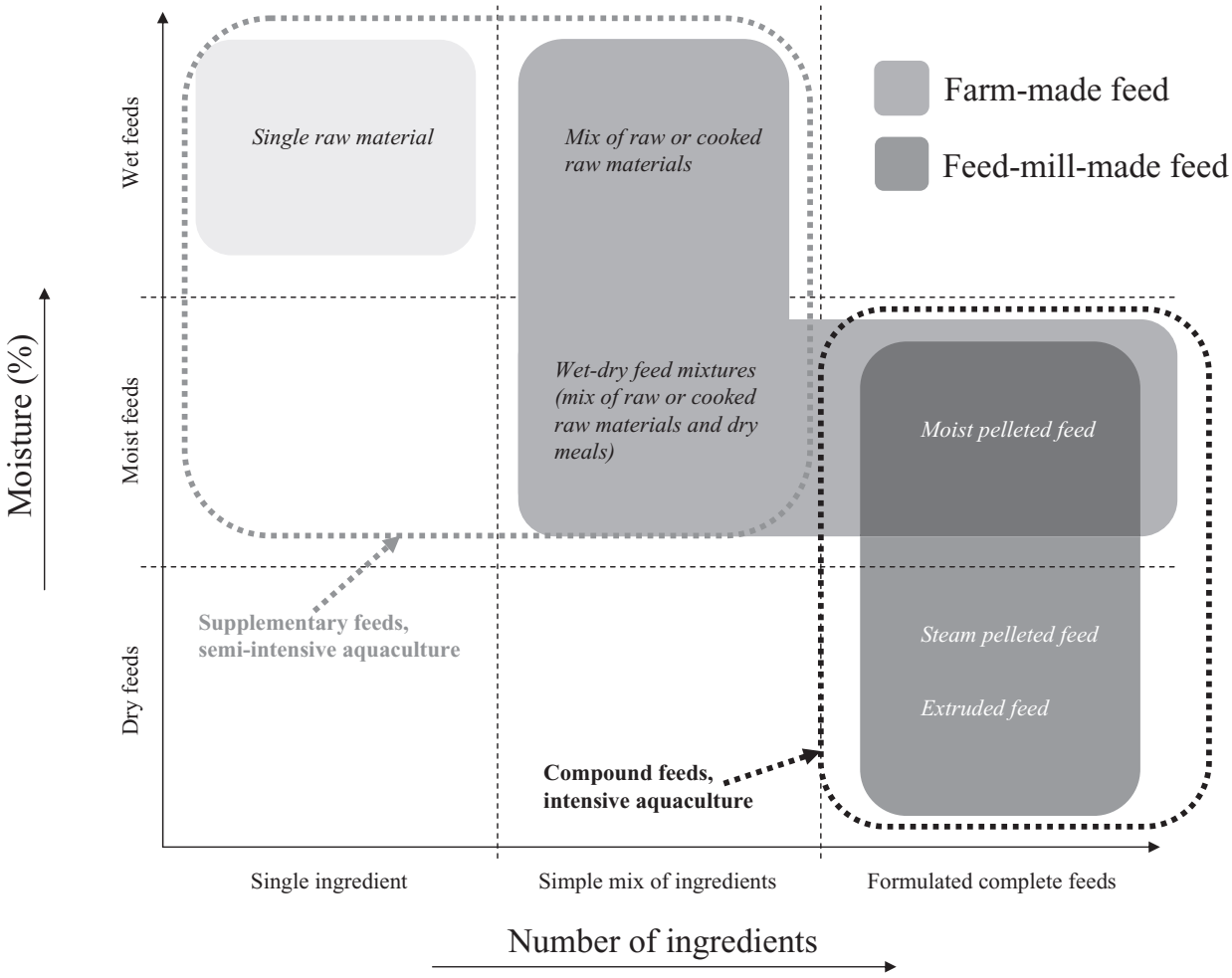


Fig. 8.4 Schematic representation of different types of feed grouped relative to the number of ingredients used and the moisture content of the final product.

centralised industrial feed mill plants. As such, aquafeed can be classified into wet and moist feeds, commonly farm-made, and dry feed. Dry pelleted feed can be further classified into steam-pelleted or extruded feeds, according to the technology used, and these are commonly produced in specialised commercial feed mill manufacturing plants (Fig. 8.4).

8.4.1 Farm-made feeds

Based on the fact that the great bulk of aquaculture is in Asia, which is rural, small scale and generally semi-intensive, there is a high degree of dependence on farm-made or home-made feeds. Details on farm-made feeds, the extent of usage, ingredients used and methods of preparation have been dealt with previously (Hasan *et al.*, 2007). The efficacy of farm-made feeds, although controversial, is best exemplified from an earlier study on shrimp in India, where it was demonstrated that the farm-made feed was equally or more effective than compounded feeds formulated on the basis of the nutritional requirement of the cultured stock and prepared using the most appropriate techniques available at that time (Wood *et al.*, 1992).

While farm-made feeds have historically been implemented in semi-intensive culture practices, the principle is also being extended to intensive culture practices, such as the culture catfish (popularly known as tra catfish or

striped catfish; *Pangasianodon hypophthalmus*) in the Mekong Delta in Vietnam. The catfish industry in the Mekong Delta is most likely one of the aquaculture practices that has witnessed the highest growth globally (section 1.8.1). This practice within a decade has reached a production level of 1.2 million t, 90% of which is processed and exported, with an export value estimated at approximately US$980 million in 2007. Catfish in the Mekong Delta are cultured intensively, in deep ponds (4–4.5 m of water depth) where the average yield is 450 t/ha per crop. The great bulk of the catfish farming sector is still dependent on farm-made feeds; the feeds are made *in situ* from locally available ingredients, using simple improvised methods.

Aquaculture farming communities continue to rely on farm-made feeds for a number of reasons. Foremost of these is the cost. There is also a general perception that the cultured stock performs better on farm-made feeds. The formulation of farm-made feeds can also be adjusted according to availability of particular local ingredients, without compromising stock performance, and as such provide the farmers with greater flexibility in their operations.

The expansion of culture operations in certain regions, such as the flourishing major carp farming region in Andhra Pradesh, India, has resulted in the expansion of farm-made feed preparations to suit farmers' needs. In this region groups of small-scale farm-made feed manufacturers have set up, from whom individual farmers can obtain feeds prepared according to their own specifications, in reasonable quantities, as required. This development has provided the opportunity for farmers to be released from the burden of feed preparation and most of all to obtain feeds to comply with their specifications and needs, bringing about substantial savings in cost and time.

8.4.2 Wet, moist and semi-moist feeds

The term 'wet feed' is usually refers to fresh, frozen or ensilaged raw fish or other seafood materials which is fed, as is, to fish. The raw fish used for feed is referred to using a variety of synonyms including trash fish, low-value fish and forage fish, but none of the above accurately describe the large variety of potential fish used for this practice, and therefore it has recently been suggested to use the general term 'feed fish' (FAO, 2008a). The wet feed category also commonly includes the utilisation of a mix of different fresh or frozen raw materials, such as fish- or seafood-derived by-products, meat and poultry offal or other agricultural-derived by-products, and these feeds usually contain more than 60% moisture. Sometimes, fresh or frozen products and/or silage are combined with dry meals, including for example plant-derived meals, fish meal and animal by-product-derived meals to reduce the moisture content. Likewise, oils and/or vitamin and mineral mixtures are included to increase the overall water stability and nutritional value of the feed. These feeds are commonly referred to as wet-dry feed mixtures (Hardy and Barrows, 2002), and are similar to what has been previously described for general aquaculture practices. Wet feeds can be increasingly elaborated in a continuum to arrive at the production of semi-moist pelleted feeds.

Moist feeds or semi-moist pelleted feeds are commonly farm made, but in some regions they are manufactured in central plants and then delivered locally from feed mills to the farm on a daily basis (Goddard, 1996). These feeds are nutritionally wholesome, with the formulations including different dry and wet ingredients, plus vitamins and minerals, as required. The ingredients are ground and mixed and the dough is passed through a spaghetti/noodle-type machine and cut to specific lengths; the resulting pellets have a relatively high moisture content (30–35%) and need to be utilised rapidly. An example of such a feed is the Oregon Moist Pellet (OMP) (Hublou, 1963), and subsequent modifications, developed in the 1960s by the Oregon Department of Fish and Wildlife, which represents a milestone in salmon nutrition; its formulation is shown in Table 8.7.

8.4.3 Compounded/commercial dry pelleted feeds

Dry feeds are currently the most commonly used feeds in intensive aquaculture (Fig. 8.5); such feeds are conveniently transported for distribution, easily stored and handled, have a longer shelf-life and are generally consistent in quality (Goddard, 1996). Dry feeds commonly have less than 10% moisture, can be produced in a wide range of pellet shapes and sizes and are commonly produced by two different methods: steam or extrusion pelleting (section 9.10.2). The difference between the two methods is that in the extrusion technology the mixture of ingredients is processed at a higher pressure and temperature. Pellets obtained by the extrusion process are tougher (as the starch will be fully gelatinised), and, via the modification of the extrusion parameters, sinking or floating feeds can be produced. A further advantage of extruded pellets is the fact that very large quantities of oil can be added to the feed, up to 40% in weight, producing the high-energy diet (HED) described earlier (Goddard, 1996; Hardy and Barrows, 2002; Sargent *et al.*, 2002; Bell and Koppe, 2010). Some of the benefits and drawbacks of the

Table 8.7 The formulation of the Oregon Moist Pellet (OMP). Reproduced from Hublou (1963), with permission of Taylor & Francis.

Ingredients	Percent
Meal mix	
Cottonseed oil meal (pre-pressed solvent extracted, 44% protein)	23.00
Herring meal (*Clupea harengus pallasi*)	21.00
Crab or shrimp solubles (composed of equal parts crab shell or shrimp meal and condensed fish solubles, dried together)	6.00
Wheat germ meal	3.60
Distiller's dried corn solubles	2.40
Vitamin premix	1.50
Wet mix	
Albacore tuna viscera (*Thunnus alalunga*)	20.00
One, or mixture of any of the following three: 1. Turbot (*Atheresthe stomias*) 2. Pasteurised salmon viscera 3. Dogfish (*Squalus acanthias*)	20.00
Corn oil	1.80
Choline chloride (liquid, 70% product)	0.65
Antioxidant (Tenox IV)	0.05
TOTAL	**100.00**

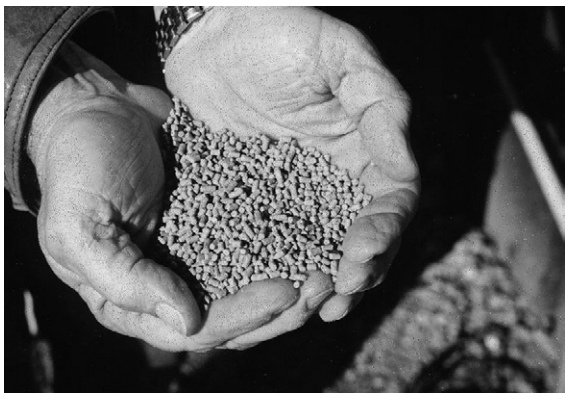


Fig. 8.5 Aquaculture feeds are formulated to satisfy the nutritional requirements of the target species. Commercially produced pelleted aquaculture feeds with species-specific formulations are now widely used. (Photograph by courtesy of Darryl Jory.)

Table 8.8 Example of formulations of salmonid diets (open formula from Ontario Ministry of Natural Resources). Reproduced from Hardy and Barrows (2002), with permission from Elsevier.

	Starter	Grower	Broodstock
Ingredient	Percentage in diet		
Fish meal	30.0	18.0	25.0
Blood meal	7.0	–	–
Poultry by-product meal	6.0	13.0	15.0
Dried whey	9.0	9.0	9.5
Alfalfa meal	–	–	11.0
Soybean meal	–	–	13.0
Brewer's yeast	5.0		
Corn gluten meal	25.0	37.6	17.0
Lysine hydrochloride	0.5	1.4	–
Vitamin premix	1.0	0.5	1.0
Mineral premix	0.5	0.5	0.5
Fish oil	16.0	20.0	8.0

extrusion process are detailed in Table 9.13. An example of dry feed formulation for salmonids is shown in Table 8.8.

8.5 SELECTING FEED INGREDIENTS AND FORMULATION

The proper formulation of aquafeed is a complex procedure. It requires knowledge of the specific dietary requirements of the species in question, knowledge of the nutritional, chemical and physical characteristics of all the ingredients and raw materials used, and knowledge of all the technical issues of the processing technologies used for feed preparation. As such, aquafeed formulation is a rather complicated process and further detailed reading is suggested (Hardy and Barrows, 2002). Briefly, aquafeed formulators start selecting the available dietary ingredients and then determine for each ingredient specific inclusion limits. The aim of the formulators is to obtain an optimal mixture of ingredients that will be easily and effectively pelletable and will produce a final product (the pellet), which will be palatable and nutritious for the specified species, but also economically viable.

Ingredients are commonly chosen based on availability and cost. In normal diet formulation the ingredients pro-

viding proteins are usually the most expensive, with fish meal likely to be the most expensive of these ingredients. Therefore, these raw materials are often chosen on the basis of price per unit protein. Once the list of all available ingredients, their chemical and nutritional values, upper and lower inclusion limits and costs are known, the process of diet formulation begins, aiming to obtain a balanced mixture which will produce a final product with the required specific chemical, physical and nutritional characteristics. This commonly involves the choice of an optimal solution between two extreme approaches: formulate solely on the basis of the nutritional values of ingredients, or formulate on the sole basis of the total feed cost. Aquafeed formulations normally fall somewhere in the middle between these two extremes.

An important aspect that affects feed mill strategies and feed formulations is the price variability of raw materials, in particular fish meal and fish oil, the production and availability of which are highly variable according to season and yearly variations of fishery catches. Consequently, the same aquafeed formulation can be economically viable at a given time, but not so a few months later, and vice versa. Therefore, in consideration of the complex technological processes involved in industrially compounded aquafeed production and the demand for consistent product quality from farmers, raw material price variation is increasingly becoming an obstacle for the feed mill industry. Further aspects of feed formulation are discussed in section 9.10.

8.6 FEED MANAGEMENT

Feed is one of the highest recurring costs in intensive and semi-intensive aquaculture, accounting for over 50% of the cost in the most intensive culture operations. The effective management of on-farm feeding practices is therefore imperative for the economic viability of an aquaculture operation, where its optimisation can have a significant impact on costs/benefits. A sound knowledge of the dietary requirements and feeding behaviour of the culture species is pivotal for effective feed management. Likewise, regular monitoring of growth rates and feed conversion ratios, key parameters of interest to farmers, is important for the evaluation of productivity in a culture venture (e.g. section 18.8.1). The evaluation of feed product quality and the potential impacts of farm effluent water are, however, of equal importance and also directly related to feed management. Suboptimal feeding practices can lead to major problems associated with water quality, leading to stress and disease, and other impacts on surrounding waterways that receive the final effluent discharges. It is therefore critical to ensure that feed is consumed quickly and completely in order to minimise the potential leaching of nutrients and also to minimise the unnecessary expenditure of energy associated with foraging.

8.6.1 Feeding

Feeding and feed ingestion in itself is multifaceted and the end result of a variety of interactions between the fish (its senses and hormonal systems) and its environment. Of considerable importance when determining the type of feed and the feeding regime is the natural feeding behaviour of the cultured stock. This is usually dictated by adaptations to its natural environment and natural feeds. These adaptations manifest in a wide range of mouth structures and digestive functioning, which, in turn, influence feed intake patterns and digestive efficiency. Fish species, however, are often able to modify their feeding habits in response to feeds provided as part of the farming practice. Appetite is controlled by complex metabolic, neurophysiological and hormonal mechanisms (Goddard, 1996). An understanding of appetite, and recognition of satiation and the various stages of feeding up to satiation is therefore important to ensure that effective feeding regimes are implemented.

Fish metabolism, growth and feeding rate are strongly influenced by water temperature, dissolved oxygen levels, salinity and other parameters. Feeding activity will generally cease when these fall outside a species' tolerance levels. Intensive feeding can prove detrimental to feed intake. This may result from the accumulation of metabolic wastes excreted by fish, which have toxic effects on fish and potentially influence feed intake.

Careful monitoring of the feeding activity of cultured stock and prompt appropriate modification to scheduled feeding plans are paramount for the success of aquaculture ventures (sections 18.8.8 and 21.9).

8.6.2 Feeding frequencies

Determining a culture organism's optimal feeding frequency is important to ensure maximal feed conversion ratios (FCR) and dress weight. Optimal feeding frequency may vary according to age, size, feed quality and water temperature. Optimal feeding frequencies in fish species are highly variable, ranging from a continuous feeding regime to day-on day-off feeding regimes. Notably, fewer meals require more feed and a greater feed rate at each meal, which may in turn result in a higher degree of feed wastage at times when fish are not feeding at an optimal

level. Additionally, it is important during the feed delivery process (section 9.11.2) to ensure a rapid rate of dispersal to avoid feed domination, particularly in agonistic species where there is belligerence between members of the same species.

There is considerable diversity in the timing of daily feeding, which is generally accepted to be linked to daily and seasonal rhythms in photoperiod. Feed delivery should aim to coincide with the natural peaks in the culture species' feeding rhythm to maximise feed intake, and avoid unnecessary and costly wastage. Further discussion of feed management in marine fish and marine shrimp culture is available in sections 18.8.8 and 21.9, respectively.

8.6.3 Compensatory growth

The term 'compensatory growth' refers to a period of enhanced growth induced by the restoration of optimal conditions following a period of growth retardation. Growth rates and FCRs during compensatory growth exceed those of fish that have been continuously fed to satiation and, therefore, feeding strategies involving prolonged starvation followed by re-feeding have potential cost saving applications in aquaculture. Starvation and other growth depressors, such as feed restriction and sub-optimal temperature, salinity and dissolved oxygen, have been reported to be capable of supporting and stimulating compensatory growth when optimal conditions are restored.

8.6.4 Mixed feeding schedules

Mixed feeding schedules in aquaculture differ from feeding the same feed in a similar quantity across the entire grow-out cycle. Mixed feeding schedules as a method of reducing feed costs were first documented by De Silva (1985) who described an apparent rhythmic variation in protein digestibility in the green chromis (*Etroplus suratensis)* and Nile tilapia (*Oreochromis niloticus*) reared under different dietary schedules. Based on this observation, De Silva (1985) hypothesised that the administration of diets with differing protein contents may be more economical than feeding continuously with constant dietary protein content. Additionally, this concept could involve the provision of daily ration alternation, alternate days of feeding and starvation, and the alternation of diets with differing protein contents. The implementation of such strategies offers feed cost saving, but also assists in the improvement of nitrogen and phosphorus balances. These potential economic and environmental advantages associated with mixed feeding schedules have been somewhat overlooked in intensive 21st-century farming practices. In consideration of the supply threats facing the fish meal and fish oil industries, the potential applications of mixed feeding schedules need be considered.

8.7 MAJOR FEED-RELATED ISSUES CONFRONTING THE AQUACULTURE SECTOR

8.7.1 Statistics

Global aquaculture production in 2007 (Fig. 8.6) (section 1.4) was 65 million t, and much of this production was fish (32 million t; 49% of total production), which in turn was dominated by freshwater fish aquaculture (27 million t; 84% of total fish production). The rank order of the top ten freshwater, brackishwater and marine cultured fish is given in Table 8.9.

The total compound aquafeed production in 2006 was between 20.2 and 22.7 million t, representing roughly 4% of total global animal feed production (Tacon and Metian, 2008). The top ten producing countries were China (12.0 million t), Thailand (1.3 million t), Chile (1.2 million t), Norway (960 000 t), Indonesia (900 000 t), USA (850 000 t), Vietnam (850 000 t), Japan (800 000 t), the Philippines (400 000 t) and Taiwan (350 000 mt). The indicated forecast for global aquaculture and related aquafeed productionis that there will be a further expansion of the industry in the near future.

Globally, unsustainable fishing practices and the resultant stagnation of global fish landings since the early 1980s have created the overwhelming need for aquaculture to meet the shortfalls in the supply of fish and seafood. Fish are known to be a nourishing and high protein food and also the primary, if not the only, readily available source of the health-promoting *n*-3 HUFA for human consumption. Thus, the global consumption and demand for fish products is on the rise (FAO, 2007) apart from the needs of increasing populations. However, fish meal and fish oil are the two basic ingredients used in aquafeed formulations and these commodities are currently derived only from wild fisheries. This observation has been responsible for a global debate on the current contribution of aquaculture to total fish production (Naylor *et al.*, 2000).

8.7.2 Fish meal and fish oil

As highlighted in the previous sections of this chapter, the fulfilment of protein and lipid requirements, and in particular, amino-acid, fatty-acid and energy requirements, is paramount for an efficient aquafeed. Consequently, it is not surprising that fish meal and fish oil, in view of their amino- and fatty-acid compositions, are the two best and

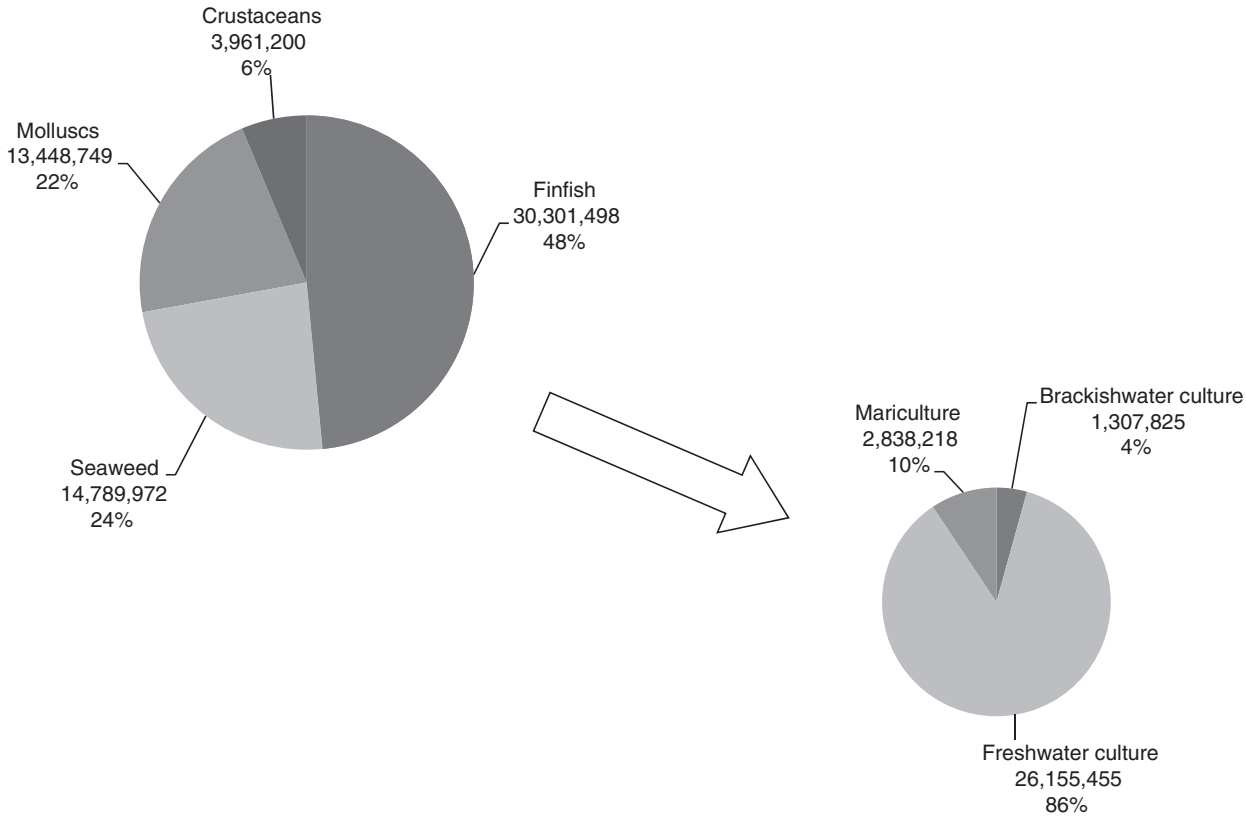


Fig. 8.6 The global aquaculture production in 2005 and percentage contribution of major groups (FAO, 2008b). The fish production has been split according to the environment, clearly illustrating that global aquaculture is dominated by freshwater fish species.

Table 8.9 The rank order of the top ten freshwater, brackishwater and marine cultured fish and their production (t) in 2005. FAO FishStat Plus (2008b).

	Freshwater culture		Mariculture		Brackishwater culture	
1st	Silver carp	4 152 506	Atlantic salmon	1 216 791	Milkfish	497 322
2nd	Grass carp	3 904 799	Marine fishes nei[1]	251 389	Nile tilapia	212 335
3rd	Common carp	3 043 712	Rainbow trout	183 575	Flathead grey mullet	157 881
4th	Bighead carp	2 208 678	Japanese amberjack	159 798	Freshwater fishes nei[1]	146 325
5th	Crucian carp	2 086 311	Coho salmon	116 737	Cyprinids nei[1]	114 465
6th	Freshwater fishes nei[1]	1 653 433	Gilthead seabream	102 301	Barramundi	26 138
7th	Nile tilapia	1 490 790	Japanese seabass	90 594	Mozambique tilapia	21 029
8th	Catla	1 235 992	Silver seabream	82 083	Tilapias nei[1]	20 000
9th	Roho labeo	1 195 965	Lefteye flounders nei[1]	76 884	Atlantic salmon	18 461
10th	White amur bream	552 922	Large yellow croaker	69 641	Groupers nei[1]	16 119

[1]nei, not identified in available statistics.

most commonly utilised sources of proteins and lipids, respectively, for aquafeed. However, the apparent scarcity of these raw materials is colliding with the ever-increasing demand for them as a result of aquaculture's expansion, impelling the aquaculture feed industry to find suitable alternatives (Turchini *et al.*, 2010). Fortunately, the environmental and ethical concerns surrounding the unsustainable use of these finite resources are not the only driver, as the ever-increasing and highly unpredictable fluctuation in fish meal and fish oil prices is also playing a key role in pushing the feed industry towards evolving possible alternatives.

A review of current fish meal and fish oil utilisation by the global aquafeed industry has highlighted some interesting situations and forecasts of future directions (Tacon and Metian, 2008). There is a surprisingly wide variation within and between countries in the inclusion of fish meal and fish oil in aquafeed formulated for the same species. For example, in aquafeed formulated for salmonids, fish meal inclusion varied between countries from 15% to 55% and fish oil from 3% to 40%. Similarly, in shrimp feeds, fish meal inclusion varied from 5% to 40% and fish oil from 0.5% to 10%. The variations partly reflect differences in the production systems employed and differences among species and breeds. However, a major role in determining such variations is played by the following:

1. The local fish meal, fish oil and other feed ingredients: availability, cost and quality;
2. The different national legislative regulations relative to imports and/or ingredient use;
3. The intended market and market value of the cultured species (Tacon and Metian, 2008).

There are significant differences between species in the average amounts of fish meal or fish oil utilised in their diets. For example, eel feeds contain up to 60% fish meal, while there is only 1.5% in some tilapia feeds. Similarly, fish oil can vary from being completely absent in aquafeed formulations up to 28% inclusion in salmonid feeds produced in the United Kingdom. Importantly, the average fish meal inclusion in aquafeed for a specific farmed species has been significantly reduced in the past decade, decreasing from 28% to 18% in shrimp, from 50% to 30% in marine fish in general and from 45% to 24% in salmonid feed. These promising trends clearly reflect not only the major strides achieved by the fish nutrition science sector, but also that this knowledge gained is then considered by feed mill formulists. Despite the general reduction of fish meal and fish oil percentage inclusion in aquafeeds, there has still been a significant increase in current global fish meal and fish oil exploitation (Tacon and Metian, 2008). This is primarily due to increased global trends in aquaculture production, particularly in shrimp production in Asia and South America, marine fish and catfishes in China and Vietnam, several other species in China, and an increased proportion of tilapia production based on formulated feeds.

8.7.3 Direct use of trash fish and low-valued fish

Fish can be utilised in aquaculture as a feed source in a variety of ways. This takes place through use of fish meal and fish oil in formulated feeds, and also by direct utilisation of whole or chopped raw fish in wet form or as live fish. The latter is not common and the overall amounts used are relatively small (De Silva and Turchini, 2009).

Forage fish (also termed trash fish or low-valued fish) are used for fish culture in fresh-, brackish- and marine waters, as well as for some crustacean species, such as mud crabs, and a few mollusc species (De Silva and Turchini, 2009). Estimates of the amounts used directly (as raw ingredients not previously reduced into fish meal) are variable (De Silva and Turchini, 2009):

1. About 5 million t of trash fish globally.
2. About 4 million t in China alone in 2001 (D'Abramo *et al.*, 2002).
3. 9.8 million t in the Asia-Pacific region. However, only a small proportion is used directly in animal feeds.
4. 2.1–3.8 million t in the Asia-Pacific region (De Silva and Turchini, 2009). They also estimated a reduction in the near future, with this amount contracting to 1.8–2.7 million t by 2010.

The main countries in the region that use fish as feed directly in mariculture are China, Indonesia, Malaysia, Thailand and Vietnam, together with bluefin tuna farming operations in South Australia.

The range in estimates is substantial, which is unavoidable considering the nature and diversity of farming practices and the difficulties in obtaining information. Nevertheless, these estimates provide a figure that can be utilised in planning and development activities and enable recognition of the research needs to reduce the dependence on trash fish resources. As recently pointed out (FAO, 2008a) there is a clear need to move, where possible, from the direct use of raw fish in wet or semi-moist farm-made feeds to industrially compounded aquafeeds. In fact, it is

envisaged that this will increase the overall sustainability of the sector, particularly through:

- greater efficiency in terms of fish-in fish-out of compounded feed compared to raw fish feed;
- moving away from the higher rate of feed waste and related environmental hazards of raw fish feed;
- reduction of the risk of transmission of diseases to cultured stocks and the surrounding environment.

It has been suggested that this trash fish or low-valued fish resource can be more efficiently used directly for human food consumption. However, this raw material is mostly landed in areas where there are other suitable fish commodities for human consumption. Direct usage of the trash fish or low-valued fish resource would involve some degree of value-adding and transportation costs, which are most unlikely to deliver the consumer an acceptable and affordable price, particularly in remote rural areas. In such a scenario, the exploitation of this perishable resource, used directly or indirectly as a feed source to produce a consumable commodity, makes sense and appears to be the most logical use of a biological resource for overall human benefit. Most of all, it contributes to food security through increasing income generation opportunities and hence contributes to poverty alleviation. Another factor that needs to be taken into account is that, particularly in the Asia-Pacific region, there are large numbers of artisanal fishers, who harvest this raw material to maintain their livelihoods (De Silva and Turchini, 2009).

8.7.4 Principles

Important principles for the future directions of aquafeed development have come out of the FAO workshop on the use of wild fish and/or other aquatic species as feed in aquaculture (FAO, 2008a). The main outcome of the workshop was that the use of fish as feed is acceptable, but should be governed by 10 important principles that have been identified:

1. Aquaculture should not utilise resources from unsustainable fisheries.
2. Guidelines for responsible fisheries should be employed where wild aquatic organisms are harvested for use as feed.
3. Reduction fishery and feed fishery operations should not significantly impact the environment or create significant negative ecosystem-level impacts, including impacts on biodiversity.
4. Using fish as feed should not adversely impact the livelihoods and compromise food security of poor and vulnerable groups.
5. The use of fish as feed should not be permitted to be governed by market forces alone.
6. Formulation of policies related to the use of fish as feed should not exclude other users of this primary resource.
7. Aquaculture should be encouraged to make a progressive move away from using wet fish as feed to formulated/compound feeds.
8. The use of fish as feed should not compromise food safety and quality of aquaculture products.
9. The use of alternative raw materials (both animal and plant) should not compromise food safety and quality of aquaculture products.
10. Management of fisheries requires a sound knowledge base and a decision-making process based on the participation of different stakeholders (e.g. capture fishery operators, traders, fishmeal producers, aquaculture operators).

One of the final recommendations of the workshop was that, in order to encourage fish farmers to move away from fresh or non-formulated feeds towards formulated feeds, there is a need to better inform farmers and the aquaculture sector regarding the opportunities provided by formulated feeds and the limitations concerning the direct use of fish as feeds (FAO, 2008a). However, within this context, while it can be empirically supported, the theory that industrially compounded formulated feed can provide a series of advantages to farmers, particularly in terms of conversion efficiency, food safety and impacts on the environment, there is little substantiated scientific evidence to support this contention. In particular, there is a need for specific information regarding the pros and cons of wet feed versus dry compounded feed. This needs to take into consideration the lack of reliable nutritional information for several farmed species, the peculiarities of some aquaculture practices in operation, for example, in remote and not easily accessible areas, and the actual costs associated with the two different practices, which will be significantly different from other realities in different regions with different technological/industrial backgrounds. On the other hand, the opposite is true, for example, for the Southern bluefin tuna industry in Australia. Compounded aquafeeds for tuna have been successfully developed, but it is common knowledge that the industry still prefers to use raw fresh or frozen forage fish directly, merely due to economic advantages, likely derived from legislative gaps

as tuna farmers and forage fish fisheries licence-holders are often one and the same.

8.8 CONCLUSIONS

The science of aquaculture nutrition is progressing fast and major strides have been made since its birth in the 1950s. Our comprehension of the nutritional requirements and metabolic responses of fish and other aquatic species is growing. From the pioneering phase of basic chemical evaluations we have moved into more complex biochemical and biomolecular studies and now into the nutrogenomic era. We are now entering into the '-omic' revolution phase and the tools available are numerous and powerful. Fish meal substitution is theoretically and technically possible. Several studies have shown that fish meal inclusion can be substantially, if not completely, reduced in aquafeed formulation. The drawbacks, however, in our current knowledge are limited only to slightly reduced growth performances of cultured stocks. As such, fish meal exploitation is largely an economic problem and biological nutritional knowledge needs now to merge with business and economic science to generate viable solutions for the industry. Similarly, the inclusion of fish oil in aquafeed can be significantly reduced as well. In this case, however, the drawbacks are not relative to growth performance, but to final quality of farmed fish that could result in a potential deterioration of final nutritional qualities. Unfortunately, a definitive solution to this problem is still to be found, although outstanding progress has been made and it is envisaged that efficient strategies to substantially replace fish oil in aquafeed will soon be available (Turchini *et al.*, 2010). Unfortunately, the industry is often reluctant to apply the knowledge gained by the aquaculture nutrition research sector, a point alluded to by De Silva and Davy (1992) some time ago.

Within this context, it is important to remember that fish nutrition is not solely related to the development of the perfect blend of raw materials and ingredients to provide optimal performance, but should also aim to encompass other fundamental aspects. Environmental impacts are paramount and a more holistic approach is needed. In addition to the outputs of nitrogen and phosphorus, additives and contaminants, processes involved in raw material production and transformation, related transport and carbon emissions are also having a detrimental impact on the environment.

We believe that even the best aquafeed can perform very poorly if not properly managed on farm. Feed management is indeed paramount and can make the difference between an economically viable and environmentally friendly farm and an unsustainable farm from an economic, environmental and social viewpoint.

Needless to say, it should be evident by now that the use of fish meal and fish oil in fish feeds remains a major global issue and a point of contention. The issues relating to usage of these are not only technical and environmental, but are also ethical, with the latter gaining momentum in view of the possibility of channelling the base resources for direct consumption to the malnourished millions on this planet.

Fish meal in particular is also used extensively in husbanded terrestrial animals such as poultry and pigs. Research over the years on these animals has led to major reductions in fish meal usage in their feeds. In this respect fish feeds lag behind.

The ethical question of the channelling of fish resources to animal feeds has been further exacerbated with the recent revelation of use of these resources for non-human food production, primarily for the pet food and fur animal industries (De Silva and Turchini, 2008). All in all, nutrition and issues pertaining to it regarding the culture of aquatic organisms cannot be considered in isolation. Aquaculturists need to have a more holistic approach, paying attention to global issues that are of major relevance to the sector as a whole.

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9
Foods and Feeding

Paul C. Southgate

9.1 INTRODUCTION

Food is one of the major costs for aquaculture operations, typically making up between 30% and 60% of the total operating budget, depending on the intensity of the operation. Clearly, the suitability of the food used, the efficiency with which it is utilised for growth by the culture animals, and the feeding practices used are major factors determining the profitability of an aquaculture operation.

There are two general types of aquaculture foods:

1. Hatchery foods are used to feed larval stages (and their prey items) during hatchery culture. They are usually cultured on-site at the hatchery and include microalgae, rotifers and brine shrimp.
2. Nursery and grow-out feeds are usually man-made and formulated to satisfy the known nutritional requirement of the culture animal.They are usually manufactured as pellets at specialised facilities away from the grow-out site and this requires an understanding of the nutritional requirements of cultured aquatic animals.

Development of effective aquaculture feeds for hatchery, nursery and grow-out requires an understanding of the nutritional requirements of culture animals. Research which generates greater knowledge of the specific nutritional requirements of aquaculture species is the basis for development of more efficient aquaculture feeds and reduced feed costs.

9.2 FOODS FOR HATCHERY CULTURE SYSTEMS

Intensive rearing of the larval stages of fish and shellfish currently relies on the availability of live food organisms. The two major types of live foods used for larval rearing are microalgae and various types of zooplankton.

9.3 MICROALGAE

Cultured microalgae have a central role as a food source in aquaculture. Microalgae are used directly as a food source for larval, juvenile and adult bivalves, and for early larval stages of some crustaceans and fish. They are also very important as a food source for rearing zooplankters, such as rotifers and brine shrimp, which, in turn, are used to feed crustacean and fish larvae.

The golden-brown flagellates (Prymnesiophyceae and Chrysophyceae), the green flagellates (Prasinophyceae and Chlorophyceae) and the diatoms (Bacillariophyceae) (Fig. 9.1) are the most widely used microalgae in aquaculture. As their name suggests, the flagellates possess one or more flagellae, which give the cells motility, whereas diatoms lack a flagellum and are non-motile. Diatoms contain silica in their cell walls and may possess siliceous spines. Some species of diatoms exist as single cells (e.g. *Chaetoceros gracilis*), and other species have cells joined to form chains (e.g. *Skeletonema costatum*). The generalised morphology of microalgae used in aquaculture is shown in Fig. 9.1.

9.3.1 Culture methods

The simplest method of microalgal production is to 'bloom' local species of phytoplankton in ponds or tanks. This is achieved by filling a pool or pond with local water, which is filtered to remove zooplankton, detritus and other unwanted particulates while retaining the smaller phytoplankton. With the addition of fertiliser (usually an inor-

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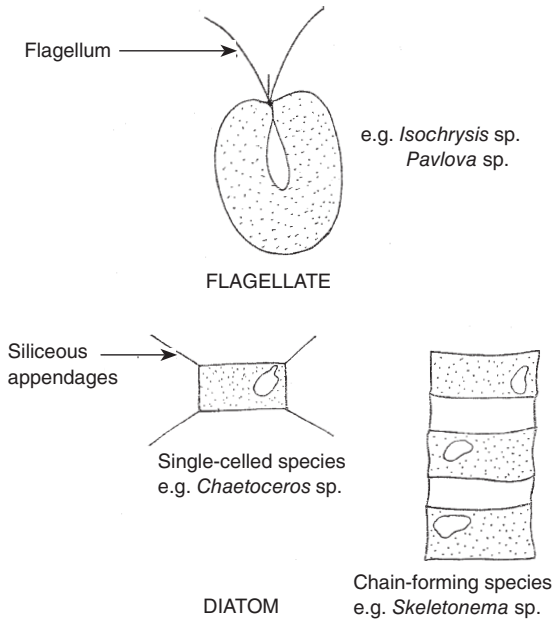


Fig. 9.1 Diagram showing general morphology of a flagellate and diatoms.

ganic fertiliser), adequate light and aeration, blooms of natural phytoplankton will develop. Although inexpensive, this method can be unreliable: the bloom is not guaranteed, and there is little control over the species composition of the bloom. As such, the nutritional value of microalgae produced in this way is unpredictable.

More commonly in aquaculture, monospecific cultures of microalgae are intensively reared in systems where efforts have been made to minimise or exclude bacterial contamination. Monospecific axenic (bacteria-free) 'starter' cultures of many species of microalgae are available to the aquaculture industry from specialised laboratories. These are the basis for microalgae production, which involves scaling-up the culture volume and the density of algal cells by maintaining favourable conditions for algal growth. An example of a suitable scale-up procedure is shown in Fig. 9.2. Stock cultures are maintained under controlled conditions of temperature and light. During the scaling-up process, microalgae are usually transferred from container to container under axenic conditions using a laminar-flow or sterile transfer cabinet. Culture vessels receiving the inoculant contain seawater that has been filtered (usually to 0.2–0.45 µm) and sterilised by autoclaving. Inoculating microalgae cultures under these conditions reduces the possibility of bacterial contamination. However, it is impractical to use this method for culture vessels with volumes greater than ca. 20 L. In larger bag and tank cultures (Fig. 9.3), efforts are made to reduce the bacterial population in the culture water by fine filtration (0.2–0.45 µm); this may be followed by passage of the water through an ultraviolet (UV) steriliser.

Flasks, bags or cylinders of microalgae may finally be transferred to indoor or outdoor ponds or pools where a large aquaculture operation requires large volumes of microalgae.

For growth, microalgal cultures require:

- aeration;
- a suitable nutrient medium;
- light.

At each transfer, a clean vessel containing filtered (preferably sterile) seawater, to which culture medium has been added, is inoculated with microalgae. The newly inoculated vessel is provided with a filtered (0.2–0.45 µm) air supply to maintain microalgae cells in suspension and to supply sufficient carbon dioxide (CO_2) for their growth. The air supply may also be supplemented directly with CO_2 gas to further stimulate growth. Microalgae cultures are maintained under a controlled light and temperature regime, e.g. suitable conditions for most species of microalgae are provided by a photoperiod of 12–16 h, providing irradiance of 70–80 mE/m^2/s at 20–25 °C. The growth of microalgae follows a distinct pattern and consists of a number of different phases (Fig. 9.4):

1. A lag phase occurs following inoculation and is characterised by a steady cell density.
2. The exponential or log phase is marked by a rapid increase in cell density within the culture. This is the time when microalgae generally have optimal nutritional value.
3. The stationary phase is reached as nutrients in the culture medium start to become limiting, and increasing cell density results in reduced light intensity within the culture; the rate of cell division slows and cell density reaches a plateau.
4. The death phase is reached when, eventually, cells within the culture begin to die as nutrients in the culture medium become exhausted and the culture enters the phase characterised by declining cell density.

The cell density of a microalgae culture is usually determined using haemocytometer counts or high-speed electronic particle counters. The volume of microalgae culture

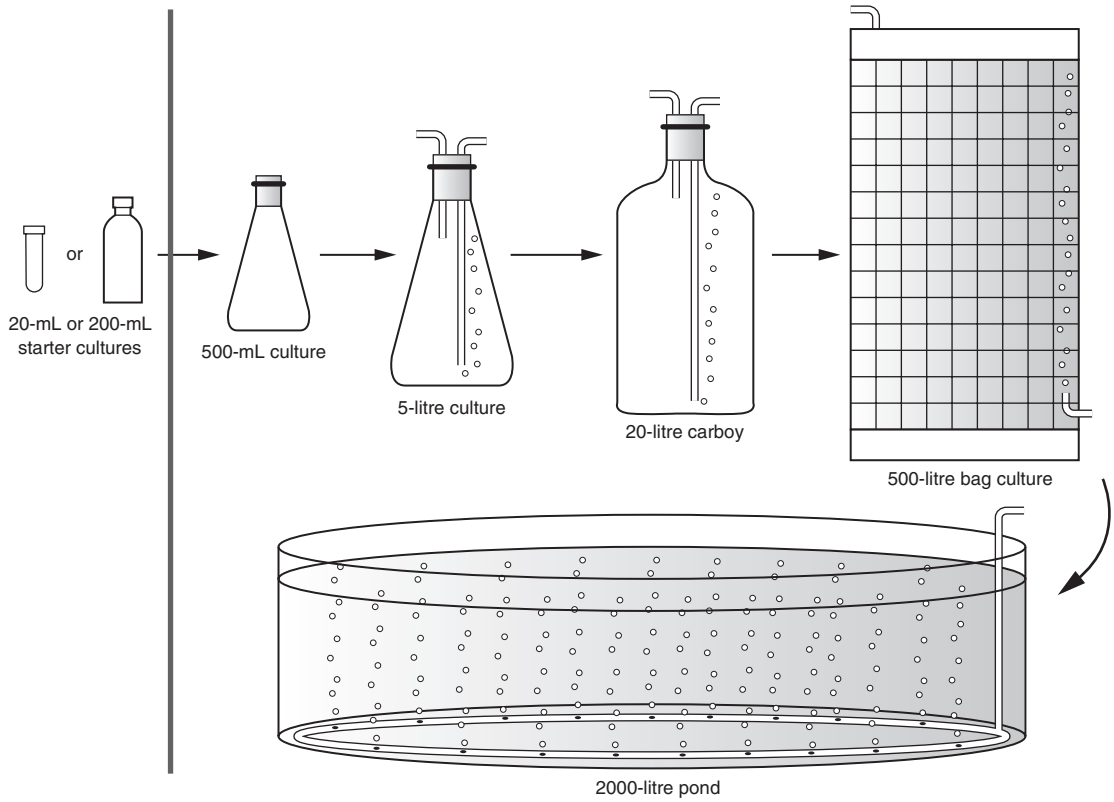


Fig. 9.2 Typical scale-up of microalgae cultures from starter cultures. Reproduced from Brown *et al.* (1989) CSIRO Marine Laboratories Report 205 – *Nutritional Aspects of Microalgae Used in Mariculture: A Literature Review*, with permission from Dr M. R. Brown/CSIRO Marine and Atmospheric Research.



Fig. 9.3 Microalgae culture in 200-L bags and 1000-L tanks.

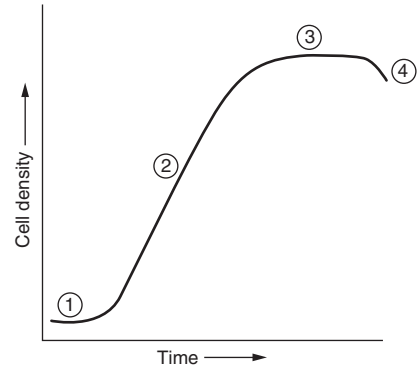


Fig. 9.4 General pattern of changes in cell density over time in microalgae batch cultures. 1, Lag phase; 2, exponential or log phase; 3, stationary phase; and 4, death phase.

that needs to be added to larval rearing tanks is calculated using the equation described in section 9.5.1.

There are three main methods for culturing microalgae:

1. Batch culture. This is when a microalgae culture is grown to a point at which it is completely harvested.
2. Semi-continuous culture. This is when partial harvesting of culture vessels is done periodically and culture vessels are 'topped up' with new water and fresh nutrient medium.
3. Continuous culture. This is when microalgae are harvested on a continuous basis and the volume removed from the culture vessel is continually replaced by new water and fresh nutrient medium.

The objective of continuous and semi-continuous cultures is to maintain maximal growth rate (exponential phase) which maximises production and reduces variability in the biochemical composition (and nutritional value) of the microalgae. In batch cultures, the biochemical composition can vary widely according to the growth phase and age of the culture.

9.3.2 Nutrient media

Many nutrient media have been developed for microalgae culture. They generally contain macronutrients to provide nitrogen and phosphorus (e.g. sodium nitrate, sodium glycerophosphate), trace metals and vitamins. A commonly used medium is the 'f/2' medium of Guillard (1972), whose composition is shown in Table 9.1. Nutrient media are made up from distilled water to which nutrients are added. It is convenient to make up concentrated standard stock solutions of media, which are then added to microalgae culture vessels to provide appropriate nutrient levels. In general, 1–3 mL of stock nutrient solutions is added to each litre of culture water. Given the structural importance of silica in diatoms, a source of silica (usually sodium metasilicate) should be included in nutrient media used to culture diatoms. A stock solution is prepared by dissolving sodium metasilicate (40 g) in 1 L of distilled water, and 0.2–0.4 mL of the resulting solution is added per litre of culture water. Nutrient media are generally added to smaller culture containers (e.g. glass flasks) before they are sterilised by autoclave. Once cooled, containers are inoculated to begin new microalgae cultures.

Table 9.1 The composition of 'f/2' medium for microalgae culture. Reproduced from Guillard (1972) with kind permission from Springer Science+Business Media B.V.

Nutrient	Concentration/L
$NaNO_3$	75 mg
NaH_2PO_4	5 mg
*Na_2SiO_3	15–30 mg
Trace metals	
Na_2EDTA	4.36 mg
$FeCl_3 \cdot 6H_2O$	3.15 mg
$CuSO_4 \cdot 5H_2O$	0.01 mg
$ZnSO_4 \cdot 7H_2O$	0.022 mg
$CoCl_2 \cdot 6H_2O$	0.01 mg
$MnCl_2 \cdot 4H_2O$	0.18 mg
$Na_2MoO_4 \cdot 2H_2O$	0.006 mg
Vitamins	
Cyanocobalamin	0.5 µg
Biotin	0.5 µg
Thiamin HCl	100 µg

*Required for diatom cultures only.

9.3.3 Nutritional value of microalgae

When considering the suitability of various species of microalgae as a food source, the first concern is their physical characteristics. Factors such as:

- cell size;
- thickness of cell wall;
- digestibility;
- presence of spiny appendages; and
- chain formation (e.g. diatoms)

influence the nutritional value of a particular species. Clearly, microalgae must have suitable physical characteristics to enable ingestion and, once ingested, must be digestible. Cultured invertebrate larvae vary in their feeding and digestive mechanisms, and this greatly influences the sizes and kinds of microalgae that can be ingested and digested. For example, shrimp larvae have a complete set of setous mouthparts adapted to feeding on chain diatoms. However, such diatoms cannot be captured and ingested by the ciliated feeding structures of bivalve larvae.

Assuming suitable physical characteristics, the nutritional value of a given microalga is determined by its biochemical composition. Biochemical composition varies greatly between species (Table 9.2) and according to growth phase. Composition is also influenced by abiotic factors such as:

Table 9.2 The gross nutritional composition of microalgae commonly used in aquaculture. Data compiled from Parsons *et al.* (1961), Utting (1986) and Whyte (1987).

	Composition (%)		
Species	Protein	Carbohydrate	Lipid
Golden-brown flagellates			
Isochrysis clone T-ISO	44	9	25
Isochrysis galbana	41	5	21
Pavlova lutheri	49	31	12
Diatoms			
Chaetoceros calcitrans	33	17	10
Phaeodactylum tricornutum	33	24	10
Skelotenema costatum	37	21	7
Green flagellates			
Dunaliella salina	57	32	9
Tetraselmis suecica	39	8	7

- light (photoperiod, intensity and wavelength);
- temperature;
- nutrient medium (composition and concentration);
- salinity;
- nitrogen availability;
- CO_2 availability.

For example, protein levels decrease while lipid and carbohydrate levels typically increase during the stationary phase of a culture. Similarly, the protein content of microalgae is greatly influenced by the nitrogen content of the culture medium. Culture conditions also influence levels of micronutrients such as fatty acids and vitamins in the resulting microalgae. As such, the conditions under which microalgae are grown and the stage at which they are harvested may greatly influence their nutritional value.

The nutritional requirements of cultured aquatic organisms were discussed in Chapter 8. Numerous growth trials, using different species of microalgae as food, have shown that differences in the food value of microalgae are related primarily to their fatty-acid and carbohydrate compositions. As detailed in section 8.3.3, marine fish and shellfish larvae have an essential dietary requirement for *n*-3 highly unsaturated fatty acids (HUFAs). As such, *n*-3 HUFA content is an important factor in determining the nutritional value of microalgae, and it is generally accepted that species containing the essential fatty acids eicosapentaenoic acid (EPA, 20:5*n*-3) and docosahexaenoic acid (DHA, 22:6*n*-3) will be of high nutritional value for cultured animals.

Table 9.3 shows the *n*-3 fatty-acid compositions of various species of microalgae, which vary widely between species. Golden-brown flagellates and diatoms generally contain relatively high levels of essential fatty acids (EFAs), whereas others, notably species of green algae, contain low levels of EFAs or none at all.

Differences in the carbohydrate compositions of microalgae are another important factor in determining their nutritional value. Assuming that dietary EFA requirements are met, research has shown that growth and condition of bivalve larvae are correlated with dietary carbohydrate content. Dietary carbohydrate is used primarily as an energy source and is considered to spare dietary protein and lipid, which can then be utilised for tissue growth (section 8.3.6). Diets consisting of more than one species of microalgae are generally considered nutritionally superior to a single-species diet. They are thought to provide a better balance of nutrients by minimising any nutritional deficiencies present in any of the component species.

Choosing an appropriate species of microalgae for use in an aquaculture hatchery requires careful consideration of their suitability for culture and use under local conditions. This is particularly important when microalgae are cultured in outdoor tanks. The three most important factors to consider for outdoor culture are temperature, salinity and light intensity. For example, in the tropics, the ability of microalgae to tolerate fluctuating salinity and temperature is particularly important in areas where cultures may be subjected to periods of high rainfall and high temperatures. Microalgae vary in their optimal temperature and

Table 9.3 The *n*-3 fatty acid compositions (% total fatty acids) of selected species of microalgae used in aquaculture. Reproduced from Volkman *et al.* (1989) with permission from Elsevier Science.

	Fatty acid		
Species	18:3*n*-3	20:5*n*-3	22:6*n*-3
Golden-brown flagellates			
Isochrysis clone T-ISO	3.6	0.2	8.3
Pavlova lutheri	1.8	19.7	9.4
Diatoms			
Chaetoceros gracilis	–	5.7	0.4
Chaetoceros calcitrans	Trace	11.1	0.8
Thalassiosira pseudonana	0.1	19.3	3.9
Green flagellates			
Tetraselmis suecica	11.1	4.3	Trace
Dunaliella tertiolecta	43.5	–	–
Nannochloris atomus	21.7	3.2	Trace

Table 9.4 Salinity and temperature tolerances of microalgae used in aquaculture. Reproduced from Jeffrey *et al.* (1992) with permission of NSW Fisheries, Australia.

Species	Salinity tolerance (‰)	Temperature tolerance (°C)
Golden-brown flagellates		
Isochrysis sp. (T-ISO)	7–35	15–30
Pavlova salina	21–35	15–30
Pavlova lutheri	7–35	10–25
Diatoms		
Chaetoceros calcitrans	7–35	10–30
Chaetoceros gracilis	7–35	15–30
Thalassiosira pseudonana	–	10–20
Skeletonema costatum	14–35	10–20
Green flagellates		
Tetraselmis suecica	7–35	10–30
Dunaliella tertiolecta	7–35	10–30
Nannochloris atomus	7–35	10–30
Nanochloropsis oculata	7–35	10–30

salinity ranges (Table 9.4). Light intensity also affects the growth rates of microalgae and may alter their biochemical composition and therefore their nutritional value (Jeffrey *et al.*, 1992). Again, this is of particular importance in areas of high natural light intensity such as the tropics.

On the basis of their known ranges of tolerance to these parameters, microalgae can be categorised according to their suitability for culture and use in different environments. For example, Jeffrey *et al.* (1992) divided a range of microalgae species according to their temperature tolerances into:

1. Excellent universal species, which show good growth at 10–30 °C (e.g. *Tetraselmis suecica*, *T. chuii*, *Nannochloris atomus*).
2. Excellent tropical and subtropical species, which show good growth at 15–30 °C (e.g. *Isochrysis* clone T-ISO, *Chaetoceros gracilis*, *Pavlova salina*).
3. Good temperate species, which show good growth at 10–20 °C (e.g. *Chroomonas salina*, *Skeletonema costatum*, *Thalassiosira pseudonana*).

9.3.4 Recent developments in microalgae production

Despite its central nutritional role in aquaculture hatcheries, production and use of cultured microalgae presents a number of potential disadvantages for aquaculture hatcheries:

1. On-site production of microalgae is labour intensive and is associated with high running costs (up to 30–50% of hatchery operating costs).
2. On-site production of microalgae requires specialised facilities and dedicated personnel. There are substantial establishment costs, and microalgae culture requires hatchery space that could otherwise be devoted to larval production.

3. Microalgae cultures can 'crash' through failure of the culture system or infection with contaminant or pathogenic organisms. Either case may result in a shortage of food for the culture organisms.

Research to overcome some of these problems resulted in development of microalgae products that have become commercially available to aquaculture hatcheries:

- microalgae concentrates;
- dried microalgae.

Both allow microalgae to be stored in concentrated form until required, thereby alleviating the need for on-site microalgae culture.

Microalgae concentrates are prepared by removing the culture medium from the microalgae culture to produce a thick paste of concentrated algal cells. The resulting microalgae cells are intact and remain in suspension with minimal agitation of the culture water to which they are added. Microalgae concentrates have very high cell densities (billions of cells per mL) and can be stored for months or years in a refrigerator or frozen. A number of species of microalgae are now available as concentrates from commercial suppliers including those from the genera *Tetraselmis*, *Isochrysis*, *Pavlova* and *Nannochloropsis*. Mixed species concentrates are also available commercially as foods for shellfish and zooplankton. Dried microalgae preparations have been produced from microalgae grown heterotrophically. This technique involves growing microalgae in the dark, using sugars rather than light as an energy source (Gladue and Maxey, 1994). Growth under these conditions produces microalgae with a considerably different biochemical composition from that of the same species grown using conventional culture methods with light. Although the number of microalgae species that can be produced in this manner is limited, some have been produced commercially. A number of studies have shown the value of dried microalgae as a food source for crustacean and fish larvae (Biedenbach *et al.*, 1990; Cañavate and Fernández-Díaz, 2001; Navarro and Sarasquete, 1998) and for the larvae and spat of bivalves (Knauer and Southgate, 1999; Laing and Millican, 1992).

The major advantage of using concentrated or dried microalgae is that the microalgae is cultured at a central facility and distributed to hatcheries. This system eliminates the need for hatcheries to have microalgae culture facilities which could result in considerable cost savings.

9.4 ZOOPLANKTON

Hatcheries that culture fish and crustacean larvae also rely on zooplankton as a larval food source. The two major organisms cultured for this purpose are rotifers and brine shrimp. However, recent years have seen considerable research effort directed towards the development of mass culture techniques for copepods and their use as live feeds in aquaculture.

9.4.1 Rotifers

Rotifers (*Brachionus* species) are widely used in aquaculture as a food for the larvae of fish (section 18.6) and crustaceans (section 21.6.3), and their use in aquaculture was reviewed by Fenggi (1996). The species typically used in marine aquaculture hatcheries are:

- *Brachionus plicatilis* (known as large or L-type), which is ca. 130–340 µm in length;
- *B. ibericus* (known as small or s-type) which is ca. 100–230 µm in length; and
- *B. rotundiformis* (known as super small or ss-type), which is ca. 90–190 µm in length.

Clearly, an important consideration in rotifer culture is selection of a strain best suited to the mouth size of the culture species.

Rotifers consist of a lorica or body shell from which the foot extends ventrally and the head extends dorsally (Fig. 9.5). The head has two bands of cilia used for the capture of food particles and for locomotion. The life cycle of the

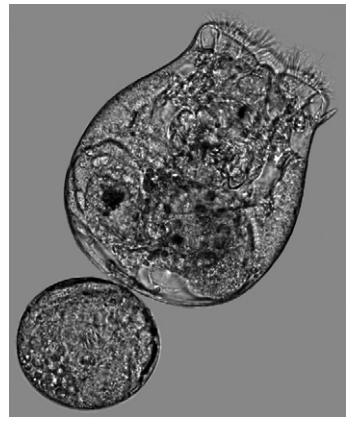


Fig. 9.5 A female rotifer with a single egg (at bottom). The body is enclosed within a shell or lorica and the ciliated head is visible at the top of the animal. (Photograph by courtesy of Richard Knuckey.)

rotifer (egg–juvenile–adult) takes 7–10 days. Cultures may contain both males and females, but males are rare and considerably smaller than females. Rotifers reproduce sexually or asexually depending on culture conditions. Under favourable conditions, reproduction is asexual and the female produces diploid amictic eggs, which she carries until they hatch into females:

Female rotifer → amictic egg ($2n$) → female rotifer → amictic egg ($2n$) → female rotifer, etc.

Most reproduction in cultured rotifer populations occurs by this method.

Under unfavourable conditions, reproduction occurs sexually; females produce smaller, haploid mictic eggs that hatch into males if not fertilised. If fertilised, they become resting eggs that have a dehydration-resistant outer shell. They can remain dormant for several years and hatch into females when conditions become favourable. The presence of males in rotifer cultures therefore indicates poor culture conditions.

9.4.2 Rotifer culture

Rotifers are hardy and are easily mass cultured on a wide variety of foods. Mass culture of rotifers is usually initiated by inoculating a culture of microalgae with rotifers. Under suitable conditions, the rotifers consume the microalgae and their population rapidly increases. Consumption of microalgae must be monitored regularly and more microalgae added when required; it is important to ensure constant food availability. A portion of the culture water is usually removed from the rearing vessels on a daily basis and replaced with a similar volume of microalgae culture. Water can be removed by siphoning through a 60-µm sieve, which prevents removal of rotifers. Bakers' yeast (*Saccharomyces cerevisiae*) and commercially produced modified yeast are also commonly used as a food for rotifers, either singly or in combination with microalgae. Various species of microalgae are used to culture rotifers, including *Nannochloropsis*, *Tetraselmis*, *Isochrysis* and *Pavlova*. The microalga *Nannochloropsis oculata* and bakers' yeast are considered excellent foods for maintaining rotifer cultures. Rotifers are generally cultured using either batch, semi-continuous or continuous methods.

Brachionus plicatilis, *B. ibericus* and *B. rotundiformis* are euryhaline and productive at salinities between 4‰ and 35‰. Optimal water temperature varies between species, with *B. rotundiformis* and *B. plicatilis* most productive at high (30–35 °C) and low (15–25 °C) water temperatures, respectively. Rotifer cultures are generally maintained at a salinity of 20–35‰, within a temperature range of 20–30 °C and with gentle aeration. Successful rotifer culture requires the maintenance of constant conditions. Water quality must be maintained by regular cleaning to prevent the build-up of detritus and faecal matter. Conventional rotifer cultures can be very productive and may reach densities of 700–1000 individuals per mL; however, ultra-high-density culture methods with 10 000–30 000 rotifers/mL have been developed in Japan (Yoshimura *et al.*, 1996).

The health of rotifer cultures can be assessed by monitoring the swimming activity and the number of eggs present. Healthy cultures will contain females that are active and rapidly swimming, with many carrying more than one egg (Fig. 9.5). The presence of male rotifers indicates imminent production problems as sexual reproduction occurs only when environmental conditions become unfavourable.

The nutritional value of cultured rotifers is largely determined by their food. For example, rotifers reared on bakers' yeast, which is deficient in EFAs, are themselves deficient in these fatty acids. For this reason, rotifers reared on yeasts or other foods with low levels of EFAs are usually fed microalgae or artificial feeds high in EFAs prior to feeding to fish larvae. This process is generally known as enrichment and is outlined in section 9.4.7. Use of commercially available microalgae concentrates as a food source for cultured rotifers is becoming increasingly common. In this case, concentrates composed of a single species (e.g. *Nannochloropsis* or *Chlorella*) or a mixture of microalgae species containing EPA is chosen.

Marine rotifers are generally unsuitable as a food for freshwater fish larvae because of their limited duration of survival in freshwater. The freshwater rotifer, *Brachionus calicyflorus*, has shown potential as a larval food source in freshwater culture systems. It can be continuously cultured and shows variation in sizes between strains (section 25.12.7.1. Culture of *B. calicyflorus* and its application in fish larviculture were discussed by Arimoro (2006).

9.4.3 Brine shrimp

Brine shrimp (*Artemia* species) are found worldwide in salt lakes and similar habitats. Their inactive dry cysts can be harvested in large quantities and stored in a dry state for many years. When immersed in saline water, the cysts rehydrate and become spherical, and the embryo inside begins to metabolise. The cyst ruptures after ca. 24 h and a free-swimming nauplius emerges (Fig. 9.6). This first larval stage (instar I) is generally 400–500 µm long and brown-orange in colour. It has a single red eye and three sets of appendages, which have sensory, locomotory and

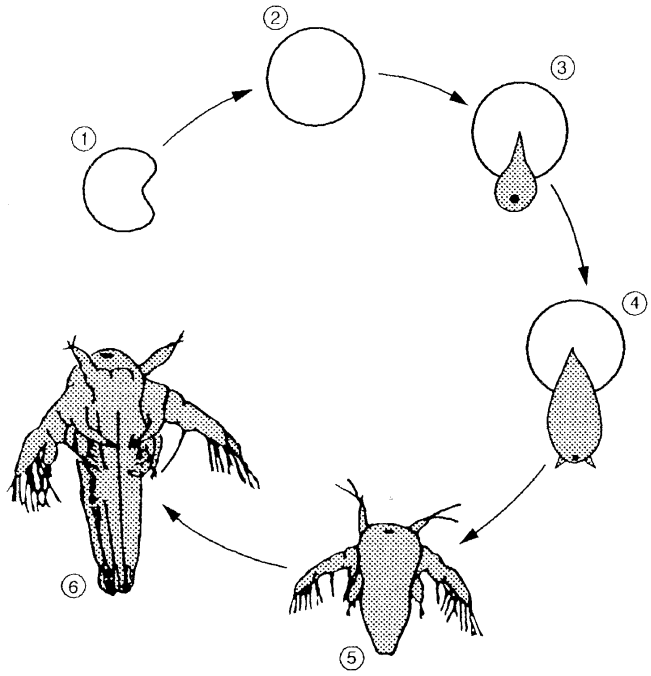


Fig. 9.6 Hatching and development of brine shrimp (*Artemia* species). 1, Dry cyst; 2, hydrated cyst; 3, breaking; 4, hatching; 5, nauplius; 6, larger metanauplius. Reproduced from Southgate and Lou (1995) with permission of Elsevier.

feeding functions. Instar I larvae do not feed as their digestive tract is not yet functional. After ca. 12 h, the nauplius moults to the instar II stage, which has a functional gut and begins to ingest small particles such as microalgae.

Brine shrimp undergo ca. 15 moults over 8–14 days to produce mature adults 10–20 mm in length. Like rotifers, they can reproduce sexually or asexually. Under favourable conditions, females produce free-swimming nauplii (ovoviviparous reproduction); however, under unfavourable conditions, such as high salinity and low oxygen, the shell glands of the female become active and secrete a thick shell around the developing gastrula, which enters a dormant state (diapause). These embryos are released by the female as cysts. Under optimal conditions, brine shrimp can reproduce at a rate of 300 nauplii or cysts every 4 days. Production of cysts has obvious advantages for aquaculture. Dry cysts can be easily stored and live feed (in the form of nauplii) can be produced when required.

9.4.4 Hatching brine shrimp cysts

Although cysts can be successfully incubated in full-strength (35‰) seawater, the hatching rate is generally superior at low salinities and a salinity of 5‰ is optimal. Cysts are incubated at densities up to 5 g/L culture medium, which is maintained at 25–30 °C with vigorous aeration. Dissolved oxygen content must be maintained above 2 mg/L and, to facilitate good aeration and water movement, culture vessels are usually V-shaped or conically based. Cultures require a pH of 8–9 and constant illumination at the water surface. Culture conditions must be constant during incubation. Within 24 h, the majority of cysts will have hatched.

To harvest hatched nauplii, aeration is stopped, causing the cyst shells to float to the top of the culture vessel. Nauplii are positively phototaxic, and this behaviour can be used to concentrate them prior to harvesting by siphon. It is important that the number of cyst shells accompanying the nauplii is limited. Cyst shells have the potential to introduce disease and bacteria into larval cultures and can cause digestive disorders in fish larvae. Contamination with cyst shells can be minimised if the cysts are decapsulated prior to incubation.

9.4.5 Decapsulation of cysts

The process in which the outer shell or chorion is removed from hydrated brine shrimp cysts is decapsulation. This is

achieved by treating hydrated cysts with hypochlorite solution, which dissolves the chorion without damaging the embryo inside. Prior to decapsulation, dried cysts are rehydrated in freshwater for 60–90 min at the rate of 1 g of cysts per 30 mL of water. Approximately 20–30 mL of liquid bleach (sodium hypochlorite – NaOCl) is added per gram of cysts, and the solution stirred continuously. The colour of the solution changes as the chorion is dissolved, and decapsulation is complete within 2–4 min when the solution becomes orange in colour. The solution is then poured through a sieve to remove the chlorine solution. The decapsulated cysts retained on the sieve are washed thoroughly with seawater or freshwater until no further chlorine smell can be detected. Any residual chlorine can be removed from decapsulated cysts by washing in 0.1% sodium thiosulphate solution for 1 min. The decapsulated cysts are then washed and placed into a medium for hatching, or they may be stored at 4 °C for a short period before hatching. Decapsulation offers major advantages in limiting potential digestive and disease problems caused by cyst shells; it disinfects brine shrimp embryos and improves hatch rate. Decapsulated cysts can also be offered directly as a larval food source, with a major advantage being that, prior to hatching, embryos have their maximum energy content.

9.4.6 Culturing brine shrimp

For the production of large or adult brine shrimp, nauplii are reared in tanks at an initial stocking density of ca. 1000–3000 per litre. Nauplii are initially fed cultured microalgae at a density of approximately 5×10^5 to 1×10^6 cells per mL; the feeding rate is adjusted as the brine shrimp grow and more food is required. Best growth of brine shrimp cultures occurs with:

- good aeration;
- good water quality;
- a readily available food supply;
- low light conditions;
- 25–30 °C;
- 30–35‰ salinity.

Culture tanks must be cleaned regularly to remove detritus and faecal matter to maintain water quality. Under suitable conditions, production rates in the order of 5–7 kg (wet weight) of brine shrimp per cubic metre are achievable using batch culture techniques (Sorgeloos *et al.*, 1986).

Brine shrimp nauplii have their greatest energy content at hatching and, because instar I nauplii do not feed, there is a 24% reduction in organic content and a 27% reduction in lipid content between instar I and instar II (Sorgeloos *et al.*, 1986). Although the lipid and fatty-acid content of brine shrimp varies according to geographical origin of the cysts (Webster and Lovell, 1991), they are generally considered to be deficient in essential fatty acids. The nutritional value of instar II nauplii, particularly their fatty-acid content, can be significantly improved using an appropriate enrichment procedure.

9.4.7 Enrichment of rotifers and brine shrimp

Rotifers and brine shrimp are extensively used in aquaculture, primarily because they are amenable to mass culture, not because they are an ideal food source (Table 9.5). Rotifers and many strains of brine shrimp have very low levels (or a total lack) of certain EFAs that are required for normal growth and development of marine larvae (section 8.3.3). To overcome these deficiencies, the EFA content of rotifers and brine shrimp has to be manipulated using fatty-acid enrichment techniques. This process involves feeding a nutrient source rich in EFAs to the rotifers or brine shrimp prior to feeding them to the cultured larvae. Various materials can be used for enrichment,

Table 9.5 Some potential problems associated with use of rotifers and brine shrimp.

Disadvantage	Comments
Nutritional deficiency	Both brine shrimp and rotifers have inadequate fatty-acid compositions for marine larvae. They have to be artificially 'enriched' prior to their use as food, which adds to the expense of food production
Nutritional inconsistency	Live feed organisms may vary in their nutritional composition according to the source of stock (i.e. source of *Artemia* cysts) and the nutritional composition of their food
Reliability of supply	Hatcheries in most parts of the world rely on a supply of brine shrimp cysts from North America. Availability may fluctuate from year to year (Lavens and Sorgeloos, 2000) and imports may be subject to quarantine problems

Table 9.6 The fatty acid compositions of unenriched and enriched brine shrimp nauplii. Reproduced from Southgate and Lou (1995) with permission from Elsevier Science.

	Composition (% total fatty acids)	
Fatty acid	Unenriched	Enriched
18:2*n*-6	5.0	4.5
18:3*n*-3	33.1	25.4
18:4*n*-3	6.6	5.4
20:5*n*-3 (EPA)	2.3	4.7
22:6*n*-3 (DHA)	0	2.5
Total EPA + DHA	2.3	7.2

including microalgae, oil suspensions, microencapsulated diets and yeasts (Leger *et al.*, 1986), and a number of enrichment preparations are available commercially. The benefit of enrichment is clearly shown by increases in the EFA content of the live food organism (Table 9.6). Enrichment of rotifers and brine shrimp boosts dietary EFA intake, resulting in improved survival and growth of larvae.

The degree to which EFAs are incorporated into rotifers and brine shrimp during enrichment is influenced by the duration of the enrichment procedure, the density of rotifers or brine shrimp, and the density and EFA content of the enrichment material. Although enrichment procedures were developed to improve the fatty-acid composition of live food organisms, this process is also used to improve dietary intake of other important nutrients such as vitamins, and as a means of presenting therapeutic compounds.

9.4.8 Copepods

Copepods occur in all aquatic systems and are natural prey for virtually all fish larvae. There are over 10 000 known species, with most planktonic forms ranging between 0.5 mm and 2.5 mm in size. Given their importance as prey for wild fish larvae, there is clear potential for the use of copepods in aquaculture, and developments in this field were reviewed by Nellen (1986). Research with fish has shown that using copepods as food can improve larval survival, growth rates, pigmentation and gut development. Research has focused primarily on the harpacticoid and calanoid copepods:

- Harpacticoid copepods (Fig. 9.7) are distinguished by a very short pair of first antennae with biramous second antennae and a joint between the fourth and fifth body segments. They are generally epibenthic.
- Calanoid copepods (Fig. 9.8) are distinguished by first antennae that are at least half the length of body with biramous second antennae and a joint between the fifth and sixth body segments. They are generally pelagic.



Fig. 9.7 Harpacticoid copepod adult female with eggs from which small nauplii will hatch. (Photograph by courtesy of Tomas Camus.)



Fig. 9.8 Calanoid copepod (*Parvocalanus* sp.) female. (Phototograph by courtesy of Tomas Camus.)

Copepods are readily digested by fish larvae and are superior to rotifers and brine shrimp in terms of nutritional value. In particular, they contain high levels of *n*-3 HUFAs. The small size of copepod nauplii (note egg size in

Table 9.7 Some copepods used as food for fish larvae.

Type	Species	Type	Species
Calanoid	*Acartia clausi*	Harpacticoid	*Euterpina acutifrons*
	Acartia tonsa		*Nitokra lacustris*
	Acartia sinjiensis		*Tigriopus japonicus*)
	Acartia tsuensis		*Tigriopus californicus*
	Acartia tranteri		*Tigriopus brevicornis*
	Centropages spp.		*Tisbe furcata*
	Eurytemora affinis		*Tisbe holothuriae*
	Gladioferens imparipes		*Amphiascoides atopus*
	Paracalanus sp.		
	Parvocalanus sp.		
	Pseudocalanus sp.		
	Pseudodiaptomus spp.		
	Temora spp.		

Fig. 9.7) makes them an ideal food for the early larvae of species with a small mouth gape, such as the groupers and a wide range of copepod species have been used as food for marine fish larvae (section 18.6.2.4; Table 9.7). However, much of our knowledge of the nutritional value of copepods is based on small-scale (experimental) culture. Where copepods are used as a larval food on a larger scale, they are often obtained serendipitously from blooms in local water bodies or from blooms in dedicated ponds following fertilisation (e.g. *Pseudodiaptomus* spp. in Taiwan; section 18.6.2.4). This is because copepods are generally difficult to mass culture with variable and unreliable production. Their density in mass culture conditions is generally low and is influenced by factors including cannibalism (e.g. *Acartia* spp.) and appropriate system design for epibenthic species. However, intensive culture techniques have been reported for some copepod species where efforts were made to reduce cannibalism by regular removal of nauplii (Payne and Rippingale, 2001). It is likely that copepods will assume increasing importance as a hatchery food as more reliable mass-culture techniques are developed and more species are investigated for culture potential.

9.5 FEEDING STRATEGY FOR LARVAL CULTURE

9.5.1 Feeding protocols

A generalised feeding protocol for marine fish larvae begins with rotifers at first feeding followed by brine shrimp nauplii and larger brine shrimp as larvae increase in size (Fig. 9.9). Formulated diets are then introduced and larvae are weaned from live food organisms. Fish hatcheries also culture microalgae as a food source for rotifers and brine shrimp and, as such, they generally culture three different live foods to feed the larvae of a single target species. Shrimp hatcheries generally begin feeding with microalgae (usually a diatom such as *Chaetoceros* species), which are usually followed by rotifers and brine shrimp or just brine shrimp as the larvae grow. Bivalve hatcheries rely exclusively on cultured microalgae as a larval food source.

The volume of a microalgae, rotifer or brine shrimp culture that has to be added to larval rearing tanks to obtain the desired density of food organisms is calculated as:

$$A = (B \times C)/D$$

where A is the required volume (L) of the live food culture; D is the density of the live food culture (number/mL); B is the required density of microalgae, rotifers or brine shrimp in the larval tank (number/mL); and C is the volume of the larval tank (L).

The feeding regimen is an important aspect of hatchery management. Overfeeding is wasteful and expensive. It also compromises water quality, which can lead to disease and affect larval growth. Underfeeding reduces growth rates, thereby increasing hatchery running costs. It is important to monitor the presence of food in larval tanks to avoid these problems.

9.5.2 Some disadvantages of live feeds organisms

A number of disadvantages are common to intensive culture of microalgae, rotifers and brine shrimp. Some of

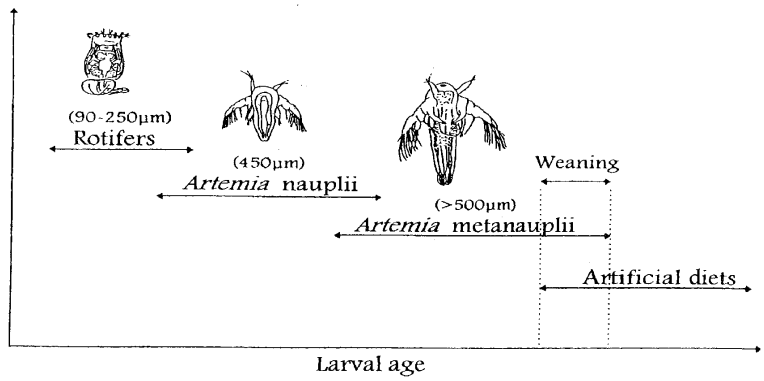


Fig. 9.9 A generalised feeding protocol for marine fish larvae begins with rotifers at first feeding followed by brine shrimp nauplii and larger brine shrimp as larvae increase in size. Larvae are then weaned to artificial formulated feeds. Reproduced from Southgate and Partridge (1998) with permission from Academic Press.

these have been outlined for microalgae production in section 9.3.4, but they also apply to rotifer and brine shrimp production. Other potential problems relating specifically to rotifers and brine shrimp are listed in Table 9.5.

In response to these problems, in particular the high costs associated with live food culture, there has been considerable research and commercial interest in developing compound hatchery feeds as alternatives to live foods.

9.6 COMPOUND HATCHERY FEEDS

9.6.1 Advantages

The high cost of live food production in aquaculture hatcheries could be reduced by cheaper production of live foods and earlier weaning to formulated feeds in the case of crustaceans and fish. Complete or significant replacement of live foods would considerably reduce hatchery running costs, provide 'off-the-shelf' convenience and nutritional consistency. Perhaps the greatest potential advantage of appropriate compound larval diets is that, unlike live foods, the size of the food particle and diet composition can be adjusted to suit the exact nutritional requirements of the larvae. However, they must satisfy a number of criteria (Table 9.8).

Various materials have been assessed for their potential to replace live microalgae as a food for bivalves. These include dried and concentrated microalgae (section 9.3.4), dried and pulverised macroalgae, yeasts and cereal products (Knauer and Southgate, 1999). They also include formulated diets, such as microbound diets and microencapsulated diets, which are often referred to as 'microdiets'

Table 9.8 Desired characteristics of compound feeds for aquatic larvae.

Characteristic	Comments
Acceptability	Must be attractive and readily ingested. Diet particles must be of suitable size for ingestion and must elicit a feeding response from the larvae. Diet particles must remain available in the water column
Stability	Diet particles must maintain integrity in aqueous suspension and nutrient leaching must be minimal. Some nutrient leaching may be beneficial in enhancing diet attractability
Digestibility	Diet particles must be digestible and their nutrients easily assimilated
Nutrient composition	Diets must have an appropriate nutritional composition. Material added to the diet as binders or the components of microcapsule walls should have some nutritional value
Storage	Diets must be suitable for long-term (6–12 months) storage with nutrient composition and particle integrity remaining stable

or 'microfeeds'. Although dried microalgae have also been used in the culture of crustacean and fish larvae (Biedenbach *et al.*, 1990; Cañavate and Fernández-Díaz, 2001), much of the research to develop alternatives to live feeds has focused on microdiets.

9.6.2 Microbound diets

In microbound diets (MBDs), nutrients (both particulate and dissolved) are bound within a particle matrix consisting of a binding material such as agar, gelatin, alginate or carrageenan. Dietary ingredients are mixed with the binder to form a slurry, which is then dried, ground and sieved to produce food particles of the desired size. MBDs allow precise manipulation of dietary contents and, for this reason, have been used extensively in research to determine nutritional requirements of larvae; particularly crustacean larvae (e.g. Genodepa *et al.*, 2004; Holme *et al.*, 2007). However, because MBDs have no barrier between dietary ingredients and the culture water, there is potential for nutrient leaching and they are susceptible to direct bacterial attack.

9.6.3 Microencapsulated diets

Microencapsulated diets (MEDs) consist of dietary materials enclosed within a microcapsule wall or membrane. This greatly reduces nutrient leaching and the susceptibility of the diet to bacterial attack. MEDs have been used with some success as a replacement for microalgae for bivalve spat and larvae (Knauer and Southgate, 1999). MEDs have been commercially available for shrimp larvae for a number of years and are widely used in hatcheries (section 21.6.3). It is generally accepted that a combination of the formulated diet and live feeds, a practice known as co-feeding, supports superior growth and survival of shrimp larvae than either feed alone. It is now possible to completely replace live feeds with formulated diets in penaeid shrimp hatcheries (section 21.6.3). The development and use of formulated diets for crustacean larvae was reviewed by Jones (1998) and Holme *et al.* (2007). Despite the development of successful artificial diets for shrimp larvae and their routine use in shrimp hatcheries, similar success has not been achieved with fish larvae.

9.7 DEVELOPMENT OF ARTIFICIAL DIETS FOR FISH LARVAE

9.7.1 Limited success

Numerous studies have been conducted to assess the nutritional value of microdiets for marine fish larvae (e.g. Koven *et al.*, 2001; Southgate and Partridge, 1998). In general, they have resulted in lower survival and poorer growth of larvae than those fed live foods and they often lead to a higher incidence of deformity. These results indicate that total replacement of live prey with artificial diets is still not possible for the larvae of most marine fish. Despite this, partial replacement of live foods can result in cost savings, and some studies have shown that between 50% and 80% of a live feed ration can be replaced with a microdiet without affecting larval growth (Kanazawa *et al.*, 1989; Koven *et al.*, 2001). Weaning fish larvae to a formulated diet at the earliest possible age is another means of reducing feed costs and is a major goal for commercial fish hatcheries (section 18.7.5). It has been estimated that weaning European bass 15 days earlier enables savings in brine shrimp production of up to 80%.

9.7.2 Constraints to developing formulated diets for marine fish larvae

The relatively poor performance of formulated diets in studies with marine fish larvae is thought to result primarily from reduced rates of ingestion and poor digestion.

Successful formulated diets must be ingested at a similar rate to live foods. This is a particular problem with carnivorous fish larvae, which require the visual stimulus of moving prey to initiate a prey capture response. Attempts to overcome or reduce this problem have included:

- inclusion of various chemicals (using light refraction) into diet particles to impart a sense of motion;
- incorporation of food dyes into diets to simulate the colour of brine shrimp nauplii; and
- amino acids that naturally emanate from live food organisms have been used to enhance larval feeding response and may be incorporated into artificial diets to improve attractability.

Most marine fish larvae are poorly developed at hatch, and in many species the digestive tract does not develop fully until after 'metamorphosis'. Marine fish larvae also have low gut enzyme activity compared to adult fish and, again, secretion of some enzymes begins only after metamorphosis when a functional stomach is present. Marine fish larvae generally improve in their ability to digest artificial food particles with age. Live food organisms consumed by larvae assist digestion by 'donating' their digestive enzymes either by autolysis or as zymogens, which activate endogenous digestive enzymes within the larval gut. The digestive enzymes contributed to fish larvae by live food organisms and the implications for artificial diet development were outlined by Kolkovski (2001). The inclusion of digestive enzymes (particularly proteases) in

artificial diets has been shown to improve nutrient assimilation by up to 30%, resulting in superior larval growth. Similarly, inclusion of digestive system neuropeptides in microdiets may also improve nutrient assimilation and growth.

9.7.3 Weaning diets

Larvae reared on live feeds during the hatchery phase require weaning to artificial feeds towards the end of the larval period (Fig. 9.9; section 18.7.6). The weaning process usually involves providing live feeds together with formulated food particles over a period during which the live feed component of the diet is gradually reduced and the formulated component is increased. The duration of the weaning process varies, but weaning is usually completed within 30 days. A wide variety of weaning diets are available commercially in the form of MBDs, MEDs, flake diets, crushed pellets (crumbles) and yeast-based diets. Development of successful formulated diets for fish larvae would eliminate the need to wean larvae from live to formulated foods: larvae could simply be fed larger food particles as they grow.

9.7.4 Practical problems with formulated hatchery feeds

Formulated hatchery feeds are negatively buoyant and this presents problems maintaining food particles in suspension. This may reduce the availability of food to the larvae, and food particles that settle at the bottom of larval culture tanks may pollute culture water and enhance bacterial activity. In contrast, live foods generally remain motile in larval culture tanks, and this maximises their availability to larvae and reduces contamination of the culture water from uneaten food. Tank design and aeration systems are important in maximising particle buoyancy and for maintaining particle movement. The use of compound hatchery feeds requires careful consideration of tank design and aeration, and regular monitoring of feeding rates. Adding small quantities of food a number of times per day optimises water quality and maximises the food available to the larvae.

9.7.5 Further development of formulated hatchery feeds

As described above, use of commercially produced formulated hatchery feeds is commonly practised in shrimp hatcheries (section 21.6.3). However, the situation is not as good for marine fish and bivalve hatcheries. Although metamorphosis of fish and bivalve larvae fed artificial diets has been achieved in the laboratory, commercial fish and bivalve hatcheries still rely exclusively on live food production for the majority, if not all, of the larval culture period. Development of formulated hatchery feeds for fish and bivalves has been hindered by our lack of knowledge of their nutritional requirements and by problems relating to the attractability and digestion of formulated food particles and their use in culture systems. Development of more suitable formulated diets for marine fish larvae will require further research in the following areas:

- improved ingestion and digestion of artificial diets;
- greater understanding of the nutritional requirements of larval stages;
- development of more appropriate culture system designs.

The potential cost savings offered by the use of suitable artificial diets will ensure that research in this field is ongoing.

9.8 HARVESTING NATURAL PLANKTON

Natural sources of zooplankton represent a large, relatively untapped, potential food source for aquaculture hatcheries. A large portion of this plankton is composed of copepods, which can occur naturally at densities up to 10 000 per cubic metre. Utilising this potential food source requires efficient extraction of zooplankton from very large volumes of water. This can be achieved by pumping water through sieves that collect zooplankton. Plankton harvesting machines have been developed that harvest and grade plankton by size. One of the drawbacks of harvesting from natural waters is that plankton densities may vary between locations and, as such, the reliability of the food supply is questionable. However, this may be overcome by harvesting from dedicated plankton ponds in which high plankton loads can be encouraged by fertilisation. The use of harvested natural zooplankton as a food source for aquaculture is not widespread; however, the variety of organisms composing natural zooplankton would undoubtedly provide a nutritionally superior diet to the standard rotifers/brine shrimp diets used routinely in aquaculture hatcheries.

9.9 POND FERTILISATION AS A FOOD SOURCE FOR AQUACULTURE

Extensive and semi-intensive pond culture of herbivorous and omnivorous species is usually based on food production through pond fertilisation (section 2.3.4). Fertilisation of ponds for semi-intensive culture of tilapia, for example, is outlined in detail in section 16.8.2. Although this system is more commonly used for grow-out, pond fertilisation has also been used successfully for larval fish culture

(section 18.6.2). The fertilisers used for this purpose may be inorganic or organic in nature, or a combination of both.

9.9.1 Fertilisers

Inorganic fertilisers are chemical fertilisers that contain at least one of the primary nutrients nitrogen (N), phosphorus (P) and potassium (K). Commercially available agricultural fertilisers such as ammonium sulphate and superphosphate are widely used in aquaculture. Animal manures are probably the commonest organic fertilisers used in aquaculture, although decomposed plant materials are also widely used. Use of organic fertilisers in aquaculture is an ancient practice and is an economical means of increasing production in aquaculture ponds. There is greater reliance on organic fertilisers in developing countries as they are more readily available than chemical fertilisers. They are also more economical to use and more efficient if pond culture is integrated with crop or animal production. In developing countries, terrestrial and aquatic animals (usually fish) are often reared together in integrated systems (section 2.3.6).

9.9.2 Production in fertilised ponds

Fertilisation encourages primary productivity and promotes a succession of organisms within the pond. Initially, fertilisation results in blooms of protozoa and bacteria, which are generally followed by blooms of algae and then zooplankton. The natural food organisms present in ponds can be divided into a number of categories:

1. Bacteria and protozoans.
2. Plants (phytoplankton, periphyton, macrophytes).
3. Animals (mainly invertebrates: zooplankton, zoobenthos, small nekton).
4. Fish.

Fertilisation increases the biomass of potential food organisms present in a pond. For example, Schroeder (1974) reported zooplankton levels of $<0.055\,g/m^3$ and $3.3–424\,g/m^3$ in non-manured and manured ponds, respectively.

Ecological conditions within a pond determine which organisms are present, the proportion of each and their abundance. All the listed organisms in the pond form the biocenosis (self-regulating ecological community) of the pond, which therefore contains all potential food sources for cultured organisms. However, a given species will only feed on a certain portion of the biocenosis and this is dictated by its feeding habit (e.g. carp species; Fig. 2.10). The specific portion of the biocenosis consumed by an organism is its trophic basis. The trophic basis of a particular species may differ during its life cycle, e.g. the larvae of many herbivorous fish eat zooplankton.

The finite biomass of natural food in a pond can only support a finite standing crop of animals under culture. When the standing crop is low, the amount of available food exceeds the requirements of the culture population and so each animal is able to find sufficient food to support its energy requirement for maintenance and maximum growth. However, an increase in the culture population brings about an increase in their food requirement. At a certain population density, the amount of food required by the culture population for maintenance and growth exceeds that available in the pond. Since the maintenance requirement must be satisfied, the amount of food that can be utilised for growth is reduced and growth rates decline as a result. This standing crop is termed the critical standing crop (CSC). A continued increase in the standing crop further limits the amount of food that can be utilised for growth, and the point at which the natural food available in the pond is sufficient to support only the maintenance energy requirement of the culture crop is known as the carrying capacity of the pond. Fertilisation increases the CSC and carrying capacity of a pond, and growth rates of the culture population can only be increased above these levels if supplementary feed is added to the pond (Table 9.9).

Supplementary feeds are generally classified into simple feeds and compound (or compounded) feeds. Simple feeds may be of animal origin (e.g. trash fish, slaughterhouse waste and fish meal), or of plant origin (e.g. forage, oil meals, rice bran and sorghum). Many simple feeds are relatively cheap agricultural by-products. The simple feeds used by a given aquafarm are dictated by what is locally available. As would be expected, the nutritional composition of simple feeds varies greatly. Those of plant origin

Table 9.9 The effect of fertilisation and supplemental feeding on critical standing crop and carrying capacity of freshwater ponds. Reproduced from Hepher (1988) with permission from Cambridge University Press.

Treatment	Critical standing crop (kg/ha)	Carrying capacity (kg/ha)
No feeding, no fertilisation	65	130
No feeding, fertilisation	140	480
Feeding and fertilisation	550	2500

are usually rich in carbohydrate, whereas others, such as oil meals and animal products, are rich in protein. Compound feeds consist of mixtures of ingredients that are commonly bound together to form doughs or pellets.

An aquaculture system that utilises supplementary feeds is classed as semi-intensive (section 2.3.4), and the densities of culture animals are higher than in equivalent extensive systems that rely on natural foods alone. In many developing countries, however, the use of supplementary feeds may not be feasible because it raises the cost of production. These countries may also have a limited capability to manufacture or import compound feeds.

9.9.3 Pond culture of fish larvae

Freshwater and marine/estuarine species can be reared extensively in earthen ponds with blooms of natural plankton as their food source (section 18.7.1.2). The larvae of barramundi (*Lates calcarifer*) are a good example of larvae reared in this manner. This method has proved to be very successful, and achieves ca. 50% survival through larval rearing and growth rates greater than those achieved using intensive culture systems.

9.10 COMPOUND FEEDS

9.10.1 Formulation of compound feeds

Semi-intensive and intensive aquaculture, especially the latter, require formulated feeds, which are usually obtained as uniform particle or pellet sizes from a commercial source. These feeds are typically used for rearing carnivorous and omnivorous fish and crustaceans, i.e. the highly valuable products of aquaculture in developed countries (Chapter 1). As previously indicated, the use of these compound feeds begins for many culture species when the larval stages have been weaned off the initial live feeds.

The major aim in developing commercial diets for aquaculture is to formulate a diet that satisfies the nutritional requirements of the target species for the minimum possible cost – 'least-cost formulation'. The information required before diet formulation can begin includes:

- a list of available raw materials and information on their compositions and costs;
- knowledge of the nutritional requirements of the target species;
- the specifications of the diet to be made (i.e. desired levels of protein, lipid, amino acids, fatty acids, etc.);
- knowledge of the special suitability of available raw materials for the target animal.

Feed composition tables provide data on proximate composition (see following) of feed ingredients, and they are useful for the initial choice of potential raw materials for aquaculture diets. Information on the proximate composition of feed ingredients is given according to six major components:

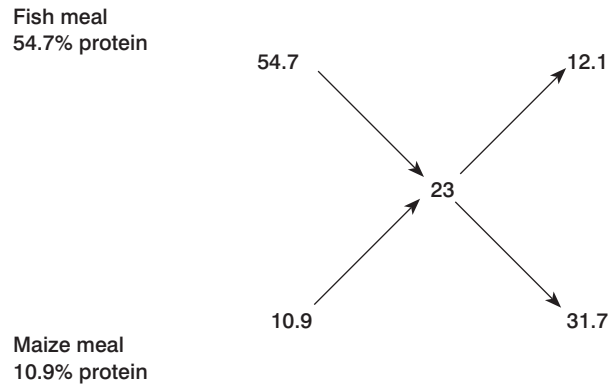
1. Moisture is a measure of the water content of the feed or feed ingredient.
2. Crude protein (CP) is a measure of the protein content, estimated after total nitrogen analysis.
3. Ether extract (EE) is a measure of the lipid- and fat-soluble vitamin content.
4. Crude fibre (CF) is a measure of the insoluble polysaccharide content (e.g. cellulose).
5. Nitrogen-free extract (NFE) is equivalent to the carbohydrate content.
6. Ash is a measure of the inorganic content.

Other compositional data important in feed formulation are the calcium/phosphorus ratio, the available phosphorus content, the amino-acid content (particularly lysine, methionine and cysteine) and the polyunsaturated fatty-acid contents. An example of a feed composition table is shown in Table 9.10. The information from feed composi-

Table 9.10 Feed composition table showing the composition of selected feed ingredients.

	Composition (%)						
Ingredient	CP	EE	CF	NFE	Ash	DE (kcal/kg)	Cost ($/t)
Fish meal	54.7	5.3	4.0	6.1	29.9	3121	580
Shrimp meal	30.6	9.7	0.3	2.3	57.1	2150	400
Groundnut meal	46.9	7.7	6.4	31.7	7.3	3032	330
Maize meal	10.9	5.0	3.1	76.9	4.1	3119	185
Rice bran	13.7	5.4	18.1	48.9	13.9	2417	130

CF, crude fibre; CP, crude protein; DE, digestible energy; EE, ether extract; NFE, nitrogen-free extract.



Required proportions

Fish meal	12.1 ÷ (12.1 + 31.7) × 100 = 27.6%
Maize meal	31.7 ÷ (12.1 + 31.7) × 100 = 72.4%

Fig. 9.10 Balancing the crude protein level in a feed using the square method. In this example, fish meal (54.7% protein) and maize meal (10.9% protein) are to be included in a diet with a desired protein level of 23%. This is achieved with a combination of 27.6% fish meal and 72.4% maize meal.

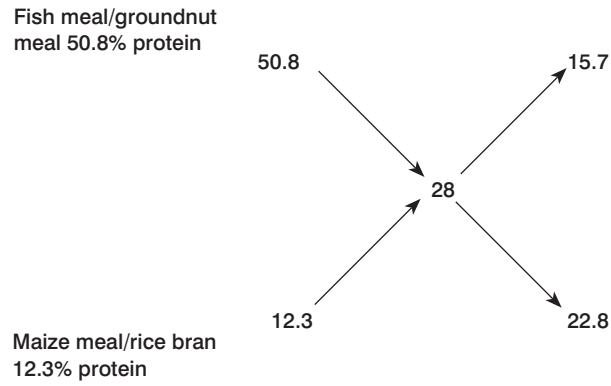
tion tables can be used to construct a table detailing the proximate compositions and costs of complete diets following formulation. The list of potential ingredients often reflects what is locally available.

Protein is usually the largest and most expensive part of an aquaculture diet and, as such, the protein composition of the diet is usually considered first when formulating a new diet. The crude protein level in a feed is most commonly balanced using the 'square method'. A worked example of this method using two ingredients is shown in Fig. 9.10. In this example, fish meal, with a protein content of 54.7%, and maize meal, with a protein content of 10.9%, are to be included in a diet in which the desired protein level is 23%. This desired value is inserted into the middle of the square and the protein content of the two protein sources placed at the left-hand corners of the square. The latter values are subtracted from the value in the centre of the square (ignoring minus signs) and the values obtained are placed on the diagonally opposite right-hand corners of the square.

The values obtained allow us to calculate the proportions of each of the protein sources required to obtain the desired protein level. This is calculated on a percentage basis by dividing the value obtained for each protein source by the sum of both values and multiplying by 100. Thus, a combination of 27.6% fish meal and 72.4% maize meal would provide the desired protein level of 23% (Fig. 9.10).

In practice, aquaculture diets are usually formulated with more than two ingredients. A more complicated example in which four nutrient sources are included in the formulation is shown in Fig. 9.11. In this example, a mean protein content is calculated for combined nutrient sources. From the example, the protein content of a 1 : 1 mixture of fish meal (54.7% protein) and groundnut (peanut) meal (46.9% protein) is calculated as (54.7 + 46.9)/2. The resulting mixture contains 50.8% protein. If the desired proportion of groundnut meal to fish meal was 2 : 1, the protein content of the mixture would be calculated as (54.7 + 46.9 + 46.9)/3; the resulting mixture would contain 49.5% protein.

Using the example shown in Fig. 9.11, two other combined ingredients, maize meal and rice bran, are also to be included with the fish meal and groundnut meal in a diet containing 28% protein. A 1 : 1 mixture of maize meal (10.9% protein) and rice bran (13.7% protein) has a protein content of 12.3%. A combination of 20.4% fish meal, 20.4% groundnut meal, 29.6% maize meal and 29.6% rice bran will give the desired level of 28% protein (Fig. 9.11). The formulation can now be examined in terms of its cost



Required proportions	
Fish meal/groundnut meal	15.7 ÷ (15.7 + 22.8) × 100 = 40.8%
Maize meal/rice bran	22.8 ÷ (15.7 + 22.8) × 100 = 59.2%
Final combination is	Fish meal 20.4% Groundnut meal 20.4% Maize meal 29.6% Rice bran 29.6%

Fig. 9.11 Balancing the crude protein level in a feed containing: (1) a 1:1 mixture of fish meal (54.7% protein) and groundnut meal (46.9% protein) with a protein content of 54.7 + 46.9)/2 = 50.8%; and (2) a 1:1 mixture of maize meal (10.9% protein) and rice bran (13.7% protein) with a protein content of 10.9 + 13.7/2 = 12.3% protein to achieve a desired protein content of 28%. The final formulation is shown in Table 9.11.

and also with regard to its content of other nutrients, such as lipid (Table 9.11). This information can be calculated from data in the feed composition table (Table 9.10). The method outlined above is clearly not restricted to balancing the protein content of a diet. It can be used to calculate the combination of ingredients required to provide a desired level of other nutrients such as lipid.

In practice, diet formulation may be far more complex than the examples shown above and further reading is recommended (e.g. Hardy and Barrows, 2002). Clearly, ingredients must be chosen for inclusion in aquaculture diets so that the completed diet provides the target animal with the required nutrient balance. The completed diet must provide all essential nutrients; dietary protein must provide essential amino acids (EAAs) and dietary fat must provide essential lipids and fatty acids. Other considerations in diet formulation are energy content and a suitable mineral balance and vitamin content. When selecting ingredients from a wide range of available raw materials, it is useful to make a crude comparison of them on a best-value basis. For example, which of the potential ingredients offers the best value per unit protein (cost per unit protein)? Their amino-acid compositions must also be considered; for example, fish meal may have a better EAA profile than plant proteins, but the latter may have higher

Table 9.11 Composition of the diet formulated in Fig. 9.11.

Ingredient	Inclusion (%)	Protein	Lipid	Inclusion cost ($/mt)
Fish meal	20.4	11.16	1.08	118.3
Groundnut meal	20.4	9.56	1.57	67.3
Maize meal	29.6	3.23	1.48	54.8
Rice bran	29.6	4.05	1.59	38.5
Total	100.0	28.0	5.72	278.9

protein contents. The majority of pelleted feeds for marine fish and crustaceans are based on marine protein meals such as fish meal, squid meal and shrimp meal. Although of high nutritional value, marine meals are expensive and may be difficult to obtain in a regular supply. There are also sustainability issues relating to the use of fish meal and fish oil in aquaculture feeds (section 8.7). Increasingly, plant meals (in particular soybean meal) are being studied as a partial replacement for marine meals in aquaculture feeds. Plant meals are considerably cheaper than marine meals, although they are generally deficient in the sulphur amino acids (methionine and cysteine) and they may contain anti-nutritional factors such as a trypsin inhibitor in soybean meal. The use, potential and limitations of using plant meals in aquaculture feeds is discussed in relation to nutrition and marine fish culture in Chapter 8 and sections 18.8.7, respectively.

Aquaculture species differ greatly in their nutritional requirements. Clearly, aquaculture feeds must cater for these differences and, where there is sufficient information about nutritional requirements, commercial diets are usually formulated on a species-specific basis. Broad knowledge of the nutritional requirements of a species may permit formulations to suit specific parts of the culture cycle; this is the case with salmon (Table 8.8). Examples of compositions and ingredients of commercial pelleted aquaculture feeds for marine shrimp and marine fish are shown in Table 9.12, and detailed formulations for salmon are shown in Table 8.8. Their compositions reflect the nutritional requirements of the target species. For example, unlike fish, shrimp are unable to synthesise sterols, which must be supplied in the diet (section 8.3.3). A source of sterols (usually cholesterol) and phospholipids (e.g. lecithin) is usually included in diets for shrimp (Table 9.12). These are not usually supplied as individual nutrients in diets for fish, although sterols and phospholipids are present as components of other ingredients.

9.10.2 Pellet manufacture

9.10.2.1 Compressed pellets

There are two major steps involved in the manufacture of compressed pelleted aquaculture feeds:

1. Grinding involves a reduction in the particle size of coarse ingredients using a hammer mill or roller. This process helps efficient mixing of ingredients and aids the pelleting process. It also increases pellet stability and digestibility of the final product. Once weighed, dietary ingredients are thoroughly mixed to produce a homogeneous blend prior to pelleting.
2. Pelleting converts this mixture into a compressed water-stable form and involves forcing the feed mixture through holes in a metal die plate. Exposure to steam during the process increases the temperature of the diet mixture to ca. 85 °C and its moisture content to ca. 16%. This process causes gelatinisation of some of the starch in the diet, which helps pellet formation and nutrient binding. Pellets of the desired length are cut off as they emerge from the die. Newly manufactured pellets are cooled using a cooler–drier, in which the pellets are moved on a belt through a stream of cold air. The resulting pellets have a moisture content of ca. 10%.

Pellet quality is greatly influenced by the fat level of the diet. Ideally, the fat content of the dietary mixture must be between 2% and 10%. Fat in the mixture aids lubrication of the diet through the die and helps reduce dust. However, excess fat in the mixture can result in insufficient compression and a reduction in pellet hardness. The fat content of pelleted feeds can be increased by top-dressing. This

Table 9.12 Examples of the typical ingredients and general compositions of commercial aquaculture feeds for grow-out of a carnivorous marine fish and shrimp.

Animal	Ingredients	Composition (%)		
		Crude protein	Fat (lipid)	Fibre
Fish	Fish meal, cereals, oilseed meals, protein meals, fish oil, vitamin, minerals	43	10	5
Shrimp	Fish meal, shrimp meal, squid meal, fish/squid oil, wheat flour, soybean meal, broken rice, cholesterol, phospholipid (lecithin), vitamins, minerals	37	2.8	3

involves spraying fat onto the surface of the pellet after manufacture. Top-dressing can also be used to apply heat-sensitive nutrients, such as vitamins, with the fat.

9.10.2.2 Extruded pellets

Extrusion is a process whereby the dietary mixture is exposed to controlled conditions of high temperature (125–150 °C), increased moisture content (20–24%) and high pressure during the pelleting procedure. This process causes the water in the feed to evaporate, which leaves air spaces in the resulting pellets. This can be controlled to regulate the bulk density of the pellet, enabling production of floating or sinking pellets, depending on design. The benefits and disadvantages of the extrusion process are shown in Table 9.13.

Extruded pellets are superior in nutritional value to pellets of the same formula made by conventional steam pelleting. Extruded feeds have a better food conversion ratio and protein efficiency ratio (ratio of wet weight gain to amount of protein consumed) than conventional pellets (i.e. less feed is required for the same growth rate). This has been estimated to allow a 7–8% reduction in production costs for rainbow trout. However, the extrusion process also has some drawbacks (Table 9.13).

9.10.3 Storing pelleted feeds

Aquaculture feeds are used in large amounts in aquafarms and this necessitates on-site storage (Fig. 9.12). Storage conditions can have considerable influence on the nutritional value of pelleted feeds and a number of factors are important in this regard:

1. Age of the feed. Feed must be as fresh as possible when it arrives at the aquaculture site for storage.
2. Environmental factors such as:
   (a) moisture (of feed and humidity of storage area);
   (b) temperature;
   (c) light;
   (d) oxygen.

These affect the stored feed directly or influence other factors causing deterioration, such as fungal contamination and insect infestation.

Regardless of the initial moisture content of pelleted feeds, they will gradually absorb moisture and achieve equilibrium with atmospheric humidity. Because of this, feeds cannot be stored as long in tropical areas because of the high humidity. Ideally, feeds must be stored with a 10–12% moisture content.

Atmospheric oxygen is required for the development of oxidative rancidity of dietary fats and for growth of fungi and insects. However, it is impractical to exclude oxygen from storage areas. Although most fungi are killed during feed processing (by heat), their spores may remain viable and can re-infect the feed if conditions become suitable. Fungal damage is a major concern in the tropics, where high temperature and humidity encourage their growth. Fungal infection causes increase in temperature and moisture, off-flavour and discolouration, and may also produce mycotoxins. These include aflatoxins, which are toxic to fish. Plant products in feeds are particularly prone to contamination with mycotoxins.

Table 9.13 Some benefits and drawbacks of extrusion processing.

Benefits	Drawbacks
Starches are gelatinised, rendering them more digestible	Increased production costs
A high fat content (≤40%) is possible to produce 'high energy diets'	Destruction of thermo-sensitive ingredients such as vitamins.
Gelatinisation also aids pellet binding	Corrected by over-fortifying with these ingredients or by spraying them onto the pellet surface after manufacture
Proteins are rendered more digestible	Reduced availability of certain nutrients (e.g. amino acids)
Anti-nutritional factors are eliminated	
Control over pellet buoyancy	



Fig. 9.12 A feed storage shed at a large shrimp farm.

9.10.4 Chemical changes during food storage

The major chemical changes that can occur in aquaculture feeds during storage include:

- oxidation of lipids;
- fermentation of carbohydrates;
- loss of vitamin activity;
- loss of pigment colour.

Lipids may undergo irreversible autoxidation when exposed to air, causing rancidity. Rancidity results in a number of compounds that have toxic properties and the peroxides are probably the better known of these. Feeds containing ingredients with high levels of polyunsaturated fatty acids (PUFAs), such as fish meals and oil cakes, have a greater risk of oxidation. Grains contain natural antioxidants that protect them from rapid deterioration and antioxidants may also be added to feeds to slow deterioration. Antioxidants commonly added to aquaculture feeds include butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and ethoxyquin (EQ). Fermentation of carbohydrates in aquaculture feeds produces alcohols and volatile fatty acids. Oxidation and fermentation reduce the nutritional value and the palatability of aquaculture feeds and can produce toxic chemicals that may depress growth.

The potency of vitamins may decrease during storage, with vitamin C (ascorbic acid) and vitamin B_1 (thiamin) being particularly susceptible. More stable forms of ascorbic acid, such as calcium ascorbate, L-ascorbyl phosphate and ascorbyl palmitate, are better suited to use in aquaculture feeds. The vitamin content of pelleted aquaculture feeds can be improved immediately prior to use by coating or dipping the feed in a vitamin-rich material.

In order to ensure the maximum nutritional value of stored aquaculture feeds a number of guidelines should be followed:

1. The storage time must be minimised. Food must be used as quickly as possible using the oldest feed first. The feed must be ordered in appropriate quantities.
2. The feed must be protected from the elements (rain, light, high temperature). Efforts must be made to reduce humidity and allow adequate ventilation.
3. The storage area must be kept clean to minimise pest populations.
4. Storage under refrigeration or air conditioning is ideal.

9.10.5 Other components of aquaculture feeds

A number of non-nutritional or undesirable materials are either deliberately incorporated into aquaculture feeds (e.g. to improve feed utilisation and prevent deterioration) or are present incidentally as components of diet ingredients.

The materials present incidentally may seriously affect the growth and survival of culture animals (e.g. toxins), and some may be passed on to humans through consumption of contaminated aquaculture products. Many of these components are naturally occurring and are associated with protein-rich plant materials, fungi and bacteria. They include:

1. Protease inhibitors have been isolated from a number of feed ingredients from plants including soybean meal and wheat flour. Inhibition of enzyme activity (e.g. trypsin) results in poor feed utilisation and depressed growth rates.
2. Gossypol is a toxic pigment present in cottonseed meal. It binds to protein, thereby reducing amino-acid availability and causing reduced growth rates and poor utilisation of feed.
3. Saponins are water-soluble plant toxins. Toxicity is believed to result from destruction of erythrocytes. Aquatic animals vary in their sensitivity to saponins, with fish generally being more sensitive than crustaceans (which do not have erythrocytes). This is used to advantage in the culture of shrimp when saponin-containing material is added to ponds as a piscicide to eliminate fish predators.
4. Cyanogens are plant materials containing cyanide. Their toxicity results from the release of free cyanide following enzymic hydrolysis.
5. Mycotoxins are metabolites produced by fungal contamination of feeds or feed ingredients.
6. Aflatoxins are mycotoxins produced by *Aspergillus flavus* that can be extremely toxic to aquatic organisms. Aflatoxins have been shown to cause tumours and death in fish at very low levels, although sensitivity varies greatly between species. Levels of aflatoxin in feeds lower than 1 mg/kg have been shown to cause death in rainbow trout within days.
7. Bacterial contamination of aquaculture feeds can cause disease and mortality of aquaculture animals. For example, *Mycobacterium* species associated with trash fish fed to cultured Pacific salmon have been reported to cause disease. A bacterium of particular note is *Clostridium botulinum*, which produces a toxin (botulinum toxin) causing death in fish.
8. Chemical contaminants may be present in aquaculture feed ingredients in many forms. The most common contaminants are residues of agricultural chemicals

Table 9.14 Some non-nutritional additives to aquaculture feeds.

Additive	Function
Antioxidants	Oxidation leads to losses of vitamins, amino acids and fatty acids from the feed, and to the production of toxic by-products. Natural antioxidants such as α-tocopherol and lecithin or artificial antioxidants such as BHA (2,3-tert-butyl-4-hydroxyanisole), BHT (3,5-di-tert-butyl-4-hydroxytoluene) and ethoxyquin are often included in the vitamin or mineral premix used in aquaculture feeds
Antifungal agents	Fungal growth leads to production of toxins such as aflatoxins. Examples of antifungal agents used in aquaculture feeds are potassium sorbate, which is a preventive only and does not kill existing fungi, and polypropylene glycol, which prevents fungal growth and kills existing fungi. Neither will remove aflatoxins already present in the feed
Binders	Binders increase the water stability of the feed and reduce nutrient leaching. Examples of binders used in aquaculture feeds are alginate, guar gum, gelatin, starches, lignosulphonates, hemicellulose and carboxy-methylcellulose, sodium and calcium bentonites and polymeric binders. Binders are generally considered to have limited or no nutritional value but they may influence feed intake and digestibility
Antibiotics	Antibiotics may be used at high levels for short-term treatment of disease. Used at low levels, antibiotics may improve growth and feed conversion in terrestrial animals by facilitating nutrient uptake
Hormones	Hormones influence the growth rate of an animal and hormone supplements in feeds can improve growth and feed utilisation. Major problems may be the accumulation of residues in tissues
Pigments	Chemicals included in aquaculture feeds to influence tissue colour (e.g. carotenoid pigments in salmonid feeds). Carotenoids such as astaxanthin or cantaxanthin also function as antioxidants, in provitamin A activities, in immune enhancement and in reproduction and larval and early-feeding stages
Flavourings	Gustatory stimulants such as animal extracts and certain amino acids may increase feed consumption and palatability of aquaculture feeds

BHA, butylated hydroxyanisole; BHT, butylated hydroxytoluene; EQ, ethoxyquin.

such as organic pesticides and herbicides, and heavy metals from industrial wastes. These materials can have a direct toxic effect on culture animals, but may also accumulate in their tissues, rendering the animals unsuitable for human consumption.

Some non-nutritional additives may be deliberately added to aquaculture feeds (Table 9.14) and some of the additives commonly included in shrimp feeds and marine fish feeds are outlined in sections 21.9.1 and 18.8.5, respectively.

9.11 DISPENSING AQUACULTURE FEEDS

9.11.1 Feed ration

The amount of food presented to culture animals has a major influence on the productivity and running costs of an aquaculture venture. Overfeeding wastes expensive feed and promotes water quality problems, whereas underfeeding reduces growth rates, overall yield and profitability. Species-specific feeding tables have been developed as a guide to the appropriate rations to feed culture stock (Table 9.15). They are available to farmers from commercial feed manufacturers. Feeding tables take into account the size of culture animals and the water temperature, both of which influence optimal ration size. Effective use of feeding tables requires knowledge of animal size, the number of animals in a culture unit and the water temperature. However, there are some problems in relying on feeding tables (section 21.9.7) and they should be used in conjunction with observation of factors such as feeding activity, water quality and uneaten food. In pond culture of shrimp, for example, in which feeding activity is difficult to observe directly, feeding trays are commonly used to monitor food consumption (section 21.9.8). Feeding

Table 9.15 An example of a feeding table for a cultured tropical fish.

	Water temperature (°C)								
	20	21	22	23	24	25	26	27	28
Fish weight (g)	Daily food allowance (kg per 1000 fish)								
50	0.51	0.72	0.95	1.12	1.30	1.48	1.60	1.75	1.84
60	0.65	0.87	1.06	1.29	1.44	1.62	1.78	1.89	2.01
70	0.76	1.01	1.20	1.41	1.59	1.75	1.90	2.03	2.13
80	0.88	1.33	1.52	1.70	1.87	2.03	2.13	2.24	2.34
90	0.99	1.22	1.44	1.63	1.82	1.97	2.11	2.24	2.36
100	1.09	1.32	1.54	1.73	1.92	2.07	2.22	2.35	2.45
150	1.52	1.75	1.97	2.17	2.35	2.52	2.65	2.79	2.89
200	1.89	2.12	2.33	2.53	2.71	2.87	3.02	3.15	3.24
250	2.20	2.44	2.65	2.85	3.02	3.19	3.33	3.46	3.56
300	2.48	2.72	2.93	3.13	3.30	3.47	3.61	3.74	3.83
400	2.97	3.21	3.42	3.63	3.8	3.96	4.10	4.23	4.33

tables should be used as a guide only, and rations should be adjusted on the basis of observation if required.

Dividing the daily ration over a number of separate feeds results in more efficient use of feeds and reduces waste. A successful feeding protocol should also account for the natural feeding habits of the culture species. For example, shrimp farms in Asia provide the majority of the daily feed ration for *Penaeus monodon* at night, to better suit the feeding activity of this species (section 21.9.6).

9.11.2 Methods of feeding

Aquaculture feeds are commonly dispensed to tanks and ponds by hand. This allows the farmer to observe feeding activity and inspect culture stock on a regular basis. On larger land-based farms (e.g. shrimp farms), the feed is usually transported to tanks or ponds using trucks or tractors. The feed can then be thrown or shovelled into ponds or can be distributed using machines such as air-blowers, which can propel feed a considerable distance from the pond bank (Fig. 21.20). In ocean-based aquaculture (e.g. cage culture of fish), the feed can be distributed from air-blowers mounted on boats or from water cannons (Fig. 9.13).



Fig. 9.13 Using a water cannon to feed pellets to Atlantic salmon at a farm in Tasmania, Australia. (Photograph by courtesy of John Purser.)

However, manual distribution of feeds (whether machine assisted or not) is costly, labour intensive and inappropriate on large farms. In these cases, feeders can be mounted 'permanently' over a pond, tank or cage and can operate as demand feeders or use timers to control feed release (Fig. 9.14). All have hoppers to hold large amounts of food (Fig. 15.8). Demand feeders have a rod or feeder pendulum, which runs down from the hopper to a depth of ca. 20–30 cm below the water surface. Movement of the pendulum when touched by fish brings about the release of a small quantity of food. Computerised control of feeders is now common on large aquaculture farms. This allows automation of the amount of feed dispensed, the number of feeds per day and the timing of feeds. To improve the efficiency of automated feeders, new technologies are being developed to more closely match the supply of feed to demand (section 15.5.2). Modern 'adaptive feeders' often include some method of feedback by which the amount of feed dispensed is adjusted in response to the



Fig. 9.14 Automatic feeding devices are commonly used in tank-based nursery culture of fish. This type of feeder allows the amount of food dispensed and the frequency and time(s) of feeding to be programmed.

feeding activity of the fish. This approach minimises wasted feed and has obvious environmental benefits (section 4.2.1).

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10
Diseases

Leigh Owens

10.1 INTRODUCTION

To put the impact of disease into perspective, it is interesting to evaluate the future of the human race and its needs. It has been estimated that for humankind to maintain its consumption of seafood at current levels, aquaculture production will have to double by 2020. There probably is not enough land or suitable maritime areas for this to occur without massive disruptions to multiple ecosystems. However, 40% of all aquaculture production is lost to disease, so by just removing or limiting disease impacts, humankind can almost meet seafood requirements without changing any land-utilising practices.

Disease can be defined as 'any process that limits the productivity of a system' and is one of the most seriously limiting factors in aquaculture (Kabata, 1985). Economic evaluations of disease problems are relatively rare, but where they have been determined, the costs are staggering. The best-known example is the US$3 billion loss of cultured marine shrimp in 1994 (Lundin, 1997). Similar losses include:

- US$0.5 billion crash of cultured black tiger shrimp (*Penaeus monodon*) in Taiwan in 1987–88;
- a loss of FF1.8 billion francs over an 8-year period due to protozoan infections in French oysters (*Ostrea edulis*), and a further FF1.3 billion lost in associated industries;
- US$33 million loss caused by Hitra disease (*Vibrio salmonicida*) in Norwegian salmon farms in 1985.

Diseases include both infectious (see Table 10.1) and non-infectious (environmental, nutritional and genetic) problems. The non-infectious diseases are solely due to management practices and are often limited to particular farms. However, infectious diseases have the potential to threaten whole industries and will therefore form the basis of this chapter.

10.2 GENERAL PRINCIPLES OF DISEASES IN AQUACULTURE

10.2.1 Interaction between host, pathogen and environment

The Sneizko three-ring Venn diagram of the interactions between host (the aquacultured species), pathogen and environment is well known (Fig. 10.1). It illustrates the fact that, to occur, most infectious disease is a three-way interaction needing all components:

1. Pathogen.
2. Host.
3. Environment.

Various modifications of this model have been made to illustrate specific points. Non-infectious disease is an interaction only between the host and environment. The area of overlap between pathogen and host represents obligate pathogens: the most threatening group as they do not need environmental stress to cause clinical disease. Epizootic haematopoietic necrosis virus in redfin perch and crayfish plague (*Aphanomyces astaci*) in signal crayfish are two such examples of obligate pathogens producing disease in the most pristine conditions.

For specific diseases of cultured species, the three-ring model can be modified by changing the size of the

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Table 10.1 Some major pathogens of aquaculture species.

Group	Genera, etc.
Viruses	Bacilliform viruses, herpesvirus, iridovirus, nodavirus, rhabdovirus, coronavirus, birnavirus
Bacteria	Rickettsiales, *Aeromonas*, *Enterococcus*, *Flavobacterium*, *Flexibacter*, *Pseudoalteromonas*, *Pseudomonas*, *Streptococcus*, *Vibri*
Fungi	*Aphanomyces*, *Branchiomyces*, *Lagenidium*, *Saprolegnia*, *Sirolipidium*
Protozoa	Amoebae: *Neoparamoeba* Flagellates: *Hexamita*, *Ichthyobodo* Ciliates: *Ichthyophthirius*, *Trichodina* Sporozoans: *Bonamia*, *Loma*, *Marteilia*, *Perkinsus*
Helminths	*Dactylogyrus*
Nematodes	
Annelids	*Polydora*
Crustaceans	Fish 'lice': Isopods Fish 'lice': Branchiura: *Argulus* Copepods: *Lernaea*, *Ergasilus*, *Mytilicola* Crabs: Pinnotherids
Gastropods	Pyramidellids

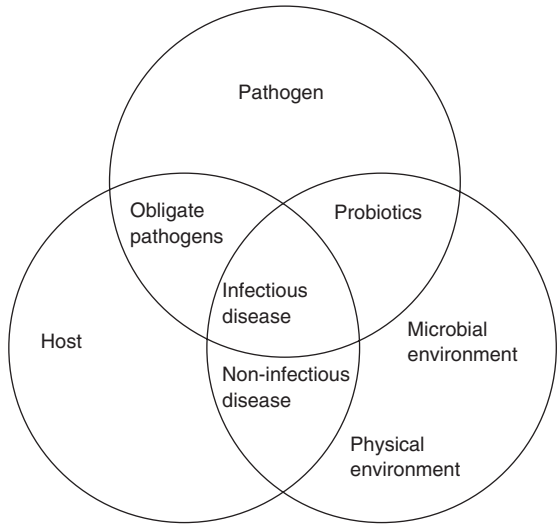


Fig. 10.1 A modified Sneizko three-ring model depicting the interaction between host, pathogen and the environment.

rings to reflect the relative importance of the various components.

10.2.2 Density and disease

The spread of pathogens is a density-dependent process and is therefore affected by stocking rates. The higher the density, the smaller the distance between neighbours. This leads to a higher likelihood of pathogens crossing the distance between hosts in a viable state. Immobile pathogens such as:

- viruses;
- non-motile bacteria;
- sporozoans; and
- parasite eggs

basically follow the diffusion laws and, therefore, in still water conditions a concentration gradient of the pathogens will be formed around an infected individual. Other pathogens, such as:

- bacteria;
- fungal zoospores;
- protozoa; and
- metazoans,

generally have active but variable dispersal capabilities. As distance increases, fewer pathogens will be able to reach susceptible hosts to establish or continue a disease epizootic (outbreak). As there is natural attrition of pathogens in the environment, if the pathogen does not reach a susceptible host in a defined period of time, the chance of establishing a new infection is almost zero.

Higher densities lead to genetic selection of mutant pathogens that are virulent:

1. In the natural environment, a newly mutated pathogen does not 'know' where the next susceptible host will be encountered. In such a case, pathogens that slowly release progeny are selected for because this will give a random-moving infected host a chance to spread the pathogen in the environment and to the next host. That is, a pathogen does not 'want' to destroy its current host, but rather allow it to function as close to normal as possible, thus increasing the chance of an encounter with another susceptible host.

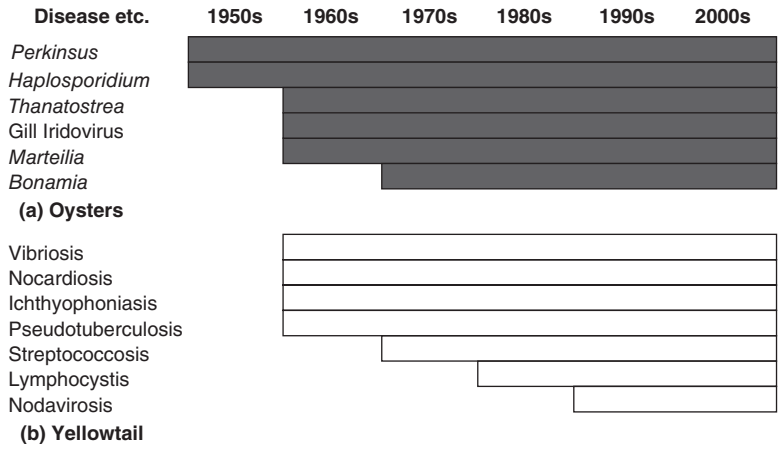


Fig. 10.2 Sequential appearance of microbial diseases of cultured animals. The dates are approximate. (a) Atlantic oysters (*Crassostrea virginica* and *Ostrea edulis*), (b) Japanese yellowtail (*Seriola quinqueradiatus*).

2. However, under culture conditions the next host is very close. A mutant that uses all the available resources of a host to produce pathogenic progeny, no matter what the consequences to the host, will be selected for (i.e. a virulent organism). One of its large number of progeny is more likely to infect the next host as opposed to a conservative, slow-release adapted pathogen (1, above). Once a virulent, resource-utilising pathogen arises by mutation, it is very quickly selected for in an aquaculture environment. A single mutation in a nucleotide that begins a codon for a single amino acid can change virulence from non-virulent to highly virulent. This may contribute to the sequential rise of pathogen problems that beset so many aquaculture industries (Fig. 10.2).

10.2.3 The effect of aquaculture on life cycles of pathogens

By stocking facilities with monocultures, aquaculturalists exclude both predators and competitors of the species being cropped. A large number of the prey items of the cultured species are also excluded. Exclusion of cohabiting animals results in removal of intermediate hosts and definitive hosts from the aquacultural ecosystem. This effectively breaks the life cycle of many of the multihost helminths (flatworms and nematodes) which consequently have less of a role in disease in aquaculture than in wild populations. Seacages are much less effective at breaking these life cycles than are ponds or recirculation systems.

10.3 THE PHILOSOPHY OF DISEASE CONTROL

Disease control in aquaculture is usually attempted on the assumption that an absence of pathogens is the desired state. However, the chance of beginning an aquaculture venture without any potential pathogens in the system are very slim and the question arises as to whether it is cost effective to reach the pathogen-free state. This 'total elimination of pathogens' strategy is the classic approach to disease control: the pathogenocentric approach. This may not always be the best strategy. If, for instance, the chance of re-infection from the local environment is very high, the infection starts to re-establish immediately after control is completed. Clearly, the eradication philosophy is untenable in such areas.

There are a number of factors to consider when deciding on control measures:

1. The cost of the control measure. Some pathogens make culture uneconomical in their presence and must be totally removed from the culture system (e.g. highly pathogenic organisms such as crayfish plague). Others might be self-limiting and only reduce standing stocks by a small percentage (e.g. peritrichous ciliates on crustaceans). In this latter case, living with the pathogen is more cost effective than trying to eradicate it. In most cases, however, there are no accurate cost estimates of losses due to a pathogen. Rather, a general and often very inaccurate 'feel' for the costs associated with a disease agent are the best estimates available. However,

this should not stop the aquaculturalist from trying to economically evaluate the cost of the control method and the downstream benefits. A classic example of the inaccuracy of the 'feel' of an impact of pathogens occurs in the freshwater crayfish industry of Australia. This industry has three 'orphan viruses' (viruses that cause no overt mortalities, so they are not considered important): *Cherax* bacilliform virus, *Cherax* giardiavirus and *Cherax* reovirus. Typically, crayfish with these viruses grow to 35 g in 7 months – standard practice. However, once these viruses were removed by hatchery raising of surface sterilised eggs, the average weight of 6-month-old crayfish was 70 g!

2. The likelihood of re-infection. Ideally, there should be almost no chance of the pathogen being re-acquired from the environment or from wild stocks in the vicinity. Alternatively, infection with a pathogen and subsequent treatment will often allow the vertebrate immune system to be primed and thus further infections are limited (e.g. white spot, *Ichthyophthirius multifiliis*, on fish). However, sometimes the environment is supersaturated with the pathogen from adjoining farms suffering the same problem and, unless the farm can be isolated, treatment may be almost useless (e.g. vibriosis in south-east Asian shrimp farms). In seacage situations, wild stock will often congregate outside the cages where they can easily re-contaminate caged stock that have been treated. Therefore, an understanding of the probability of re-infection is needed to correctly assess the control strategy. Again, this information is usually lacking.
3. An adequate assay for the pathogen. It must be possible to accurately identify the pathogen to be able to assess the effect of the control measures on the pathogen (Sindermann and Lightner, 1988). In the first instance this relies on an accurate diagnosis and later on a sampling regime that will determine true positives (sensitivity) and true negatives (specificity) (Table 10.2). The sampling regime is constructed by assuming a certain prevalence which is the maximum allowable for that pathogen and the confidence level that is acceptable by the aquaculturist. If the test is insensitive, the sample size or frequency of sampling must be increased to compensate for the low discriminatory ability of the test. If the test is non-specific, then animals will be assessed as infected when they are not, and a pathogen control or treatment regime may be discounted when it had worked at the level required. The numbers needed for accurate sampling of low prevalences are large and few commercial ventures willingly part with the necessary numbers without monetary compensation or legislation that makes compliance mandatory.

Table 10.2 Relationship between a diagnostic test and the real presence of a pathogen.

Result of test	Pathogen present	Pathogen absent
Positive	A	B
Negative	C	D
	A + C	B + D

Sensitivity (%) = A/(A + C) × 100.
Specificity (%) = D/(B + D) × 100.

10.4 GENERALISED DISEASE MANAGEMENT TECHNIQUES

The most important factor for the movement and introduction of pathogens to farms and, indeed on any geographical scale, is the movement of animals. This includes:

- live broodstock in particular;
- live larval forms for stocking;
- live alternative hosts, such as hobby fish;
- frozen carcasses for human consumption;
- aquaculture feeds;
- bait.

Probably 99% of new introductions of pathogens to uninfected systems are due to the unrestrained movement of contaminated animals. Sometimes this is unavoidable as aquaculture does not exist without either broodstock or live juveniles for stocking. However, the bio-security of broodstock, the number one contaminator, has been greatly neglected and should be the first point considered, i.e. are pathogen-free broodstock available? If not, what is the pathogen status of the broodstock that is being used? For example, the number one scourges of marine finfish aquaculture and shrimp aquaculture, viral encephalopathy and retinopathy and whitespot syndrome virus, respectively, are both spread vertically from broodstock to larvae and then distributed through infected postlarvae and juveniles to farms.

While it is impossible to have strategies that will work for all pathogens, there are a number of procedures that can help limit pathogens within culture systems.

10.4.1 Batch culture

Batch culture works on the 'all in, all out' principle. Continuous culture eventually suffers from the early

batches acting as pathogen 'factories' which contaminate the environment to levels where later batches cannot be raised (e.g. viral encephalopathy and retinopathy or 'barramundi nodavirus'). Furthermore, young animals are often susceptible to levels of a pathogen that only mildly affect older stages. This is due to a naive immune response in young animals and, having fewer cells in a target organ, the animal is more compromised when cells lose their function due to a pathogen. Drying out and sterilisation of the culture system and associated items between batches stops the amplification in numbers of pathogens for later batches. This is widely practised in hatcheries, but it is often not used in grow-out systems, where individuals from a number of spawnings and of different ages may be mixed to stock up to an optimum density. However, batch culture during grow-out and techniques to reduce pathogens between batches, such as drying out ponds and leaving ponds or seacage areas fallow, are employed by the marine shrimp (section 21.4.2) and salmonid industries (section 15.4).

10.4.2 Incoming water treatment

Treatment of incoming water is essential in recirculating culture systems and more useful in hatcheries than grow-out situations due to the sheer volume of water involved in the latter. Water treatment includes chemical sterilisation (chlorine, iodophores, ozone) and physical sterilisation (UV light). All are greatly enhanced by the inclusion of good settlement ponds preceding the treatment. Particulate matter, producing high turbidity, offers a substrate, nutrition and protection for pathogens, particularly bacteria, which prefer to be attached to substrates (benthic) rather than pelagic. It has been shown that such settlement for 9 days causes *Vibrio* bacteria to die out and be replaced by oligotrophic, less pathogenic, species. The bodies of each generation of bacteria are used as nutrient for a progressively smaller biomass of bacteria, so that the numbers and biomass spiral downwards. This phenomenon is called 'self-cure'. Settlement ponds reduce the particulate load of incoming water. Sterilisation is most effective against obligate pathogens that cannot use an alternative life cycle to build up their numbers (e.g. viruses, rickettsia, chlamydia, sporozoans). However, aerosols arising from vigorous aeration are very common in hatcheries. They allow facultative pathogens to bypass the sterilisation processes, build up to threshold numbers and then cause problems. For example, bacteria in marine shrimp hatcheries have been shown to travel 8 m as aerosols to contaminate other tanks.

Chlorine is very dangerous to use as a sterilising agent in organically polluted waters due to the formation of chloramines. These are highly reactive, have a long half-life and are not neutralised by chemicals used to remove free chlorine. Many aquaculturists have neutralised free chlorine and subsequently watched in horror as their fry proceeded to die from chloramine toxicity when the water was used.

Ozone is a particularly useful sterilising chemical, especially for recirculation where its application can be tied into reduction-oxidation (redox) measuring devices. When reduction potential is high, anaerobic bacteria easily produce electrons from electron-donating substances and electron acceptors are relatively rare. These electrons are highly reactive and can cause cellular damage as a means of stabilising. At high oxidation potential, electron acceptors are abundant and free electron damage is low. For aquaculturists, oxidative conditions are preferable and ozone produces such conditions. Ozone is also safe as it and its ozide derivatives have a very short half-life and the final product, oxygen, is very useful.

10.4.3 Lower stocking density

By lowering the stocking density, the average 'interfishal' distance is increased and the probability of a pathogen reaching the next host is reduced on an exponential scale. On theoretical grounds, epizootics (disease outbreaks) will fall to extinction unless a threshold number of hosts is present in a given area. Simplistically, each infected host must infect at least two other hosts as it succumbs or the epizootic will not propagate. Furthermore, lowering stocking densities will also decrease the level of sibling-interaction-induced stress and competition for space and food.

10.4.4 Single spawning stockings

Differential growth is a good indicator of poor health in a captive population. Runts are very useful to screen for diseases as they are either stunted by pathogens, or behaviourally and nutritionally stressed by being at the bottom of a pecking order (Fig. 10.3). Such stressed animals will also express pathogens. If a mixed spawning population is used to stock a culture system, the differential growth due to age, genetics or variations in hatching conditions will obscure pathogen-caused, differential growth. Thus, stocking with a single spawning is of particular benefit for an aquatic pathobiologist. This technique is not quite as useful in many finfish species where size grading is a normal part of culture (e.g. eels, salmon and trout), but it

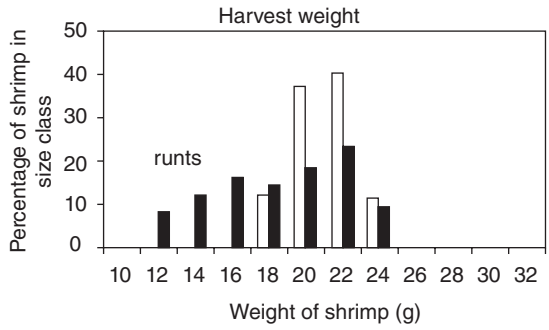


Fig. 10.3 A typical proportion of runts in a population of shrimp carrying a suite of diseases. Note the spread of the sizes of the infected shrimp (■) across more size classes compared to the uninfected shrimp (□).

works well for invertebrates (e.g. freshwater crayfish). This technique also highlights the problem of a very common practice among fish farmers. At harvest, most fish farmers will put the runts that are too small to meet market needs into a pond to allow them to grow to market size. This overlooks the most likely reasons for their failure to reach market size: they are compromised by having a disease. So the farmer, in reality, is keeping a reservoir of diseased individuals on the farm to infect the next stocking.

10.4.5 Specific pathogen-free broodstock

Most pathogens are more virulent to the younger stages of a host. By producing offspring from broodstock free of specific pathogens, the offspring have a good chance of growing to a non-susceptible size before being infected and thus a crop can be produced even in an enzootic area. This can also work if all life stages are equally susceptible, but by late infection of the host, the crop can be grown to harvest before the epizootic has a chance to establish. This is the approach taken for infectious hypodermal and haematopoietic necrosis virus in marine shrimp (*Litopenaeus vannamei*) culture.

Use of the words 'specific pathogen-free' (SPF) is greatly misunderstood by farmers and scientists alike who in their mind hear the words 'free of all diseases'. The term SPF should be followed by the pathogens that it has been tested for and found free of as part of the definition, to start to clear up the misinterpretation that abounds in society and the literature.

10.4.6 Vaccination

Vaccination basically works on the premise that an immunological memory exists and that prior exposure to a pathogen will allow a stronger and quicker immune response. Vaccination success against bacteria in Norwegian salmon has been almost solely responsible for the increase in production during the early 1990s.

Vaccination works reasonably well in vertebrates with both specific and cross-reactive antigens (Ellis, 1988), but immunological memory has not been demonstrated in invertebrates. Evidence is accruing that adaptive immunity does perhaps exist in invertebrates. Enhanced stimulation of invertebrate haemocytes (blood cells) by beta-glucans or killed *Vibrio* cells in the diet has been shown to confer some protection. This is thought to work by blocking the binding sites for the pathogens or perhaps increasing the production and maturation rate of haemocytes, a premise for which there is little evidence. On theoretical grounds, not every fish has to be fully vaccinated to achieve protection of the entire population. If about half the population in each pond is protected, this is equivalent to halving the stocking density of susceptible fish and, in many cases, this will prevent epizootics from progressing.

In most cases, the only practical method of delivery of a vaccine is via immersion at the nursery stage (Table 10.3). Furthermore, many factors affect the success of vaccination (Table 10.4).

Immunity and vaccination are covered more comprehensively in Chapter 19.

10.4.7 Stress reduction

Stress is often used as an excuse for problems when no other logical explanation is available. Despite this nebulous usage of the concept of stress, it does have a real physiological basis and consequences (Pickering, 1981). Unfavourable conditions lead to an adaptive response and a new level of homeostasis is achieved. If this is not achieved, then exhaustion follows and the over-production of the stress hormones (including, in fish, the corticosteroids cortisol, cortisone, corticosterone, 11-deoxycortisol and adrenocorticotrophic hormones). High plasma levels of corticosteroids:

- induce lymphopenia;
- reduce phagocytosis;
- reduce access of lymphocytes to inflammatory sites;
- deplete vitamin C reserves and consequent wound repair;
- increase protein catabolism (gluconeogenesis) leading to muscle wasting, low antibody and collagen synthesis, which again restrict wound repair.

Table 10.3 The effect of the route of vaccination on the immune response.

Route	Systemic antibody response	Problems
Immersion	None	Only the integument is protected by monomeric IgM. Systemic infections can still occur through skin breaches. Antigen might possibly enter the rectum, and give some systemic protection
Oral	None	The acid stomach often denatures the antigen, so large doses are often needed to reach the lower intestine 'Peyer's patch-like' accumulations of macrophages where stimulation will occur. Memory cells are sensitised
Inoculation	Yes	Best protection as both memory cells and antibodies are produced, but practically it can be very costly to handle fish individually. Handling stress is increased. Swim bladder inoculation is superior to muscle, which is better than intraperitoneal

Table 10.4 Factors affecting vaccination responses.

Factor	Response
Temperature	The higher the temperature the better the response, within physiological limits
Size	The larger the animal the better the response, independent of age. Earliest size is 0.5 g but 10 g is better
Diet	Vitamins C and E are most important and high protein diets increase response
Stress	High stress can prevent an effective response
Dose of antigen	Larger is not necessarily better. A small primary dose followed by a larger secondary dose is most often the best
Timing of booster vaccination	If the booster is given when the primary response is waning, the secondary response is better
Age	The older the better. Ova may have maternal antibody which will neutralise the antigen and prevent an immune response. Non-specific immunity (interferon, transferrin and lysozymes) is present in the very young. Cell-mediated immunity is present within 2 weeks; antibacterial antibodies within 4 weeks and antiprotein antibodies within 8 weeks
	Vaccines have not been extensively tested with larval invertebrates
Antibiotics	Antibiotics can suppress the immune system by 90%, particularly oxytetracycline which lowers protein (antibody) production at the ribosomal level

Levels of the stress hormones do not correlate well with levels of stress as some fish pass through the high levels of secretion to a new, highly stressed state with no secretion of the hormones. The two most practical ways of limiting stress are to double the aeration, thus alleviating any oxygen stress that may be occurring, particularly during hot summers, and to lower the stocking density, as already mentioned above. In Australian prawn ponds, keeping the morning dissolved oxygen above 4.44 ppm was the single biggest factor able to limit viral mortalities and keep survival at 85%.

10.5 MAJOR DISEASES

This topic is impossible to cover adequately in a general text. The following rendition only gives a broad brush to the various aquaculture industries.

10.5.1 Molluscs

10.5.1.1 Edible oysters

Worldwide, protozoan parasites are the most significant cause of losses to bivalve industries. This predominance of protozoan parasites is reflected in a guide to diseases

for the mollusc farmer (Elston, 1990). Of the eleven 'notable oyster diseases' described in this guide, seven are caused by protozoans:

1. *Perkinsus marina*.
2. *Haplosporidium nelsoni*.
3. *Haplosporidium costalis*.
4. *Bonamia mackini*.
5. *Bonamia ostrea*.
6. *Marteilia refringens*.
7. *Hexamita nelsoni.*

Although this guide to diseases is based primarily on the experiences of the North American and European cultured bivalve industries, similar diseases affect bivalve industries around the world.

For example, *Marteilia sydneyi* (Acetospora) causes summer mortality in Sydney rock oysters (*Saccostrea glomerata*) in Australia. A flushing of rivers usually brings on an outbreak of the diseases (epizootic) with deaths in about 6 weeks. The digestive gland is attacked, first in the Leidig tissue and later the epithelium. The gland becomes pale yellow and watery and gonadal condition may be greatly reduced. No control is known, but growers try not to have oysters on their leases over the wet summer months. The intermediate host appears likely to be a polychaete that scavenges the tissues of dead and dying oysters. *Marteilia refringens* causes similar problems in *Ostrea edulis* in France and Spain, with infection occurring first in summer and most deaths in winter.

The *Bonamia* group of intracellular parasites cause problems worldwide, infecting both the cupped and flat oysters. They are characterised by causing mortality in oysters 3 years or older when the oysters enter the female stage of their life cycle. *Bonamia roughleyi* causes winter mortality in Sydney rock oysters and requires high salinity (30–35‰). Small yellow to brown pustules occur on the gills, palps and mantle. *B. mackini* occurs in North America and causes similar problems in the Pacific oyster (*Crassostrea gigas*).

Bonamia ostreae is a major problem in the cultured flat oysters of Western Europe. It appears that it was originally taken to France with shipments of oysters from the western United States, similar to the route of the iridovirus causing oyster velum viral disease (OVVD). *Bonamia exitiosus* has also caused local problems in New Zealand bluff oysters (*Ostrea chiliensis*), where it destroyed over 80% of the industry over a 6-year period. In Australia in 1991, the Victorian flat oyster industry also lost 90% of its production to *Bonamia*. Early this century, boats took live bluff oysters from New Zealand to Hobart and then to Melbourne, because the local fisheries had collapsed. Speculation suggests that this spread the *Bonamia* from New Zealand to Australia, but the question of what first caused the collapse of these oyster industries needs to be answered. Furthermore, the occurrence of *Bonamia* in Western Australia supports the premise that the parasite is native to Australia.

Bonamia first infects the tissues below the gut and later invades the haemocytes that are involved in resorbing unspent gonadal material, especially the ovaries. *Bonamia* is a density-dependent disease. Reducing the stocking density and growing the oysters on hanging lines to keep them off the bottom should allow marketable crops to be grown in areas where *Bonamia* is endemic.

It is not just protozoans that cause disease in molluscs, however; viruses and bacteria are also involved.

A herpesvirus (131 nm diameter) has been associated with larval mortalities in Pacific oysters in northern New Zealand. Feeding ceased between days 3 and 4, and 60 to 100% mortality occurred in days 7–11. The cells infected were the fibroblasts and presumptive phagocyte precursors, which displayed enlarged, marginated nuclei with both intranuclear and cytoplasmic inclusion material. Herpesviruses have been reported from oysters in the northeastern United States, northern Wales and northern France. Predisposing conditions for all herpesvirus-related disease included elevated temperatures and crowding.

Iridoviral infections cause disease of the larval velum in Pacific oysters in western United Sates (OVVD), and gill necrosis and haemocytic diseases in adult *Crassostrea angulata* and, lately, Pacific oysters in France. There has been speculation that the same iridovirus is responsible for all diseases and was imported into France from the United States with Pacific oysters.

Bacterial infections have from time to time caused mortality in Tasmanian oyster hatcheries. *Vibrio* (13 strains), *Alteromonas* (10 strains), *Pseudomonas* (8 strains) and *Flavobacterium* (3 strains) were all involved. Five of the *Vibrio* and two of the *Alteromonas* strains caused mortality at 10^7 and 10^5 bacteria per mL, respectively, with the lower doses taking longer. All strains were lethal at 10^8 bacteria per mL. Bacterial virulence for oysters has been strongly associated with a low molecular weight (500–1000 kDa), heat-stable toxin. The toxin stops cilia movement (ciliostasis), which in turn reduces feeding and swimming in larvae and inhibits the cleaning function of the gills of older oysters.

Some diseases affect the oyster's shell and not the soft tissues. The shell may be much weakened structurally in

extreme cases, thus exposing the oyster to predation. In lesser infections there is malformation of the shell, making the oyster less presentable or unacceptable to the market, which is as good as killing it from the viewpoint of the industry.

There is a shell disease caused by a fungus that grows as filaments through the shell, weakening the shell and causing dark raised 'warts' on the inner surface of the shell. It occurs in the European flat oyster, where it has caused heavy mortalities in some regions. Similarly, boring sponges, *Cliona* species, riddle the shells of some bivalves, including clams and pearl oysters, with very deleterious effects on marketability. However, *Cliona* is apparently not a problem with table oysters.

Boring polychaete worms (*Polydora* species) invade the shells of oysters, some other benthic bivalves (e.g. scallops) and even abalone. They have a widespread distribution, including Norway and France. *Polydora* is responsible for the use of intertidal stick culture of Sydney rock oysters in northern New South Wales. These 'mudworms' bore through the shell, causing blisters on the inner surface. By culturing oysters in the intertidal zone, the racks dry out during low tides and newly settled polychaete larvae are killed. *Polydora* also affects abalone farms in South Australia. Similarly, *Polydora* causes problems in benthic mussels, particularly in Victoria, Australia. However, rafted mussels are almost free of infection, except for minor problems in Port Jackson.

10.5.1.2 Other molluscs

There is a large family of ectoparasitic gastropods, the Pyramidellidae, and some parasitise oysters and other bivalves. Pyramidellids are small gastropods which suck the blood or other tissue fluids of the host by using a long, penetrating proboscis. In mild infections the host has reduced energy for growth. In heavy infections, the host may die of tissue and fluid loss. The pyramidellid, *Boonea impressa*, is an important parasite of the American oyster.

Epizootics have been observed in cultured pearl industries. There were considerable long-term mortalities in the blacklip pearl oyster (*Pinctada margaritifera*) in French Polynesia during the late 1980s, but no causative agent was identified. In the silverlip pearl oyster (*P. maxima*) industry in Australia, a bacterium, *Vibrio harveyi*, was isolated from the haemolymph of dying oysters. Cold water temperatures (19 °C) apparently predisposed the pearl oysters to infection, and crowded transportation with very little water circulation allowed the bacteria to establish. Of some interest is the association of *V. harveyi* with mortalities of scallop spat in temperate, central New South Wales.

A protozoan parasite, *Perkinsus*, has been associated with mortalities of pearl oysters in northern Queensland and a haplosporidian has been found in Western Australian pearl oysters.

Die-back of abalone in South Australia has been associated with *Perkinsus olseni*. When water temperatures are elevated, the cellular immune system of the abalone cannot encapsulate and destroy the *Perkinsus*. Treatment consists of lowering the water temperature by 8 °C in a holding tank. This will stop even a progressing disease outbreak in 2 days. A related species, *Perkinsus marinus*, has caused kills of table oyster in southeastern United States during summer.

Several families of decapod crustaceans contain species that live in the mantle cavities of large bivalves, such as large clams, mussels and oysters. These are species of the shrimp family, Pontoniidae, and the crab family, Pinnotheridae. These crustaceans often live in pairs within the host. They usually occur on the gills, where they feed from the host's food grooves. They cause mechanical damage to the host's gill tissue. There is no evidence that these parasites cause mortality, but presumably they have some minor adverse effect on the host.

10.5.2 Crustaceans

10.5.2.1 Marine shrimp

Viruses have caused hatchery mortalities and considerable grow-out problems in marine shrimp (Chapter 21). The most devastating virus known to date is whitespot syndrome virus (WSSV). It started in 1993 in China where it destroyed almost US$1 billion worth of *Fenneropenaeus chinensis.* When infected kuruma (*Marsupenaeus japonicus*) prawns were imported from China and Korea to Japan, the virus was spread with devastating consequences. Further spread of WSSV to Taiwan, then to Thailand and the rest of southeast and central Asia ensued. Later the virus was moved to Texas, USA, presumably via frozen commodity shrimp. Central and South America followed, so by 1999 only the Philippines, Australia and Oceania were known to be free of this virus. Unfortunately, illegal movement of broodstock from the Indo-Malaysian archipelago to the Philippines infected that country. On a regional scale, the spread of this virus was always with live animals or frozen commodity shrimp. Interestingly, other rod-shaped viruses have been described from crabs, yet crabs do not seem to be as susceptible to the virus as marine shrimp. The original outbreak of this virus coincided with the upsurge in the practice of feeding raw

crustaceans, particular mud crabs (*Scylla* species), to broodstock shrimp as a maturation diet.

Marine shrimp stocks have become partially resistant to WSSV so that carrier animals are common. Carriers can be detected biochemically using nested polymerase chain reactions. The most highly infected animals are detected at the first stage of the reaction and are discarded. If these progeny are stocked, the risk of crop failure is 95%. Progeny of broodstock that are positive on the second stage of the reaction and those that are negative (ideally) are used to stock shrimp ponds. The risk of crop failure from these marine shrimp is 31%. The risk of crop failure is enhanced by stresses (osmotic, pH, oxygen) associated with the wet season. Failure rate might be around 19% during the dry season, but it may leap to 70% with the onset of the wet season. These same crop failures, which are enhanced by the wet season, occurred in Australia, where they were called midcrop mortality syndrome (MCMS). Stresses from infections with other pathogens also greatly affect the outcome of an infection with WSSV.

Taura syndrome virus has been responsible for widespread mortalities in *Litopenaeus vannamei* in the Americas after initial outbreaks near the Taura River, Equador. Taura syndrome was initially believed to be due to the toxicity of fungicides used to control black sigatoka disease on banana crops. The washing of the fungicides into the waterways allowed it to accumulate in shrimp ponds. The fungicides were very similar to crustacean moult inhibitors and this is believed to be the mode of action. A massive lawsuit against the European chemical companies that produced the fungicides was launched for compensation. Subsequently, cell-free bioassays and electron microscopy unequivocally demonstrated the presence of a picornavirus which produced the characteristic hypodermal, buck-shot lesions and mortalities. The disease seems to be most aggressive in *L. vannamei* so that *Litopenaeus stylirostris* had become an alternative crop in infected areas. Unfortunately, survivors of the epizootic are chronic carriers and infected *L. vannamei* have been introduced to Taiwan, China and Indonesia.

Monodon-type baculoviruses (MBV) can infect all species of marine shrimp, although their ability to produce disease differs between shrimp species. MBV has been implicated as one of the causative agents of the mass mortalities that swept through Taiwanese shrimp farms in 1987/88 and resulted in the crash of that industry. This may not be correct as yellowhead ronivirus (initially called a baculovirus, then a rhabdovirus in the literature) (YHV), which was undiagnosed at the time, seems to have had a very prominent role. All life stages of the host after mysis 1 are susceptible to MBV, but it is a disease of hatcheries rather than later stages, which are largely asymptomatic when infected. In an Australian study, MBV was found in 8 of a total of 13 monitored hatcheries for *Penaeus monodon*. However, it has been largely managed out of existence in *P. monodon*, but it has been a major problem with the establishment for aquaculture of *Fenneropenaeus merguiensis*. Control, developed in Tahiti, involves separating floating eggs from the spawning female, surface sterilising them and then using the phototactic response of the nauplii to separate the larvae from the egg shells and moribund larvae.

Infectious hypodermal and haematopoietic necrosis virus (IHHNV) has all but destroyed the aquaculture industry based on *Litopenaeus stylirostris* in the Americas. Interestingly, as *L. stylirostris* was not susceptible to Taura virus, IHHNV-resistant strains made a temporary comeback as an alternative crop to *L. vannamei* before the virus mutated and started killing *L. stylirostris*. Intranuclear inclusions were prominent throughout all tissues except hepatocytes. However, the inclusions from Australian shrimp did not cross-hybridise with an IHHNV gene probe that was 90% specific for the American IHHNV strain. A Philippine IHHNV strain did cross-hybridise with the American gene probe, suggesting that the Australian IHHNV is a different strain. Nor did the gene probe react with lymphoidal parvovirus which may be a variant in expression of IHHNV. Similarly, an American-produced hepatopancreatic parvovirus (HPV) gene probe based on virus from Korean *Fenneropenaeus chinensis* did not cross-react well with the Indonesian and Australian shrimp strains nor with HPV from Malaysian freshwater prawns (*Macrobrachium rosenbergii*). HPV has not been found to be a problem in the aquaculture of Australian *Penaeus monodon*, even though it is common in wild stocks of Australian banana shrimp (*Penaeus merguiensis*) and causes 28% losses in farmed *P. merguiensis*. However, recent information about *Penaeus monodon* in Thailand demonstrated HPV to cause severe stunting and therefore considerable loss of income to farmers.

Spawner mortality virus (SMV) (a parvo-like virus) in conjunction with gill associated virus (ronivirus) have caused major losses in *P. monodon* broodstock and grow-out. This midcrop mortality syndrome (MCMS) has become a major grow-out problem in northern Australia with most farms losing 50% of their stock. A$44 million was estimated to be lost over the years 1995–97. The disease outbreak is slow in its onset and shrimp grow to around 12–15 g before dying. Much time, labour, feed and money are thus invested in a crop which will be devastated if not harvested. A symptom of MCMS is the presence of

sick, weak and reddened shrimp at the edge of grow-out ponds. The number of days the stock is in ponds is a major risk factor, with the probability of epizootics increasing dramatically after 120 days. Partial harvests to decrease stocking density allow some animals to be grown beyond 120 days.

Bacteria are still a major constraint to hatchery production with very few batches of postlarvae able to be produced without antibiotics. Strategic use of antibiotics during larval and early postlarval development (nauplii VI to protozoea I and again at mysis III to postlarvae I) is usual. The Vibrionaceae predominate in isolations from marine shrimp hatcheries, with 8 of 37 strains producing significant mortality in larval bioassays. *Vibrio* species, including *V. harveyi* and *V. tubiashi*, and *Photobacterium damsela* are involved. On a worldwide basis, the non-sucrose utilising bacteria (those producing green colonies on thiocitrate bile salt agar; TCBS) are often involved in disease in crustaceans. Application of sucrose can alter the bacterial balance, favouring the less pathogenic bacteria and hence reducing losses.

V. harveyi has been involved in massive mortalities in Ecuador and major mortalities in two grow-out farms in Australia. In one study in Australia, over 50% of the bacteria at the completion of grow-out were *V. harveyi* and *V. alginolyticus*. Strains of *V. harveyi* in Australia and the Philippines have been shown to have very strong antibiotic resistance with one virulent Australian strain having a plasmid coding for four different antibiotic resistances. *V. penaecida* has been associated with mortalities in ponds in Japan (*Marsupenaeus japonicus*) and New Caledonia (*L. stylirostris*); and *V. nigripulchritudo* has been associated with mortalities in ponds in New Caledonia.

In the early years of marine shrimp culture, the fungi *Lagenidium callinectes* and *Sirolipidium* were frequently involved in causing larval mortality, especially in hatcheries where there was excessive use of antibiotics. Lobster eggs have been shown to have a surface commensal *Pseudoalteromonas* species which protects against fungi. Antibiotics inhibit this commensal, allowing the fungi to become established. A *Pseudoalteromonas* species has been found associated with shrimp eggs and so the mechanism is presumed to be similar. Use of the Agent Orange-based herbicides have effectively controlled fungi in the hatchery.

While the fungus *Fusarium solangi* is present in grow-out ponds in Australia, it is only seen when moulting frequency is slowed down due to cool water temperatures or another disease problem. It is thus a good indicator of retarded growth. Similarly, the peritrich ciliate protozoans also indicate suboptimum conditions and, at their highest fouling rates on the shrimp's gills, can cause death by suffocation if any further stress is added. Peritrich ciliates need high organic loads to feed. So by lowering the amount of uneaten food with water changes or by restricting supplemental feeding, the peritrich numbers can be reduced within a week.

10.5.2.2 Freshwater crayfish

The European noble crayfish (*Astacus astacus*) industry, which was a major aquaculture industry, has been decimated by a fungus, *Aphanomyces astaci*. Furthermore, in 1986/87 the crayfish industry in Turkey was also devastated by the introduction of this fungus across the natural barrier of the Bosphorus Straits. This led to the introduction into Europe of the signal crayfish (*Pacifastacus leniusculus*) from the USA, because this latter species is one freshwater crayfish that is highly resistant to the fungus.

WSSV has decimated the Louisiana crayfish ranching industry in the last few years. While the route of introduction of WSSV is unknown and since crustaceans in the Gulf of Mexico waterways were not known to be infected with the virus, commodity shrimp are suspected.

With the exception of the devastating crayfish 'plague' in Europe and WSSV in USA, freshwater crayfish are generally considered to be relatively disease free. This reflects the extensive culture methods used for crayfish, whereby only very low stocking densities (5 per m^2) are used. With the development of more intensive culture methods, disease will become more of a problem. A list of pathogens already documented from farmed crayfish in Australia is presented in Table 10.5. The most important agents causing mortalities so far are:

- the rickettsia *Coxiella cheraxi*;
- *Cherax* baculovirus/ bacteraemia;
- bacterial erosion of the eyeballs associated with *Aeromonas hydrophila* and *A. sobria*.

The rickettsia and the baculovirus have been introduced, certainly into Equador, and the baculovirus into the USA through trans-shipments of crayfish from Australia. Emerging pathogens include microsporidiosis in Western Australia and a systemic parvovirus in the redclaw crayfish, *Cherax quadricarinatus*. More information on the diseases of freshwater crayfish is provided by Copland (1981).

Table 10.5 Pathogens found in farmed Australian freshwater crayfish.

Type	Pathogen
Viruses	*Cherax* bacilliform virus, *Cherax* giardiavirus, parvovirus, reovirus, picornavirus
Bacteria	*Coxiella cheraxi*, *Aeromonas hydrophila*, *A. sobria*, *Citrobacter freundii*, *Klebsiella pneumoniae*, *Plesiomonas shigelloides*, *Pseudomonas*, *Shewenella putrifaciens*, *Streptococcus*
Microsporidia	*Agmasoma* (= *Thelohania*), *Vavraia parastacida*
Fungi	*Achlya*, *Mucor*, *Psorospermium*
Temnocephalids	*Craspedella spenceri, Diceratocephala, Didymorchis, Notadactylus, Temnocephala dendyi, T. minor*
Ciliates	*Corthunia, Epistylus, Lagenophrys, Tetrahymena, Vorticella, Zoothamnium*

10.5.3 Finfish

10.5.3.1 Carp

As outlined in Chapter 14, the carp and their relatives are the largest group of finfish or shellfish produced from aquaculture. They made up 38% of all finfish and shellfish production, and 66% of all finfish and shellfish from freshwater, respectively, in 2007. The Disease section in Chapter 14 refers the reader to monographic treatment of diseases in carps and their relatives by Hoole *et al.* (2001).

10.5.3.2 Marine fish

The finfish group that dominates the aquacultural literature on a worldwide basis is the salmonids because, unlike carp, they are cultured to a considerable extent in many developed countries around the world. Over twenty viral diseases are recognised in salmonids and approximately half are considered to have moderate to high virulence.

1. Infectious haematopoietic necrosis virus is one of the most studied as it infects a range of salmon species including Atlantic salmon, rainbow trout, sockeye salmon, chinook salmon and coho salmon. While this rhabdovirus is endemic to the north-eastern Pacific, it has been spread across the northern Pacific as far south as China and Taiwan, eastern United States, France and Italy. It primarily affects fish less than 6 months old and, as it is found in the sexual secretions of both male and female broodstock, it has ready access to young fish for infection.
2. Viral haemorrhagic septicaemia (VHS) of rainbow trout was once restricted to Europe, but has been introduced to the United States. It only grows at temperatures below 16 °C so its geographic range is limited and losses can be ameliorated by increasing temperature. Recently, VHS has been found in wild Southern Californian pilchards and mackerel that are used extensively for bait and fodder to other aquaculture industries such as Bluefin tuna.
3. The Aquabirnavirus, e.g. infectious pancreatic necrosis virus, and similar viruses are found worldwide. These viruses have a great propensity for replicating or surviving in hosts other than fish. These other hosts include bivalves, crayfish, marine shrimp, leeches and the intestines of fish-eating birds. Therefore, it is very difficult to eradicate. Luckily, it is easily managed by recognising that there will be fairly predictable losses to the virus. Up to 40% excess of eyed ova above what is believed to be necessary for that year's stocking may be purchased in anticipation of 40% being killed by the virus.

Bacteria are major pathogens of salmonid culture (Fig. 10.4) (Austin and Austin, 1999). Antibiotics have been widely used to control outbreaks, but they are becoming less and less acceptable in food animals due to concerns about antibiotic resistance spreading to pathogenic bacteria of humans. Bacteria in the sediments of abandoned aquaculture sites have been assayed for antibiotic resistance ten years after the culture operation ceased and they were found to still have major resistance against oxytetracycline. This type of problem has led to the production of effective vaccines (see Chapter 19). The 30% rise in Atlantic salmon production in Norway is due to control of *Vibrio salmonicida* and *V. anguillarum*. Similarly, non-specific stimulation of the immune system using yeast cell wall ß-1,3 and ß1,6 M-glucans against *Yersinia ruckeri* has gained popularity. The transfer of *Aeromonas salmonicida* from Scotland to Norway led to a massive slaughter campaign to control the bacterium. This cost $US100 million in losses. In fact, *A. salmonicida* is probably the most



Fig. 10.4 Wounds on market-sized rainbow trout (2–3 kg) infected with *Vibrio* and *Flexibacter* species bacteria. (Photograph by courtesy of Dr John Purser.)



Fig. 10.5 Mediterranean sea bass *(Dicentrarchus labrax)* infected by the Gram-negative bacterium *Vibrio anguillarum* (vibriosis) causing haemorrhagic septicaemia. (Photograph by courtesy of Dr Panos Varvarigos.)

important finfish pathogen in all marine aquaculture industries.

Enterococcus seriolicida (= *Lactococcus garviae*) is still the major problem of yellowtail culture in Japan despite being diagnosed in the 1960s. Streptococcosis is also a problem of rainbow trout in South Africa and southern Australia, and of barramundi (Asian sea bass) in northern Australia. Part of the problem is the bacterial A-layer which defeats the ability of complement to attach to the bacterial cells. Hence, macrophage engulfment is compromised and host antibody production is slowed. Carrier status in the antibody havens of the brain and gonads ensues, allowing sporadic outbreaks whenever stress eventuates.

Fig. 10.5 shows another example of the effects of bacterial infections on fish in seacage farming. The Mediterranean sea bass is the basis of a major industry in this region.

There are numerous and diverse protozoans that parasitise aquatic vertebrates in freshwater and marine habitats and these cause various levels of debilitation. For example, the amoeboid protozoan, *Neoparamoeba* species, is an opportunistic pathogen that infects the gills of fish under some conditions. On a coho salmon farm in Washington State, USA, Kent and Margolis (1995) observed 25% mortality that was attributed to *Neoparamoeba* species. The amoebae occurred on marine farms and were quickly eradicated from the fish gills by exposing them to freshwater, e.g. towing the fish cages from seawater into freshwater or by treating the fish in freshwater baths. However, the *Neoparamoeba* are developing resistance to the freshwater treatment and it is now only effective for about 30 days.

Kent and Margolis (1995) listed eleven parasitic protozoans causing significant disease conditions and mortality in salmonids reared in seawater. Virtually all the fish's tissues may be infected by the range of parasites, but there is a tendency for these protozoans to parasitise the gills and skin.

10.5.3.3 Tropical and subtropical finfish

Epizootic ulcerative syndrome (EUS) is caused by the fungus *Aphanamyces invadens*. EUS came into prominence in the 1980s as a devastating pathogen of snakehead and other fish of southeastern and central Asia. Snakehead fish are used for protein supplementation in the diets of the rural poor. Destruction of these fish led to widespread malnutrition, starvation and socio-economic disruption of subsistence lifestyles. After many competing hypotheses on the aetiological agent, including rhabdoviruses and *Aeromonas hydrophila* bacteria, *A. invadens* was identified by a group of researchers in Australia. In the fullness of time, EUS has been traced back to early outbreaks in the 1970s in Australia and Papua New Guinea. The earliest outbreak was in Japan in 1970. One interesting outbreak occurred only in the fishpond at the Sri Lankan international airport, where presumably a passenger had thrown some fish scraps from a meal into the pond. Lately, EUS has become a problem in the establishment of the jade perch (*Scortum barcoo*) as an aquaculture species.



Fig. 10.6 Barramundi infected with 'white spot' (*Cryptocaryon irritans*). (Photograph by courtesy of Professor Bob Lester.)

Barramundi (*Lates calcarifer*) are the only fish seriously cultured in the tropics of Australia. While most farms have intermittent problems from time to time, the most common pathogens are *Cytophaga johnsonae* causing saddleback, which may lead to dirty yellow ulcers, and white spot from *Cryptocaryon irritans* in seacages (Fig. 10.6). Problems are associated with the onset of cooler temperatures, usually in May. *Streptococcus iniae* is emerging as the most important disease in barramundi in estuarine seacage operations. The initial episode starts after displacement of soil in the watershed where the barramundi are being grown. Losses approach 30% and are not ameliorated by winter water temperatures. Also, *Vibrio harveyi* is emerging as major enteric disease in barramundi hatcheries.

Politically, there have been major problems associated with nodaviruses which causes viral encephalopathy and retinopathy of larval barramundi and kill young, crowded larvae, especially in later batches through a hatchery. While the virus was definitely associated with vacuolate brain and retinal tissues in larvae from 8 of 111 batches (it was not looked for in each batch), it has been responsible for the placement of certification procedures for movement of larval barramundi between states in Australia. Despite this, the virus has been found in South Australia in uncertified batches of larvae. The growing of barramundi outside its natural range in the Murray-Darling watershed and the potential impact of the nodavirus on the endemic freshwater fish has been hotly debated. A similar, if not identical, virus has been found in barramundi in Tahiti and Singapore and in most fish species (about 30 species at present) around the world. Almost all fish raised in marine hatcheries end up being found infected with this virus, once the correct tools for investigation have been used.

Other pathogens seen from time to time include lymphocystis virus associated with dirty seacages, vibriosis, epitheliocystis and *Epieimeria*. Experimental infections with Bohle iridovirus, a systemic ranavirus, has shown barramundi to be acutely susceptible in both fresh and marine waters. Total mortalities usually occur within 10 days. As the virus appears to be endemic along the tropical coast, it is only a matter of time before major mortalities occur.

The diseases of channel catfish in the southern USA are outlined in section 17.8.

10.5.3.4 Australian temperate finfish

Salmonids and in particular Atlantic salmon (*Salmo salar*) are one of the main aquacultured fish in temperate waters, although some silver perch and aquarium species are being cultured. Fattening operations for southern bluefin tuna are also increasingly important. Again pathogens have had a major impact on current aquaculture practices even though this is often not recognised by growers. Most culinary experts prefer rainbow trout to Atlantic salmon and accordingly the Australian seacage industry started with rainbow trout. The bacterium *Enterococcus* (previously *Streptococcus* biovar I) killed 30% of rainbow trout in freshwater and as high as 60% in marine waters associated with the acclimatisation stress. Therefore, the industry swapped *en masse* to Atlantic salmon, which is highly resistant to this bacterium.

Yersinia ruckeri serovar III (the Australian strain) causes some problems, mainly in young Atlantic salmon, particularly when water temperatures are above 15 °C. Some fish may develop the pathognomonic haemorrhages in the eye and, in chronic cases, bilateral exophthalmia and anorexia may predominate. The bacteria can be spread in the faeces of carrion feeding raptors. *Vibrio anguillarum* serovar C or 01 has been isolated from Tasmanian rainbow trout and Atlantic salmon. Clinical signs include hyperaemic prolapsed rectum, congestion at the bases of the fins and reddened ulcers on the flanks. The muscles may contain enclosed lesions and severe peritonitis can occur. Overseas, virulence in *V. anguillarum* has been linked to a mostly plasmid-encoded siderophore (iron sequestering) system with the most virulent strain known having four separate siderophores. Both of these conditions can be controlled by efficient vaccines.

The typical, non-motile strain of *Aeromonas salmonicida* usually forms large boils or furuncles in salmonids. This obligate pathogen exists in Victoria, Australia, as the

atypical, non-salmonid infecting, motile strain in goldfish, *Crassius auratus*. The bacterium seems to have been imported to Victoria from Japan in 1974. Despite there being a restriction on the movement of goldfish from Victoria to Western Australia and Tasmania, there was an isolation of a typical strain of *A. salmonicida* from flounder in Tasmania. Similarly, a silver perch farm in New South Wales was quarantined due to this bacterium.

Since the eradication of infectious haematopoietic necrosis virus during the initial outbreak, only three viruses have been found in the Australian salmons. The chum salmon *Aquareovirus* was isolated from ovarian fluid of Atlantic salmon and it apparently only causes disease in fry of chum and chinook salmon. This virus has more significance as a trade barrier for exclusion of competing Australian products by importing countries than as a disease threat. Infectious pancreatic necrosis virus was isolated from seacaged Atlantic salmon displaying pinhead.

Neoparamoeba caused problems at the outset of the salmonid seacage industry in Tasmania. Fish in their first year in seawater develop a proliferative gill disease, especially if seacages are fouled or temperatures are above 15 °C. Up to 2% of fish per day will die if untreated. Chemical baths are very ineffective but freshwater baths of a few hours or moving the whole seacage to freshwater works very well. Once the cycle is broken, the immune system prevents further heavy infestations. Unfortunately, there is strong evidence that the freshwater treatment is becoming less effective. Instead of a one-off bath, bathing frequency has been increased up to four times per year.

10.6 CONCLUSIONS

The nature of aquaculture and the best anti-disease technologies are the direct nemeses of each other. Aquaculture is about increasing profits by growing more animals in smaller and smaller volumes of water. Anti-disease methodologies include keeping densities as low as possible to break the fish-to-fish cycles. As profit is the major goal in aquaculture, densities will always be maximised, and therefore, disease problems amplified. As such, aquatic pathobiologists will always be in demand and, as the job is perpetually full of challenges, it makes a very interesting career path for students.

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11
Post-harvest Technology and Processing

Allan Bremner

11.1 INTRODUCTION

Post-harvest technology encompasses areas of harvest, handling, slaughter, processing, packaging, manufacturing, storage, transport, distribution and display, through to the point of sale or the time of consumption. This chapter can therefore only be considered as an introduction to the topic and the reader should look also to monographs and proceedings of conferences that are listed in the References for further depth of detail (Bremner, 2002; Bremner *et al.*, 1996; Huss *et al.*, 1992; Kestin and Warriss, 2001; Martin, 1992; Nesheim and Yaktine 2007; Sato *et al.*, 1999; Sikorski *et al.*, 1994).

The scope of post-harvest technology is broader in aquaculture than in capture fisheries, since the selection, breeding, rearing, feeding and farming conditions can all be controlled to affect the properties of the product and must be recorded into cross-referencing databases. The appearance, odour, flavour and texture of aquaculture products have a huge bearing on their marketability and acceptability by the consumer. The odour and flavour of either raw or cooked product is a result of the inherent biochemical composition, the accumulated results of bacterial and enzymic action and the effects of processing.

- Characteristic odours of a product are the result of volatile compounds.
- Flavour is due to both volatile and non-volatile compounds.

Inherent non-volatile compounds of importance in this regard are salts, amino acids and other non-protein nitrogenous compounds and, in particular, the breakdown products of the purine nucleotides. The levels of these factors in aquaculture products can be affected by harvesting and processing. Volatile components arise from feeds; many are lipid based in origin and they are products of either enzymic or chemical oxidation. Hence, both nutritional practices, and processing and storage, can have profound effects on the flavour and stability of the product.

The texture of the product is of considerable importance too, and harvest stresses and post-harvest practices are factors that combine to affect textural characteristics.

In this chapter, emphasis is given to the interaction between the pre-harvest practices and the post-harvest product, since this is an area of vital concern to aquaculturists in which considerable research is needed. The material presented relates mostly to aquaculture products grown for human consumption. However, the considerations of harvest practices that involve low stress are relevant to species reared for other purposes such as restocking of waterways for ecological, aesthetic or recreational purposes.

11.2 BASIC CHARACTERISTICS

There are several basic characteristics that influence post-harvest technology and processing, and these must be determined for each cultured species. These are:

1. The gross proximate composition (water, crude protein, fat, crude fibre, ash and nitrogen-free extract) (section 9.10.1).
2. The yields of edible product.

Aquaculture: Farming Aquatic Animals and Plants, Second Edition. Edited by John S. Lucas, Paul C. Southgate.
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3. The composition of the edible product.
4. The gross morphology of the edible parts.
5. Alternative processing forms.
6. The distribution of by-product and off-cuts and their composition.
7. The chilled life of the product (0 °C).
8. The frozen storage characteristics of the product.
9. The pattern of rigor mortis.
10. The inherent bacterial flora.
11. The nature of the spoilage bacterial flora.
12. The inherent nucleotide composition and its rate of change during storage.

Although all these characteristics are affected to some extent by harvest regimes, selection, husbandry practices, feed and a host of other factors, it is important that they are determined. It is also important to determine the proportions of red to white muscle in a fish species, the position of lipid deposits, the structure of the muscle and the skeletal structure.

11.3 SAFETY AND HEALTH

Aquacultured foods generally have a good safety record (Nesheim and Yaktine, 2007). Most of the major food-poisoning organisms of public health significance, at least in the developed countries, do not occur in the aquatic environment unless it has been polluted by human activity. The run-off from farming, animal excrement, human sewage and sullage are the main sources of viral and bacterial contamination, and industrial contamination contributes metallic and chemical impurities. Since the environment of the aquaculture production cycle is controlled for the most part, it could be expected that the product would be free from harmful organisms. It is the task of the industry to ensure this potentially beneficial situation is not eroded. However, even in the relatively controlled environment of brackishwater ponds, cultured shrimp can be contaminated with bacteria such as *Salmonella* species and *Vibrio cholerae*. For example, of 304 samples of shrimp and mud/water samples from 131 shrimp ponds, *Salmonella* species were present in 16% of shrimp and 22% of mud/water samples, and *V. cholerae* occurred in 1.5% of shrimp and 3.1% of mud/water samples.

In some instances, aquaculture forms part of an existing farming activity and runoff containing faecal material from terrestrial farm animals and contamination from waterfowl may introduce unwanted organisms. The practice of fertilising ponds with manures and other organic material (Chapters 2, 4, 9 and 14) can also introduce unwanted organisms. Most manufactured feeds are likely to be free of problems, but intensive culture practices that lead to wasted feed and high faecal loads provide an excellent environment for proliferation of undesirable bacteria.

Failure to remove dead animals can even lead to outbreaks of botulism in fish as a result of cannibalism. The result is that ponds need to be completely drained and the soil totally removed before the area is fit for use again.

Problems of harmful micro-organisms are relatively less important in extensive systems or in situations in which there is a high rate of water exchange. However, a number of problems can occur with seafoods from estuarine environments, which are partly due to the nature of the animals involved. For example, because bivalve molluscs, such as oyster and mussels, are filter feeders, they remove bacterial and viral particles from the surrounding water. This situation is worsened because bivalves are often eaten raw and the whole animal, organs and all, is consumed. Bivalves are readily contaminated by organisms of faecal origin from raw or improperly treated sewage (section 23.5.3). Prime agents appear to be Norwalk or Norwalk-like viruses that cause viral gastro-enteritis. *Vibrio* organisms are also involved and one species, *Vibrio vulnificus*, can be particularly virulent. Outbreaks often occur after rainfall, since the resulting decrease in salinity releases organisms from the sediments into the adjacent water column where they can be ingested by the animal. In addition, rainfall floods extra water into sewage systems, causing flushing of untreated sewage.

Another major cause for concern is natural blooms of microalgae. In freshwater, blooms of blue-green algae may cause toxins which can be passed along the food chain. In estuarine and marine environments, paralytic shellfish poisoning (PSP), diarrhoeic shellfish poisoning (DSP) and amnesic shellfish poisoning (ASP) can occur with consumption of shellfish contaminated as a result of algal blooms (Nesheim and Yaktine, 2007; section 23.5.3). When these occur, there is no option other than to close the site to harvest. Monitoring programmes and closures are now employed worldwide to prevent diseases from this source. Although the toxic algal blooms are ‘natural’, there is growing evidence for the spread of these species in the ballast-water of cargo ships.

Other ‘natural’ water-borne bacteria, such as *Aeromonas* species, *Vibrio vulnificus* and *Vibrio parahemolyticus*, have been associated with poisoning, particularly in the summer months in temperate areas. The infective doses are not known for each organism but they are small.

Consumption of raw shellfish must always be approached with care.

The process of depuration, that is treating the shellfish for several days in clean, running water, has been adopted in some areas (section 23.5.3). This process is partly effective in reducing bacterial numbers, but viruses are not easily removed. Far more effective is a properly designed and enforced quality assurance system in which growing sites are selected to be free from problems in the first instance and in which water quality is continually monitored to ensure that no deleterious changes have occurred.

These 'shellfish sanitation' programmes can be integrated into broader industry programmes involving total quality management (TQM) and the hazard analyses critical control point (HACCP) systems (Martin *et al.*, 1997; Nesheim and Yaktine, 2007). Schemes such as these are generally a requirement for export. A general code of practice for aquaculture drawn up by the Codex Alimentarius Commission of the World Health Organization and the Food and Agriculture Organization provides a very good guide to practices required to ensure a safe product (FAO, 1994).

Recently the process of subjecting shellfish to high pressure in a chamber is being commercially explored as a batchwise means of both significantly reducing bacteria, e.g. *V. vulnificus*, and aiding in releasing the meat from the shell. The results of high pressure on viruses are less well known so far.

Chemical contamination invariably occurs through exposure to toxic wastes dumped or leached into the aquatic environment. The potential for contamination because of previous activities in the area or from adjacent application must also be considered. However, some natural accumulation of heavy metals, such as mercury, can occur if mercuriferous ores (e.g. cinnabar) are found in the region. Again, considerable attention needs to be paid to the siting of aquaculture ventures. The application of more advanced methods of analysis for heavy metals has shown that many values recorded for fish in the older literature are in error and are far higher than the levels found by these more accurate methods.

In general, aquacultured fish are less likely to be infected with parasites, but this cannot be taken for granted. Many parasites inhabit portions of the fish, such as the head, guts and belly flap areas, which are discarded during processing. Others, however, such as the anisakids, are found in the flesh. These parasitic nematode worms are highly infective for humans, but are destroyed by thorough cooking and on freezing. More recently, the practice of eating raw fish or lightly preserved or pickled fish dishes, such as ceviche, has become more common in Western societies. It is therefore very important that the product is monitored for the presence of parasites.

11.4 NUTRITIONAL ASPECTS

The products of aquaculture are health giving and nutritious, being good sources of vitamins, minerals and protein with a low total-fat content. Marine products represent by far the best natural source of the highly unsaturated fatty acids (HUFAs) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). These fatty acids have important roles in membrane functioning and as precursors for biologically active compounds. They are considered beneficial in human nutrition in reducing plasma cholesterol and triglyceride levels, and in reducing the risk of cardiovascular disease. They are used in treatments for arthritis and other ailments (Nesheim and Yaktine, 2007). They are essential for development of the brain and nervous system in infants, as a high proportion of DHA is found in the grey matter and nerve tissues.

As steps are taken to decrease the proportion of fish meal and fish oil in aquaculture feeds (section 8.7.2), so the levels of EPA and DHA in cultured fish will decrease unless alternative sources are found. The level of these fatty acids is one of the major desirable selling points for aquatic produce. Clearly, a balance needs to be struck between maintaining high levels of EPA and DHA and decreasing product costs by altering feed composition.

Marine aquaculture products are a good source of selenium, an essential element, which is a co-factor for the antioxidant enzyme glutathione peroxidase. Oysters and other shellfish are also good sources of iodine.

11.5 THE BALANCE BETWEEN SAFETY AND NUTRITION

The general consensus of medical and technical opinion is that, in general, the benefits of eating aquaculture products far outweigh any potential risks (Nesheim and Yaktine, 2007). The broad range of nutrients and trace minerals, the contribution of the essential HUFAs, the low glycaemic index, the high-quality protein, and the high levels of free amino acids and related non-protein nitrogen products coupled with ready digestibility make aquaculture products a most nutritious natural food.

11.6 AQUACULTURE AND FISHERIES PRODUCTS

It is difficult to directly compare aquaculture products with wild-caught products. The variability in genetics, in feed composition and in growth conditions makes comparisons

difficult to interpret except in the broadest sense. Preferences for wild or farmed products will vary greatly according to season and location of capture. In general, aquaculture products tend to provide flesh that in comparison with wild-caught products is:

- softer in texture;
- less strong in flavour;
- often of different hue;
- with a higher but more uniform oil content.

Atlantic cod reared in pens have higher contents of sarcoplasmic proteins and different water-binding capacities compared with wild cod. Both factors affect the texture of the cooked flesh. Wild fish have to swim more than captive fish, and the myoglobin content of their red muscle is often higher. Lower levels of free amino acids have been found in cultured coho salmon, red seabream and ayu than in their wild-caught counterparts. Similarly, other non-protein nitrogen compounds appear to occur in lower concentrations in cultured species. Variations in composition and distribution of tissue constituents occur both with changing seasons and with maturation. There are also considerable seasonal variations in the mineral content of both wild and cultured fish, and textural changes are also apparent.

11.7 HARVESTING

There are stresses during harvesting that may affect the final product. Crowding of fish in a net or in raceways during harvest leads to:

- rises in blood cortisol;
- an increase in metabolic rate;
- utilisation of glycogen with resulting:
  - increases in lactate levels;
  - decrease in tissue pH.

The provision of good aeration can help relieve this situation, but every effort must be made to reduce obvious stress factors during harvest (Robb, 2001).

In rested fish, cortisol levels in the blood are generally below 10 ng/mL, but values of up to 10 times this level are common in stressed or exercised fish. Similarly, lactate levels in the blood and muscle of rested fish are many times lower than in stressed or exercised fish. There is some evidence that chronic stresses, such as those experienced during harvest because of overcrowding or poor water quality, result in a different mix of biochemical indicators from those that may result from imposition of a sudden stress such as physical exertion due to capture.

In crustaceans and molluscs, the expenditure of energy in struggling for survival is met from arginine phosphate and from glycogen. In crustaceans, the main end product is generally lactate, but octopine is formed from the reaction of pyruvate with arginine in many bivalves and gastropods. In the gastropods, tauropine, alanopine and strombine are similarly formed. Tauropine is formed in abalone, and levels of these compounds have been suggested as indicators of harvest and transport stress.

11.8 LIVE TRANSPORT

Many markets prefer their products to arrive live to ensure freshness and to allow consumers to select their own dish or, in some instances, to eat the product within seconds or minutes of death or, as in the case of shellfish, while still live (Fig. 11.1). The returns for these markets are often

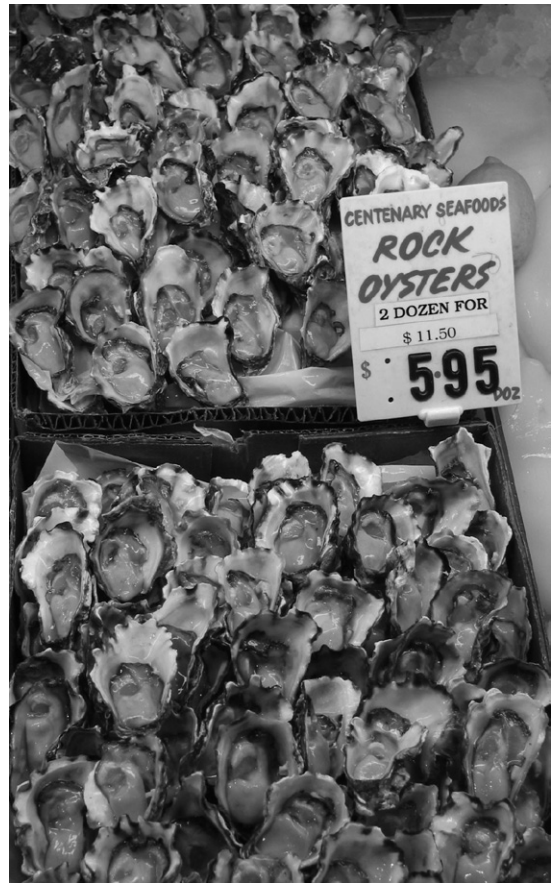


Fig. 11.1 Table oysters are transported live to the point of sale and shucked.



Fig. 11.2 Quiescent kuruma shrimp being examined at the market.

higher, but the risks are greater since the associated costs of transporting animals out of their natural environment can be considerable.

Live kuruma shrimp (*Marsupenaeus japonicus*) have fetched prices of approximately ¥18 000 per kilogram in the Tokyo market (Fig. 11.2). This market is supplied by domestic sources, and from Taiwan, France and Spain. The source of the largest quantities by far, however, is China. Getting the product to the market in a live state requires appropriate harvest practices. The shrimp are active only at night and must be harvested then by the gentlest means possible. Simple tangle nets or tunnel nets set near paddle wheels are appropriate. The shrimp are gently laid on to floating wooden trays that stack one on the other and are transferred in these to a chilling tank to bring their temperature down to ca. 16 °C. The tanks are taken from the ponds to the factory, where the temperature is further reduced nearer to 12 °C. At this temperature the shrimp become inactive and can be handled readily without becoming excited. They are then size-graded and packed in chilled damp sawdust in corrugated cardboard boxes. About 10 boxes are placed in an outer insulated cardboard box in a manner that allows airflow between them. Chilled coolant gel packs are placed strategically in the box to absorb ingressing heat during transport and to maintain a uniform temperature in the box during transportation. The weights and positions of the coolants are calculated carefully so that the temperature is stable in transit from the farm to the airport and during the flight. It is timed such that the temperature of the shrimp will rise to about 16 °C when the packs are opened for inspection at auction. The shrimp will have revived from their inactive state and will be capable of jumping out of the box when the lid is raised. Thus, there must be adjustments for flight times, cargo hold temperatures and seasonal factors. Low-cost disposable temperature recorders are included in each consignment. High survival rates of over 95% have been achieved using these methods.

Crustaceans such as redclaw crayfish (*Cherax quadricarinatus*) can be transported by similar methods. Other species of shrimp can also be transported in this way over short distances, but no other species seems to show the same characteristics as kuruma shrimp. The black tiger shrimp (*Penaeus monodon*) is excitable at temperatures of 12 °C and is not docile to handling. In live kuruma shrimp stored immersed in sawdust at 12 °C, adenosine triphosphate (ATP) levels are maintained (presumably from arginine phosphate), whereas in other cultured species, such as *P. monodon*, ATP is rapidly depleted.

Similarly, there are lucrative markets for live fish but in these instances the fish must be transported in a minimum amount of water to keep them alive. The systems required for live fish transport must include features to:

- prevent injury to the fish;
- prevent fouling of the water;
- maintain an even (generally chilled) temperature;
- allow for sufficient aeration to achieve at least 80% O_2 saturation and removal of CO_2 produced by the fish.

The water must be circulated to achieve these requirements.

Oxygenation is best achieved by direct blowing of air or even oxygen into the water to maintain sufficiently high levels. This also serves to purge CO_2 from the water. CO_2

must then be vented to prevent re-dissolution. Supersaturation with oxygen must be avoided, to prevent high CO_2 levels in the blood, with consequent shift towards an acidic pH. Acidosis is lethal, for the oxygen-carrying capacity of the blood is much lower at low pH.

Low (chill) temperatures are commonly used to reduce metabolic activity, respiration and hence production of ammonia (section 3.3.1). The optimum temperature varies according to species and their environmental temperatures, e.g. some species of flatfish become so inactive that they can be transported in just an amount of water sufficient to keep them wet. Containers for air transport range from the relatively simple bag-in-box type to highly specialised containers with pump-operated recirculating units that use oxygen production from solid chemical pills. Containers may also include internal spray systems to keep the fish moist. Because of the corrosive potential of saltwater in aircraft, airlines are very strict as to the nature of the containers they will accept. Clearly, the package must not leak.

To maintain fish in a docile and inactive state, and to reduce respiration and production of metabolic by-products, anaesthetics can be used during transport. When the fish are for human consumption, the use of anaesthetic is questionable and the search for food-grade anaesthetics has resulted in the use of isoeugenol, a major constituent of oil of cloves, being incorporated into a harmless dispersing system. The usage rate of active ingredient is low, ca. 20 ppm, but, although the chemically measured residue is negligible, the faint odour of cloves persists and alters the characteristic smell of the product. It is appropriate to starve the fish prior to transportation to allow their gut to empty and, thus, reduce strain on the transport system.

Although stress will occur in the process of transporting animals out of their natural environment, all steps during handling and transport must be designed to minimise stress. Operations leading to sudden stresses must be avoided if possible since fish are often slow to recover. Once the technical parameters have been overcome, the logistics of transport, and the match of land-freight and airline schedules with customs clearance, must be carefully organised.

11.9 MUSCLE STRUCTURE: RIGOR AND TEXTURE

11.9.1 Muscle structure

The major edible portions of aquaculture products consist of muscle. The muscles of fish and other marine animals are similar in basic structure to other members of the animal kingdom. The muscles are fibrous in nature, contractile and are attached to the shell or skeleton by connective tissue. This connective tissue, in differentiated form, also surrounds each individual muscle fibre to form an interconnecting network (Bremner, 1999).

Muscle fibres in fish are arranged between connective tissue sheets (myocommata) in a roughly parallel fashion, and rhythmical contractions of portions of the body musculature are transmitted through the myocommata, causing flexure of the skeletal system, resulting in a swimming motion. Each individual fibre is surrounded by a network of connective tissue linked into the myocommata. Within the fibre, the major contractile elements are a system of myofibres in which two major proteins, actin and myosin, form the bulk. Contraction of each muscle segment occurs as a nerve impulse results in release of calcium ions into the cell from the surrounding sarcoplasmic reticulum. Relaxation occurs as the calcium is pumped back into the sarcoplasmic reticulum, a process fuelled by ATP. In death, ATP is no longer present to pump the calcium ions back into the sarcoplasmic reticulum and the muscle remains in the contracted state and rigor mortis is said to have set in. The recruitment of fibres into the rigor process is not uniform and many fibres undergo passive contraction. Muscle in rigor, with contracted fibres, is much tougher than post- or pre-rigor muscle.

The muscle softens after rigor, but the processes by which this occurs are not well understood. Protease enzymes are thought to act at junction areas within each fibril. The actomyosin complex remains intact. Using electron microscopy, evidence of general disintegration can be seen, mostly as a loss of definition in the structures. In addition, it is now clear that changes in the interstitial connective tissue can precede the resolution of rigor mortis within the muscle in some species. Further breakdown in the fine connective tissues of the extracellular matrix leads to a progressive softening of the texture (Bremner, 1999).

11.9.2 Rigor mortis and nucleotides

The extent of rigor mortis, and its duration and speed of onset, are important factors in determining how quickly processing has to occur. However, these are largely dictated by the conditions existing before, during and after harvest, and are thus under the control of the processor. The speed of onset and duration of rigor at any given temperature are controlled by the amounts of ATP and creatine phosphate or arginine phosphate in the muscle. This depends on the feed and the pre-harvest stress, but

the major influence is the degree of stress during the harvesting procedure. As described before, when fish struggle violently, ATP is depleted and lactate levels in the muscle increase. Rigor processes set in quickly and the duration of rigor is less.

Fish in rigor are difficult to process. Indeed, they should not be handled in the rigor state, since this results in tearing the muscle and consequent downgrading of the flesh. This is a serious problem with high-priced materials, such as salmon, which are filleted for smoking or production of gravad lax (in which the raw salmon flesh is salted and lightly pickled by the action of naturally occurring lactobacilli), when perfect flesh is very desirable. It is common practice for fish to be harvested and bled, then chilled and stored in ice for at least a day until rigor mortis resolves, when the flesh loses its stiffness and can be handled without tearing. Newer methods of rested harvest and the use of rapid mechanical stunning machines, coupled with bleeding, provide opportunities for pre-rigor processing.

The method of slaughter also affects the rigor process. For example, the use of CO_2 as an anaesthetic for salmon results in shorter times for onset and duration of rigor mortis, and it is probable that this also holds true for other species. Stunning with CO_2 is common practice, but it is far from ideal since considerable struggling occurs in the stun tank. Whatever method of slaughter is chosen, it is important that the process is accomplished in a manner that is as stress free as possible and that the fish are chilled as soon as possible after slaughter (Robb, 2001).

In the post-mortem state, oxygen is no longer available to the tissue to regenerate ATP by the normal cycle and it is broken down through a sequence of compounds, including inosine monophosphate (IMP), inosine (INO), adenosine (ADO) and hypoxanthine (HX). Anaerobic glycolysis is used to regenerate ATP, leading to production of lactate, which decreases the pH of the tissue. In different orders, genera and species of fish, these processes occur at different rates and through different intermediaries.

The pattern of nucleotide breakdown appears to be consistent for each species, and the relative concentrations of the intermediates are controlled by the activities of the various enzymes. Completely unstressed marine animals commonly have a total adenylate nucleotide pool in the region of 10 µmol/g, of which over 90% is present as ATP. In the post-mortem state, the total adenylate nucleotide pool generally remains at or near this level, even for up to 15–20 days in the case of product chilled to 0 °C. This occurs until the hypoxanthine is converted enzymically to xanthine (XA) and uric acid (U) or until bacteria metabolise the inosine or hypoxanthine.

In general, the steps leading to formation of IMP (also called 5′-inosinic acid) occur rapidly. This is an important phenomenon as far as the product is concerned since IMP belongs to a group of compounds known to enhance flavour. Thus, high concentrations of IMP are desirable. The next major breakdown products are inosine (INO) and hypoxanthine (HX). Inosine is said to have no flavour impact, but hypoxanthine is bitter. It has been suggested that the balance between the relative levels of IMP and hypoxanthine in the tissue plays a large role in determining the flavour acceptability of the product. Fish have been classified according to whether they are inosine producers or hypoxanthine producers.

The ratio of the unphosphorylated to the phosphorylated nucleotides has been expressed as a ratio known as the *K*-value.

$$K\text{-value} = \mathrm{INO} + \mathrm{HX}/(\mathrm{ATP} + \mathrm{ADP} + \mathrm{AMP} + \mathrm{IMP} + \mathrm{INO} + \mathrm{HX}) \times 100$$

where ADP is adenosine diphosphatase and AMP is adenosine monophosphatase; the other abbreviations have been defined earlier.

In many species this value has been found to be useful as a time–temperature indicator that integrates product life and it is an index of the state of the product. Sashimi-grade products have a low *K*-value (below 20%), whereas high *K*-values tend to indicate that the product either has been stored for some time or has experienced a high temperature. This rule cannot be applied across all species, and it is essential to know the pattern and rates of change for the species in question and whether it is an HX or an INO producer.

A fish that maintains a high level of IMP in its tissues is likely to have a higher flavour acceptability for a longer period, whereas one that converts it through to HX is likely to lose consumer acceptability quite rapidly.

In the live state, the adenylate energy charge (AEC) of an animal is an indicator of its vital status.

$$\mathrm{AEC} = (\mathrm{ATP} + 1/2\,\mathrm{ADP})/(\mathrm{ATP} + \mathrm{ADP} + \mathrm{AMP})$$

Harvest stress can force the tissues of shrimp into having an AEC so low (ca. 0.5) that they would be considered dead by this criterion alone, yet many survive if resuscitated in oxygenated water. If these animals are then killed they have high IMP levels and high *K*-values at the start of their storage life, and these indicators are of little value as estimators of post-mortem storage life.

11.10 STUNNING AND POST-MORTEM PROCESSING

The percussive stunning method is recognised by all world authorities as the optimal method for humane slaughter:

- World Society for the Protection of Animals (WSPA);
- The Royal Society for the Prevention of Cruelty to Animals (RSPCA);
- Defra (UK Department for Environment, Food and Rural Affairs);
- The International Fund for Animal Welfare (IFAW);
- USDA (United States Department of Agriculture);
- The European Commission's Standing Committee of The European Convention For The Protection of Animals Kept For Farming Purposes;
- The HSA (Humane Slaughter Association).

With fish, processing can be considered to start at harvest, at the point when they are killed (Rørå *et al.*, 2001). Modern methods involve the use of automated stunning and bleeding equipment, which has been found to have many advantages for the fish, the operators and the product. The advantages are that the fish are stunned and bled within seconds of arriving in the small holding tank and thus have minimal stress. The process is automatic, fewer staff are required, injuries, strain injuries and knife stickings are eliminated and the fish are not handled by personnel. Consequently, they are do not get bruised, ATP levels are high, bleeding is fast and clean, and the flesh has superior properties. There are three stages:

1. A flow-through holding tank that elicits natural escape behaviour, causing the fish to swim into a short steep chute leading in under a second to
2. A percussive bolt triggered by the fish's passage from the chute through the machine and into an inversion chute which turns the fish on its axis, exposing the gills for
3. The automated blade to make the bleed cut.

The momentum carries the fish, stunned and bleeding, onto the bleed table and thence onto a moving belt for further processing. The whole process from live to stunned and bleeding takes about 2 seconds. Generally, about four channels operate and the count and rate of fish processed is automatically monitored, recorded and can be transmitted by wireless to the office or process plant.

Prior to development of the automated equipment, common approaches have been to anaesthetise the fish with CO_2, or by plunging them into iced water (Fig. 11.3) or to club them on the head with a 'priest'. The gills were then slit by hand to allow them to bleed as a result of the action of the heart and the contractions of the muscle. All these methods result in stress to the fish, slow processing and potential injuries to staff.



Fig. 11.3 An ice slurry bin for chilling Atlantic salmon. (Photograph by courtesy of Dr John Purser.)

After the bleeding period, which lasts about half an hour, the fish are chilled in ice slurry or in ice and transported to the factory for processing. If the factory is near the farm, the fish may be processed straight away before rigor sets in. Otherwise they are generally held in chillers for up to 2 days to allow rigor to resolve.

Processing continues with washing and grading, followed by preliminary separating of prime edible materials, generally the flesh, from inedible materials such as the head and gills, and other by-products such as the gut, part of which may be further processed.

Washing can be carried out in vertical or horizontal machines that use the friction of a water jet, the abrasion between the fish and the action of the machine structure, to remove outer debris and, at times, scales. Scaling by hand or by machine with blunt, rotating blades is the next step before gutting and removal of the fillets. This is commonly done by hand, but machines are available to handle large throughput.

Some machines may gill and de-head the fish in the same operation. The gut can then be removed by suction in small fish. Filleting can be done by hand or by machines in which the fish is conducted end to end past rotating circular knives that cut the flesh from the backbone.

Machines differ in configurations: some species are best cut from the head end, whereas others can be cut from the tail. Several types of mechanical skinners are available. In one type, the fillet passes over a refrigerated drum that freezes the skin layer, allowing it to be cut off with a band knife. In another type, the skin side of the fillet is carried on a belt and pressed by a roller against a stationary flat knife.

In planning a processing operation, careful consideration must be given to the proper structure and flow of the process line to prevent build-up of partly processed product at any stage. A logical sequence of procedures to allow steady flow of product must result. Care must be taken to prevent cross-contamination and to keep the product chilled at every opportunity.

Fish are generally gilled and gutted, and the belly cavity thoroughly cleaned out and inspected for parasites or defects. This may be done before rigor when the process area is adjacent to the farm. The cleaned fish is then ready for market, for processing or for freezing and further processing at a later date. For many fish, such as the salmonids, processing involves filleting and removal of small bones, and trimming the fillet prior to dry salting or brining. Salt is applied for 1 day, then the sides are brushed clean and cold smoked to produce highly desirable smoked salmon. Cold smoking is carried out at a temperature near 22 °C. Above 25 °C the collagen of the connective tissue begins to decompose and the resulting product does not have sufficient integrity to be sliced thinly. Smoked salmon is sold as whole sides or as slices, generally vacuum packed onto a board or plastic tray, and distributed and sold chilled or frozen. Other products such as terrines and patés are made from the trims and off-cuts of the filleting and smoking processes.

Shrimp are harvested by net or by drainage of the pond into pounds (section 21.8.5). Good practice then dictates that they are chilled in ice or ice slurry and transported to the processor. Either at the farm site or on receipt, they are generally treated with a dip in compounds to prevent melanosis (blackspot) developing on the shell. The most common dip is a form of sulphur dioxide (SO_2) supplied by either sodium metabisulphite or sodium sulphite solution. Dip times and concentrations are calculated to be effective yet leave residual levels to comply with domestic and export/import regulations. In most countries this level is 30 mg SO_2 per kg of shrimp, calculated on the weight of whole shrimp. The SO_2 is effective, since it inhibits the polyphenoloxidase enzyme responsible for melanosis. Other dips that consist of acidifying agents, such as sodium acid pyrophosphate, work by acidifying the tissue out of the active range of the enzyme. Recently, compounds such as 4-hexyl resorcinol have become commercially available and these act at very low levels (e.g. 4 mg/kg of shrimp) as substrate analogues to inactivate the enzyme. Other sacrificial analogues, e.g. hydroxytyrosol from olives and extracts from grape seed, are being explored for this use.

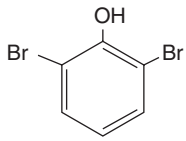
Shrimp may be sold raw in whole or peeled forms, chilled or frozen, or they may be cooked. They are normally cooked in vigorously boiling water, either potable or seawater or with salt added, for up to 5 min to ensure that the enzymes of the digestive gland are thoroughly denatured by the heat. If denaturation is not thorough, enzymes from the gut can digest the tail flesh, leading to staining and off-flavours. After cooking, the shrimp are chilled in fresh cool water, then in ice slurry, and then packed in ice before grading and packing into containers for market or further storage. Size grading is either by hand or by machines. Grading machines consist of rollers or inclined slats with gaps between; the gaps increase in size along the length of the machine.

11.11 EFFECTS OF FEED ON THE PRODUCT

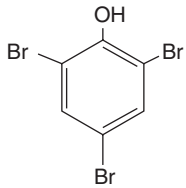
Aquaculturists can favourably affect the flavour characteristics of the final product through manipulation of feed ingredients. In order to do this it is essential that the industry knows the characteristics required by the market. In general, good husbandry practices and care for the feed will result in better products than if these factors are neglected. Most feeds are relatively high in lipids and these oxidise rapidly if exposed to heat, light and oxygen. Not only does this destroy the nutritional value of the product, but the long-chain fatty acids can be broken down into compounds that impart undesirable ‘fishy’ taints. Thus, the level of antioxidant in the feed and the antioxidant status of the fish are important factors.

Most of the effects of feed on flavour that have been reported in the literature concern changes in the diet from customary ingredients such as fish meal to protein sources from grains and legumes, which are cheaper and more environmentally sound (section 8.7.2). The resulting change in flavour can be subtle but of significance to the consumer. The analogy with wines, with the loss or gain of volatile compounds resulting in lower status, price and marketability, is quite apt.

Recently, the importance of the bromophenols in imparting seafood flavour has become apparent (Fig. 11.4). These compounds occur naturally in marine invertebrates, such as bryozoans, and it has been established that even small amounts of 2,6-dibromophenol in the µg/kg range can have a profound effect on the perception of seafood



2,6-Dibromophenol



2,4,6-Tribromophenol

Fig. 11.4 Chemical formulae of the two major bromophenols that impart 'seafood' flavour.

flavour (Whitfield, 1990). The threshold level at which the flavour of 2,6-dibromophenol can be detected in water is extremely low, at 0.00005 µg/kg, whereas in shrimp flesh it is 0.06 µg/kg. The equivalent threshold for 2,4,6-tribromophenol in water is 0.6 µg/kg.

Similarly, terpene compounds from seeds, grasses and algae move their way up the food chain to impart flavour to the wild product. There would seem to be considerable scope to incorporate premixes or minor ingredients rich in these compounds into aquaculture diets to overcome some of the perceived problems of bland flavour. Synthetic or other natural sources could be used.

11.12 SPECIALISED NICHE MARKET PRODUCTS

Recent work on the incorporation of selenium into garlic to make a selenium-enriched product that, in turn, is included into fish feed has shown that Se is taken up by the fish. This opens up the concept of using aquacultured fish that are enriched as particular 'natural' dietary supplements.

There are demands for 'organically' raised aquaculture products in which the feeds are 'organically' grown and only approved medicinals are used in treatments. This is a sector experiencing an increasing demand in some cultures that are prepared to pay a premium for the product.

Similarly, ethical protocols and practices are being developed to result in products that can be labelled as being ethically produced through all stages from hatching through to final processing into food items. Consumer surveys of attitudes on ethically produced and of aquacultured products have been conducted to explore this new market.

11.13 FLAVOURS AND TAINTS

Flavours arise from the complex of compounds in the tissue and, within a species, these are controlled by the environment and the feed ingredients (Lindsay, 1990). Wild-caught fish and shrimp have stronger 'natural' flavours than their cultured counterparts because of the greater diversity of their diet. Replacing natural feeds with the blander cereal- and legume-based artificial diets thus perpetuates this lack of diversity.

One major problem prevalent in freshwater aquaculture is the presence of taints from blue-green algae and actinomycetes, which lead to the compounds geosmin and 2-methylisoborneol accumulating in the lipid portion of the tissue. The taints are variously described as muddy, earthy or musty. Most of the work on these problems has been carried out in the USA on channel catfish, *Ictalurus punctatus*. These taints can be removed by purging the live fish in clean water for several days. The time of purge obviously is dependent on the degree of contamination, and each situation needs to be determined on its merits. Exposure of catfish to a concentration of 0.5 µg/kg methylisoborneol in the water leads to rapid uptake of musty flavour within 1–2 h, as detected by a sensory panel. Significant taint still existed in fish even after 48 h of purging in clean charcoal-filtered water. Purging is currently the only remedy since algal outbreaks will always occur in intensive systems. Steam pre-cooking to leach the volatiles, followed by canning, has been found to neutralise the flavours to some extent with trout.

Contamination of water from domestic and industrial sources can also occur. Traces of the highly odorous musk xylols used as perfumants in commercial detergents have also been detected in freshwater species in Europe, and breakdown products from petroleum can taint both marine and freshwater species. Other taints may arise from oxidised or mouldy feeds, but these are due to bad management practices (section 9.10.3) and will not be considered further here.

Much of the flavour of aquatic foods, and hence of farmed products, is derived from the non-volatile, non-protein nitrogen compounds, including:

- free amino acids;
- urea;
- betaines;
- peptides;

- nucleic acids;
- nucleotides;
- trimethylamine oxide;
- other amines;
- salts and minerals.

The concentration of the free amino acids, glycine, taurine and alanine, are important in determining the flavour of abalone. Glycine, proline, arginine, serine, threonine and alanine are particularly important in determining the flavour of shrimp, and their relative content is affected by the salinity of ponds: higher levels occur at higher salinities. Glycine is quite sweet and the major contributor to the sweet flavour of shrimp and scallops. It is also readily soluble in water and can be leached out during processing: raw shrimp have a different, much sweeter, flavour than those that have been boiled. Indeed, cooking the product by different methods can also create a different range of heat-induced volatiles.

The main storage carbohydrate in aquatic products is glycogen, which in itself does not contribute to flavour, but its breakdown products of glucose and glucose-6-phosphate are important. Because of the higher content of carbohydrate in artificial diets, most cultured animals tend to have higher glycogen levels than in the wild. Exercise increases glycogen levels in the muscle. The importance of glycogen in determining flesh properties lies in its function as an energy reserve for anaerobic glycolysis when the fish is stressed or in the post-mortem state.

The volatiles in fresh fish flesh are mostly alcohol and carbonyl compounds, which arise from the action of specific enzymes on individual long-chain fatty acids (see Table 11.1). These lipoxygenase enzymes are involved in the production of feed and culture and through post-harvest practices.

Table 11.1 Typical compounds derived enzymically from polyunsaturated fatty acids.

Compound	Aroma
Hexanal	Heavy, green[1], aldehyde-like
t2-Hexanal	Green, stinkbug-like
c3-Hexanal	Green, leafy
1-Octen-3-ol	Raw mushrooms
1-Octen-3-one	Cooked mushrooms
1,c5-Octadien-3-ol	Heavy, earthy, green, mushroom
1,c5-Octadien-3-one	Crushed geranium leaves
t2,c6-Nonadienal	Cucumber
t2,c6-Nonadienol	Cucumber, melon
(2Z)-Octen-1-ol	Weak green, fishy
2,5-Octadien-1-ol	Fried fish
(2E)-Pentenal	Green apple
(1,5Z)-Octadienal	Geranium leaves
(1,3E,5Z)-Undecatriene	Balsamic
(1,3E,5Z,8Z)-Undecatetraene	Seaweed

[1]Green denotes cut grass or herb-like aromas.

11.14 TEXTURE

After death, glycogen in muscle may be broken down by either phosphorolytic or hydrolytic pathways to result in the production of lactic acid, which in turn determines the ultimate pH of the flesh. pH is the major determinant of texture in flesh, with the inherent buffering capacity of amino acids and peptides playing a role in its control. Low pH normally results in firm-textured flesh, but too rapid a reduction in pH tends to cause a soft texture even at a low ultimate pH. Thus, the biological conditions, as reflected in glycogen levels and buffering capacity, dictate the flesh texture within a species.

The texture of flesh in various species is a consequence of its structure. In the raw state, the relative proportion of collagen dictates the resistance of the muscle to shear, since the myofibrillar components are present as a soft gel. Those species with low levels of connective tissue are too soft for sashimi; those with too high a level are too tough. The situation reverses in cooked muscle and the myofibrillar components become tough while the collagen molecules shrink, unfold, gelatinise and soften. As a result, the flesh readily separates into flakes, each of which is a muscle block. Thus, the texture of cooked fish is dependent on the state of the myofibrillar proteins. If the proteins are denatured and have lost their ability to hold moisture then the cooked product may taste dry and stringy, since the moisture will only be loosely held. The oil content and the amount of gelatinised connective tissue contribute to the sensation while chewing.

11.15 CONCEPTS: QUALITY, FRESHNESS, SHELF-LIFE AND QUALITY INDEX

Quality and freshness are concepts that are best restricted to the context of sales and marketing. Unfortunately, the terms are often used in technology and are applied ambiguously, even within the one sentence or paragraph, to convey both the concept and a particular property or set of properties. If the terms are to be used meaningfully then

Table 11.2 Relative rate of spoilage and potential change in shelf-life at various temperatures with reference to storage at 0 °C.

Temperature of storage (°C)	Rate of spoilage relative to 0 °C	Shelf-life (%) relative to 0 °C
−1.5	0.7	140
0	1.0	100
4	2.0	50
10	4.0	25
15	5.25	19

it is necessary to conduct a structured definition of them, in terms of measurable quantities (Bremner, 2002).

Freshness is often applied to unfrozen product to convey an idea of the integrated time-temperature history since harvest. It is customary to use 0 °C, the temperature of melting ice, as a reference for chilled storage work. This is because no mechanical or electrical devices are required for control of temperature, just the ice itself, and because spoilage at this temperature can be readily related to that at other temperatures (Table 11.2) through the equation:

$$r = (1 + 0.1t)^2$$

where r is the relative rate of spoilage and t is temperature in °C.

Thus, compared to storage at a temperature of 0 °C, spoilage at −1.5 °C occurs 30% more slowly, leading to a 40% increase in shelf-life. In comparison, temperatures of 4, 10 and 15 °C result in increases in the rate of spoilage of two times, four times and over five times, respectively, and a substantial decrease in shelf-life (Bremner *et al.*, 1986). These results strikingly display the need to chill product to 0 °C and to keep it as close to that temperature as possible. The above relationship is valid up to about 16 °C and means that any combination of time and temperature in this range can be calculated into equivalent days at 0 °C (ice days or ice hours) by multiplying the period of time at a particular temperature by the relative rate. The individual ice day results can then be summed to give the overall equivalent ice days or ice hours. This is then a specific measure rather than a vague expression of freshness, that can be understood throughout the chain.

The term 'quality' brings in many other aspects other than time and temperature, and it is necessary to construct a structured definition to establish communications that are readily understood.

Shelf-life and storage life depend entirely on the criteria used for judgement. It is essential to have a good estimate of the chilled storage life of an aquaculture product in order to determine the scope for distribution. Products that remain marketable for over a fortnight in ice can obviously be distributed more widely than those in which undesirable changes occur in a week. The criteria by which to measure the marketable shelf life are determined by:

- the market;
- the suppliers;
- some sector of the industry.

There are markets for produce that is not in optimum condition or of a high number of ice days, as there are the products into which such material can be made. Generally, the aquaculture producer is aiming at the fresher (low ice days) end of the market, where prices are higher. Thus, the pattern of change in the product in these first few days can be more important than the total shelf-life in determining the market and distribution.

The typical rates of change seen in products are shown in Fig. 11.5. In general, the best product is available for only a relatively short period (often only 3–4 days), after which the product slowly declines. It is in these first few days that the product could be regarded as suitable for sashimi, i.e. to be eaten raw. The formation of spoilage products is a consequence of bacterial growth. A typical situation is set out in Table 11.3, in which an initial count of only 10^3 bacteria per cm^2 is assumed to be present on the product surface and the reasonable assumption is made that counts will double each day. Bacterial counts are low in the early stages: in the first 4 days, counts increase by only ca. 20 000 per cm^2 of surface. However, in the 24 h from day 12 to day 13 the increase is 12 million per cm^2! The methods used to determine the storage life may be either direct or indirect. Direct methods may involve the use of systematic scoring systems or sensory assessment of the cooked or raw material by trained panellists.

A systematic scoring system that is simple, direct and non-destructive, and has been shown to be capable of integrating time–temperature effects (Bremner *et al.*, 1986), is now widely used in research and industry. Known as the quality index method (QIM), the common parameters related to appearance, odour and texture are scored on a systematic basis as demerit points that are summed to a total. This total provides a straight line relationship with ice days and an inverse relation with sensory evaluation of the cooked flesh by trained taste panels. QIM is therefore a very rapid and valuable technique for estimat-

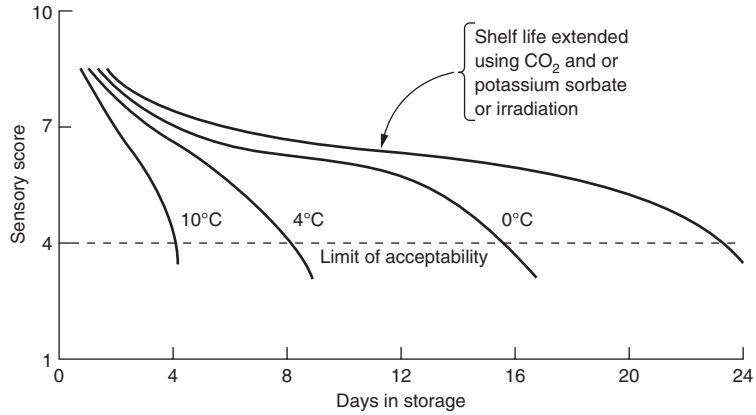


Fig. 11.5 Typical pattern of spoilage in chilled fresh seafood product.

Table 11.3 Hypothetical examples of calculated bacterial counts on the surface of fish flesh if the bacteria double in number each day. These bacterial counts and percentages can be considered equivalent to the relative concentration of spoilage compounds, assuming that the initial organism has spoilage potential.

Storage period in ice (days)	Calculated bacterial counts ($\times 10^3$ bacteria per cm^2)	Bacterial counts as % of count at 13 days[1]
0	3	0.01
1	6	0.025
2	12	0.05
3	24	0.10
4	48	0.20
5	96	0.39
6	192	0.78
7	384	1.56
8	768	3.13
9	1536	6.25
10	3072	12.5
11	6144	25
12	12288	50
13	24576	100

[1]End of acceptable shelf-life.

ing ice days elapsed, remaining storage life and an evaluation of sensory score.

Sensory assessment by trained panellists is very effective, but it requires considerable skill and organisation and suitable facilities.

Sensory methods describe the product when it is eaten and are thus more closely related to consumer appreciation. Full consumer trials must be undertaken to relate the results of trained sensory panels to 'the assessment of the marketplace'.

Indirect methods include:

- microbiological counts;
- measurement of flesh metabolites, such as nucleotides;
- measurement of microbial metabolites, such as trimethylamine or products of oxidation.

11.16 MICROBIOLOGY, SPECIFIC SPOILAGE ORGANISM (SSO) AND OTHER SPOILAGE PROCESSES

The microbiology of chilled storage of flesh foods is dominated by the pseudomonad group of bacteria. In temperate marine situations, the major spoilage organism is a heterotrophic facultative Gram-negative bacterium, *Shewanella putrefaciens* (formerly *Alteromonas putrefaciens*). This organism can metabolise a number of free amino acids and sugars to produce a range of odorous compounds, including hydrogen sulphide (H_2S). Indeed, this property alone is often linked to spoilage and enumeration of H_2S-producing bacteria correlates well with spoilage, even though they may represent only a proportion of the total flora. Other pseudomonad bacteria contribute to spoilage and, in tropical and subtropical waters, *Pseudomonas fragi* is often the organism that results in off-odours and off-flavours reminiscent of rotting tropical fruits.

It is important for any given situation to understand the nature of the spoilage microflora and to know which specific spoilage organism (SSO) is involved. In any given

Table 11.4 Important international websites for post-harvest technology and processing.

Site	Content
www.difres.dk/micro/sssp	Seafood safety and shelf-life predictor. A free downloadable program describing current knowledge on seafood microbiology and specific spoilage organisms
www.seafoodplus.org	Largest EC project information on seafood safety, seafoods and human health, consumer preferences, traceability, new products, ethical aquaculture, genetics and hormones and product characteristics
http://www.fda.gov/oc/bioterrorism/bioact.html	US bioterrorism act
http://europa.eu.int/comm/food/food/foodlaw	European food law
www.thaiqualityshrimp.com	Thai Department of Fisheries (2003) *Good Aquaculture Practice*. Marine Shrimp Culture Research Institute
www.scottishsalmon.co.uk	Scottish Salmon Producers Organisation (2006) *Code of Good Practice for Scottish Finfish Aquaculture*
http://seafood.ucdavis.edu/index.html	A prime site for matters regulatory, technical and for HACCP
www.codexalimentarius.net/web/standard_list.jsp	CAC/RCP (2005) *Code of practice for fish and fishery products*. FAO Rome. 98p
www.cenorm.be/catweb/67.120.30.htm	CEN (2003) Traceability of fishery products – Specification of the information to be recorded in farmed fish distribution chains. CEN publication date 2003-02-19. Reference number CWA 14659:2003
www.eurepgap.org	Eurepgap (2005) General Regulations Integrated Aquaculture Assurance 2.01. **Food**plus GMBH, Köln. 32p
www.tracefood,org	Tracefood (2007) Tracefood Specification Document – Tracecore XMLv2rc1.doc
www.fishbol.org	The Barcode of life project on fish. In June 2008 it contained information on 5042 species and 26 330 barcodes of mt DNA segments useful for identification purposes

set of circumstances (temperature, species, mobile water, pH, atmosphere), one organism, the SSO, dominates the microflora and causes spoilage. The SSO must therefore be established in order to take steps to inhibit its growth. The range of known SSO and the inhibitory steps are covered in the website at www.difres.dk/micro/sssp (Table 11.4). Often the SSO is present initially in very low numbers, as in the example in Table 11.3, and may even be undetectable at point of harvest.

It is common for bacterial counts to decrease in number when warmwater species are placed on ice. For example, tropical shrimp that show a total count of 10^6 organisms per gram when first harvested may only have counts of 10^4 per gram after storage on ice for a day or two (Fig. 11.6). This is because the microflora (bacteria and fungi) capable of growth at chill temperatures (mainly psychrotrophic organisms) are initially present in only very low numbers and are often introduced with the ice or alternative chilling medium and on boxes or utensils. The native warm-loving (mesophilic) microflora cannot survive the chill conditions and die. For this and other reasons, tropical aquaculture products can exhibit a longer chilled shelf-life than species from colder waters, in which the psychrophilic microflora are already present in abundance.

11.17 FREEZING AND FROZEN STORAGE

Although much aquaculture produce is sold fresh, factors of seasonal production mean that a large proportion is frozen for further processing or as a final product for orderly distribution to the market.

In essence, the process of freezing is one of substantially reducing temperature by absorbing heat from the product and immobilising the bulk of the water in the form of ice. The preservative effect of freezing is due to two factors:

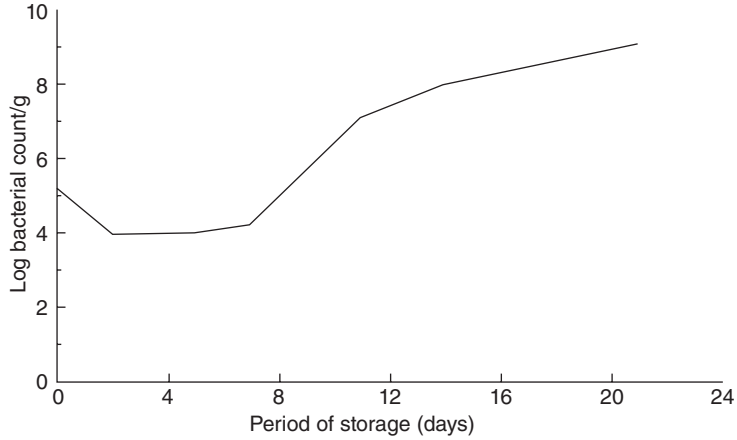


Fig. 11.6 Total bacterial counts of tropical products (e.g. shrimp) often decrease in the initial period of chilled storage.

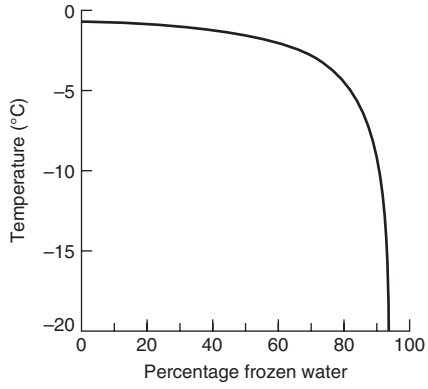


Fig. 11.7 The percentage of water in fish tissue frozen at different temperatures. Note that even at –20 °C not all the water is frozen.

1. The reduced temperature slows chemical and biochemical reactions, in some cases to imperceptible rates.
2. Low temperature completely retards microbial and fungal growth.

The reduction of mobile water with freezing reduces the water activity to further retard enzymic and chemical reactions, but it is important to note that not all the water is frozen at temperatures commonly used for commercial storage (–20 °C). A concentrated solution of salts, amino acids and other soluble constituents still exists in a mobile phase distributed throughout the tissue (Fig. 11.7), and many biochemical and chemical reactions still continue at appreciable, though reduced, rates. The means of freezing depends on the nature of the product:

1. Blast freezing. Freezing using refrigerated air in a blast freezer is by far the most common and versatile method used, since the same equipment can accommodate different product volumes, shapes and sizes. In addition, continuous blast freezers have been developed to freeze individually quick frozen (IQF) product. In blast freezing, refrigerated air is blown at high speed around the product. Air will take the path of least resistance, and it is important that it passes over the product, not around it in the voids. The velocity must also be sufficient to create a turbulent flow of air at the product surface, otherwise heat transfer from the product to the air will not be efficient. Fans with aerofoil sections must be used to achieve sufficient airspeed under the pressure head.
2. Plate freezing. Horizontal plate freezing, in which the product is frozen between plates arranged in banks, is quite efficient and is commonly used for finished product, e.g. fillets, scallops and shrimp, for which uniform packs are produced. Vertical plate freezing is employed mostly for whole fish or shrimp destined for further processing and is used more at sea than for aquaculture products.
3. Immersion freezing. Freezing by immersion in refrigerated brine gives efficient heat transfer with consequent rapid freezing times and is best suited to small

individual products such as shrimp or scallops. Such products may then be packed as IQF items.

4. Cryogenic freezing. Freezing with liquid nitrogen tunnels or solid CO_2 'snow' are also used for IQF products. These techniques have the advantage of low capital cost (equipment can be hired), but the disadvantage of high operating costs.

There are newer exploratory methods in which freezing is done under pressure. This approach, pressure-shift freezing at pressures of about 200 MPa and temperatures of −20 °C, is said to produce smaller ice crystals which cause less physical damage to the product and hence result in less fluid loss as drip during thawing.

Products are commonly frozen to about −20 °C, but −30 °C should be used. The rate at which the product reaches this temperature is important since the aim is to produce small ice crystals within the cell structure rather than large jagged crystals, which pierce and disrupt the cell membrane. All frozen product is eventually thawed for consumption, and product in which the structure has been disrupted will be of poorer texture and will release more of the tissue fluids when thawed. These fluids are important contributors to the taste and succulence of the final product and its nutritive value. Furthermore, they represent a considerable loss of revenue if valuable product ends up being discarded.

Much of the heat load on the refrigeration system is due to the latent heat of freezing of water. When a time–temperature profile of freezing is plotted, it shows a plateau area on the curve known as the zone of thermal arrest (Fig. 11.8). In general, this spans the temperature region from −1.5 °C to about −5 °C, but the exact values depend on the water-soluble salts and minerals present in the product, which vary according to seasonal and nutritional circumstances. The aim is to drive the temperature of the product through this zone of thermal arrest as rapidly as possible.

The storage temperature of the frozen product is also very important. All products have a finite life that is governed by the temperature of storage and the stability of this temperature. As a very broad guide, a storage temperature of −20 °C provides twice the shelf-life of −10 °C, whereas −40 °C provides twice (or more) the shelf-life of −20 °C. Equally important is the stability of the temperature. If the temperature warms up from −20 °C to −10 °C, a portion of the ice in the tissue will melt and, if the product is cooled again, this will refreeze to build even bigger ice crystals. Continual temperature cycling will exacerbate this effect, with consequent destructive effects on the product.

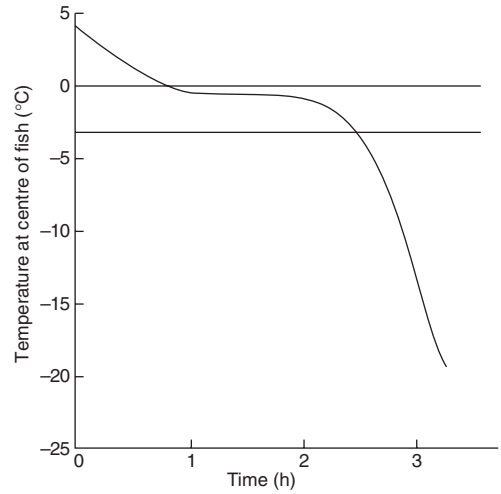


Fig. 11.8 The rate of freezing of tissue is slow as the latent heat of freezing is removed in the 'zone of thermal arrest', which is demarcated by the two horizontal lines.

Although no bacteria will grow at temperatures lower than about −7 °C, enzymes previously released from them may still be active if large numbers of bacteria were allowed to proliferate on the product before freezing. Thus, product that is poor due to bacterial growth can deteriorate even further in frozen storage, as undesirable products of bacterial metabolism diffuse through the whole product from the surfaces where the bacteria grew. Inherent enzymic activity also takes place during frozen storage. Lipases are active to produce free fatty acids, which are then prone to oxidation. In marine species, trimethylamine oxidase can split the osmoregulatory compound trimethylamine oxide (TMAO) to dimethylamine and formaldehyde. The formaldehyde is highly reactive and migrates in the aqueous phase either to react with proteins or to destabilise the layer of water molecules that form buttressing structures around the proteins. This results in denatured proteins, which are less able to rehydrate when the tissue is thawed. This leads to more fibrous, and often stringy and dry, texture in the product.

The most common cause of deterioration in frozen product is oxidation of the constituent lipids with the oxygen of the air (Table 11.5). Oxidised lipids react with protein, leading to denaturation and textural change. More importantly, oxidation results in off-odours and flavours variously described as straw-like, nutty, paint solvent and stale. Small, fatty fish are notoriously prone to oxidation,

Table 11.5 A general outline of the process of oxidation.

Phase	Reactions	Results
Initiation step	$-RH \rightarrow R + H^+$	Free radical formation
	$-O_2 + RH \rightarrow R + OOH$	
Propagation	$-R + O_2 \rightarrow RO_2$	Peroxide and hydroperoxide formation
	$-RO_2 + RH \rightarrow ROOH + R$	
Termination	$-2R \rightarrow R\text{-}R$	Terminal non-propagating products
	$-R + RO_2 \rightarrow ROOR$	

R is a highly unsaturated fatty acid (HUFA) that may be a free fatty acid (FFA) or attached as part of a phospholipid or a triglyceride.

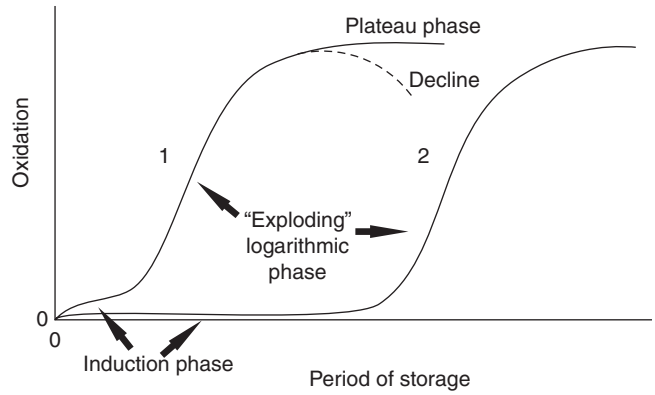


Fig. 11.9 Typical patterns of oxidation in stored fatty fish: 1 represents the situation for a product with little antioxidant protection and 2 represents that for a product with significant antioxidant protection.

but within any species the antioxidant status of the flesh and the lipid composition are critical factors.

Fish oils containing highly unsaturated fatty acids readily react with any available oxygen. The reaction starts with an induction phase during which oxygen is absorbed and free radicals are initiated, but little detectable change is noted. This is followed by the propagative self-catalysed phase, known as autoxidation, in which the reactions are rapid and self-sustaining.

In lipids and oils with low levels of antioxidants, such as vitamin E, the induction period may be slight and the autoxidative phase starts almost immediately (Fig. 11.9). As shown in Table 11.4, peroxides are intermediate products of lipid oxidation. A common method of determining oxidation in aquaculture products is to measure the peroxide content or 'peroxide value'.

The hydroperoxides themselves have little odour or flavour, but they break down to give a wide range of compounds that have quite strong odours and flavours. For example, the compound hept-*cis*-4-enal has been shown to be the main cause of the 'cold storage odour and flavour' found in some species and it contributes nutty 'cardboard' flavours.

Products of oxidation in combination with trimethylamine (produced bacterially in marine species from trimethylamine oxide) are regarded as being responsible for the production of fishy odours that occur during storage.

Thus oxidation in stored products is a problem that causes:

- downgrading of product;
- lower acceptability by the consumer;
- lower price;
- a decrease in the product's nutritional value.

Oxidation can be minimised by ensuring that the product is fresh when frozen and that the precursors of oxidation

as a result of the activity of oxidase enzymes, or potential exposure to air, light or heat, are not allowed to form. Thus, frozen product can be protected either by glazing with water (which may contain antioxidants such as ascorbic acid) or by packing in a plastic film with low permeability to oxygen. Vacuum packing is very effective and the inclusion of oxygen-absorbent materials in the packs to harvest any residual oxygen provides even greater protection.

Product can also dehydrate in frozen storage as water evaporates from the surface in the low humidity of the cool store and gets deposited on the evaporator coils. In exposed product, this leads to areas of white, denatured tissue known as 'freezer burn'. Freezer burn is irreversible and causes downgrading of the product.

In packaged products, temperature cycling leads to condensation of water vapour on the inside of packs and its subsequent refreezing into crystals in the bag or box. This represents loss of yield and is an indicator of poor storage conditions and poorer product.

11.18 PACKAGING

Packaging of the final product is important for its preservation and presentation. The technical requirements of a package for frozen stored product are that:

- oxidation is minimised;
- dehydration from the product's surface does not occur.

There are a number of options for the chilled product brought about by continual improvements in packaging films and in equipment.

Vacuum-packed products are now common. The product is placed in these packs on a tray or in a pouch (or the tray is placed in the pouch), a vacuum is drawn and the pouch is then sealed by heat, or with a clip or, alternatively, a film is sealed over the edges of the tray. This type of pack provides excellent presentation since the plastic seals in the juices, the product itself can be viewed and the pack can be decorated with brand, advertising and other product information.

Vacuum packing in oxygen-impermeable films is often used for processed materials such as smoked and salted eels and salmonids. A considerable chilled shelf-life can be achieved for these products, since the conditions in the pack slow the growth of spoilage bacteria. These conditions of low pH, low oxygen tension, low water activity (due to salt and other curatives) and a low initial microflora (smoke has antibacterial action) all combine to help select a microbial flora that is not spoiling in nature and generally consists of lactobacilli.

Vacuum packing of fresh product in oxygen-impermeable films does not improve the shelf-life of the product to any worthwhile extent as the main spoilage organisms are not inhibited. It does provide more presentable packs, but this can be achieved using less costly oxygen-permeable films. Vacuum packing in films of low oxygen permeability is not necessary for fresh-chilled product.

In modified atmosphere packing (MAP), the product is placed in a tray or pouch, a vacuum is drawn, then the atmosphere is replaced with another gas or gas mixture generally containing at least 40% CO_2 by volume. The pouch is then sealed or a gas-impermeable film sealed over the tray. The gas to product ratio is in the region of 2 or 3 to 1. In these packs, the CO_2 dissolves in the aqueous portion of the flesh of the fish, leading to a reduction in pH due to formation of carbonic acid. The preservative action that occurs is thought to be due to both the lower pH and the amount of undissociated carbonic acid present. This undissociated acid can readily cross bacterial cell membranes, following which it dissociates to release H^+ ions. The decrease in pH and disruption of cell metabolism result in inhibition and death of the bacteria. Thus, spoilage organisms are destroyed or inhibited and can grow only slowly in these packs. The lower pH and low oxygen tension encourage the growth of lactobacilli, which themselves are inhibitors of Gram-negative spoilage bacteria. A doubling of the normal chilled shelf-life can be expected for such products, but it is notable that endogenous enzymes are still at work within the flesh and that deleterious changes still occur slowly. Also, SSO *Photobacterium phosphoreum*, a bacterium with a great capacity to form trimethylamine, thrives and spoils marine products, especially in the Northern hemisphere. If *P. phosphoreum* is present then other measures must be taken, such as a short period of freezing the flesh, or addition of low levels of an antibacterial such as potassium sorbate.

The degree to which the system works for any particular product is a function of the partial pressure of CO_2 in the pack and the relative volume ratios of product to gas. Fish flesh will absorb more than its own volume of CO_2 so the initial pack must be over-inflated to allow for this. The disadvantages of MAP are:

- the bulkiness of the packs;
- the fact that the product is loose (unless held by some internal film);
- the pack can fog as a result of condensation;

- unsightly drip liquor from the product may be high (due to the low pH), making the product less attractive;
- the potential for botulism if low temperature is not maintained.

MAP has been used with part-cooked product and products enrobed in batter to prevent some of these problems.

One of the most serious considerations with MAP is that conditions in the packs can lead to the growth and production of toxin from the botulism bacterium (*Clostridium botulinum* type E) if the temperature is not strictly held at, or below, 3 °C. This organism produces the deadliest natural toxin known. The concern is that the longer shelf-life obtainable with MAP will provide the opportunity for the organism to produce toxin before there are any overt signs of spoilage which would otherwise warn the consumer not to use the product. Cooking readily destroys the toxin, but it could be inhaled as an aerosol when the pack is opened and smelled. Also, it could contaminate knives, boards and utensils, and be passed on to other products that may not be cooked or to foods that have already been cooked.

MAP is widely used in Europe, but has been considered too potentially dangerous in the USA. This probably reflects a difference in culture and approach to safety and food regulatory matters rather than to any real risk. The key to safety is to ensure low product temperatures throughout the distribution system. There are some fears that even vacuum packing could provide conditions suitable for botulism. However, this will not occur if the film used is permeable to oxygen and there is no real need to use oxygen-impermeable films with chilled product, although it is excellent for frozen products.

With smoked salted products, such as those made from trout and salmon, *C. botulinum* cannot grow if the concentration of salt in the water phase of the product is 3%. This level thus provides a critical control point and its measurement must be standard practice in quality control (Fig. 11.10).

More recently the use of carbon monoxide (CO) in MAP mixtures has been used to 'stabilise' the myoglobin in the flesh and thus retain its colour by formation of the stable carboxymyoglobin. This prevents oxidation to the grey/brown metmyoglobin (Fig. 11.11). This is very effective for both highly pigmented species, such as tunas, and lighter-coloured species alike. Rather than use CO gas, a



Fig. 11.10 Vacuum-packed smoked Atlantic salmon.

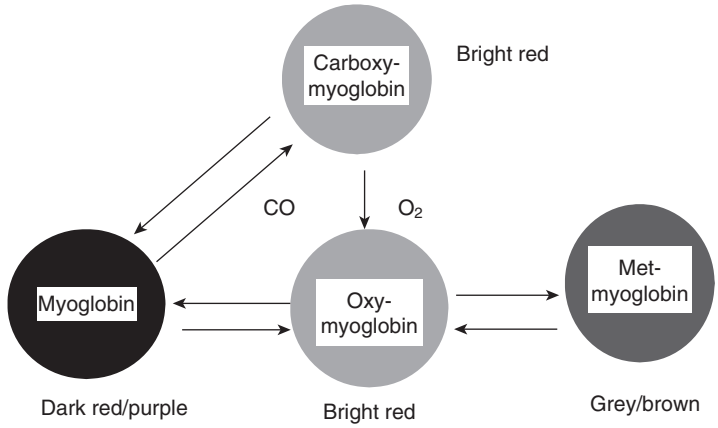


Fig. 11.11 Diagrammatic representation of colour states of myoglobin.

'tasteless smoke' produced from condensed smoke from which many smoke components have been removed, but which retains dissolved CO, has been employed to achieve the same effect. These techniques are often used with frozen packaged cuts rather than with chilled product.

11.19 QUALITY CONTROL, QUALITY ASSURANCE, HACCP AND RISK ASSESSMENT

Quality control (QC) is the practice of determining the properties of products to ensure they are within some agreed range. Quality assurance (QA) is the adoption of practices that will guarantee that the quality attributes of the product lie within that range. Thus, quality assurance is focused on the process, whereas quality control is focused on the product. The two are complementary and QA arises from an understanding of QC and is superior to it.

For processors, QA begins with decisions on the correct site for the factory and its layout, and the processing techniques to be used. A proper QA plan will include schedules for equipment and plant cleaning, the instruction of staff in the use of detergents and sanitisers, and in personal hygiene and food-handling practices (Fig. 11.12). There must be a general maintenance plan and protocols for a full sanitary control programme.

QC procedures generally revolve around measurement taken on raw materials, incoming goods, process operations and finished product. In aquaculture:

- The raw material must always be up to standard since this is controllable.
- Incoming goods, such as packages and cans, must be inspected to ensure that they are of correct specification.
- Process parameters, such as temperatures and times, must be measured and logs kept.
- Finished products must be inspected to ensure they meet company standards.

These standards must be laid down unequivocally. In large operations, QC staff, independent from production operations, must be trained in proper inspection procedures.

Hazard analyses critical control point (HACCP) schemes are mandatory in most countries and are required by importing countries. These schemes are based on:

- evaluation of the hazards involved;
- identification of critical points in the process to control the hazards;



Fig. 11.12 Training in hygenic processing of marine shrimp. (Photograph by courtesy of Darryl Jory.)

- adoption of procedures to control the hazards;
- recording and verification that these procedures have been carried out correctly (FAO, 1994; Martin *et al.*, 1997; Nesheim and Yaktine 2007).

Risk assessment is a new discipline rendered possible by the power of latest-generation computers, development of manipulative software and construction of databanks of information on products, safety and microbial growth that are interactive. Risk assessment models are used by export/import regulators and health authorities to estimate potential risks and hazards from various degrees of food contamination in combination with processing, storage and transport. Risk analysis is a developing technology that covers evaluation of the degree of risk from particular threats or hazards. It fits closely with concepts embodied in HACCP to help define the risk associated with specific hazards and to provide broad assessment of general risk to

consumers from particular hazards, e.g. *Listeria* and *Salmonella*. It is mainly a tool for national food safety authorities to aid their decision making and includes the three elements of risk analysis, risk assessment and risk communication. The document *Food Safety Risk Analysis* (WHO/FAO, 2006) covers these matters in great detail.

Risk analysis does not supplant HACCP or GHP, but is used to cover those situations where more detailed information of the microbiological degree of risk is required. Issues of risk may arise in several ways such as the risk for specific target groups, for the general population, in setting priorities among different safety problems, for a specific public health issue, for justification of a new technology or inspection system or determining an equivalence.

11.20 TRACEABILITY, IDENTIFICATION AND ORIGIN

Traceability of aquaculture products has become of increasing importance in world trade. The ability to trace a product back through its distribution and processing history to the whole animal, its feed history, its culture environment, its medicinal treatments, the hatchery and the broodstock is available through suppliers of traceability software packages, e.g. TraceTracker (Fig. 11.13).

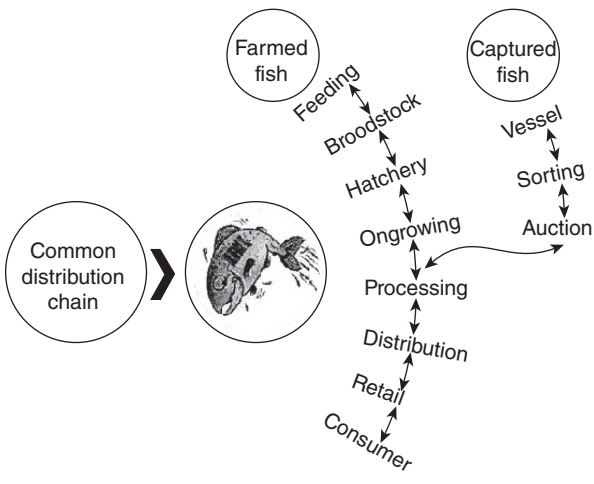


Fig. 11.13 Main steps in the chain where traceability must be maintained. As the product moves from its source in the farm or fishery to the consumer, it can be traced back through successive intermediate sources. (Adapted from Figure 10 of *Traceability of Fish Guidelines EAN International.* November 2002.)

Several integrated research projects, the forerunner being TraceFish, have surveyed industry and producers to determine which parameters:

- shall be recorded as essential 'shall statements';
- should be recorded – 'should statements' provide further information;
- may be recorded – 'may statements', e.g. ones to share with trade partners, and which can be used for publicity, advertising, marketing and for public information.

This information was incorporated into a European standard from which a core standard software for all food commodities was identified (Trace Core XML).

The payoff for installation of electronic traceability systems is in significant increases in efficiency both within a company and its trading partners. The system must be part of, or relate to, the ERP (enterprise resource planning) computing business system of the organisation. Companies with registered traceability systems obtain reductions in insurance premiums.

Identification is the science of identifying the particular species involved in a product or product mixture. This starts with classical taxonomy but has moved onto the placement of photographs, drawings, indices, X-rays, measurements, descriptions and whole catalogues being available online and being connected interactively. This facilitates identification as access to collections can be done remotely. This process of identification is backed up with a battery of genetic tests for species ID, often using mitochondrial DNA fragments. The largest collection is on www.fishbol.org in which the aim is to cover the world's fish resources.

Authentication is the science of determining the area of origin or production of the goods, firstly through species identification on which is laid the compositional matrix of microchemical, micro-mineral and radioisotope signatures to see if they match the known patterns. This is an infant technology for most aquaculture products as the database required can be enormous.

Origin, including country of origin, is similar to authenticity and involves similar techniques, but may not include artefacts due to process or maturation.

11.21 CANNING

The canning of seafood products is well understood, and mostly it is only wild-caught, not cultured, material that is used. The process is one of hermetically sealing the product in a rigid can under partial vacuum and heating the contents sufficiently to ensure destruction of

micro-organisms (FAO, 1988). The heat process required is calculated to destroy spores of the botulism bacterium, should they be present, and the times and temperatures required depend on the size of the can and the nature of the pack. For example, a solid tuna pack requires over 60 min at 121 °C, whereas shrimp in brine require only 16 min because of the greater convection in the fluid in the can and the more rapid thermal conductivity.

Basic canning operations involve the following stages:

1. Preprocessing steps, e.g. cutting, dicing and slicing, are carried out.
2. Some species are pre-cooked, then picked over, selected or separated before packing into the cans.
3. The seafood is then packed and weighed into cans.
4. Vacuum is then drawn either by passing the can through a vacuum chamber or by purging the can headspace with steam before closing of the lid.
5. Filled cans are loaded on trolleys or baskets and placed into a steam-heated chamber for processing.
6. The air is purged from the retort (a steam-heated horizontal or vertical cylinder) and the temperature rises as steam is introduced.
7. Careful monitoring of the temperature is required for each batch.
8. The cans are then cooled by the introduction of clean water.
9. They are moved from the retort and allowed to stand for their surfaces to dry.
10. Labelling occurs several hours later when the can has 'settled'.

11.22 SMOKING

The smoking process has been used for many years to preserve and impart a characteristic smoky odour, flavour and texture to fish products (Doe, 1998). Smoke is invariably generated from wood by direct combustion of logs, sticks, chips or sawdust or by friction of grinders on logs. A huge range of organic compounds have been identified in smoke, including:

- phenolics (>80);
- acids (>30);
- alcohols (>10);
- esters (10);
- aldehydes (>20);
- ketones (>60);
- hydrocarbons (>20);
- aromatic hydrocarbons (>60);
- lactones, ethers, furones and nitrogenous compounds.

These constituents are all volatile in the smoke zone and are carried in the air and deposited on the surface of the product.

It is the phenolics that supply the major characteristic flavour to smoked fish, and the compounds syringol, guaiacol, 4-methylguaiacol, eugenol, 4-methylsyringol and 4-allylsyringol have been reported to be most important (Miler and Sikorski, 1991). These phenolics and the other smoke constituents are antibacterial, so the product surface at the point of exit from the smoker is close to sterile.

There are two main types of smoking, designated cold smoking and hot smoking. Cold smoking is mainly used for fillets, hot smoking for gilled and gutted whole fish. In cold smoking, the product is not cooked and the temperature of the air in contact with the product rarely reaches 30 °C. Temperatures in the 22–25 °C range for 6–8 h are common. The salt (natural and added) present in the fish, the preservative action of the smoke and the slight drying that occurs give the product microbial stability. It is then ready for eating without the need for cooking. In hot smoking, the product is cooked during smoking. The process lasts only 1–2 h and reaches temperatures above 65 °C. The product is eaten either cold or reheated.

It is very important that the product is not smoked at an intermediate temperature that is insufficient to pasteurise it, because in the heating and cooling process bacteria in the interior of the product may well have proliferated to make the product dangerous.

The chill-stored life of hot-smoked fish may be up to 2 weeks. Cold-smoked fish are more heavily smoked and salted, and may have a chilled shelf life of up to 6–8 weeks. It depends greatly on the salt content and the degree of smoking, and it is advisable for each producer to check the results from their own system before marketing a product and publicising a use-by date.

11.23 CONCLUDING REMARKS

Many factors impinge on the attributes of the raw material and finished products from aquaculture, and to further complicate the picture they interact highly with one another.

This has been but a brief introduction to post-harvest technology of aquaculture products. There are many other issues not covered that can properly be considered in this area but are beyond the scope of this chapter. These other important issues involve:

- traceability from harvest to retail sales;
- quality chain management;
- information technology;

- product development;
- total utilisation;
- genetic and seasonal influences on the properties of the product;
- extraction and development of by-products;
- biotechnologies for preparation of bioactive materials from aquaculture products.

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12
Economics and Marketing

Clem Tisdell

12.1 INTRODUCTION

Economics plays an important role in the survival and development of aquaculture. Technical ability is a precondition for the aquaculture of a given species, but this will fail to develop and survive (in any meaningful sense) if it is commercially uneconomic. Economic failure of an aquaculture project may stem from production, technical or cost problems, or from marketing problems (Fig. 2.1). Therefore, those who want to have a commercially successful aquaculture enterprise must pay considerable attention to economics, including marketing issues. Furthermore, aquaculture's economic value often needs to be assessed taking into account its social or community-wide impacts.

Aquaculture economics and marketing is now a specialised subject, and its whole range cannot be covered in depth in a single chapter such as this. Therefore, the purpose of this chapter is to highlight, for non-specialists in economics, selected important issues that need consideration:

- in developing aquaculture commercially; and
- in assessing aquaculture from a community-wide economic perspective.

Readers who require further in-depth coverage of this subject can consult specialised books on aquaculture economics such as Shang (1990), Hatch and Kinnucan (1993) and Jolly and Clonts (1993).

Aquaculture economics can be applied in many different contexts. It can be used to improve the business performance of individual aquaculture businesses (e.g. their profitability), to assess the economic performance prospects of sectors of the aquaculture industry (e.g. economic prospects for edible oyster production), to determine the value of aquaculture from a national perspective and can even be used to evaluate aquaculture from a global point of view.

In western countries, in particular, profitability is likely to be the major economic concern of an individual business, whereas overall net national benefit should be the main focus from the national viewpoint. The profitability of a business or industry does not necessarily measure net national economic benefit. For example, if an industry has adverse environmental effects, profits in the industry are likely to overstate its national economic benefit because the social costs of its production exceed the costs paid by individual firms.

If aquaculture businesses are be profitable in market economies, most must actively market their product and do so effectively. For established products, this can be relatively easy because existing marketing networks can be tapped. Established food processors, transport and distribution channels may be used. Engle and Quagrainie (2006) provide a useful overview of marketing channels for aquaculture. Marketing of new aquaculture products can, however, be quite difficult, especially in the absence of appropriate existing marketing networks (Tisdell, 2001).

Profitability is influenced not only by the market for a business's product but also by the firm's costs of production. The latter depends on, among other things, the culture

Aquaculture: Farming Aquatic Animals and Plants, Second Edition. Edited by John S. Lucas, Paul C. Southgate.
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Table 12.1 Explanation of some economic terms.

Discounted benefits	Future benefits from a project reduced to equivalent present values
Discounted costs	Future costs of a project reduced to equivalent present values
Discounted realisable value	The sum, reduced back to its equivalent present value, that could be realised by a business selling out at the end of its planning period
Discounted value of future profits	This is the sum of profits during the firm's planning period with future profits reduced below their actual future values. The reduction of future profits reflects the fact that a dollar available in the future is worth less than a dollar available now because a dollar available now can be invested at a going *nominal rate of interest* to earn more than a dollar in the future. So a future dollar is equivalent to only a fraction of a dollar now
Equity	The proportion of a firm's assets or capital belonging to the owner(s) of a business
Internal rate or return (IRR)	Indicates the percentage rate of return on funds employed by a business or a project. It is a useful indicator of the degree of profitability of a business or a project. Estimates of IRR take into account the time-pattern of returns
Market transaction costs	Costs involved in arranging market exchanges, e.g. cost incurred in searching for potential buyers in arranging contracts, agency costs and so on for a sale of aquaculture products
Nominal rate of interest	This is the rate of interest payable, not adjusted for price inflation. The nominal rate of interest tends to rise with the rate of inflation
Present discounted value	The sum of money that, invested now, would accumulate with the addition of interest to a stated future sum of money
Real rate of interest	This is the rate of interest reduced for price inflation. The greater the rate of inflation, the larger the reduction in the rate of interest needed to obtain the real rate
Spillovers (externalities) from business activity	These are side-effects of the activities of a business on other businesses or entities for which no economic payment (e.g. compensation) are involved. They can be favourable or unfavourable

techniques used and the costs of inputs to the production process. These costs vary according to whether the business is involved in the hatchery phase, the grow-out phase or both and whether aquaculture is performed in artificial enclosures requiring pumping of water or in natural water bodies.

Modelling the economics of aquaculture is complex, but some insights can be obtained by considering simple economic models. Thus, this chapter will successively consider models analysing:

- the profitability of a business;
- the market;
- the nature of production costs;
- methods by which a firm can assess and cope with business risk and uncertainty;
- the social economic evaluation of aquaculture.

Economic terms used in this chapter which are not explained in the text are highlighted (in italics) and explained in Table 12.1.

12.2 PROFITABILITY FROM A BUSINESS VIEWPOINT (FARM MODELS)

For a single period, say a year, a firm's profit can be obtained by taking the difference between its revenue and its total costs. Its revenue is equal to its volume of output multiplied by the price at which units of this output are sold.

$$\text{Profit} = \text{total revenue}$$
$$(= \text{volume of output} \times \text{price/unit of output}) - \text{total costs}$$

If the market in which the firm sells its product is very competitive, the firm will need to sell at the going market

price per unit of the product. This, for example, is likely to be the case for the sale of table oysters and for shrimp on the international market. Clearly, other things being equal, the higher the price for the aquaculture product, the higher will be the profit of the business. Sometimes, however, there may be few or virtually no competitors in the market for the cultured product and, up to a point, an aquaculture business selling this product will be a price-maker (as opposed to a price-taker, e.g. the above examples of businesses, selling table oysters and shrimp). Price-making has been true of the Japanese cultured pearl industry, but is no long the case (Tisdell and Poirine, 2000). It is currently true in Australia for producers of pearl-oyster seed, and the two dominant Australian suppliers of Pacific pearl oysters now have some market power in the sale of South Pacific pearls (Tisdell and Poirine, 2008).

Note that the economic concept of cost differs from that of accounting costs. The latter considers only actual costs (including purchases, salaries and depreciation). Economic cost takes account of opportunity cost, that is, the economic benefit forgone by not choosing the best alternative to the choice actually made.

$$\text{Economic costs} = \text{actual costs} + \text{opportunity costs}$$

For example, if family labour is supplied to an aquaculture business free of charge, this would not be included in the accounting cost of the business. However, if that family labour could earn an income if employed elsewhere, the highest income that it can earn elsewhere is its opportunity costs. In order to calculate economic cost, this opportunity cost would be included and ascribed to the family labour employed.

Often, but not always, opportunity costs are zero. Then actual costs and economic costs do not differ. This is likely to be so when all inputs for an aquaculture operation are purchased at market prices, as is frequently the case in western market economies.

In general, we are interested in the profitability of an aquaculture business not only in a single period, but for an interval of time spanning several periods, and economists usually assess the firm's profitability for a planning period covering several time intervals, e.g. for a 10-year period covering 10 annual intervals. The appropriate planning period is likely to vary with the enterprise at hand. However, a very long planning period, say 50 years, is likely to be too long because the *discounted value of future profits* (Table 12.1) and uncertainty will mean that events 50 years hence have little consequence for current decisions.

The optimal business strategy from the point of view of an aquaculture business is, according to standard economic theory (Tisdell, 1972), that which maximises the business's net present value. It is the *present discounted value* of its stream of profits over its planning period plus the *discounted realisable value* of the business (compare Shang, 1990).

A unit of currency (e.g. a dollar) available in the future can be expected to be less valuable to a business than a dollar available now. There are two reasons:

1. If there is a price inflation, the purchasing power of a dollar in the future is less than now.
2. A dollar available now can be invested at the going *nominal rate of interest* with relative safety to earn income from interest and so, in the future, returns the initial capital invested plus interest. Even in the absence of price inflation, this makes it more valuable than a future dollar that has not been invested.

Furthermore, the higher the market rate of interest, the lower is the net present value of a dollar available in the future.

Usually the market rate of interest on government bonds or similar safe investments is used to take account of the minimum opportunity cost (economic actual costs) of committing funds to a business. This takes account of a relatively safe alternative profit that is forgone in committing funds to the business. For some businesses, however, the opportunity costs are higher than the returns from this alternative investment, or, if they are borrowing funds, they may also have to pay a higher rate of interest. Therefore, a higher rate of discounting of future monetary amounts would be appropriate. Some judgement is required in determining the appropriate discount rate to apply. Here it is only possible to bring attention to this issue, which forms a part of a study of finance. Nevertheless, it should be clear that discounting of future income flows is appropriate when determining the financial returns of an aquaculture business.

If the net present value of a project is positive, it is profitable from an economic point of view because it earns more than the relevant rate of interest. This principle is applied to cost–benefit analysis. If the net present benefit from investment in a project is positive after being reduced using the appropriate rate of interest, it is economic. This also implies that its *discounted benefits* divided by the *discounted costs* exceed unity, or, in other words, that its benefit–cost ratio exceeds unity (Shang, 1990; Allen *et al.*, 1984).

If the net present value of a project or enterprise is zero, then it is marginal because it earns only the going rate of interest; if this value is negative, the project is unprofitable because it returns less than the going rate of interest on the finance required for it. In the latter case, an actual loss will be made if the aquaculturalist borrows to finance the project; or if self-funding is practised, income will be forgone by investing the funds in this project rather than elsewhere at the going rate of interest.

Alternatively, the profitability of aquaculture project or enterprise can be specified by its benefit–cost ratio. Benefit–cost ratios have the advantage that they make for easy comparisons of the relative profitability of different projects and enterprises, but, at the same time, they are a reflection of the **net** present value of a project or enterprise. This follows because the net present value of a project or enterprise equals the discounted value of its benefits **less** the discounted value of its costs. Consequently, if the net present value of a project is zero, its discounted benefits equals its discounted cost and therefore, its benefit–cost ratio is one. Hence, unity is the critical value of the benefit–cost ratio for determining the profitability of a project. If this ratio exceeds one, the project is profitable; it is marginal if the ratio is unity; and it is unprofitable if the ratio is below one.

Weston *et al.* (2001) completed a study of the profitability of farming six selected species for aquaculture in Australia and reported the most likely benefit–cost ratios for representative farms assuming a 20-year planning period. For discounting financial flows, they employed an interest rate of 6%, which would have been realistic at the time of the study. For abalone farms producing 100 tonnes annually, they reported a most likely benefit–cost ratio of 1.48 and for farms producing 200 tonnes per year, 1.58. For typical farms supplying mussels they estimated that farms producing 100 tonnes of mussels per year would most likely have a benefit–cost ratio of 1.07 whereas those supplying 200 tonnes annually would most likely have a benefit–cost ratio of 1.37.

A number of implications follow from these results. First, abalone production is predicted to be substantially more profitable than the farming of mussels. Secondly, profitability in both cases tends to rise with the scale of annual production. Furthermore, a mussel farm producing 100 tonnes of mussels annually is expected to be barely profitable.

An alternative (but compatible) procedure for determining a farm's profitability (or the profitability of an investment project) is to calculate its *internal rate of return (IRR)* (see, for example, Shang, 1990; Jolly and Clonts, 1993). As in the case of benefit–cost ratios, the use of IRRs enables comparative analysis of profitability to be completed easily. However, IRR specification has the additional advantage that the net profitability of a project can be readily compared with different levels of the rate of interest whereas if benefit–cost ratios are used, they have to be recalculated when different rates of interest apply. If the IRR of a business exceeds the relevant rate of interest, the business is profitable, and it is more profitable as the IRR increases in relation to the rate of interest.

It is not uncommon for economists to make estimates of internal rates of return for the culture of different species. Treadwell *et al.* (1992) estimated the IRR from cultivating various aquaculture species in Australia. To make these estimates, they considered model (or representative) aquaculture farms and specified their annual operating costs and capital cost. These costs, together with predicted levels of revenue, provided the basis for estimating net benefits and subsequently the IRR values for the different types of farms. An aquaculture business's planning interval was assumed to be for a 20-year period. A similar approach was adopted by Weston *et al.* (2001) in their later study of the profitability of aquaculture of different species in Australia but they estimated benefit–cost ratios rather than IRRs.

Only one of the cultured species (mussels) was assessed for its profitability by Treadwell *et al.* (1992). They estimated the mean internal rate of return (IRR) on a model mussel farm in Australia to be 12.3% with a wide likely range of 1–22.7%. For this species, the profitability of farms of different sizes were not calculated by Treadwell *et al.* (1992) but Weston *et al.* (2001) found that the benefit–cost ratios of such farms rose with their size. However, this was done by Treadwell *et al.* (1992) for two other cultured species. The mean IRR for small grow-out (saltwater) crocodile farms was estimated to be 10% and for large ones 14%. In the case of Atlantic salmon grown in 60-m diameter cages, the predicted mean IRR for a model farm with 40 000 smelt was 5.5%, and for those with 150 000 smelt, 12.5%. In both these cases, the relative profitability of model farms increased with their size.

The above types of analysis of profitability mostly assume that the capital (finance) market is perfect and give limited attention to uncertainties. Ways of allowing for uncertainties are a focus of section 12.5. In practice, a firm may need to give special attention to its liquidity (cash availability) to ensure its continuing viability. It may, therefore, be concerned about how quickly a business enterprise can pay back the investment (Shang, 1990;

Jolly and Clonts, 1993) in a project and about how large its debt may become during the implementation period: the larger its debt, the greater are its risks.

12.3 MARKETS AND MARKETING

The markets for most aquaculture products are influenced by supply and demand conditions and changes in these (Allen *et al.*, 1984; Engle and Quagrainie, 2006). For products of aquaculture businesses that are price-takers rather than price-makers, the standard economic analysis of purely competitive markets is relevant. Most suppliers of aquaculture goods are price-takers but there are exceptions, as in the case of South Pacific pearl oysters (Tisdell and Poirine, 2008). Consider the standard economic analysis.

The quantity demanded of an aquaculture product depends on many factors. Factors such as its price per unit, the income levels of buyers, price of substitutes, tastes and so on, can all be expected to have an influence. Usually, as the price of a commodity is reduced, the demand for it increases, if all other factors remain constant. This can be illustrated diagrammatically. However, it should be noted (in advance of the following diagrammatic outline) that, in illustrating market relationships, economists conventionally put the independent variable on the *Y*-axis and the dependent variable on the *X*-axis. This convention is followed here and differs from the convention in natural science. So in the discussion which follows, the independent variable, in this case price per unit of the aquacultured product, is shown on the *Y*-axis and the market quantity of the product is shown on the *X*-axis.

Normally the demand curve in a market is downward-sloping (Fig. 12.1, D_1D_1), indicating that buyers purchase more of the product as its price is lowered. The market supply of the product is usually upward-sloping, indicating that greater supplies only become available if producers are paid higher prices (Fig. 12.1, S_1S_1). The quantity demanded of a product as a function of its price represents the market demand curve for a product, all other things being constant. The quantity supplied of a product as a function of its price represents its market supply curve, all things, other than its price, being held constant. The point at which these two curves cross represents the market's equilibrium and the corresponding price is the equilibrium price and the corresponding quantity traded is the market equilibrium quantity. In Fig. 12.1 for instance, the demand curve D_1D_1 might represent the demand for shrimp in Japan in July 2012 and S_1S_1 might represent the supply curve of shrimp. Market equilibrium would be established

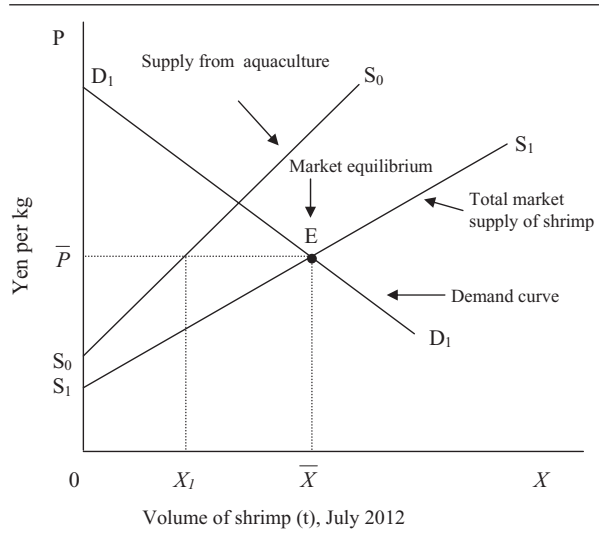


Fig. 12.1 A theoretical market model for marine shrimp in Japan illustrating market equilibrium and dividing supply into capture and culture components. As mentioned in the text, economists conventionally place the price variable, in this case the independent variable, on the *Y*-axis and the dependent variable, in this case the quantity of the product demanded or supplied, on the *X*-axis. This differs from the normal convention in the natural sciences.

at point E, with the equilibrium price of shrimp being $\bar{P}$/kg with $\bar{X}$ of shrimp being supplied. Supplies may be drawn from cultured shrimp (the supply curve for these may be as indicated by the curve marked S_0S_0) and from captured shrimp, the supply function of which is the difference between curves S_1S_1 and S_0S_0. In the case shown, in the market equilibrium X_1 of supply comes from cultured shrimp and $\bar{X} - X_1$ from captured shrimp.

It is clear from Fig. 12.1 that, if the demand curve for shrimp moves upwards (and everything else remains constant), the equilibrium price and quantity traded will rise. It becomes more profitable for businesses to supply shrimp. Other things held constant, the market demand curve for shrimp may rise, for example, if:

- incomes in Japan rise and, more generally, incomes in market outlets for shrimp rise;
- the prices of shrimp substitutes rise;
- the human population increases;
- tastes alter in favour of the product.



Fig. 12.2 Sushi-style golden rolls of tempura shrimp and mango (photograph by Jon Maybury).

It is important to be able to predict such trends and their influences on demand.

Sometimes, the demand curves for aquaculture products are stated in terms of the average consumption per head of population or per household. Miyazawa and Hirasawa (in Liao *et al.*, 1992) reported the relationship between the consumption of shrimp in Japan and the price of shrimp on the basis of annual data for 1980–89. A fall in price led to a substantial increase in the consumption of shrimp. Consumption per Japanese household rose from ca. 2.4 to ca. 3.4 kg/year as the cost of shrimp per 100 g declined from ca. 280 to 220 yen. Other data also showed that a rise in Japanese incomes led to a significant rise in the per capita consumption of shrimp in Japan (Fig. 12.2).

A shift downward in the supply curve (that is, increased supply for any given price), other things unchanged, tends to lower the equilibrium price for the aquaculture product, in this case shrimp. Other things constant, the supply curve of an aquaculture product may shift downwards, because, for example:

- The price of one or more inputs falls, e.g. fish food.
- New technologies are discovered that lower production costs, e.g. techniques that greatly reduce food wastage, such as have been developed for the culture of Atlantic salmon (Asche *et al.*, 1999).
- Improved methods may be found to reduce the incidence of pestilence or disease in aquaculture.
- Genetic selection and breeding may raise the productivity of cultured organisms such as tilapias (Dey, 2000) (section 7.4).
- High returns in the industry may result in new businesses entering and investing in the industry, thereby raising supplies.

As indicated above, most aquaculture products compete with supplies of substitutes from the capture fishery. Sometimes, these are perfect or near perfect substitutes. Hence, a reduction in supplies of substitutes from the capture fishery usually raises demand for the farmed product. A rise in supply from the competing capture fishery has the opposite effect. Nevertheless, there is evidence that increased supply of aquaculture products is not completely at the expense of sales of the capture fisheries because market segmentation exists between farmed and wild-caught products (Asche *et al.*, 2001).

Trends or expected variations in relation to all of the above-mentioned demand and supply matters need to be considered in predicting future prices and markets for aquaculture products. To do so accurately can be very difficult, especially if a long planning period is being used.

There are also marketing decisions to be made at the business level. These include the quality of the product to be supplied and how far to process it. In established industries, middlemen are often present to facilitate marketing and distribution (Engle and Quagrainie, 2006), but one of the difficulties sometimes encountered in developing a market for a new aquaculture product is the absence of suitable networks for its distribution and sales. For example, the sale of giant clam for human consumption in Australia was hampered by the absence of suitable distribution networks for this. On the other hand, the sale of Australian cultured giant clams as aquarium specimens initially progressed quite rapidly because of the existing network of wholesalers and distributors of aquarium specimens. In the absence of suitable distribution and marketing networks, considerable costs of marketing activities will fall on the innovating aquaculture business. These costs will include advertising the product, its presentation, search for market opportunities and information transfer (Tisdell, 2001).

Many cultured species progress through a typical product cycle (Fig. 12.3 and Table 12.2) as, for example, outlined by Chaston (1983). In the early stages of this cycle, new production techniques are developed, and to a large extent the market is uncertain. Only innovators or adventurers enter the industry at this stage. At the next stage, sorting of techniques tends to take place, with the least effective ones being discarded, and market penetration may proceed rapidly. The industry goes from a position of earning low and uncertain profits to one of high

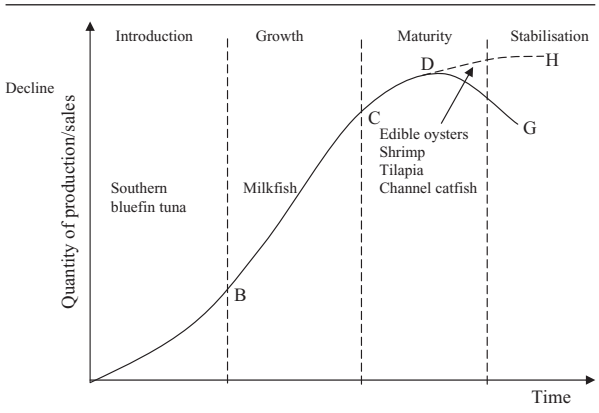


Fig. 12.3 Product cycle showing typical stages which aquaculture industries pass through if they succeed economically and the approximate stage in which some of these industries are now. The demand and volume of supply for all these industries does not decline but stabilises for some as upper limits to production and demand are approached.

profit if the new product is well accepted. This induces followers to enter the industry and eventually the industry becomes well established with 'appropriate' techniques settled and potential markets fully tapped. This is the mature stage in which profitability tends to fall to the average level of business profitability in the economy. Channel catfish culture in the USA is in the mature phase (Chapter 17). Atlantic salmon culture is in the mature phase in Europe. The culture of southern bluefin tuna in Australia is still in a relatively early stage. Redclaw crayfish culture in Australia was also in an early stage in the 1990s and since returns in 1991 seemed relatively high for little risk, one would have expected considerable entry into the industry, resulting eventually in a fall in returns due to increased supply. However, returns may not fall substantially at first because demand might also expand as consumers become more aware of this product and it gains greater acceptance. There are a number of instances in which this has occurred. For example, when tilapia culture was first introduced to Fiji, local demand for this introduced fish expanded slowly. However, it is now a sought-after fish.

When a market needs to be developed or a business plans to supply a new market, a variety of methods may be used to determine the nature of the market and to foster

Table 12.2 Product cycle stage (see Fig. 12.3) for some aquaculture industries. Based on details from John S. Lucas.

Stage	Aquaculture industry
Introduction	Southern bluefin tuna[1]
	Spiny lobsters[1]
	Sea cucumbers
	Sea urchins
	Short-finned eel[1]
	Turbot
	Giant clams
Growth	Groupers
	Abalone
	Mullet
	Pangasid catfishes
	Sargassum seaweed
	Swimming crabs
	Whiteleg shrimp
	Chinese mitten crab
	Atlantic salmon
	Soft-shelled turtle
	Freshwater crayfish[2]
Maturity	Milkfish
	Tilapias
Stabilisation	Rainbow trout
	Carps
	Gilthead seabream
	Mussels
	Scallops
	Oysters
Decline	Black tiger shrimp[3]

[1]Three 'holy grails' of aquaculture – to close their life cycle. They are very high value, but present great technical difficulties in culturing the early developmental stages. They are currently cultured by collecting developed stages from the field and rearing them to market size.

[2]When China enters an aquaculture industry it will typically go into a phase of very rapid growth regardless of its previous state, e.g. global freshwater crayfish production varied between 8 and 35×10^3 t in an erratic fashion during the 1990s; then China entered this industry and production rose to 378×10^3 t by 2007.

[3]Being replaced by culture of whiteleg marine shrimp in some countries (section 1.8.2).



Fig. 12.4 A dish using cultured giant clam prepared by a Japanese restaurant in Australia for market testing (photograph by Linda Cowan).

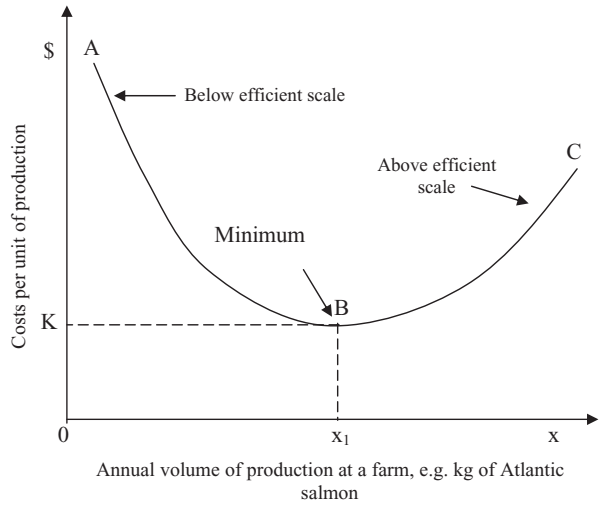


Fig. 12.5 U-shaped average cost of production curve. Businesses having a level of production less than the minimum efficient scale can reduce their costs per unit of production by expanding their level of production.

it. These include trials of the product such as taste-testing of a new aquaculture product, pilot or trial marketing, interviews and various types of surveys and examination of the demand for substitute products (Shang, 1990; Meade, 1989). Because giant clam farming was so new in the 1990s, it was necessary to use all these methods to assess potential demand for cultured giant clams for eating (Tisdell *et al.*, 1994). Fig. 12.4 shows a dish using giant clams (one of several) prepared by a Japanese restaurant in Australia for experimental taste-testing. An aquaculture business also has to make economic decisions about how to distribute its product, promote and present it.

As a market expands, it becomes increasingly necessary to standardise the cultured product, or its grades, in order to reduce *market transaction costs* and increase market penetration. Supermarkets, which have become the dominant form of retailing in developed countries, demand standardised products (see Young, 2001). The industry may itself set standards or a government marketing body may do so. Furthermore, large retail chains often specify the standards they require. There can be an economic benefit to an aquaculture industry in imposing financial levies on its businesses in order to have its product promoted by a 'government' or a co-operative marketing authority (Engle and Quagrainie, 2006). This is so even though members of the industry as individuals would not be prepared to spend so much on promotion, because others would benefit considerably by their promotion of a relatively generic product, e.g. Atlantic salmon, Pacific oysters, channel catfish.

12.4 ECONOMIES OF SCALE AND SIMILAR FACTORS

The costs per unit of production of an aquaculture business are likely to vary with the size of the undertaking (Shang, 1990). There are economies of scale or decreasing costs per unit of production for many species, up to some annual volume of output. After this point, costs per unit of production may begin to rise with greater volume of output (Fig. 12.5) or they may rise after remaining stationary over a range.

The scale (volume of annual production) at which a business obtains its minimum cost per unit produced is called its minimum efficient scale. If a business is operating below this level and is a price-taker, it will usually be at an economic disadvantage compared with businesses operating at their efficient scale. Consequently, its rate of profit can be expected to be lower than that for the latter businesses. Nevertheless, in some market conditions, the most profitable level of production by an aquaculture farm can be for a level of production less than that corresponding to minimum efficient scale. This occurs, for instance, when markets are not perfectly competitive and individual suppliers of products have downward-sloping demand curves for their products. In such cases, the limited size of

their market restricts the ability of firms to take advantage of economies of scale in production.

Economies of scale are likely to be significant in land-based aquaculture operations involving the pumping of water to tanks, raceways or ponds and requiring water circulation. This is mainly because of engineering relationships, e.g. the volume tends to increase at a faster rate than the circumference of a container, but there may be other economies of scale, for example in being able to use more effectively the services of specialised personnel who can be employed. Economies of scale can also be present for farming *in situ*, as the case of Atlantic salmon farming indicates, for example. The minimum efficient scale (size of production operations) of an Atlantic salmon farm has tended to increase with the passage of time. Significant economies of scale in production exist for hatchery/nurseries engaged in land-based production, e.g. in the supply of giant clam seed (Tisdell *et al.*, 1993). However, in the case of seaweed production in developing economies, economies of scale do not appear to be significant.

The above discussion (centred on Fig. 12.5) implies that it is desirable when considering the economies of the scale of operation of an aquaculture farm not only to consider cost economics but also to take into account market conditions. Therefore, a more general approach to assessing the economics of the scale of operation of an aquaculture farm is to take account of how its internal rate of return or its benefit–cost ratio varies with its scale of operation. The farm's scale of operation can be measured in different ways but commonly it is measured by the volume of its annual production.

As noted earlier, Treadwell *et al.* (1992) found (for the scales of production which they assessed) that the internal rate of return for crocodile breeding farms and for crocodile grow-on farms, as well as for Atlantic salmon farms, increased with their annual volume of production. Similarly, Weston *et al.* (2001) found that the benefit–cost ratios for abalone farms and for mussel farms in Australia rose with their annual volume of production, for the production range assessed. Consequently, smaller farms tended to be less economic than larger ones for the production ranges considered. The likely economic situation of very large farms (outside the range examined) is unspecified.

The general situation can be illustrated by Fig. 12.6, assuming that the IRR of an aquaculture farm at first rises with its value of production but falls if its annual volume of production becomes quite large. In Fig. 12.6, the curve ABC represents the rate of change of the IRR of a farm in relation to its annual level of production of an aquaculture

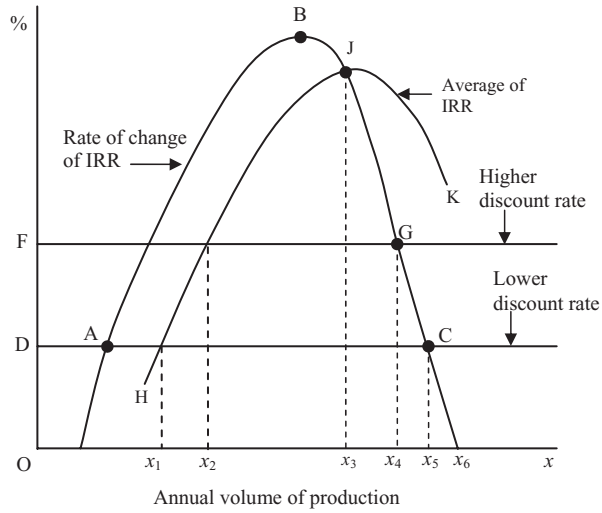


Fig. 12.6 Diagram illustrating a case in which the scale of aquaculture production by a farm alters its profitability.

product, and curve HJK represents the farm's IRR per unit of its output (IRR/x) for its planning period. This implies that the IRR of the farm is at a maximum when its annual value of production is x_6. However, the net profitability of the farm depends on the discount rate (the rate of interest). The discount rate is independent of the level of output. If the discount rate is OD, the farm will not be economic unless it operates at a scale of at least x_1, and its most profitable scale of operation will be for an annual volume of output of x_5. At x_5, the benefit–cost ratio for the farm is at a maximum. Should the discount rate rise, for example, from OD to OF, other things held constant, the minimum scale of production at which the farm will just break even rises from x_1 to x_2 and the annual volume of production for which it maximises its net return falls from x_5 to x_4. Note that the level of output that maximises profit per unit of output, x_3, is not the firm's most profitable level of output.

Given the type of IRR relationship shown in Fig. 12.6, very small scales of production are likely to be unprofitable. However, there can also be economic disadvantages of the firm being too large. Diseconomies of scale may eventually occur for several reasons. For example, the coordinated management of a large enterprise may become more difficult and it may be necessary to begin using sites for expansion that are ecologically inferior, more distant from markets or more expensive to acquire. In the case shown in Fig. 12.6, if a firm produces more than x_4 annually when the rate of interest is OF, it will be forgoing

profit. If it produces a large enough volume of output, it can actually make a loss. Note that the above model is a simple one because it assumes steady states, as does the implicit modelling done by Treadwell *et al.* (1992) and by Weston *et al.* (2001).

Apart from economies of producing a greater volume of a particular species, other types of economies may exist. These include economies of scope (or diversification) and economies of specialisation. To a large extent, these are the opposite sides of the same coin. To take advantage of economies of scope, if they exist, the firm engages in the supply of multiple products or services and this can include polyculture (section 2.3.5). There may be biological synergies (complementarity) in the production of more than one species so that mixes of aquaculture of species are the most profitable. In land-based facilities, it may be possible to spread overheads, e.g. those involved in pumping water, or the employment of specialists, by producing different species in different ponds or containers of various kinds.

Economies of diversification, however, have to be balanced against possible economies from specialisation. Frequently, if specialisation by production of species is absent, there is often specialisation by stages in the culture of a species. For example, some businesses may specialise in the hatchery/nursery stage culture of a species, whereas others may confine themselves to the grow-out stage, or even just a part of it. This pattern has been developed, for instance, in Taiwan, with a series of small businesses specialising in successive stages of fish aquaculture. As a result, the industry can take advantage of maximum economies of scale at different stages in the culture of a species.

Economies of scope or of diversification seem to be very important at the hatchery/nursery stage from an economic viewpoint. Casual observation indicates that a large number of hatcheries/nurseries supply a range of aquaculture species or varieties of these, even though the range may be restricted to closely related species.

Possibilities for economies of scale, scope and specialisation are limited by the available techniques of production and by resource availability. The appropriate choice of technique from those available is partly an economic matter. In countries in which labour is cheap relative to capital, labour-intensive techniques are likely to be more appropriate from an economic profit standpoint than capital-intensive ones. However, in developed countries, where labour is relatively expensive, the reverse can be expected.

It should be noted that the location of an aquaculture business is likely to have a significant influence on its cost of production and profitability. The location of an aquaculture business's facilities will affect its cost of access to markets, its availability of inputs and their costs. A good location ecologically may be uneconomic if it is distant from markets and lacks available human resource or services for its support.

12.5 ALLOWING FOR AND COPING WITH BUSINESS RISK AND UNCERTAINTY

Uncertainties about economic prospects are a major consideration for all aquaculture farmers. Farmers need to consider how they should allow for uncertainty in their economic planning and how they ought to adjust their business operations to best cope with it, given that some level of economic uncertainty is unavoidable. In the planning process, it is useful to identify the sources of uncertainty that are likely to impact on the business prospects of a farm (Shang, 1990). These may be essentially of an economic nature (such as uncertainty about the levels of prices, wages or the rate of interest) or of a non-economic type such as the likelihood of different levels of morbidity and mortality occurring in farmed stocks, or the likelihood of unfavourable weather patterns prevailing. Variations in the latter variables alter the productivity of aquaculture and, consequently, the productivity of a farming enterprise.

Once the sources of uncertainty affecting the business profits of an aquaculture farm are identified, decisions need to be made about how and to what extent efforts should be made to predict the likely values of the uncertain variables. How far to go in this regard is partly an economic decision. This is because costs are incurred in improving predictions and the anticipated extra economic benefit of improved predictions should be compared to the extra cost involved in sharpening the predictions. Even if certainty is theoretically possible, it is rarely economic to achieve it (Tisdell, 1996). The basic rule is that predictions (about variables of economic relevance) should only be improved up to the point where the extra cost incurred equals the extra benefit obtained.

In any case, the process of improving predictions will halt at some point, namely, the point at which an actual business commitment must be made. The question then arises of how best to specify the remaining uncertainties. Sensitivity analysis can be used to provide information on the range of possible economic payoffs, taking into account the estimated range of possible uncontrolled (exogenous) events. In effect, sensitivity analysis specifies a payoff matrix of the type commonly used in game theory (von Neumann and Morgenstern, 1944). This can be useful to a decision-maker, but it stops short of specifying the

probability of the uncontrolled events or 'states of nature' judged to be possible.

Considerable debate exists about how accurately the likelihood of uncertain events can be specified and about how best to estimate the probability of these events, if these probabilities can be meaningfully estimated at all (Tisdell, 1968). Economic events (and path-dependent events in general) often fail to satisfy the statistical conditions needed to estimate objective probabilities. In these circumstances, some analysts recommend the use of subjective (personal) probabilities as an alternative and suppose that these accord with the usual statistical probability axioms (Savage, 1951). This approach leaves open the possibility of applying risk analysis using objective probability distributions if they are available, or subjective probability distributions if objective estimates are unavailable. In the latter case, the accuracy of predictions will depend on the accuracy of the subjective probability distributions used for the analysis.

Treadwell *et al.* (1992) and Weston *et al.* (2001) used risk analysis to specify the economic risks faced by farmers involved in the aquaculture of different species in Australia. Treadwell *et al.* (1992) consider the probability distributions of internal rates of return for model farms and Weston *et al.* (2001) did this for benefit–cost ratios. The latter specify the estimated probability of a model (or representative) farm having a benefit–cost ratio of less than unity, that is of failing to break even. An interesting result from these empirical studies is that larger farms appear to be less likely to make a loss than small farms when scale economies are significant. For example, Treadwell *et al.* (1992) report that a 100-tonne mussel farm with an annual capacity of 100 tonne has a 10% chance of failing to break even whereas, for a farm with 200-tonne capacity, the chance of this is only 2%.

While risk analyses have the appearance of being very accurate because of their precise quantitative statement of predictions, caution should be exercised in drawing conclusions from them. For example, the underlying probability distributions used to make the predictions may be subject to significant error or shift. Furthermore, fundamental uncertainties may exist that are not amenable to specification in terms of statistical probabilities. When this is the case, it may be necessary to rely on decision-making criteria that do not make use of probability distributions. These include the minimax gain criterion and the minimax regret criterion (Tisdell, 1968).

Uncertainty about economic variables, such as future prices, and about levels of productivity (which can arise from possible environmental changes, disease, etc.) makes aquaculture a risky business. Most aquaculture businesses need to adapt to such uncertainties to survive and minimise their possible losses. Some methods of coping with uncertainty include:

- product diversification (not relying on a single product);
- diversification in techniques used for production (e.g. if some techniques are unproven or more variable in their productivity than others);
- incorporation of flexibility into the capital equipment or facilities used in order to keep options open (e.g. installing equipment that has multiple uses rather than a single use);
- expanding cautiously into a new business area to leave time for learning-by-doing;
- making sure that the business has limited liability;
- increasing the number of shareholders or partners in the business;
- making sure that the business's debt to *equity* (or ownership) ratio does not become so high as to jeopardise its ability to repay loans if its economic performance is below expectation;
- ensuring that the fixed costs of the business are low so that a substantial economic loss can be avoided if the price of, or demand for, the aquaculture product falls, or if production is below that planned, or if the cost (e.g. price of an important input) is above expected levels.

Fixed costs tend to be high when a production technique is capital-intensive, that is, uses a lot of equipment and fixed investment relative to other resources. When capital-intensive aquaculture techniques are adopted by a business, the business must make sure that economic conditions are favourable for this. For example, conditions are more likely to be favourable if the product is of high value or there is a high volume of demand for the business's product or the technique considerably reduces per unit operating costs. Also, the risk of production falling markedly below planned levels should be low, for instance as a result of environmental occurrences.

Fig. 12.7 shows a relatively capital-intensive white eel pond at a cooperative in Shenzhen in China. This type of intensive production is risky because the cooperative relies on high-price fish meal (of good quality) imported from the USA, and the price of this meal fluctuates considerably from year to year. Nevertheless, output is of high value and exported to Japan and California. The operation is profitable on average, even though in some years losses have been recorded due to sudden increases in the price of fish meal.



Fig. 12.7 A pond producing white eels under intensive conditions at Shenzhen, China, for export. Production is risky but the product is of high value.

An intensive shrimp farm on Okinawa, Japan, produces very high-value shrimp and can operate profitably even though its capital, overhead and operating costs are high. A semi-intensive shrimp farm near Shenzhen in China feeds its shrimp by collecting shellfish from a nearby bay. Both its operating and its capital costs are lower per hectare than in the Japanese case. The shrimp are exported, but the price received is lower than for the Japanese-reared shrimp. A seasonal extensive prawn farm (*Macrobrachium* species) in Bangladesh requires very little capital investment and has even lower operating costs. The economics of operation of the farms is hampered by the occurrence of typhoons, which result in the escape of shrimp stocks in some years, causing an economic loss. In the Bangladeshi case, both capital costs and operating costs are extremely low because the prawns are not given supplementary feeding, but rely on organisms naturally present in the water, which is interchanged with the nearby brackish river system. In this case, the business risks are relatively low.

Diversification of production is a common risk-aversion strategy. If returns from different products are not perfectly correlated, this will tend to reduce the variability of the business's total returns. The same is true of production using a range of techniques, e.g. juvenile giant clams may be cultured in onshore tanks as well as in floating cages, so reducing the likelihood of a major loss of supply if adverse weather conditions occur. Capital equipment used to farm a range of species needs to be flexible or adaptable. It may be more sensible, taking into account business risks, to use such equipment in culturing a species, than to use equipment specifically designed for the species. Although specialised equipment results in lower cultivation costs for a given species, it may have little alternative use. Should the culture of the species prove to be uneconomic, flexible equipment can be used to cultivate other species and will have a higher resale value.

Businesses engaging in the culture of a species unfamiliar to them generally go through a period of learning-by-doing. With the passage of time and with the experience gained, their productivity and economic performance in cultivating the species improves. In the early stages, therefore, they might do well to proceed cautiously, for example, to use small-scale or pilot plants, and install flexible or cheap, short-lived capital equipment (section 2.9). A late start can be a particular disadvantage for a new entrant to an aquaculture industry in which substantial economies of scale exist. If the entrant tries immediately to produce at the minimum scale of efficient production, this involves considerable risk since it does not allow time for learning by the business.

Institutional arrangements such as the limited liability form of company ownership can reduce personal business risks, and if risk is shared among a large number of shareholders or partners in a business, losses are easier to bear. In addition, the management needs to give continuing attention to the debt–equity ratio of the business. The higher this ratio is, the greater the risk to the business in the event of unfavourable economic performance. This ratio (debt–equity) is sometimes called the firm's gearing ratio, and if equity is low relative to debt the firm is said to be highly geared. A highly geared business can have a high risk of not surviving. On the other hand, a firm with a high IRR in relation to the rate of interest may be unnecessarily forgoing profitable business opportunities if its debt–equity or gearing ratio is low.

12.6 ECONOMIC ASSESSMENT FROM A SOCIAL STANDPOINT

Although an aquaculture business may be privately profitable and an aquaculture industry may be economically thriving, this does not necessarily indicate its economic value from a social point of view. The social value of production by the industry will, for example, depend upon whether social costs of production are greater than private costs. If they are, private gains overstate social net benefits (Tisdell, 2009a).

Social costs will exceed private costs of production by businesses if the aquaculture industry results in unfavourable environmental *spillovers* (externalities) that impose costs on others for which they are not compensated. For example, consider shrimp farming in some less developed countries. For example, in Ecuador, Thailand, the Philippines and parts of Bangladesh, wetlands are impounded to create ponds for the cultivation of shrimp. Vegetation (such as mangrove trees) is lost and the breeding grounds and food supplies of wild fish stocks are destroyed, with an adverse impact on local fishing communities. The results of studies of the environmental economic losses associated with shrimp farming in Thailand are outlined in Barbier and Sathirathai (2004). When ponds are stocked with captured young shrimp, as in Bangladesh, this may subsequently reduce the population of large shrimp available to the capture shrimp fishery. Furthermore, by converting coastal areas that play an essential role in the life cycle of wild shrimp populations to private shrimp ponds, the aquaculture farms further reduce wild stocks. Eventually, there is a risk of the capture fishery collapsing altogether and supplies of seed shrimp from the wild drying up, as happened in Ecuador (Tisdell, 2005).

By contrast, aquaculture can sometimes give rise to favourable spillovers and, when this happens, the profits of fish farms understate the social economic benefits of their activity. The activity might then be on a smaller scale than is socially optimal. Waste from marine fish farms causes nutrient enrichment of surrounding waters. Up to some level, this may enhance the growth of surrounding wild fish or benefit mollusc production. But, beyond some point, this positive effect can become negative (section 4.2.1). Nevertheless, there are also circumstances in which nutrient depletion occurs and the consequences can be assessed by means of economic analysis (Tisdell, 2003).

The economic theory underlying this matter is illustrated in Fig. 12.8. Curve OAB represents the profit from farming a fish species, e.g. sea bass, in a region as a func-

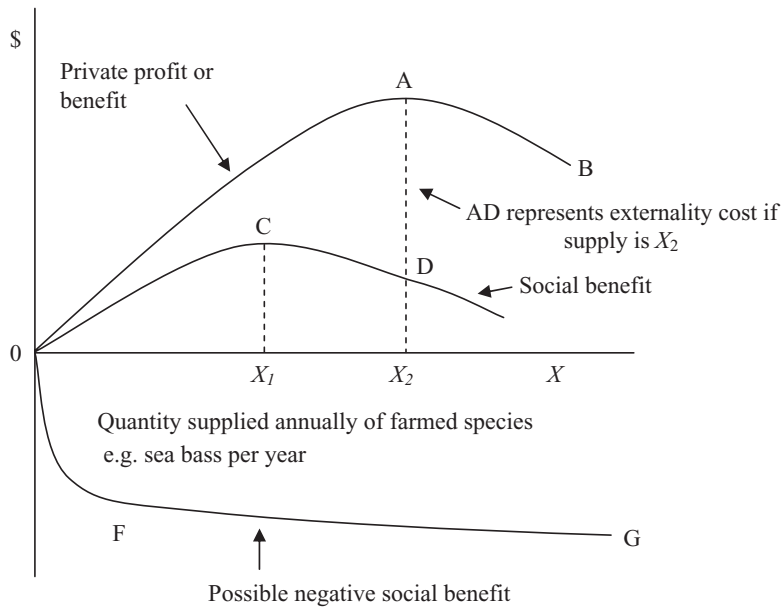


Fig 12.8 Environmental spillovers from fish farming sometimes result in private decisions being at odds with social economic benefits from these decisions (see text for explanation).

tion of the quantity produced annually. In this region, however, the farming of the species gives rise to negative environmental effects so the social benefit curve is OCD. This curve is lower than curve OAB and the difference represents environmental costs (curve OFG) not paid for by the sea bass farmers. In order to gain maximum profit, fish farms will produce X_2 of the farmed fish in the region annually. This is an excessive amount from a social economic viewpoint. Social net benefit is maximised when only X_1 of the species is produced each year. Therefore, because of the occurrence of adverse environmental effects, the market mechanism fails to ensure a social economic optimum. Hence, it may be desirable for the government to adopt policy measures to restrict production of this farmed species, or to limit the methods used to farm it.

The opposite situation can arise if the farming of a species generates favourable environmental effects. In Fig. 12.8, the environment curve OFG becomes positive, OCD becomes the profit curve and OAB is the social benefit curve.

It is also important to recognise that in some cases the adverse spillovers generated by the aquaculture of particular species can be so great that its culture should not be tolerated. For example, in Fig. 12.8, although curve OAB may represent the private benefit to producers from farming a species, the social benefit from doing so may be as shown by curve OFG. It is negative. For example, the introduction of a new species to a region can pose significant risks to wild species in the region. Escaped farmed species may compete with other wild species or become predators of them. The risks and potential costs to natural ecosystems of introduction of new species and attendant economic losses may be so great as to make it desirable from a social economic point of view to ban their introduction. Escapees from aquaculture potentially pose several types of environmental risk (Bridger and Garber, 2002).

Different methods or techniques of aquaculture can give rise to different magnitudes of external costs. It may, therefore, be desirable to introduce public policies that limit the use of some techniques or ban these altogether. The regular feeding, for example, of antibiotics to farmed fish can give rise to a number of serious environmental consequences (section 4.4.3.1). These include the growing resistance of disease-creating organisms to antibiotics and the reduced natural resistance among the farmed stock, and possibly where there are escapees, reduced resistance of wild stock to diseases (Tisdell, 1993). Therefore, some governments may consider it to be desirable to ban the use of environmentally 'dangerous' antibiotics in aquaculture or to restrict their use.

Clearly, different methods of husbandry in aquaculture can have significantly different environmental consequences. Nevertheless, it is frequently the case that technological progress reduces the magnitude of environmental effects. For instance, between 1980 and 1997 the average feed conversion ratio in Norwegian salmon aquaculture fell from just under 3 to just over 1 (Asche *et al.*, 1999). This means that less waste per kilogram of fish produced goes into the surrounding environment. The food used nowadays, for example, sinks more slowly through the water, and improved techniques are available to monitor feeding so that the quantity of food supplied to the fish can be adjusted more accurately to consumption (Asche *et al.*, 1999; section 9.11.2) in addition, innovations have resulted in a substantial reduction in use of antibiotics by the Norwegian salmon industry (Asche *et al.*, 1999).

As pointed out by Tisdell (1999), adverse environmental spillovers are often the source of lack of sustainability in production and can result in an activity eventually becoming uneconomic. Although environmental spillovers are not the only source of lack of sustainability in economic production (Tisdell, 1999), they should not be overlooked as a potentially important source. Bardach (1997) gives particular attention to the sustainability of aquaculture, and Shang and Tisdell (1997) concentrate on the economic dimensions involved.

In addition, some forms of aquaculture raise questions about income distribution. Large-scale aquaculture, which displaces small farmers or adversely impacts on the incomes of poor fishing and subsistence communities, has an adverse income distribution effect. This has happened for shrimp aquaculture in some less developed countries, for example in Bangladesh (Alauddin and Tisdell, 1998). On the other hand, seaweed farming in Indonesia appears to have reduced rural income inequality, at least in some villages (Firdausy and Tisdell, 1991, 1993).

Some forms of crustacean culture raise additional sustainability issues (see Be *et al.*, 1999, for some examples) and, in essence, pose an inter-generational income equity problem. The practice has arisen in some parts of monsoonal Asia of alternating rice and shrimp/prawn production in low-lying estuarine areas, e.g. in the Sunderbans of Bangladesh. Just before the wet season, rice is planted. After the rice is harvested in the dry season, the fields may be flooded with brackishwater to create ponds for rearing shrimp or prawns (*Macrobrachium* species). These ponds are drained before the start of the next wet season and the animals are harvested. The land is then prepared for rice

and replanted. So the cycle continues. This, however, does not appear to be a sustainable practice. It results in falling rice yields in some areas due to rising soil salinity and mineralisation of the soil (Alauddin and Tisdell, 1998).

Various forms of aquaculture can result in a variety of environmental issues (Tisdell, 2003, 2004, 2007) including environmental health risks and biodiversity loss (Tisdell, 2009b). If, however, aquaculture has adverse environmental impacts, this does not mean that it should be banned from a socioeconomic perspective. Instead, policy measures, such as taxes on effluent, could be adopted to ensure that aquaculture businesses take their external costs into account in their decision-making. (A tax, for example, can result in the firm's private costs of production after tax being brought into line with its social cost.) When this is done, some aquaculture businesses will no longer be economic. Optimal economic policies to control environmental spillovers are outlined in Tisdell (2005, 2009a), but more attention needs to be given to these, specifically in relation to aquaculture. Economic theory indicates that it is not optimal, as a rule, to eliminate all environmental effects, but that government intervention to control them is sometimes justified.

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13
Seaweed and Microalgae

Seaweed: Nicholas A. Paul and C. K. Tseng[1]
Microalgae: Michael Borowitzka

13.1 GENERAL INTRODUCTION

Seaweed (macroalgae) and microalgae differ markedly in morphology and life cycles. Therefore the methods used for their culture and the purposes for which they are cultured are, for the most part, extremely different. Because of these attributes, the two kinds of cultured algae will be treated separately in this chapter.

13.2 SEAWEED

13.2.1 Introduction

13.2.1.1 World production

Seaweeds are cultured throughout the world. More than 25 countries contribute to over 20 commercial seaweeds with diverse and perhaps, for some, surprising uses (Table 13.2). Total world seaweed production from aquaculture was estimated to be 14.8 million t in 2007 (Table 13.1). Seaweed production from aquaculture has grown by more than ca. 8.5% per annum from 2000 to 2007, with a corresponding 6.6% yearly increase in value (Fig. 13.1). During the previous decade, production of seaweeds from fisheries remained static or decreased (Table 13.1), as did other production for most fisheries (section 1.3). This trend has continued into the new millennium, as the proportion of wild-harvest seaweeds continues to decline relative to aquaculture production.

Because seaweed production from fisheries constituted only 7% of global production in 2007 (Table 13.1), the seaweed sector is the most aquaculture-orientated of all marine and freshwater sectors. Continuing wild-harvest remained stable or declined from 2000 to 2007 (ca. −1% per annum (Table 13.2)). For example, the wild-harvest in 2007 of kelps in Chile (164 000 + 53 300 t), Norway (134 000 t) and Japan (72 000 t), rockweed (*Fucus vesiculosus*) from Ireland (28 000 t), and red seaweeds from Chile (77 000 t) together have declined 20% per annum since 2000, the offset coming from China's reported increase in fishery production (175 000–324 000 t). Some nations have noticeably ceased wild-harvest production altogether (including kelp harvest in the USA from 50 000 t per annum in the 1990s to 4 t in 2007). Reducing wild-harvest pressure is essential as some stocks have clearly been over-harvested, particularly the red seaweed *Gracilaria* from Chile, which had yields of >100 000 t per annum in the early 1980s followed by low annual yields (<10 000 t) for most of the 1990s (although these have stabilised in recent years, ca. 50 000 t).

Compared with other sectors, seaweed biomass constituted 23% of total world aquaculture production in 2007 (global 65 190 000 t), and slightly under 8% by value (global US$94 555 million, Chapter 1 and Fig. 1.2). This reflects a lower value per unit weight compared with most other classes of aquaculture product. Most raw seaweed produced is edible product, for which three major varieties averaged ca. US$900/t wet weight (US$650–1450 range) in 2004. This relatively low product value (compared with other aquaculture products) is offset by a predominantly extensive open aquaculture, with correspondingly low start-up and operating costs in comparison to other sectors. However, there has been an increase in relative value of seaweed products since the 1990s. This is due to a shift towards the cultivation of more valuable red seaweeds for

[1]Professor Tseng contributed this section in the first edition of this textbook, but is now deceased.

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Table 13.1 World production (thousand t) of seaweeds from aquaculture and fisheries and its value (thousand US$) in 1990, 1999 and 2007. Growth of sector is indicated by percentage change per year from 1990 to 2007.

	1990	1999	2007	Change/year (%)
Brown seaweeds	2521	4776	6536	9
Value (thousand US$)	1798	2922	3763	6
Red seaweeds	994	1880	5894	29
Value (thousand US$)	979	1338	2595	10
Green seaweeds	30	29	17	–3
Value (thousand US$)	19	4	9	–3
Total aquaculture production[a]	3752	8826	14784	17
Value (thousand US$)	2947	5278	7505	9
Total fisheries production[b]	1321	1201	1103	–1
Proportion of aquaculture production (%)	74	88	93	2

Data from FAO Fishstat Plus (2009). Average annual change represents growth or decline over 17 years from 1990.

[a]Note that total production (aquaculture and fisheries) includes unidentified aquatic plants not elsewhere included in the convention of FAO reporting. Most unidentified plants from aquaculture production are seaweeds from China (2.2 million t).

[b]Fishery production includes only marine and brackish water species, without freshwater or microalgal production.

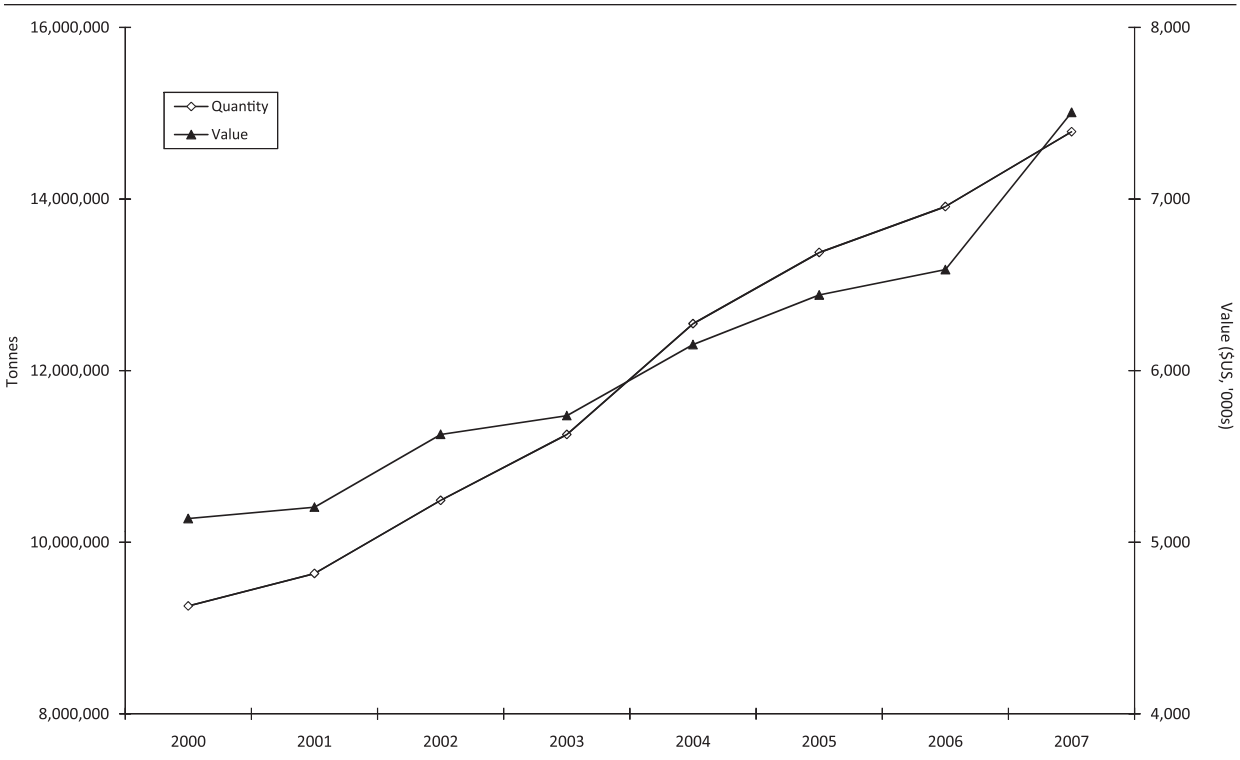


Fig. 13.1 World production of seaweeds from aquaculture and its value in millions of US$.

Table 13.2 Some uses and products of seaweeds. The gelling properties of processed material from seaweeds are used as food additives (for dairy products, sweets, processed meats and alcohol brewing) and in a variety of industrial and commercial applications (e.g. in paints, adhesives, bacterial agar, shampoo, toothpaste). Seaweed genera are listed alphabetically, not in order of importance. Seaweed taxonomic groupings are as follows: G, green; R, red; B, brown.

Use/product	Seaweed
Dried for human consumption	*Enteromorpha* (G), *Eucheuma* (R), *Fucus* (B), *Gelidium* (R), *Gracilaria* (R), *Saccharina (= Laminaria)* (B), *Porphyra* (R), *Sargassum* (B), *Undaria* (B)
Fresh for human consumption	*Caulerpa* (G), *Gracilaria* (R), *Porphyra* (R), *Ulva* (G)
Medicinal uses	*Ascophyllum* (B, muscle-related problems), *Asparagopsis* (R, antifungal, antibacterial), *Caulerpa* (G, hypertension, antifungal), *Chondrus* (R, blood anticoagulants), *Dictyota* (B, antifungal, antibacterial), *Saccharina* (B, iodine deficiency), *Sargassum* and *Ulva* (vermicides), *Undaria* (B, neutraceuticals)
Fertilisers or soil additives	*Ascophyllum* (B), *Fucus* (B), *Macrocystis* (B), *Sargassum* (B), *Saccharina* (B)
Production of gelling 'hydrocolloids'	
Agar production	*Gelidium* (R), *Gracilaria* (R)
Carrageenan production	*Chondrus* (R), *Eucheuma* (R), *Kappaphycus* (R)
Alginate production	*Ascophyllum* (B), *Fucus* (B), *Saccharina* (B), *Macrocystis* (B), *Sargassum* (B)

Table 13.3 Recent changes in total value of processed seaweed products and the relative value of the unprocessed material (wet weight). Data also include wild harvest fisheries. Pharmaceuticals include all processed use of isolated chemicals for human applications (for example, drugs, cosmetics and neutraceuticals). Data from Chopin and Sawhney (2009) and FAO Fishstat Plus (2009). The percentage annual change is approximate, calculated for 10-year periods.

Product	Value mid-1990s (US$ × 10^6)	Value mid-2000s (US$ × 10^6)	Annual change (~% per year)	Relative value of raw material (US$/t)		Annual change (~% per year)
				Mid-1990s	Mid-2000s	
Carrageenan	240	300	2.5	600	568	–0.5
Alginate	211	213	0.1	459	355	–2.3
Agar	132	137	0.4	1056	1077	0.2
Soil additives	10	30	20	18	27	5
Fertilisers	5	10	10	500	500	0
Seaweed meal	5	10	10	100	100	0
Pharmaceuticals		3	[a]		1000	[a]
Totals	603	703	1.7	2733	3628	3.3

[a]No change recorded new application or product.

hydrocolloid production (e.g. agar and carrageenan, Table 13.3). Values of the processed products of agar and carrageenan are US$18 000/t and US$ 9000/t, respectively (data from 2004, Chopin and Sawhney, 2009). New seaweed ventures should continue to occupy niches in the supply of high-value fresh seafood (in contrast to the focus on dried edible seaweeds in the previous millennium), functional foods and neutraceuticals, and in the isolation of seaweed natural products for cosmaceutical and pharmaceutical applications (Table 13.3).

Brown seaweeds (Phaeophyceae) are the major group of cultured seaweeds, constituting more than half of total production in 2007 (Table 13.1, 53%; this dominance has decreased from 71% in 1999; however, brown seaweeds comprise a significant portion of uncategorised biomass 'Aquatic plants not included elsewhere': >2 000 000 t from China). The most widely cultured species of brown seaweed is the Japanese kelp *Saccharina* (previously *Laminaria*) *japonica* ('kombu') at 4.6 million t, which made up >70% of the total cultured brown algae in 2007. Dried Japanese kelp (dried is ca. one-fifth of fresh or wet weight) is used directly as food but also is extracted for alginate, mannitol and iodine. *Undaria pinnatifida* ('wakame') accounted for most of the remaining production (1.8 million t in 2007). Reflecting the tradition of seaweed culture in Asia, by far the largest producer of brown algae (kombu, wakame and *Sargassum* spp.) is China, with ca. 9.7 million t or 65% of world brown algae production in 2007. The Republic of Korea (9% of brown seaweed production), Democratic People's Republic of Korea (7%) and, to a lesser extent, Japan (1.5%) comprise the middle tier of production, with minor contributions from other nations (e.g. Russia, France and Spain).

Production of red seaweeds (Rhodophyta) made up almost 50% of reported world seaweed production in 2007, representing a substantial increase from ca. 19% in 1999 (Table 13.3). This shift from brown seaweed dominance to red seaweed equality has been driven by meteoric increases in production from the tropical nations of Indonesia, to 2 million t in 2007, and the Philippines, to 1.5 million t in 2007 (equating to 90% and 15% per annum since 1999, respectively). The major species in culture are hydrocolloid-producing algae, specifically *Eucheuma*, *Kappaphycus* and *Gracilaria* species. Hydrocolloids ('phycocolloids') refer to emulsifying chemicals (or gums) including agar and carrageenan harvested from red seaweed, and to alginate extracted from brown seaweeds (Table 13.3). Red seaweed hydrocolloids provide structural support to the cells, through the variety of cross-links provided by components of these complex sugars. The main types of sugar ('galactans') involved are iota-carrageenan, kappa-carrageenan, and agar-agar. The binding strengths (relating to product quality/end use) and the degree of processing determine price, but traders can expect to receive around US$0.50/kg dried raw seaweed or US$12/kg for refined carrageenan. Short-term prices fluctuate substantially in response to supply chain issues and demand, but continue to rise. Before the colloid boom, 'laver' or 'nori' (*Porphyra* species used in sushi) was the main type of red seaweed under cultivation. Nori production is still significant at 1.5 million t in 2007 (US$1500 million), predominantly (>70%) from China with contributions from Japan and the Republic of Korea. Most developing nations produce red seaweeds (predominantly *Eucheuma* and *Kappaphycus*) owing to the ease of cultivation (see section 13.2.4) and the notable absence of traditional kelps and nori from tropical waters (Fig. 13.2).



Fig. 13.2 Aquaculture production of seaweeds for carrageenan in the Philippines. (Photograph by Melba Reantaso.)

Green seaweeds (Chlorophyta) comprise <0.1% of total production and are the only group of seaweeds for which production has declined since 1990 (Table 13.1). Some green seaweeds (including *Ulva* species, sea lettuce and *Monostroma*) are used as dried-food additives, and some more valuable species have small niche markets (e.g. *Caulerpa lentillifera*, known as sea grapes, are cultivated in the Philippines and the Japanese island of Okinawa). However, global production databases (e.g. FAO Fishstat+) may not necessarily provide the whole picture. For instance, *Ulva* is an exceptional seaweed for integrated (animal–plant) aquaculture (see section 13.2.4) based on its growth rates, nitrogen assimilation, life cycle and ease of culture. In South Africa, the culture of *Ulva* for abalone feed is more than 1000 t/year and part of this production would fall under unidentified aquatic plants, which totalled 2900 t in 2007. These types of integrated system are increasing and may lead to more widespread production of green seaweed as feedstock for marine herbivores in a similar manner to that in which microalgae are essential as live feeds in aquaculture nutrition.

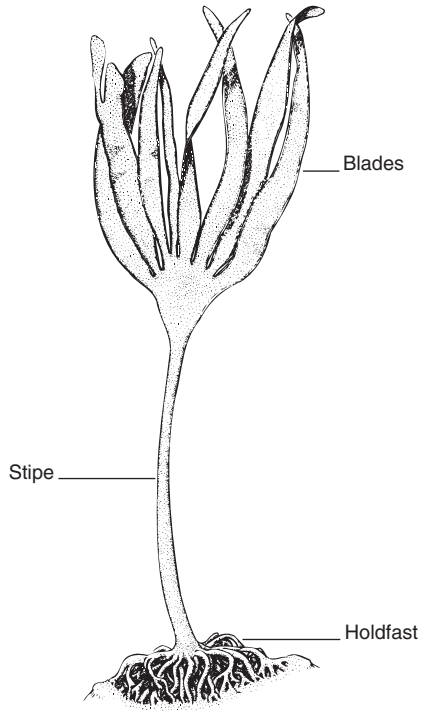


Fig. 13.3 Kelp (Phaeophyceae) showing holdfast, stipe and blades. Reproduced from Rost *et al.* (1979) with permission from John Wiley & Sons.

13.2.1.2 Morphology and habitats

Seaweeds come in a diverse array of shapes (from filaments to broad sheet-like structures) and sizes (encrusting turfs to >20 m-high stands of *Macrocystis*). The basic morphology of kelps (such as kombu and wakame) includes a discrete attachment organ or holdfast, with a stem-like stipe and a leaf-like blade (Fig. 13.3). The thallus (whole plant) is often differentiated into branches arising from the stipe, leaf-like fronds and flotation structures. Cultivated red seaweeds (e.g. *Eucheuma*, *Kappaphycus* and *Gracilaria*) are less differentiated than kelps. These seaweeds are propogated vegetatively from thallus fragments and are physically secured to ropes. Other seaweeds have little obvious differentiation, comprising only leaf-like thalli with microscopic holdfasts (e.g. *Porphyra* and *Ulva*, one- and two-cell layers thick, respectively). Seaweeds are generally benthic in nature and are restricted to solid substrata such as rock; however, many seaweeds will survive free-floating. There are also some fundamental differences to higher plants, the most obvious being limited or no internal transfer of materials within individuals. This means that seaweeds absorb nutrients over their entire surface.

In many ways seaweeds are excellent organisms for aquaculture. Seaweeds are robust and often inhabit the intertidal zone where they are exposed to the air at low tide and subject to desiccation, and deal with daily or seasonal fluctuations in temperature and salinity. The two fundamental constraints for seaweed physiology, and therefore productivity, are light and nutrients. Seaweeds require light for photosynthesis. Light quality (spectrum) and the amount of light changes with depth, and seaweeds adapt through changes in the amount and types of both primary (chlorophyll) and accessory pigments. Nutrients (especially nitrogen and phosphorus) are at times limiting in nature, and to cope with fluctuation some seaweeds store nutrients opportunistically (known as 'luxury' uptake). For practical reasons the shallow subtidal and intertidal regions support most seaweed-farming activity, and farms situated close to anthropogenic sources of nutrients will benefit from these free resources.

13.2.2 Reproduction and life cycles

The successful adaptation of seaweeds to aquaculture requires an understanding of their reproductive strategies, which can be rather complex. In brief, reproduction may be asexual (vegetative fragmentation) or sexual, and all species have a life cycle characterised by alternation of generations (diploid ↔ haploid). These species have a diploid ($2n$) spore-producing stage and a haploid ($1n$) gametophyte stage. The gametophytes can be dioceous (separate sexes) or monoecious (both male and female on the one individual). To add another level of complexity, the different stages may be morphologically different (heteromorphic alternation of generations) or may be identical but with different ploidy (isomorphic alternation). For the latter it is important to know which stage it is as it is likely that the stages have different cues for reproduction. The reproductive structures can be identified by compound microscopy. For most seaweeds reproduction is controlled by environmental factors such as water temperature, day length and light quality. Some species have winter peaks in biomass, others in summer (in most cases peak biomass correlates with reproduction). A greater understanding and control of the environmental influence on reproduction is essential to refining seaweed cultivation, in a similar way that closed life cycles and photothermal control of reproduction in finfish fulfils year-round production.

The following sections describe the reproduction and the life cycles of three important culture genera, *Saccharina*

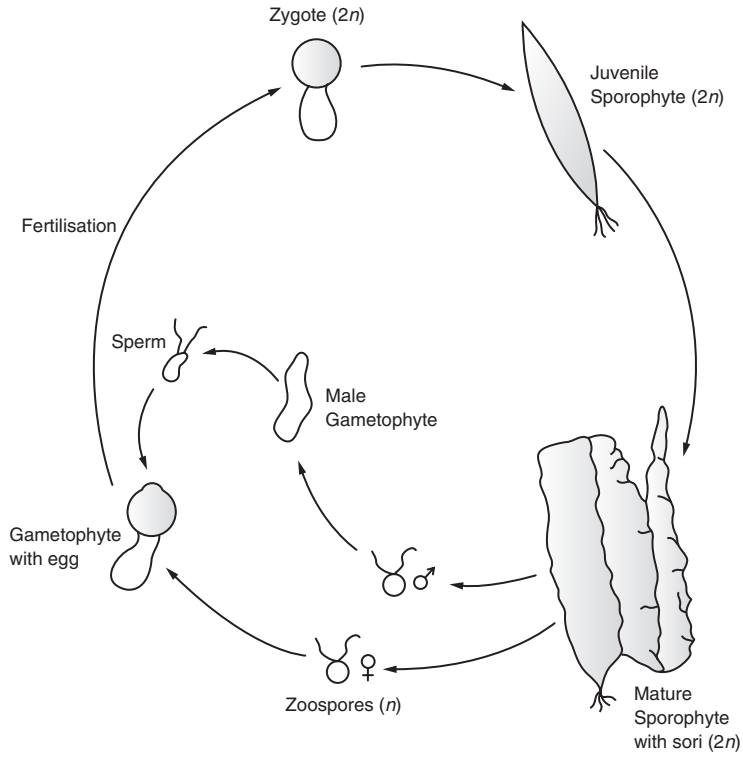


Fig. 13.4 Generalised life cycle of kelp (including *Saccharina japonica*).

(= Laminaria), *Porphyra* and *Ulva*, representing the three major culture groups, brown, red and green seaweeds, respectively. The predominant culture technique for *Eucheuma*, *Kappaphycus* and *Gracilaria* is asexual (vegetative) fragmentation, even though sexual reproduction and alternation of generations is possible. Vegetative techniques for these red algae are described in section 13.2.4.

13.2.2.1 Reproduction in kelp

The mature diploid (2*n*) sporophyte of kelp bears specialised reproductive organs, called sori, which undergo meiotic cell division to produce haploid (*n*) zoospores (Fig. 13.4). The motile zoospores settle on suitable substrata and develop into microscopic male or female filamentous gametophytes. Mature female gametophytes release eggs that secrete chemicals to stimulate the release of sperm from the male gametophyte. Motile sperm cells are attracted to the eggs chemotactically and the diploid zygote resulting from fertilisation develops into a mature sporophyte. In many species of brown seaweed, gamete production is seasonal and in temperate environments the deployment of juvenile sporophytes are timed to coincide with optimal growth conditions in warmer months.

13.2.2.2 Reproduction in Porphyra

The life history of most red algae (Fig. 13.5) is more complex than brown seaweeds as it involves an extra stage in the life cycle (the carposporophyte). Male and female gametes develop within the vegetative cells of the haploid (*n*) gametophyte stage. Spermatangia are formed from mitotic divisions in the protoplast of the male gametophyte, and the female gametes are formed as carpogonium cells on the female gametophyte. Male gametes are non-motile (non-flagellated), meaning that red seaweeds have to rely on wave and current action to carry the sperm to the carpogonium. After fertilisation the diploid cell grows into a distinct stage. This zygote or carposporophyte is actually parasitic in the female gametophyte. In other red algae it is contained within a prominent structure known as the cystocarp. The function of this distinct stage is to multiply the genetic material from a single fertilisation event into numerous carpospores (2*n*). The carposporohyte

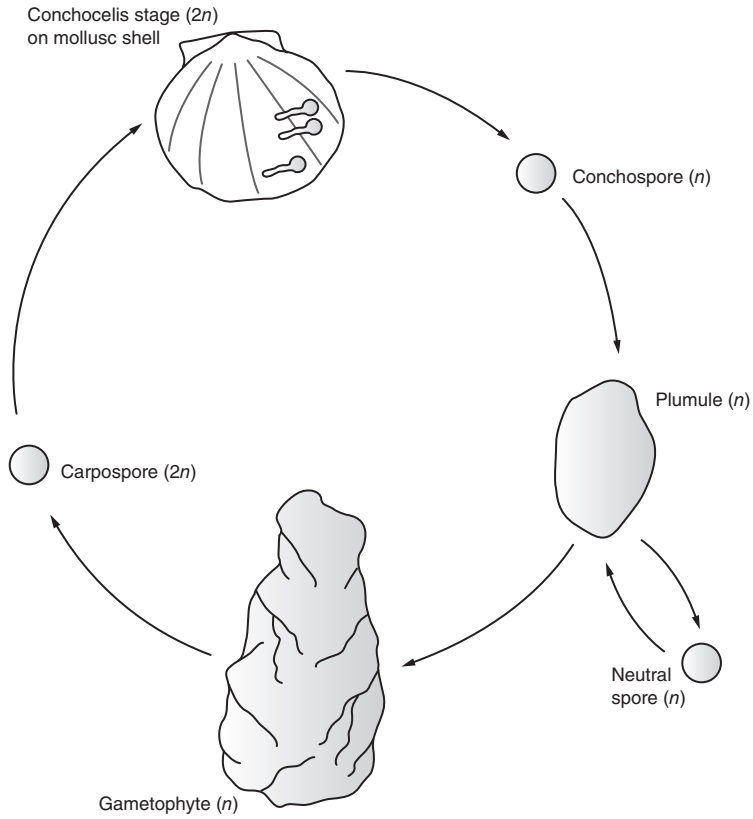


Fig. 13.5 Generalised life cycle of *Porphyra* species. The plumule is the early form of the haploid before maturing as a gametophyte. The plumule can produce neutral spores before gametophyte maturation.

releases carpospores and they settle onto appropriate substrata (e.g. scallop shells for *Porphyra*) to give rise to many minute filamentous sporophytes (2*n*), each known as a conchocelis for *Porphyra*. The sporophytes release asexually generated spores (*n*) (called conchospores in *Porphyra*). The spores settle and grow into new gametophytes (= edible stage of *Porphyra*) which, in time, mature and close the life cycle. In some species of Rhodophyta, including *Porphyra* species, neutral spores (or monospores, produced by mitosis rather than meiosis) are released from the gametophyte at certain times of the year, creating additional harvests from a single fertilisation event.

The culture of *Porphyra* initially involves seeding carpospores onto mollusc (e.g. oyster) shells. 'Seeding' of the cultivation nets with conchospores (produced meiotically by the conchocelis) is achieved by placing the nets in cultivation tanks containing the conchocelis-infested shells. The water is agitated in the tanks to enhance adhesion of the non-motile conchospores to the nets. These nets can be set in the ocean or even frozen to ensure that deployment occurs under optimal conditions. The conchospores develop into the macroscopic gametophyte generation, which is harvested for consumption.

13.2.2.3 Reproduction in Ulva

The life histories of the Chlorophyta are diverse. Some species, including *Ulva*, undergo an alternation of isomorphic generations. That is, the gametophyte and sporophyte are indistinguishable except for their microscopic reproductive structures. Their diploid (2*n*) sporophyte stage produces haploid zoospores by meiosis from the sporangia of the thallus. These spores germinate into male or female haploid gametophytes. Flagellate gametangia produced from the parent gametophyte are released, and, upon fusion of opposing gametes, a diploid zygote forms that develops into the mature sporophyte.

13.2.3 Characteristics of seaweed culture

13.2.3.1 Seaweed culture distinguished from agriculture

There are some important characteristics that distinguish seaweed culture from agriculture.

1. Macroalgae do not need a special absorption organ, as nutrients in solution can be absorbed by any part of the plant. The holdfast is sufficient to anchor the algae in place. However, the ocean is in constant motion and, as the sea level varies with tide, the amount of light reaching algae on fixed substrates varies. Use of floating rafts, where the depth of cultivation is constant, addresses this problem; raft-based culture also allows the culture depth to be changed as required. Similarly, tank-based culture using suspended ('tumbling') biomass allows light to be maintained at an optimal level through altering stock density and light penetration.
2. Seaweeds reproduce using spores that are released into open water. The spores cannot be kept alive for a long time after discharge and suitable substrata must be provided close to the parent.
3. Most cultivated seaweeds are attached to substrata. In general, seaweeds grow naturally on hard, stable substrata that are able to withstand high wave action and receive adequate light. In the early days of seaweed culture, rocks and stones were regarded as excellent substrates for cultivation. However, their limited surface area, large size and immobility, as well as the need to employ divers for harvesting the seaweeds, meant that alternatives were sought. For example, the Japanese kelp (*Saccharina* (= *Laminaria*) *japonica*) was introduced to China in the 1920s; however, the coastline lacks suitable natural substrata. Large-scale production was possible using floating rafts (Tseng, 1981), and Chinese production of this species is now many times greater than production in Japan. Now land-based seaweed aquaculture (predominantly of red and green seaweeds) is exploring free-living cultivation of selected seaweeds that do not need substrata.
4. Unlike the application of fertiliser to land plants through moisture in the soil, the application of fertiliser to seaweeds is very difficult given the dynamic nature of the ocean. Fertiliser applied to seaweed in the ocean will rapidly disperse, making seaweed culture too costly and creating the potential to pollute the coastal environment. Porous containers for fertiliser application have been developed in China, where losses of fertiliser to the open sea are minimised. For large areas cultivating large quick-growing seaweeds, such as the Japanese kelp, the required fertiliser (e.g. ammonium nitrate) can be sprayed onto the seaweed at low tide. Land-based operations, however, allow tight control of the nutrient concentrations available to seaweeds.

13.2.3.2 Problems in common with agriculture

There are also several problems in common with agriculture. Temperature is one of the most important factors in seaweed culture. Optimal and minimum temperatures for growth and development may differ, sometimes even within the same species depending on the phase of the life history and growth stage. For example, the sporophyte and gametophyte of nori *Porphyra tenera* have different optimal water temperatures. Successful culture requires information about the optimal culture requirements of all stages of seaweed development and growth, particularly for managing reproduction (including halting production).

Like land-based agriculture, seaweed culture also has a problem from weeds or nuisance algae. In kelp culture in China, for example, ropes covered with spores are placed in the ocean for grow-out in October. Spores of nuisance algae such as species of *Ectocarpus* and *Ulva* may quickly adhere to ropes and germinate, overgrowing spores and juvenile gametophytes. Therefore gametophytes are not exposed to light until December, only after the nuisance alga matures and degrades. However, this can delay development for about 2 months (Fig. 13.4). Several methods are used to combat nuisance algae. These include the collection of spores in early summer (section 13.2.4.5 'tank culture') and the cultivation of spore-covered ropes in glasshouses with artificially cooled filtered seawater. The young sporelings (called 'summer sporelings') can then be put to sea in autumn, when they are already a few centimetres tall. At this size they can out-compete nuisance algae.

The nuisance problem in the cultivation of red algae, *Porphyra* species, is more complicated. The predominant weed algae are species of *Monostroma*, *Ulva*, *Urospora* and *Licmorphora*. Some aspects of fouling by nuisance algae can be controlled. For example, in the seeding process, the attached conchospores are packed densely so that there is very little space for the nuisance spores to attach. At harvesting, large thalli are selectively collected but small thalli are left intact to limit the availability of space to nuisance algae. Another common way to control the nuisance algae is to expose the nets to direct sunlight. Nuisance algae are generally more susceptible to desiccation than *Porphyra*, and with the correct amount of exposure it can be killed, leaving the *Porphyra* intact.

13.2.4 Culture methods

Commercial cultivation of seaweeds has evolved from minor intervention to tank-based cultivation for either part or the entirety of culture. Successful commercial cultivation depends on good culture techniques that may differ to some extent according to location. For example, in the 1950s when red algae cultivation began in China, established Japanese techniques were unsuccessful because of the much larger tidal range in China. Techniques were successfully adapted to a semi-floating method of cultivation, which gave much better results than the traditional Japanese method.

There are now five major types of seaweed cultivation:

1. Natural substrate culture.
2. Long-line culture.
3. Net culture.
4. Pond culture.
5. Tank culture.

13.2.4.1 Natural substratum culture

This primitive type of phycoculture was developed several hundred years ago in the southern part of Fujian Province, China. It was first used to enhance the production of the glueweed, *Gloiopeltis furcata*. Natural rock substrates are cleaned by scraping, just before the growing season of the glueweed. The loosened material is carried away by tides and, on the following day, lime is sprayed on cleaned rocks to destroy other seaweeds and barnacles. The rocks provide natural substrata for settlement of glueweed spores, which germinate and grow to maturity before being harvested. A similar method has been used in the production of purple laver/nori (*Porphyra* species) for which, traditionally, seaweed farmers depended on nature for the 'seeds' of *Porphyra*. These days, farmers spray seawater containing conchospores over the cleaned rocks.

In the traditional method of cultivating kelps (*Saccharina* and *Undaria* species), farmers simply throw selected stones into subtidal areas when and where the zoospores of these kelps are released. The stones provide substrate for the zoospores to settle and germinate. This method was widely used to propagate kelps in the 1930s and 1940s in China and is still used today in some areas of Japan and China.

13.2.4.2 Long-line culture

Long-line culture methods (Fig. 13.6) are the usual method for commercial cultivation of kelps (*Saccharina* and *Undaria* species) in China but have also been extensively applied to shallow water cultivation of red seaweeds (*Eucheuma*, *Kappaphycus* and *Gracilaria*) in tropical regions. This type of culture technique is responsible for more than 90% of global seaweed production. Typical productivities for annual production of *Saccharina* (= *Laminaria*) *japonica* on long-lines are between 80 and 120 t/ha (Fei, 2004).

The long-line for kelp cultivation is composed of a 30- to 60-m-long synthetic fibre rope, secured by two anchor ropes. The long-line is supported by several buoys (15–20 cm in diameter). Attached to the long-line is a series of hanging ropes (or 'droppers') to which the cultivation

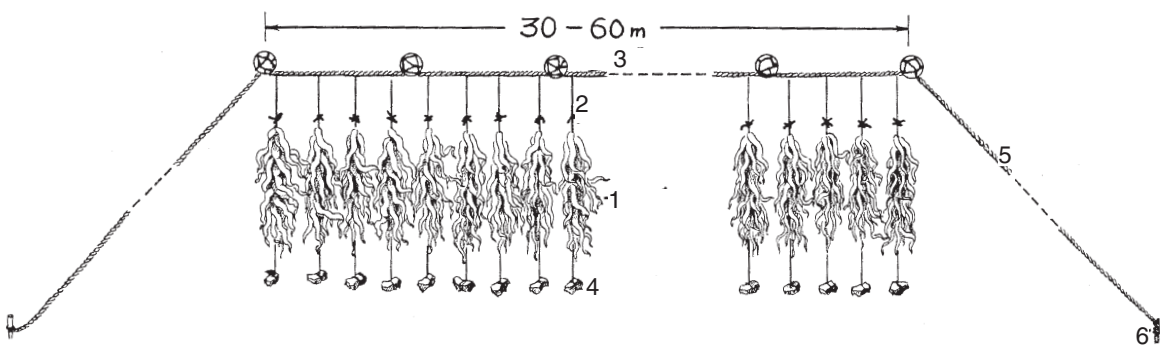


Fig. 13.6 Long-line cultivating kelp (*Saccharina japonica*) in China. Note that each cultivation rope bearing kelp (1) is attached to a hanging rope (2), which is attached to the long-line (3), and its lower end is tied to a weight (4). The anchor ropes (5) are twice as long as the depth under the long-line and they are anchored to the sea bottom using wooden stakes (6). Reproduced from Tseng (1981), with permission of Blackwell Publishing Ltd.

ropes (each about 1.2 m long, seeded with kelp) are attached with a small stone weight on the end. The distance between two adjacent cultivation ropes is 70–140 cm. Each cultivation rope holds about 30 plants, and the distance between two adjacent long-lines is about 6–7 m. This means that between 150 000 and 300 000 kelp plants can be cultivated in 1 ha, corresponding to an areal density of 15–30 individuals/m.

During cultivation there is a difference in growth rate between the upper and lower plants on the same rope. To counteract this, cultivation ropes are regularly inverted. The difference in growth rates can also be minimised by tying adjacent ropes together so that they become oriented more horizontally. Using these methods, the production level and product quality are greatly improved (Tseng, 1981, 1986).

Several other kinds of seaweed are cultivated by the long-line method, including seaweeds that propagate asexually through fragments of thalli. These include *Eucheuma* and *Kappaphycus* species, which are cultured vegetatively by attaching pieces to a long-line which may be suspended (using floats) or attached to supports driven into the substrate. Seedstock are selected (thick and sturdy portions without epiphytes) and generally tied to the long-line with a ribbon. This method is labour intensive and may result in loss of seaweed as a result of improper ties (Zertuche-Gonzalez *et al.*, 1999). However, it is the main process in many developing tropical nations where labour remains relatively cheap. In these nations similar problems may occur with nuisance algae as well as herbivores present on the reef flats. These issues can be managed through early harvesting (before known seasons of intense herbivory) or immediately subsequent to the identification of nuisance algae attached to thalli. Any 'fouled' thalli should not be used as seedstock, but can still be suitable for processing into industrial hydrocolloids (section 13.2.7).

13.2.4.3 Net culture

Open water long-line systems are primarily to cultivate large brown algae with zoospores and red seaweed from fragments, but net raft culture is better suited for the culture of small and medium-sized red algae with non-motile spores. The most established seaweed cultivated by the net raft method is purple laver/nori (*Porphyra* species). Annual productivities in China for *Porphyra* using net culture are 30–60 t/ha (Fei 2004).

Nets are first seeded in tanks containing the shells with the conchocelis (section 13.2.2 and Fig. 13.7). The tank cultures are monitored to determine the progress of conchospore formation from the shells. When the number of spores discharged reaches approximately 50 000 spores per shell per day, preparations are made for seeding the nets.

Light intensity on the surface of the tank is increased and the water is agitated. When spore discharge reaches 100 000 spores per shell per day, formal seeding begins and net rafts are placed in the tanks. The greatest discharge of spores typically occurs mid-morning, generating a minimum density of 3–5 spores/mm^2. The net rafts are then taken to the field for cultivation.

There are three variations to net cultivation from fixed height in shallow water, to semi-floating and free-floating methods. In the first, nets are fastened to pillars (vertical supports) at a defined level within the tidal range (Fig. 13.8). The semi-floating method is particularly good for cultivation of intertidal seaweed, as at high tide the net floats on the water, maximising the light available to the seaweed. Sporelings appear earlier and grow better in semi-floating systems, sometimes doubling production compared with fixed-height methods. The floating net method is used for production of purple laver/nori (*Porphyra* species) in deep-water subtidal areas. The floating nets, made of synthetic fibres, are 60 m long and 180 cm broad. They have long anchor ropes so that nets maintain their position on the ocean surface regardless of tidal height, which is an important aspect to the culture of the niche product purple nori, providing consistent conditions to sustain its unique colour. This principle is similar to the long-lines used in the cultivation of kelps (Fig. 13.6).

13.2.4.4 Pond culture

There are some seaweeds, such as *Gracilaria tenuistipitata* var. *liui*, that grow well in still ponds. In many instances seaweed cultivation in ponds has been opportunistic in using areas previously used for other aquaculture species. Sometimes seaweed may be cultivated between harvests in a similar way that agricultural cropping areas lay fallow. However, ponds are not optimal conditions for cultivation based on low water motion and only certain species thrive in these conditions (e.g. nuisance species of green tide algae).

Pond cultivation of *G. tenuistipitata* var. *liui* in Taiwan yielded, on average, 9 t of *Gracilaria* and 6.3 t/ha of grass shrimp and crab (Shang, 1976). *Gracilaria* grows most rapidly in waters of about 25‰ salinity and at a temperature of 20–25 °C. Water depth is managed to provide optimal light throughout the year, shallower 20–30 cm deep during spring to early summer (March–June) and deeper 60–80 cm deep later in summer with peak irradiance. Fragments of *Gracilaria* are seeded in spring (April)

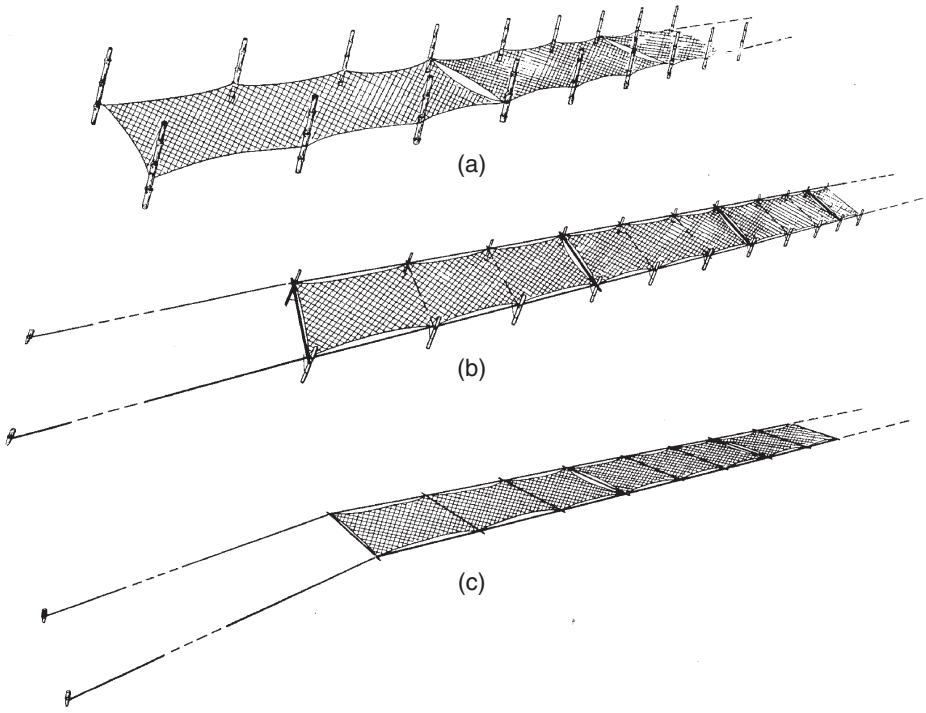


Fig. 13.7 Three methods of net raft seaweed culture practised in China for *Porphyra*. (a) Fixed type of the pillar method; (b) semi floating method; and (c) floating method. Note the short legs of the semi-floating nets. Reproduced from Tseng (1981), with permission of Blackwell Publishing Ltd.



Fig. 13.8. Seaweed aquaculture in Zanzibar at a fishing village, Uroa. (Photograph by Moongateclimber.)

at a density of 5000 kg/ha and strewn evenly. Harvesting by hand or scoop net takes place every 10 days during summer and autumn (June–November) to maintain optimal stocking density and growth. Harvested plants are washed and sun-dried, before processing into agar.

13.2.4.5 Tank culture

Tank culture can be divided into two methods: partial and complete culture. Indoor tanks are often used to cultivate juvenile seaweeds, especially those with a biphasic life history in which one phase is microscopic, for example the gametophytic phase of kelps and the conchocelis phase of *Porphyra* (section 13.2.2). These juveniles are then seeded into the ocean. However, if the seaweed is a valuable product (e.g. for pharmaceutical or premium food products), returns may justify the continued culture in tanks. Intensive tank systems have markedly different operational constraints to the extensive forms of cultivation that we have previously discussed.

13.2.4.5.1 Partial tank cultivation

In the commercial cultivation of kelp, juveniles are produced in tanks before seeding. The parent fronds with noticeably abundant sori (reproductive structures) are cleaned and hung in the air for several hours to induce artificially (by stress) the release of spores. When these fronds are placed in seawater, the pressure resulting from the quick absorption of water breaks the sporangial walls and liberates large masses of zoospores (*n*). Spore-collectors (frames with cords) are placed in the spore water and the actively swimming zoospores soon adhere to the collectors to complete the seeding process. The seeded frames may be kept in shallow indoor tanks containing seawater previously cooled to 8–10 °C and enriched with nutrients. The seeded frames remain in the cool house until autumn, when the juvenile sporophytes are about 1–2 cm high. At this time, when the ambient seawater temperature has dropped to about 20 °C, the juveniles are transferred to the farm. The production of juveniles can sometimes operate as a distinct commercial service, as in China where seed is sold to kelp farmers who cultivate these seed at their separate sites (Tseng, 1981).

The conchocelis or sporophyte phase of *Porphyra* is also microscopic and cultivated in indoor tanks in which culture nets are seeded (see section 13.2.4.3). The tanks vary considerably in size, but they are always shallow (20–30 cm deep) containing clean water (filtered and/or subject to sedimentation in the dark) to which nutrients are added. Light intensity is controlled by a series of screens to provide optimal growth for conchocelis, which varies as a function of the number of conchospores per unit area (see light equipment in section 13.2.4.5.2).

13.2.4.5.2 Complete tank cultivation

Several seaweeds (e.g. *Chondrus crispus* and species of *Enteromorpha*, *Gracilaria*, *Porphyra* and *Caulerpa*) are cultured for direct human consumption in land-based systems in indoor or outdoor tanks. Other high-value species of seaweeds, for example targets for neutraceutical or cosmaceutical applications, may also offset the high cost of tank aquaculture. Factors such as irradiance, pH, use of fertilisers and availability of carbon dioxide are of critical importance in such systems (Braud and Amat, 1996). However, these variables are interrelated and influenced by flow rates and stocking density. Pumping is continuous and flow is often high, with exchanges of at least two tank volumes per hour.

Areal (per m^2) productivity is paramount for land-based tank cultivation. Very high annual productivities (e.g. >100 g dry weight/day) are possible for some systems, when the appropriate balance of resources is supplied (see, for example, Mata *et al.*, 2010), but values of ca. 50 g dry weight/m^2/day are more realistic. Once the saturation of nutrients and carbon is ensured, light becomes the major driving force behind productivity and is thus the most important management consideration. Light changes substantially throughout the year in temperate areas and equipment such as light data loggers (measuring photosynthetically active radiation, 400- to 700-nm wavelengths) and pulse amplitude modulated (PAM) fluorescence can be used in tandem to ensure that optimal light conditions are maintain under changing conditions. The control over environmental conditions also allows tank aquaculture systems to influence competition strategically between nuisance species of algae. This can be done by maintaining a high stocking density (5 g fresh weight/L or >5 kg/m) and through pulse feeding of nutrients at night time.

13.2.4.6 Bioremediation, polyculture and integrated systems

Bioremediation in the context of aquaculture refers to the management of dissolved and particulate wastes from operation. In this respect, integrated aquaculture is based on the principle of waste utilisation to manage water quality and/or create additional products. Effluent water leaving fish farms contains high levels of nitrogen excreted by fish into the water. It has been estimated that 13% of the total nitrogen input to fish farms (primarily as protein in feed) is excreted as dissolved inorganic nitrogen. The environmental impacts of aquaculture effluents, such as

eutrophication, are discussed in section 4.2.1. Furthermore, the inadequate conversion of costly protein into fish biomass provides a financial incentive to limit effluent nutrients. One approach to minimising environmental impacts of aquaculture is to use seaweeds to remove dissolved nitrogen from aquaculture effluent (bioremediation). This has the added advantage of diversification (i.e. a second crop) through development of such systems (Chopin and Yarish, 1999). Polyculture is perhaps the crudest form of integrated aquaculture in which multiple species are cultured together (e.g. seaweed cultured in same tank as a herbivore).

Several studies propose that integrated culture of fish and seaweeds (e.g. *Saccharina* (= *Laminaria*) and *Porphyra*) can be successful in open water systems (e.g. Petrell and Alie, 1996; Troell *et al.*, 2009). The position of the seaweeds relative to the fish cages is important in determining the concentration of dissolved nitrogen available to the seaweeds, which, in turn, influences growth rates. However, there are also several practical considerations for an operation to overcome before engaging in integrated aquaculture. For example, there is the potential that mass production of seaweed required to ameliorate nutrients around cage fish culture may impede water currents, and could harbour unwanted pests of fish production (e.g. parasites). Furthermore, Petrell and Alie (1996) noted that technical and economic difficulties with fish/seaweed polyculture systems include the following:

- marketing and processing two different types of product;
- variable nutrient removal efficiencies by seaweeds;
- incompatible production rates of fish and seaweeds;
- logistical problems resulting from shared space and equipment.

Integrated aquaculture in land-based systems is more straightforward. It comprises defined (and often isolated) cultures of complementary species in a system. Seaweeds have been used to remove fish culture waste products in semi-closed aquaculture systems. For example, *U. lactuca* was reported to remove 74% of ammonia and reduce water use and nitrogenous pollution by half (Neori and Shpigel, 1999). A problem occurs as the area required to reduce nutrient levels of fish is much larger than that of the intensive production area (e.g. >100 m^2/t of fish). Essentially, fish farmers may find themselves becoming seaweed farmers with fish providing a source of nutrients. Increased productivity of seaweed production is essential to minimise culture area, but most important is the identification of value-adding seaweed that are themselves economically attractive. It should be investigated whether high nutrient waters alter the value of seaweed biomass compared with natural conditions (e.g. reducing the quality of phycocolloids).

One use of seaweeds produced by integrated aquaculture is feedstock for marine herbivores, and a system incorporating fish, seaweeds and abalone has been described by Neori and Shpigel (1999) (Fig. 13.9). In such systems, fish effluent drains back to the sea through seaweed 'biofilters' that treat the water and the resulting seaweed is used to feed abalone. A system with 1 t of fish and a seaweed pond area of 75 m^2 produced 4–5 kg of fish, 20–25 kg of seaweed and 2 kg of abalone per day (Neori and Shpigel, 1999). This same principle has been adopted by the abalone (*Haliotis midae*) growers in South Africa who culture *Ulva* (sea lettuce) to supplement diets comprising artificial feed and wild-harvest kelp (Bolton *et al.*, 2009). *Ulva* production in South Africa of >1000 t/year is the largest crop outside of Asia.

Additional research required to facilitate the uptake of integrated systems includes ensuring that seaweeds do not negatively alter growth conditions for the base unit cultures of fish. It remains to be demonstrated whether there are any positive (e.g. probiotic) effects for fish from integrated seaweed cultivation. Market research and education of the environmental and social benefits of these clearly sustainable systems will be important for the introduction and uptake of integrated aquaculture more broadly. Barrington *et al.* (2010) consider sustainability and social aspects of integrated multi-trophic aquaculture.

13.2.5 Diseases of cultured seaweeds

Because the mass cultivation of seaweeds is a relatively young and rapidly expanding industry, disease could potentially become the most important limiting factor for the domestication of seaweeds. Cultured seaweeds are affected both by physiological and pathological diseases. Most physiological diseases have environmental triggers which work to increase the susceptibility of biomass to disease. The following are examples:

1. Control of 'green rot' (caused by too little light) is achieved by inverting the cultivation ropes so that the lower, overshaded, fronds receive sufficient light. If the disease occurs during the fast-growing stage of kelp, tip-cutting may be used to increase light intensity. As much as one-third of the total length of the fronds may be cut off, greatly reducing the overcrowded condition and improving light penetration and frond health. The cut portion has a market in the alginate industry.

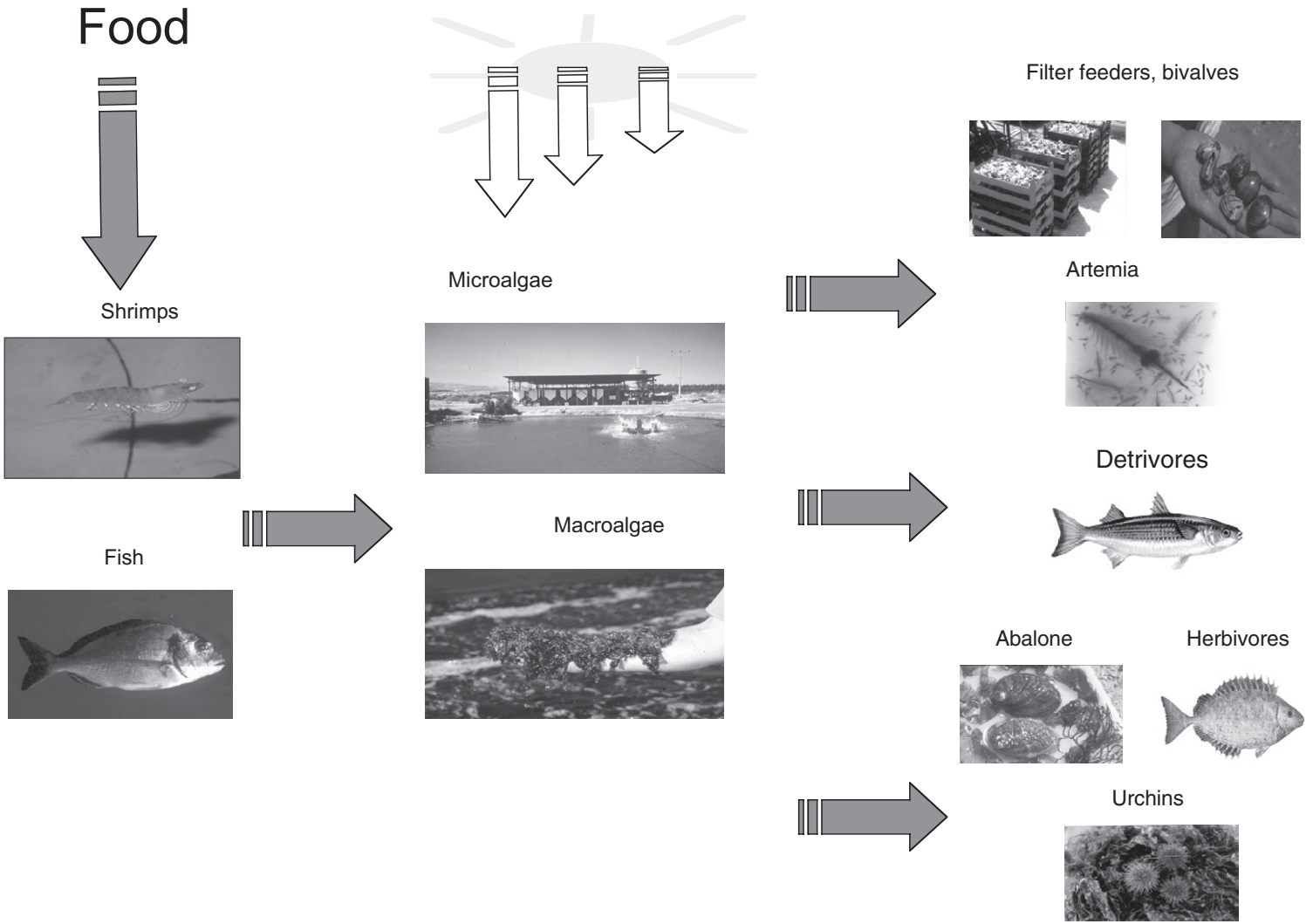


Fig. 13.9 Diagrammatic representation of a pilot mariculture system in Eilat, Israel, which uses effluent from fish/shrimp culture to provide nutrients for algae culture. In turn, algae are used as a food source for a variety of other culture animals. (Figure provided by Dr Muki Shpigel.)

2. 'White rot' disease always occurs in the fronds of the upper part of the cultivation ropes. It is believed that the three factors stimulating this condition are strong light, high water temperature and low nutrient levels. As the principal cause is believed to be strong light, treatment includes reduction of light intensity by lowering the level of cultivated algae in the water column. Fertiliser is also applied.
3. 'Ice-ice' is a common problem encountered in stressed individuals of *Eucheuma* and *Kappaphycus* (e.g. stressed from high temperature, low salinity, low light intensity). Tissue devoid of pigment is the first sign (creating an 'ice'-like appearance) and may be linked to stress from epiphyte attachment. Secondary problems stem from bacterial colonisation of these areas. Improved water quality (e.g. reducing stocking densities through harvest) can recover stock, but individuals often break at points weakened by the disease.

Several kinds of pathogenic disease have been recognised in seaweeds, but relatively few have been well documented (Correa, 1996). Bacterial disease may be more frequent in young or vulnerable parts of the cultivation cycle. For instance, the sulphate-reducing bacteria and hydrogen-sulphide-producing saprophytic bacteria, quite common in glasshouse cultivation for kelp sporelings, are causal agents in a disease characterised by plasmolysed oogonia and malformed sporophytes. Prevention measures include separating the sporeling cultivation system from the mature sporophytes and sterilising the water system with chlorine before the seeding process. Rotten and diseased fronds are periodically removed to reduce potential sources of infectious bacteria.

In 'frond-twist' disease of raft-cultivated kelp, the contagious and biotic nature of the disease was confirmed and the causal agent found to be a mycoplasma-like organism. Antibiotics such as tetracycline are effective treatments for this disease. Alginic-acid-decomposing bacteria were found to be the causal agent of a disease that causes detachment of summer sporelings. This condition can be effectively controlled using antibiotics.

In the absence of defined aetiology for diseases, some safeguards for reducing the incidence of disease include stock management (location and density), reduced pressures of productivity and maximising the genetic diversity of stock. It is likely that disease will become a particular problem for cultivation systems that rely on asexual fragmentation, as the genetic diversity of farmed stock may not be sufficient to deal with selective pressures resulting for high productivity. This means that the extraordinary production values for carrageenan-producing red seaweeds in Indonesia and the Philippines will be most susceptible. Further development of disease-resistant strains of seaweed will require more information on the mechanisms of pathogenicity and defence and on whether disease susceptibility and resistance are genetically determined traits (Correa, 1996).

13.2.6 Genetic aspects of seaweed culture

As with all farmed organisms, significant benefits can be gained through appropriate breeding programmes (Chapter 7). Research with seaweeds has sought to enhance characteristics such as yield and growth rates through genetic selection in the sexual phase of the life cycle. For example, in the 1960s and 1970s, superior strains of kelp were developed in China by intensive inbreeding and selection for specific characteristics, such as high productivity, high iodine content and increased thermal tolerance, which better met the demands of industry. Similarly, artificial seeding and strain preservation have facilitated the development of *Porphyra* cultivars, and molecular techniques have identified additional opportunities for selection through interspecific hybdridisation (Niwa *et al.*, 2009).

Whereas these developments were generally brought about through breeding programmes and strain selection, more recently major developments in this field have been brought about using modern genetic manipulation techniques or genetic engineering (e.g. Cheney, 1999). Examples of some of the modifications made to cultured red seaweeds using these techniques include increased tolerance to higher temperatures (e.g. *Chondrus crispus*, *Kappaphycus alvarezii*), increased agar or carrageenan content (e.g. *C. crispus*, *K. alvarezii*, *Gracilaria tikvahiae*) and increased growth rates (e.g. *K. alvarezii*, *Eucheuma denticulatum*, *Porphyra yezoensis*) (Cheney, 1999). Selection of unique colour morphs, particularly for red seaweeds, is possible owing to the diverse array of photosynthetic pigments in these organisms, as seen in the beautiful commercial cultivars of *Chondrus crispus* produced by Acadian Sea Plants Ltd. in Canada. Decreased productivity (e.g. growth) from cloned tissues is most pronounced where a limited number of strains or cultivars have been widely propagated for extensive periods of time. For this reason, maintaining genetic diversity as a safegauard against problematic diseases could be important, which means that those algae that are fragmented may need stock supplements or periodic control of sexual reproduction to avoid genetic bottlenecks.

13.2.7 Future developments

The high value of seaweed products and their increasing use in industrial processes, and as sources of food and neutraceuticals, will ensure continued expansion of the industry. Expansion within active regions will occur primarily from improvements to culture techniques and genetically selected stock. The previous millennium saw the development of large-scale open-ocean farming of seaweeds, particularly in China where automation of harvesting (using boats) has progressed for long-line culture. Mechanisation of other commercial stock will also increase areal productivity. Adoption of new and improved materials (e.g. plastic mesh tubing (polypropylene) versus traditional ropes as a long-line) will allow rapid seeding and harvesting of relatively large amounts of seaweed, reducing labour and cost (Zertuche-Gonzalez *et al.*, 1999). Sustained growth in production of traditional products (such as wakame and kombu) should be facilitated by the market diversification from dried food product into processed products for neutraceutical or pharmaceutical uses. For example, wakame (*Undaria pinnatifida*) is an excellent source of fucoidan, a sulphated polysaccharide, with antioxidant activity and cardiovascular health benefits (Boconegra *et al.*, 2009). High-value end-use relating to health and well-being could facilitate the adoption of intensive cultivation of edible seaweeds outside Asia.

Expansion of the industry will also result from the uptake of seaweed culture in countries without a tradition of cultivation. Because seaweeds can be cultured throughout the world (not only in Asia), we should expect increases in production beyond the 25 countries that, so far, contribute to global seaweed aquaculture. Mechanisation and the control and reliability of production are important to industry expansion into new areas. This will be in part from the uptake of traditional seaweed crops, such as in Mexico (Zertuche-Gonzales *et al.*, 1999), and in part from the development of culture techniques for new culture species. More than 30 nations harvest seaweeds, albeit some only as wrack (beached portions naturally torn from their substrata) yet half of these countries report harvests of <500 t/year. Aside from an environmental obligation to cease wild harvest of seaweeds, aquaculture can provide supplemental income from a new industry. Greater control over harvest quality and time of seeding, early growth and production all contribute to a reduced ecological footprint for culture practices.

The economic potential of seaweeds and their products is diverse. Although 66% of current production is edible species, most of the new opportunities relate to different seaweed products and applications. For example, seaweeds and their products can be used for fertiliser, paper, additives and toothpastes, and, potentially, a range of energy conversion from biomass (e.g. bioethanol through to biodiesel, depending on the types of storage compound, carbohydrate versus lipids, respectively). Land-based systems provide greater control of production and so far have been cost prohibitive. However, innovative practices have systems integrated with existing aquaculture facilities (e.g. finfish) or other industries (e.g. power plants, refineries or sewage treatment facilities) to offset the higher degree of input required for intensive aquaculture. These practices offer to change the scope and application of algal aquaculture significantly in the coming decades, particularly if financial incentives exist for environmental services (e.g. carbon or pollutant taxes) on top of valuable end-use products.

Determining value for environmental services will be facilitated by increasing pressure to minimise the impacts of aquaculture (fish) effluent. This should lead to more widespread adoption of seaweeds and other extractive organisms (e.g. molluscs, sponges) in bioremediation, becoming major components of new kinds of integrated system (e.g. integrated multitrophic aquaculture). The importance of algae more generally (being a non-additive sector) can do much to alleviate the human-induced impact on the oceans: specifically, nutrient loading of coastal waters and carbon sequestration. For instance, 15 million t per year of seaweed are produced globally, equating to the extraction of >30 000 t of nitrogen, >5000 t of phosphorus and more than 2 000 000 t of CO_2 from seaweed cultivation areas. Environmental services, and particularly contributions to carbon sequestration, clearly add to rationale for strategic mass cultivation of seaweeds in developed countries that are yet to participate. However, some caution must be shown in environmental management of cultivars and introductions between countries. Examples of past introductions include *Macrocystis pyrifera* (introduced to China from Mexico) and *Kappaphycus alvarezii* (introduced to China from the Philippines). Although crop translocation is deemed acceptable and applied broadly to agriculture, it is likely that similar approaches will be heavily scrutinised in developed nations. There is a strong incentive to explore local strains for seaweed production (e.g. *Porphyra* in the USA (Yarish *et al.*, 1998)). This research may in turn yield new and unexpected seaweed products and applications.

Creating sustainable livelihoods for developing nations represents an important economic challenge for this



Fig. 13.10 Large-scale production of *Kappaphycus* seaweed on an atoll of the Pacific nation, Kiribati. (a) Drying the seaweed; (b) bagged dried seaweed for export.

century. Developing nations contribute significantly to global seaweed production, and for these nations seaweeds are often fundamental to livelihood. For example, seaweed aquaculture represents a significant proportion of gross domestic product in Kiribati (ca. 30% of gross domestic product in 2000) and a significant portion of the aquaculture sector in the Philippines (most total aquaculture value is from seaweeds). In fact, the most efficient production per capita comes from developing Pacific nations, such as Kiribati which produced >10 000 t of red seaweed in 2000 (>100 kg per person compared with ca. 8 kg per person from China, the largest producer) (Fig. 13.10a,b). The major limitations for expansion of these industries (existing and new) relate to the development of processing capabilities and identifying weakness in the supply chain that assist in greater trading capabilities, including regional processing and refineries for carrageenan in tropical nations such the Philippines, Indonesia, Malaysia, Tanzania, Kiribati, Fiji, Kenya and Madagascar.

Food security and nutrition (e.g. the provision of protein) is a fundamental concern for many people and represents a significant social challenge for the global community. Because seaweeds are nutritious, easily dried and processed, and have long, effective shelf life, their aquaculture could be used to provide nutritional supplements in areas where essential nutrients are not easily sourced (e.g. inland Africa). Moreover, maladies from inadequate nutrition are not necessarily related to poverty. For instance, estimates are that >400 million people in China are deficient in iodine. Many seaweeds concentrate iodine and their consumption can reduce the risk of goitre and thyroid problems, re-emerging problems in developed nations where iodine intake has been reduced. Similarly in developed countries, trends towards whole food, macrobiotic and vegetarian philosophies require seaweed to replace agricultural sources of nutrients. Clearly the role of seaweeds in both traditional and contemporary foods and applications is well-defined and diverse opportunities exist for developing and developed countries alike.

13.3 MICROALGAE

13.3.1 Introduction

Microalgae are taxonomically very diverse and are found in almost every environment in nature. This great taxonomic and environmental diversity is also reflected in the range of metabolites they produce. Several species are grown commercially as sources of high-value, fine chemicals such as carotenoids and fatty acids, and as human food and animal feed (Table 13.4). Others are used in wastewater treatment and in agriculture as soil conditioners (Metting, 1988; Wong and Tam, 1998). Microalgae are also proving to be excellent sources of bioactive compounds such as antibiotics and anti-cancer drugs and,

Table 13.4 List of the major microalgae grown on a large scale and their applications (the use of algae in wastewater treatment or as biofertiliser is not included).

Alga	Product or application	Status	Countries
Chaetoceros muelleri	Aquaculture feed	Commercial	Global
Chlorella spp.	Health food	Commercial	Japan, Taiwan, Czech Republic, Germany
Crypthecodinium cohnii	Docosahexaenoic acid	Commercial	USA
Dunaliella salina	β-carotene	Commercial	Australia, Israel, China, India
Dunaliella tertiolecta	Aquaculture feed	Commercial	Global
Haematococcus pluvialis	Astaxanthin	Commercial	USA, Sweden, Israel
Isochrysis spp.	Aquaculture feed	Commercial	Global
Monochrysis lutheri	Aquaculture feed	Commercial	Global
Nannochloropsis spp.	Aquaculture feed	Commercial	Global
Pavlova spp.	Aquaculture feed	Commercial	Global
Porphyridium cruentum	Polysaccharides, pycobilin pigments	Research and development	Israel, France
Skeletonema spp.	Aquaculture feed	Commercial	Global
Spirulina platensis	Health food, phycocyanin	Commercial	Thailand, USA, China, India, Vietnam
Tetraselmis suecica	Aquaculture feed	Commercial	Global
Thalassiosira pseudonana	Aquaculture feed	Commercial	Global

Table 13.5 Estimated annual production of microalgae (based on published data, company information and estimated from market value). Algae used as aquaculture feed are not included as no data are available.

Alga	Annual production (dry weight) (t)
Chlorella	ca. 2500
Spirulina	ca. 4000
Dunaliella	ca. 2000
Haematococcus	ca. 200–300

although some of these compounds can be produced by chemical synthesis, many others will probably have to be produced through microalgae culture (Borowitzka, 1999b). Although commercial culture of microalgae is still a very new industry with only a small number of species and products, global production has grown significantly in the past 25 years. Estimated annual production for the most widely cultured species of microalgae is shown in Table 13.5. The high cost of production (Table 13.6) means that the product must also command a high sale price. It is interesting to note that the most expensive microalgae produced are those grown for use as feed for aquaculture species (Table 13.6), with the marked exception of 'green water' culture which is a mixture of phytoplankton and used in the culture of fish and shrimp (Neori, 2010). Some of the factors contributing to the high production costs are the high capital and labour costs and, for algae used in aquaculture, the rather small scale of production (section 9.3.1 and Fig. 9.2) (Borowitzka,1999a).

Despite the current high production cost, microalgae are also perceived as a future source of renewable fuels. Many microalgae have long been known to produce high levels of lipids which can be extracted and transesterified to produce biodiesel. The sugars contained in the biomass can also be fermented to produce ethanol. The potential advantages of microalgae over alternative oleagenous crops for biofuels are that they have higher productivity per unit area, do not compete with food crops for limited resources, can be grown using saline water and can be grown on land unsuitable for agriculture.

Because most algae require light for growth, a basic feature of all microalgae culture systems is that they are shallow so that light can reach all the cells. Commercial-scale microalgae culture systems may be extensive,

Table 13.6 Estimated production costs for microalgae currently grown on a commercial scale.

Alga	Estimated production cost (US$/kg dry weight)	Production system
Spirulina (Arthrospira) platensis	8–12	Raceway systems. Raceways of up to about 0.5 ha area
Chlorella spp.	15–18	Centre pivot open ponds
	>50	Closed photobioreactors
Dunaliella salina	5	Very extensive open ponds up to 250 ha in area
Haematococcus pluvialis	>40	Closed photobioreactors and open ponds
Algae for aquaculture (e.g. *Isochrysis*, *Tetraselmis* and *Skeletonema* spp.)	60–1000+	Big bags (lowest cost is for largest aquaculture facility in the USA)
Crypthecodinium cohnii	2	Grown heterotrophically on glucose in fermenters

semi-intensive or intensive. The cultures may also be either open to the air or closed.

13.3.2 Extensive culture

Extensive culture systems are very large and achieve only low cell densities of 0.1–0.5 g/L dry weight. Extensive microalgae culture is possible for a range of algae. The main microalga grown commercially in extensive culture systems is the chlorophyte, *Dunaliella salina. D. salina* is grown in extremely large shallow ponds in Australia for the production of the carotenoid β-carotene (Borowitzka and Borowitzka, 1989). It grows best at very high salinities (ca. 25% w/v NaCl) and high temperatures (30–40 °C). β-Carotene production from *D. salina* is greatest at high salinity and high light levels. The high salinity prevents almost all other organisms from growing in the ponds and competing with the it.

In the commercial systems in Australia, *D. salina* is grown in ponds of up to 250 ha in areas constructed with earthen walls on the bed of a salt lake or saline mud flats. The ponds are about 30–50 cm deep and the only water movement in ponds results from wind or convection. In such a system, the operator has little control over culture conditions other than salinity and nutrient concentrations. The ponds are usually operated in a semi-continuous mode with part of the ponds harvested at regular intervals and with the medium being returned to the ponds after microalgae cells have been harvested. Nutrients are added as required for microalgae growth, and salinity is controlled by the addition of seawater. Fig. 13.11 outlines this culture process. Because the algal density achieved in such systems is low, harvesting and further downstream processing is expensive and the final product must have a high value for the overall process to be economical. Despite this, the actual production costs for *D. salina* are among the lowest for any commercially produced microalga.

13.3.3 Semi-intensive culture

Semi-intensive culture systems are still quite large; however, mixing of the cultures and better control of culture conditions results in cell densities of up to about 1 g/L dry weight. The first commercial large-scale cultures of microalgae were developed in Taiwan in the 1950s for culturing the freshwater green alga *Chlorella*, which is used as a health food. The algae are grown in circular concrete ponds of up to about 500 m^2 surface area. The ponds have a centrally pivoted rotating arm that mixes the culture (Fig. 13.12a). This system results in uneven mixing, with the periphery of the pond being mixed much more than the centre because of the higher velocity of the mixing arm at the outer perimeter. The larger the pond, the greater is the difference between the periphery and the centre, and this limits the effective size of the ponds. Because of the inherent instability, the cultures need to be grown in batch mode. Each growth cycle is started as a small (ca. 1 L) laboratory culture (section 9.3.1) and is scaled up by a factor of 5–10 at each step.

In the 1960s, a better pond design, the ‘raceway’ pond, was developed. These ponds consist of long channels arranged in single or in multiple loops (Fig. 13.12b). Early designs used a configuration consisting of relatively narrow channels with many 180° bends and propeller pumps to produce a channel velocity of about 30 cm/s. In the 1970s, paddle-wheel mixers of various designs were introduced and found to be more effective, with reduced energy requirements and reduced shear forces on the

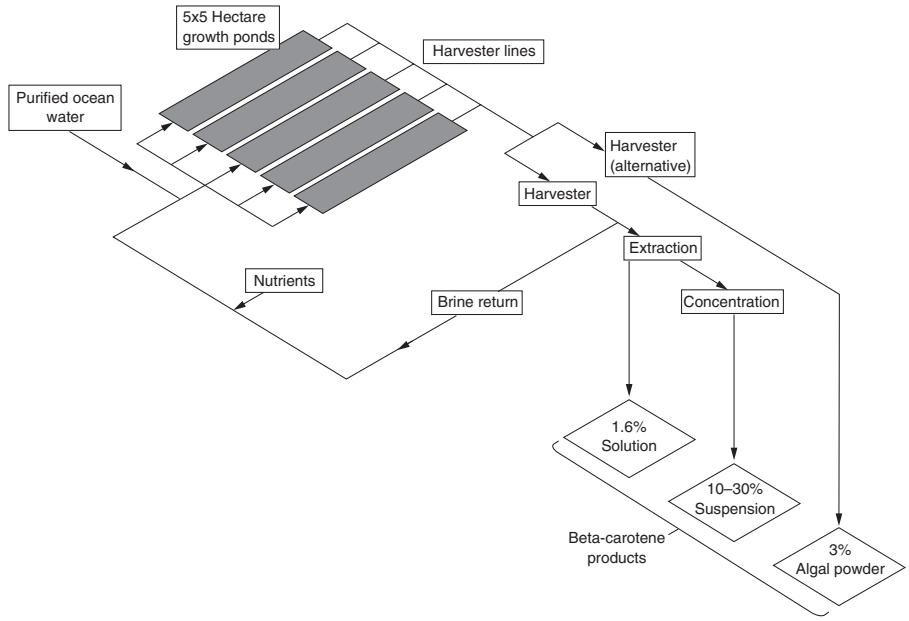


Fig. 13.11 Diagrammatic representation of the operation of the *Dunaliella salina* production plant operated by Western Biotechnology at Hutt Lagoon, Western Australia (note that not all ponds are shown).

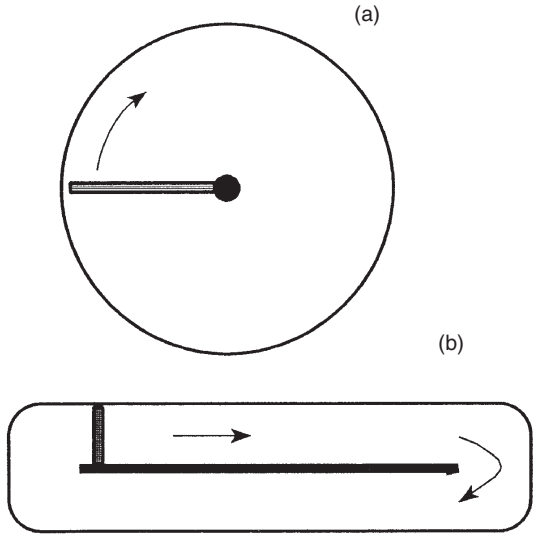


Fig. 13.12 Diagrams of the layout of semi-intensive culture systems. (a) Circular central pivot pond; (b) raceway pond (arrows indicate direction of water flow).

microalgae cells. The numerous bends in the channels of the older designs also led to hydraulic losses and problems with solids deposition. These were minimised by using a single loop (raceway) configuration, with flow rectifiers at the corners. Simple geometric optimisation has also shown that a large pond with a low length : width ratio gives the largest pond area for the least wall length, and is therefore cheaper to construct. Ponds can be up to about 6 m wide, the width being limited by the paddle-wheel design. Pond length is influenced by head loss relative to the mixing velocity and pond depth. Details of the design considerations in such systems are outlined by Borowitzka (2005).

Several factors need to be taken into account when designing the optimally sized pond. These include the following:

- optimal pond depth, taking into account the degree of light penetration;
- mixing velocity, which relates to the need to keep the algae in suspension, avoiding any dead spaces and the effects of turbulence on the pond materials;
- the energy requirement for mixing;
- materials from which the pond is constructed.

This pond design was first developed for high-rate oxidation ponds used in the treatment of wastewater, but was soon also applied to the 'clean' culture of a range of microalgae, especially the blue-green alga *Spirulina platensis*. It is also used in Israel and India for culturing *D. salina*. The raceway ponds are more efficiently mixed than the circular central pivot ponds and pond size can be up to 1 ha in area.

The ponds are either constructed of concrete, or of concrete walls or earthen walls lined with a plastic liner. The plastic liner is replaced with a concrete bottom in the region of the paddle wheel. Laboratory experiments have shown that microalgae productivity increases with increasing flow rate and that a velocity of at least 10 cm/s is necessary to avoid settlings of the cells; however, practical limitations in pond design mean that velocities in the range of 30 cm/s are optimal. To maximise productivity, the pond depth is about 20–30 cm and cell density must be controlled to minimise self-shading by the cells.

Although productivity of up to 30 g/m^2/day dry weight has been reported, actual annual average productivity is significantly lower than this with the best value of about 20 g/m^2/day dry weight reported. Several attempts have been made to improve the productivity of these ponds. Of these, the introduction of a series of aeroplane-type wings into the water flow seems to be the most promising. The wings introduce extra turbulence into the water flow, exposing the algae to more light and, therefore, increasing the growth rate by 10–20%.

Although the raceway pond design is the main culture system used for the commercial-scale culture of microalgae, its major limitation is that the system is open to the air, which may lead to contamination and infection by predators (mainly other algae, protozoa and fungi). These systems are, therefore, only suited to microalgae that grow in relatively extreme environments such as high pH (e.g. *Spirulina* species) or high salinity (e.g. *Dunaliella* species) or fast-growing algae such as *Chlorella*, *Phaeodactylum* or *Scenedesmus* species, which can outgrow most of their competitors.

13.3.4 Intensive culture

In intensive cultures, the algae are grown under highly controlled optimum conditions in closed photobioreactors, which can result in cell densities of 1–10 g/L dry weight. High cell densities have the advantage of requiring a smaller area for the reactor and, in addition, harvesting costs are also reduced significantly.

Closed culture systems include the following:

- bag culture, which is widely used for the culture of algae for aquaculture (section 9.3.1);
- alveolar panels and other flat plate reactors of various designs;
- stirred tank reactors with internal illumination;
- tower reactors with internal fibre-optic illumination;
- suspended narrow bags or tubes;
- tubular reactors.

13.3.4.1 'Big bag' systems

Probably the longest-used closed culture systems for mass culture of microalgae are the 'big bag' systems generally used in aquaculture hatcheries to feed larval fish, crustaceans, molluscs or rotifers (section 9.3.1 and Fig. 9.3). Although widely used, these systems are notorious for the instability of the cultures. This instability probably occurs because mixing in these bags is uneven, leading to build-up of cells in unmixed areas, which, in turn, leads to cell death, especially if the culture is not axenic (bacteria free). To achieve reasonably reliable cultures, it is essential to maintain axenic conditions, a feature that is not as essential for the tubular photobioreactors.

13.3.4.2 Tubular photobioreactor

Despite the range of designs listed above, few of these systems are operational at a commercial scale as yet. The two most promising designs for commercial large-scale culture are the tubular photobioreactors and the flat-panel reactors.

Many tubular reactor designs have been developed to produce cultures of relatively high density (Tredici, 2004). The first large-scale tubular photobioreactor had a solar receptor constructed of five identical 20-m^2 units made of 25-cm diameter polyethylene tubes floating on or in a large pool of water. The culture was circulated through these tubes and temperature was controlled by either floating the tubes at the water surface (to heat) or by immersing them in the water (to cool). The water in the pool also provided a convenient support for the long tubes of the solar receptor. At the end of each solar receptor, a gas exchange tower removed photosynthetically produced oxygen and CO_2 could be added. This pilot plant was quite successful in growing the red unicell *Porphyridium*; however, it was technically complex and expensive, and required a large land area.

A more efficient arrangement for the tubes of the solar receptor is to wind them helically around a tower. This is

the design of the 'Biocoil', a system developed in the UK and optimised in Australia. Several pilot-scale units of the Biocoil have been in operation in the UK and in Australia, with volumes up to 2000 L, and a very wide range of microalgae including *Chlorella*, *Spirulina*, *Dunaliella*, *Tetraselmis*, *Phaeodactylum*, *Chaetoceros*, *Isochrysis*, *Pavlova*, *Porphyridium*, *Haematococcus* and *Skeletonema* species have been grown successfully. The Biocoil system uses low-density polyethylene or Teflon tubing of 25–30 mm diameter. This narrow diameter has been shown to result in much higher productivities and reduced fouling of the inside of the tubes by the algae. The helical arrangement of the tubing also means that there are no sudden changes in direction of flow, which not only result in significant head losses, but can also lead to undesirable accumulation of algae. The maximum temperature in the reactors is controlled by evaporative cooling achieved by running water over the reactor surface. The helical design also has the great advantage of good scale-up properties. This means that the results obtained in smaller pilot experiments can be directly related to a full-scale production unit. Systems such as the Biocoil also allow for continuous culture, which results in a more consistent quality of algae produced and is cheaper. In Perth, Western Australia, *Isochrysis* (T. ISO) has been grown in a 1000-L Biocoil in continuous culture for more than 6 months.

One of the key design features of these systems is the pumping system used to circulate the algal culture. Several types of pump, including centrifugal, diaphragm, peristaltic and lobe pumps, as well as airlifts, have been used and the choice of pump depends on the degree of fragility of the algae being grown.

There are two other arrangements for the tubes that are being developed for commercial production. In these, the tubes are either arranged vertically in long rows or are laid horizontally on the ground. This is the system used in the commercial culture of *Haematococcus* in Israel.

13.3.4.3 Flat-panel photobioreactor

An alternative design for a closed photobioreactor is the flat-panel reactor first developed in the 1980s. This reactor consists of two rectangular panels of glass or Perspex spaced about 25 mm or more apart. A cheaper design in which plastic bags are placed within a rectangular framework has also been recently developed (Rodolfi *et al.*, 2009). Some of the designs have several internal baffles. The panels can be inclined to capture the optimum amount of solar irradiation and the algal culture inside is mixed by aeration or circulated by pumping. The aeration not only mixes the culture but also helps to remove photosynthetic O_2, which at high concentrations will limit productivity owing to photorespiration. CO_2 can also be added to enhance growth. The temperature is usually controlled by spraying water over the panel surface in order to cool the cultures. Some systems use a heat exchanger; however, this is usually too expensive for large-scale systems. These flat-panel reactors can be very productive, but they are very difficult to scale up to any appreciable size and are expensive to operate.

13.3.4.4 Hybrid systems

The green alga *Haematococcus pluvialis* has proved to be difficult to grow in either open raceway ponds or closed bioreactors. Commercial production of this alga, which is an excellent source of the carotenoid astaxanthin, involves growing the algae initially in a closed photobioreactor. Once sufficient biomass has been produced, the algae are then transferred to open-air raceway ponds, where the cells then accumulate the astaxanthin (Olaizola, 2000).

13.3.4.5 Heterotrophic culture

Closed fermenters have been developed for the heterotrophic production of long-chain polyunsaturated fatty acids from microalgae (Radmer and Parker, 1994). This technique uses glucose or acetate as the energy and carbon source for the algae and eliminates the need for light, which is a major cost in phototrophic microalgae culture systems. Furthermore, because cell density is not limited by light availability, microalgae can be cultured in relatively high densities with high biomass production. Heterotrophically grown microalgae have been commercially produced and shown to have value as an aquaculture feed (section 9.3.4). However, only a limited number of species can be grown heterotrophically, and therefore systems such as the tubular photobioreactors and flat-panel reactors are likely to be the main systems to be used in the future.

13.3.5 Algae for biofuels

Algae, both microalgae and seaweeds, have been proposed as sources of renewable biofuels such as biodiesel, bioethanol, H_2 and methane (Chisti, 2007). Biomass-based biofuels also have the advantage of a smaller CO_2 footprint as CO_2 is fixed during photosynthesis to produce the biomass. Some microalgae, in particular, are excellent sources of fats (lipids) with cell contents of up to ca. 30% in actively growing cells and over 50% in stationary phase

cells (Borowitzka. 1988) and these lipids can be converted to biodiesel by transesterification. The green alga *Botryococcus braunii* also produces large amounts of hydrocarbons (Metzger and Largeau 2005). Furthermore, the productivity of intensive microalgae cultures outdoors is about 5–10 times that of alternative oil crops such as canola and oil palm. The high demand for liquid transport fuels, however, means that extremely large production plants (10–100 km^2 in area) would be required. To achieve high annual productivities, such plants would need to be located in a region with high average annual solar irradiation and minimum cloud cover and rainfall, such as in the north of Western Australia and Arizona in the USA. Large saline water sources are also required so the plant must be located near the ocean or near a source of saline groundwater. A wide variety of culture systems have been suggested for algae culture from conventional raceway ponds to a wide variety of closed photobioreactors (Chisti, 2007). However, for economic reasons, open raceway ponds are the most likely system for the eventual commercialisation of algae-based biofuels. Although trial batches of algal biofuels have been produced for testing, their commercial production still requires a very significant reduction in the cost of their production for them to be able to compete with other fuel sources.

A schematic diagram of an algae-to-biofuel process in shown in Fig. 13.13. The algae are grown in open raceway ponds in semi-continuous culture with regular harvesting of part of the biomass to maintain maximum productivity. After harvesting the algae, the medium is returned to the growth ponds and supplemented with nutrients. After further dewatering, the algae oil (and possibly also the sugars) are extracted and converted to biofuel. The remaining biomass could be used either as a high protein feed supplement in aquaculture, or be fermented to produce methane, or the energy in the remaining biomass could be recovered by co-burning with coal in a power station.

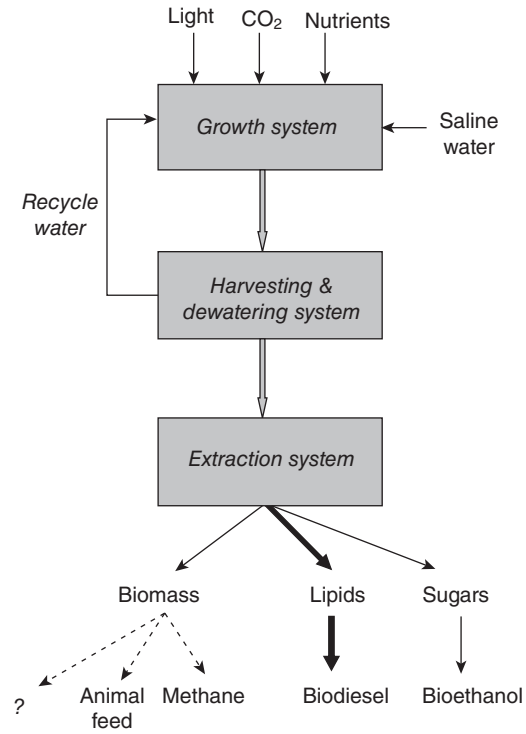


Fig. 13.13. Schematic flow diagram of the process of producing biofuels from microalgae (dashed lines indicate alternate pathways).

13.3.6 Choice of culture system

The choice of culture system depends on many factors and no single system is best for all microalgae. Table 13.7 compares some of the main characteristics of the different culture systems used for production of microalgae.

The reliability of the culture is of great importance to commercial operations and, for this reason, closed culture systems are preferred. However, many of these systems are still in the early stages of development and they are generally more expensive to construct and operate. On this basis, most commercial microalgae culture systems are open systems that can only be used with microalgae growing in a highly selective environment. As such, further developments in this field with new species of microalgae probably will require closed systems.

Large-scale photobioreactors require a high surface area to volume ratio to maximise light availability. Although this generally results in higher productivity, greater cell biomass and lower production costs, these systems do have disadvantages. For example, the high surface area to volume ratio provides susceptibility to overheating in outdoor systems. In addition, the high biomass in such systems requires turbulent flow to reduce light limitation and ensure nutrient exchange. On this basis, the number of species that can be cultured in these systems is limited as they are unsuitable for fragile species.

The final choice of culture systems will depend on the species being cultured and on commercial considerations.

Table 13.7 Comparison of the properties of different large-scale algal culture systems.

Reactor type	Mixing	Light utilisation efficiency	Temperature control	Gas transfer	Hydrodynamic stress	Species control	Sterility	Scale-up
Unstirred shallow ponds	Very poor	Poor	None	Poor	Very low	Difficult	None	Very difficult
Paddle-wheel raceway ponds	Fair–good	Fair–good	None	Poor	Low	Difficult	None	Very difficult
Stirred tank reactor	Largely uniform	Fair–good	Excellent	Low–high	High	Easy	Easily achievable	Difficult
Air-lift reactor	Generally uniform	Good	Excellent	High	Low	Easy	Easily achievable	Difficult
Flat-plate reactor	Uniform	Excellent	Excellent	High	Low–high	Easy	Achievable	Difficult
Tubular reactor (Serpentine type)	Uniform	Excellent	Excellent	Low–high	Low–high	Easy	Achievable	Reasonable
Tubular reactor (Biocoil type)	Uniform	Excellent	Excellent	Low–high	Low–high	Easy	Achievable	Easy

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14
Carps

Sena De Silva

14.1 INTRODUCTION

Carps are regarded very differently in different parts of the world. To many people in Western countries, carps are ornamental fish in ornamental ponds and aquaria. In one region, the Murray–Darling River system in south-eastern Australia, feral common carp (*Cyprinus carpio*) are regarded as a pest. Yet carps and related species of the family Cyprinidae are a major source of animal protein for millions of people in many Asian countries, as well as in Eastern Europe. World cyprinid aquaculture production in 2007 was 18 944 071 t, valued at US$20 265 million. Cultured cyprinid production in 2007 accounted for 70% of world freshwater finfish aquaculture production and 58% in value. In general, cyprinid aquaculture production over the years has increased very significantly in absolute terms and in its contribution to inland freshwater finfish production. Similar trends are evident in the total value of the produce. As such, cyprinid culture is very important to the world aquaculture industry, outweighing all the other species groups in its contribution to world aquaculture production. The trends in cultured cyprinid production from 1981 to 2005, based on 5 year averages compared with freshwater fish and shellfish production, are shown in Fig. 14.1. It is evident that cyprinids constitute the major group of all cultured amimals in freshwater. Moreover, the contribution of cyprinids to world aquaculture production has remained steady throughout the past 25 years, contributing about 30%. Their contributions to total cultured fish and shellfish production (e.g. from all environments) ranged from 44.8 to 37.6% in 1987 and 2007, respectively. For total freshwater fish and shellfish production, they ranged from 76.6 to 65.3% in 1987 and 2007, respectively. The slight decrease in cyprinid contribution from the early 1990s could be partly due to an upsurge in the culture of tilapia in freshwater and in mariculture (Chapter 16).

The family Cyprinidae is a typical freshwater group of fish with a very wide distribution; its members are collectively referred to as carps, barbels and minnows. Carps occur naturally in North America, Africa and Eurasia, but are absent from South America, Australasia and Madagascar. Distinguishing features of the group are the presence of pharyngeal teeth in one to three rows, with not more than eight teeth in any one row, lips that are usually thin and an upper jaw that is usually bordered only by premaxillae. There are about 1600 species in the family Cyprinidae, making it the largest family of fish. The taxonomy of the family is correspondingly complex, with about 11 subfamilies and 275 genera. The greatest diversity of the group occurs in Asia.

Despite the large number of species in the family, only a very small proportion are cultured commercially. Altogether, 34 species of carps are cultured globally, with the predominant species being the Chinese and Indian major carps (Fig. 14.2) and the common carp.

Out of the 34 species of cyprinid that are currently cultured, the production of 11 species exceeds 150 000 t/year, including five species whose production exceeds 1 million t/year (Table 14.1). There are only six other fish species or species groups whose production exceeds 100 000 t/year. More importantly, all species referred to in Table 14.1 have shown a significant increase in production since 1980. The importance of cyprinids in aquaculture is

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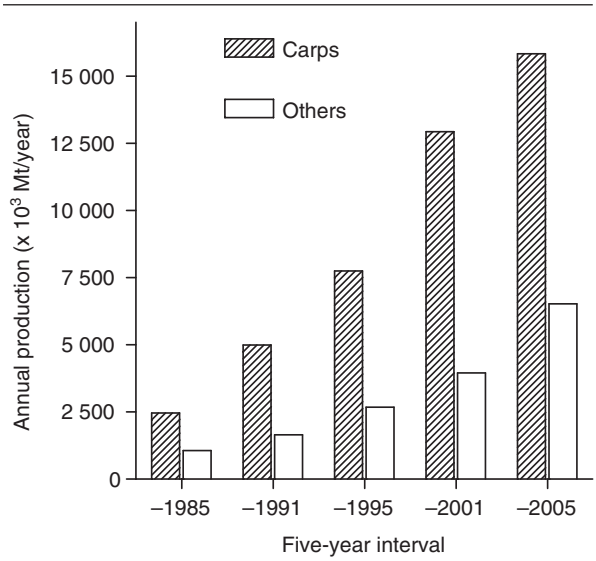


Fig. 14.1 Five-yearly average annual global production of carps and other cyprinids compared with all other cultured freshwater fish and shellfish, 1981–2005.



Fig. 14.2 Capture of Chinese carp broodstock in Romania. (Photograph by Professor R. Billard.)

further highlighted by the fact that, of all cultured fish species, the production of only 10 species exceeds 1 million t/year (2007 data) and seven of these are cyprinids, namely silver carp (*Hypophthalmichthys molitrix*), grass carp (*Ctenopharyngodon idella*), common carp (*Cyprinus carpio*), bighead carp (*Aristichthys nobilis*), crucian carp (*Carassius carassius*), catla (*Catla catla*) and rohu (*Labeo rohita*), in that order. It is also important to note that in all

Table 14.1 Cyprinid species that currently contribute more than 150 000 t/year to world aquaculture production. Production values in 1980 and 2007, and average percentage increase per year of each species over the 27-year period.

Species	Production		Mean increase per year (%)
	1980	2007	
Silver carp	449 417	3 662 810	10.4
Common carp	365 238	2 872 874	10.4
Bighead carp	198 556	2 160 627	10.7
Grass carp	155 616	3 610 318	11.1
Rohu	90 134	1 342 574	11.1
Cattla	86 751	2 274 411	11.6
Mrigal carp	46 482	287 200	10.5
White amu bream	45 100	576 341	11.1
Crucian carp	31 269	1 939 280	12.7
Black carp	27 100	331 889	11.2

the major carp species cultured, the production increases per year have averaged between 10.4% and 12.7% over the period shown in Table 14.1. In view of the culture potential of the listed species, most have been introduced to many countries that are much beyond the range of their natural distribution, particularly through Eurasia. This has happened deliberately or inadvertently or both. For example, species such as the grass carp have been introduced into the USA and New Zealand to control aquatic weed. The common carp, on the other hand, was spread across the globe with European colonisation in the eighteenth and nineteenth centuries, in a similar fashion to that witnessed for brown and rainbow trout.

This chapter deals primarily with the culture and those aspects related to culture of the Chinese and Indian major carps. The major carps include three of the Chinese species referred to previously (silver, grass and bighead carps) and three Indian carp species (catla (*Catla catla*), rohu (*Labeo rohita*) and mrigal (*Cirrhinus mrigala*)). As the name implies, these species grow to a relatively large size in nature (Table 14.2).

14.2 ASPECTS OF BIOLOGY

Lin and Peter (1991) reviewed the biology of cyprinids. Cultured carp species are all riverine, typically being found in large river systems. However, the food habits of the species differ from each other (Table 14.3). Food habits

Table 14.2 Total length (TL) and weight for four species of major carps in natural waters.

	Silver carp[a]		Bighead carp[a]		Rohu[b]		Mriga[c]	
Age (yr)	TL (cm)	Weight (g)	TL (cm)	Weight (g)	TL (cm)	Weight (g)	TL (cm)	Weight (g)
2	50.0	1803	63.0	3250	50.0	NA	51.1	1512
3	57.6	4650	74.6	10700	65.0	NA	67.0	3618
4	60.3	5340	75.1	10900	74.0	NA	79.9	6324
5	63.0	6400	77.8	11800	80.0	NA	85.8	8030
10					96.0	NA	95.8	11930

[a]Chang *et al.* (1983).
[b]Khan and Jhingran (1975).
[c]Jhingran and Khan (1979).
NA, data not available.

Table 14.3 A summary of the predominant food habits of Chinese and Indian major carps.

Species	Feeding habit(s)
Silver carp	Zoo- and phytoplankton filter feeder; prefers phytoplankton; surface feeder
Grass carp	Omnivorous; prefers higher aquatic plants and submerged grasses
Bighead carp	Predominantly zooplankton filter feeder
Chinese bream	Macrophyte feeder
Black carp	Snails, aquatic insects and crustaceans
Mrigal	Omnivorous, preferring to feed on detritus; predominantly a bottom browser
Rohu	Omnivorous planktophage; predominantly a column feeder
Catla	Plankton feeder; prefers zooplankton and surface feeder

also differ between stages of the life cycle of a given species, for example the grass carp is zooplanktivorous in the fry and early fingerling stages, and transforms into a macrophyte feeder later on.

Carps are very fecund, and most of the cultured major carp species attain sexual maturity in their third year. In the wild, they spawn once a year, generally with the onset of monsoonal floods. It is generally accepted that the interaction of many factors associated with flooding is responsible for bringing about ovulation and spawning of Indian major carps under natural conditions. The spawning of Indian major carps may be synchronised with the phase of the moon during the floods. In the case of Chinese major carps, it is believed that temperature and photoperiod provide the primary cues for maturation. Chinese and Indian major carps have not been known to spawn naturally in lake waters, nor under captive conditions without hypophysation (section 14.3). All the major carps are single spawners, in that during any one spawning season the female sheds all her mature oocytes within a very short period.

Fertilised eggs of major carps are buoyant, as opposed to the adhesive eggs of common carp and those of most of the smaller cyprinids. The fertilised eggs hatch in 72–96 h and the major carp larvae feed first on microalgae.

All the major carps grow to about 1 m in length (Fig. 1.2), and generally the Chinese major carps grow to a larger size than the Indian major carps. For example, silver and bighead carp may grow up to 1.5 m in length and exceed 10–20 kg in weight. Under culture conditions, however, all except broodstock are harvested in their second or third year, often at a weight approaching 1 kg.

14.3 ARTIFICIAL PROPAGATION

As for most cultured fish species, the single most important breakthrough in carp culture was the development of techniques for artificial propagation of the major species. Before this, carp culture depended on the availability of natural seed for stocking. Specialised fisheries developed in the flood plains of major rivers in mainland China and India to collect the natural seed.

The traditional and most commonly used technique of induced spawning in carps is injection of either:

Table 14.4 A summary of the Linpe method of ovulation and spawning of cultured carp in China.

		Treatment			
Species	Temperature (°C)	Domperidone (mg/kg)	LHRHa (μg/kg)	sGnRHa (μg/kg)	Time to ovulation (h)
Silver carp	20–30	5	20	–	8–12
		5	–	10	8–12
Mud carp	22–28	5	10	–	6
Grass carp	18–30	5	10	–	8–12
Bighead carp	20–30	5	50	–	6–8
Black carp	20–30	3	10	–	6–8
		7	15	–	6–8

Modified after Lin and Peter (1991).

- crude extract of common carp pituitary gland (or those of other mature fish species that are phylogenetically close to carp);
- partly purified human chorionic gonadotrophin (HCG).

Use of hormones and/or analogues for inducing spawning is referred to as hypophysation.

Details of the hormonal control of reproduction in fish and artificial spawning induction are given in Chapter 6 (section 6.2.1). The use of various techniques for spawning induction of Indian and Chinese major carp was discussed by Jhingran and Pullin (1985).

Initially, high efficiency of ovulation was achieved using HCG either alone or in combination with carp pituitary extracts. This treatment was superseded by the use of gonadotrophin-releasing hormone analogue (GnRHa) to stimulate reproduction. GnRHa alone, however, is not entirely effective and has to be accompanied by administration of a dopamine receptor blocker. A better understanding of neuroendocrine regulation of gonadotrophin secretion has led to the development of effective new techniques for induced ovulation and spawning of cultured fish species.

Peter *et al.* (1986) dealt with aspects of neuroendocrine regulation of gonadotrophin secretion. Basically, modern techniques of inducing ovulation and spawning use a combination of drugs, one of which blocks the inhibiting action of dopamine within the neurohormonal systems (the 'Linpe method'; section 6.2.1). Details of various effective combinations of GnRHa (LHRHa (luteinising hormone-releasing hormone analogue) or sGnRHa (salmon GnRHa)) and a dopamine antagonist (e.g. domperiodone) for induced ovulation and spawning of Chinese major carps are summarised in Table 14.4. The Linpe method is known to be more effective in many ways, ensuring the following:

- a high rate of ovulation;
- consistency between broods;
- complete ovulation;
- that the time lag between injection and ovulation is short and predictable.

Ovulation and spawning by the Linpe method do not influence subsequent reproduction cycles of the same brood fish. The Linpe method uses synthetic drugs that are cheaper and more stable and, because only one injection is needed, the brood fish are much less stressed.

Spawning induction of carps in India has been undertaken using commercially available kits (marketed under the trade name 'Ovaparim'), which use the Linpe method. Bruzuska (1999) compared the efficacy of LHRHa ((Des–Gly^{10})D-Ala^{6}, LH–RH-ethylamide) and pimozide at 15 μg and 5 mg/kg body weight, respectively, injected simultaneously, compared with carp pituitary suspension on the spawning of grass carp and silver carp. The silver carp responded better than grass carp to both forms of treatment. However, it was stressed that the LHRHa treatment involved less handling of the fish.

Another important advance in induced spawning of the major carps has been the development of the ability to spawn brood fish twice in a calendar year. A second spawning is now achieved for most major carps and is commonly practised. This development has enabled farmers to maintain fewer brood fish and has enhanced seed availability almost year round. This has almost



Fig. 14.3 A 'spawning pond' (in Sri Lanka), used for acclimatising selected broodstock before and during hypophysation.

completely eliminated the need for dependence on natural seed.

After hormonal treatment, broodstock are put into spawning ponds in a ratio of three males to two females (Fig. 14.3). They usually spawn at dawn after a second injection of HCG or pituitary extract and are removed from the spawning ponds after spawning. The floating eggs are moved from the spawning pond, by movement of the water through the tank, into a collection box. Alternatively, the brood fish may be stripped and a dry fertilisation performed. The process of stripping gametes from brood fish and dry fertilisation is described in detail in Chapter 15 (section 15.3.3 and Fig. 15.3). This method is becoming increasingly common in the culture of major carp.

Specially designed spawning tanks are now commonly used for fry production of Chinese carps. They are usually circular or elliptical cement tanks about 1.2–1.5 m deep, containing 50–60 m^3 of water. The tank bottom usually slopes towards the centre, where an outlet leads to an egg collection chamber. Incoming water is directed to create a circular flow within the pool at a rate of 200–400 L/s.

Fertilised eggs are transferred into incubation tanks or hatching pools which are circular, ca. 3.5–4.0 m in diameter and about1 m deep. Water flow is maintained at approximately 0.2–0.3 m/s. Eggs are usually incubated at a density of about 700 000–800 000 eggs/m^3. Under these conditions, a hatching rate of about 80% is achieved. In China, 150 000–200 000 eggs are incubated in 150-L clay jars or in funnel-type incubators with vertical water move-



Fig. 14.4 Fertilised carp eggs being placed into incubation jars in a hatchery in Poland. (Photograph by Professor R. Billard.)

ment. After 4–5 days, when the larvae have resorbed the yolk sac, they are removed to nursery ponds.

Glass hatchery jars are also commonly used for hatching eggs both of Indian and Chinese major carps. The jars are generally ca. 13 cm in diameter and 60 cm long, with conical bases. Each jar is supplied with water up through its conical base to create vertical water movement (Fig. 14.4). The basic concept of all hatchery designs for major carps is to provide a water current of sufficient strength to maintain the eggs in the water column and to remove metabolic waste products.

Recently, because of the emphasis on the development of culture of native species, there has been a concerted effort to artificially propagate cyprinid species that are of value to certain countries in Asia. In this regard the development of artificial propagation techniques for two, high-valued and sought after Malaysian mahseer species, *Tor tambroides* and *T. douronensis*, stand out (Ingram *et al.*,

Table 14.5 The known essential amino-acid requirement of cultured carps. The dietary protein level, where relevant, is given in parentheses.

	Common carp[a]		Catla[b]	Grass carp[c]	Rohu[d]
Amino acid	38.5%[e]	[f]	40.0%[e]	[g]	40.0%[e]
Arginine	4.3	1.6	4.8	11.1	5.8
Histidine	2.1	0.8	2.5	4.7	2.3
Isoleucine	2.5	0.9	2.4	8.8	NA
Leucine	3.3	1.3	3.7	16.5	NA
Lysine	5.7	2.2	6.2	15.5	NA
Methionine	3.1	1.2	3.6	4.5	2.9
Phenylalanine	6.5	2.5	3.7	7.8	4.0
Threonine	3.9	1.5	5.0	8.5	4.3
Tryptophan	0.8	0.3	1.0	2.4	NA
Valine	3.6	1.4	3.6	11.5	NA

[a]NRC (1993).
[b]Ravi and Devaraj (1991).
[c]Ding (1991).
[d]Murthy and Varghese (1995, 1996a,b, 1998).
[e]Expressed as a percentage of dietary protein (38.5 or 40.0%).
[f]Expressed as a percentage of dry diet.
[g]Expressed as a percentage of the total essential amino acids.
NA, data not available.

2005, 2007). This trend is being linked to the introduction of acceptable broodstock management procedures in order to maintain the genetic diversity of wild stocks, and for conservation (Nguyen *et al.*, 2006).

14.4 NUTRIENT REQUIREMENTS

Of the commonly cultured cyprinid species, the nutrient requirements of common carp are best known (see review by Satoh, 1991). This is to be expected because it was one of the earliest species to be cultured and examined experimentally. The nutrient requirements of most Chinese and Indian major carps are incompletely documented. This is not unexpected, as most cyprinids are cultured extensively or semi-intensively, and are rarely fed commercial feeds that have been compounded and formulated. Because of this, basic nutritional research on the members of the group has lagged behind that of other cultured fish species.

The protein, amino-acid and carbohydrate requirements of common carp are known (NRC, 1993). The essential amino-acid requirements of the Indian major carp, catla, are also known (Ravi and Devaraj, 1991). Also of some relevance is a study by Ding (1991), who determined the relative proportions of essential amino acids in grass carp muscle and compared them with those of three other cultured species (Table 14.5).

The dietary fatty-acid requirements of cultured cyprinids are not well known. Indeed, apart from the early work on common carp (Takeuchi and Watanabe, 1977), when it was demonstrated that this species requires equal amounts of dietary linoleic acid (18:2*n*–6) and linolenic acid (18:3*n*–3), there has not been any study on the fatty-acid requirements of carps. It is plausible that this requirement is true for all carps, and conforms to the basic notion that freshwater fish require the two base fatty acids (section 8.3.3). They then have the capability to elongate and desaturate these to longer-chain polyunsaturated fatty acids, such as eicosapentaenoic acid (20:5*n*–3), docosahexasenoic acid (22:6*n*–3) and arachidonic acid (20:4*n*–6), among others.

The dietary protein requirement of the major carps, particularly rohu, has been fairly intensively investigated. Most of these studies have investigated fry and fingerling stages, and there is considerable variation in the results of different investigators. This is mostly a result of variations in experimental protocol. Based on available information, De Silva and Gunasekera (1991) estimated that the dietary protein level that results in maximum growth of major

carps is 45% and the economically optimal dietary protein content is 31% (section 8.3.2 and Fig. 8.2).

The information available on the requirements of other nutrients is very scant and all the nutrient requirements for any one species of major carps are not known. As pointed out above, this probably reflects the mainly semi-intensive mode of culture of the Chinese and Indian major carps. As such, the need to develop complete diets does not arise. It therefore follows that there is no dire need to determine all the nutrient requirements for these species.

14.5 CULTURE

14.5.1 Larval rearing

Eggs of cultured cyprinids hatch out in 2–3 days at temperatures between 23 °C and 27 °C, and the yolk sac continues to provide nourishment for a further 3 days or so, at which time the larvae require an exogenous food supply. It is desirable, if not essential, to expose the larvae to an external food source before yolk sac resorption is complete. This entails removing the larvae from the hatchery jars and introducing them into a fry-rearing facility. The young hatchlings, which mostly move vertically, tend to change to a horizontal movement, which indicates their readiness to ingest food particles.

Larval rearing in carp culture has two distinct phases:

1. Rearing postlarvae to the fry stage, usually in nursery ponds, or in *hapas* (fine mesh enclosures) suspended in ponds or channels.
2. Rearing fry to fingerling stage, most effectively done in well-fertilised rearing ponds.

It is, however, not uncommon to combine the above two stages in one pond.

Preparation of nursery and rearing ponds often involves sowing a short-term crop of a leguminous plant (e.g. beans, clover), and ploughing and levelling the pond once the crop has grown to 6–10 cm. This process is known as 'green manuring' and is believed to enhance pond productivity. In most instances, unwanted organisms in the ponds are eradicated using a biodegradable toxicant or quicklime. This procedure is performed at least a fortnight before stocking. Commonly used toxicants include the following:

- Derris powder (4–20 mg/L);
- oilcake of the plant *Bassia latifolia* (200–250 mg/L) (= mahua oilcake);
- tea-seed cake (525–674 kg/ha);
- quicklime (900–1050 kg/ha).

The next stage is to prepare the pond to ensure a good production of small zooplankters, such as rotifers, which provide a food source for growing larvae. Ponds are often treated with either organic or inorganic fertilisers. The quantity of manure to be used is related to the toxicant used earlier. For example, if mahua oilcake was used, a dose of dry cow manure at the rate of 5000 kg/ha 2 weeks before stocking and a similar dose 1 week after hatching are desirable. However, with toxicants that have no fertiliser value, doses of 10 000–15 000 kg/ha initially, and 5000 kg/ha later, are desirable. These manuring doses are sufficient for 1.5×10^6 larvae/ha.

Fertilisation with a mix of organic and inorganic fertilisers is undesirable, as more often than not it results in harmful plankton blooms. Despite the early preparation of the ponds, undesirable predatory insects such as water spiders and water skaters may colonise the ponds. Therefore the ponds have to be regularly treated for insect control, particularly before stocking. Jhingran and Pullin (1985) recommend any one of the four treatments given below:

1. Spraying an emulsion of 56 kg of mustard or coconut oil and 18 kg of washing soap per hectare.
2. Spraying an emulsion of 56 kg of mustard oil and 560 mL of Teepol (detergent) per hectare.
3. A 0.01 ppm dose of pure gamma isomer of benzene hexachloride dissolved in ethyl alcohol.
4. Application of 0.25–3.0 ppm organophosphate such as fumadol, sumithion or diptrex.

The prepared ponds are stocked when it is certain that a substantial zooplankton population (particularly small zooplankters such as protozoans and rotifers) is established. Abrupt changes in quality and temperature between hatchery water and nursery water are avoided when stocking. Stocking is best done in the evening, which should give the larvae sufficient time to acclimatise before any possible predation.

The stocking rate depends on the proposed management practice and, if the following conditions are met, a stocking rate of 10 million/ha can be used:

- continued and repeated fertilisation to produce and maintain good plankton production;
- supplemental feeding;
- facilities to remedy oxygen deficiencies that may occur.

Postlarvae of carps are voracious grazers. Supplemental feeding and manuring, when undertaken concurrently,

Table 14.6 Some feeds and feeding rates used for Chinese carp fry and fingerling rearing.

Country	Species	Pond area (m^2)	Depth (m)	Fish length (mm)	Age (d)	SD (per m^2)	Feed	Feeding rate
China	Big head, grass carp	–	0.5–1.0	Up to 20	Up to 30	100	Egg yolk paste or soybean milk plus peanut cake after 10 days	One egg per 2500–7500 fry/day or milk from 300–500 g beans per 50 000 fry/day
	All species	–	0.5–1.0	23.1	Up to 30	–	Soybean meal	45 kg per 5000 fry/month
Hong Kong	All species	1000	0.8	8–30 (3 mg to 1 g)	Up to 25–30	150	Soybean milk and peanut cake meal	100 kg soybean milk or 200 kg peanut cake meal per month
	All species	1400	1.0	31 (1.5 g)	30–70	35	Peanut cake, rice bran or soybean cake	Start at 1.5 kg/day, build up to 5 kg/day

Reproduced from Chang, W.Y.B., J.S. Diana and W. Chuapoehutz. 1983. Workshop report to agency for international development, 19–29 April 1983. Strengthening of South east Asian Aquaculture Institutions (Grant No. DAN 5543-G-SS-2103.00).

result in better survival and growth. The commonly used supplemental feeds in carp culture in India are rice bran, and oilcakes of peanut (= groundnut), coconut and mustard. These are used in China, with soybean milk and meal, and egg yolk paste. It is very rare for carp culture to be based on complete formulated feeds, except in the case of common carp culture in some countries. Feeds are often dispersed as a crude mix, in either dry (e.g. meals or pellets) or moist form. A summary of commonly used feeds and feeding schedules for fry of Chinese carps is shown in Table 14.6. However, it should be noted that in the case of common carp, which are cultured fairly intensively on a small scale in ponds in Israel and in cages in China, formulated diets (pellet feeds) are used.

The fish in rearing ponds are harvested with sieve nets when they reach 4–6 cm. Periodical harvesting may be used to avoid overcrowding. Often, rearing postlarvae to fingerling size is undertaken in larger, earthen ponds and polyculture is practised. Stocking densities of fingerlings in polyculture range from 100 to 2500/m^2 with a mean of about 800/m^2 (Table 14.7). Size at harvesting ranges from 7 to 20 cm, with grass and black carps tending to be the largest.

14.5.2 Grow-out

Several distinctive general features are recognisable in the culture practices of major carp:

- they tend to be semi-intensive;
- almost always use polyculture;
- may be integrated with other forms of farming;
- are undertaken in earthen ponds or in pens, but rarely in cages and raceways.

However, the exception is the common carp. In certain instances they are intensively cultured in ponds and cages (Fig. 14.5).

Polyculture is thought to have originated in China, when various combinations of seven basic species with widely different food habits were cultured together, namely black carp (eat snails), grass carp (eat coarse vegetable matter), silver carp (eat phytoplankton), bighead carp (eat zooplankton and are omnivorous) and mud carp (bottom scavengers). A typical species combination used in a polyculture practice with an approximate indication of the niches occupied by each of the species is shown in section 2.3.5 (Fig. 2.10). The number of species used, and the ratio of each species, varies from region to region. Polyculture, apart

Table 14.7 Examples of stocking rates and size at harvesting of carp fingerling in polyculture.

Stocking density (×100/m^2)						Size at harvesting (cm)					
gc	bc	sc	bhc	cc	wf	gc	bc	sc	bhc	cc	wf
4–6	–	20–25	–	–	–	13–15	–	8–10	–	–	–
10–25	–	4–5	–	–	–	8–13	–	11–13	–	–	–
2–4	–	–	8–12	–	–	16–20	–	–	11–13	–	–
4–6	–	–	15–20	–	–	13–15	–	–	8–10	–	–
10–25	–	–	4–5	–	–	8–13	–	–	11–13	–	–
–	5–6	4–5	–		–	–	13–15	13	–	–	–
–	5–6	–	4–5	–	–	–	13–15	–	11–13	–	–
1	–	–	4	5–6	–	13	–	–	13	8–10	–
1	–	–	0.08–0.1	–	15–20	16–20	–	–	0.25–0.5 kg	–	7

gc, grass carp; bc, black carp; sc, silver carp; bhc, bighead carp; cc, common carp; wf, Wuchang fish (*Parabramis pekinensis*).

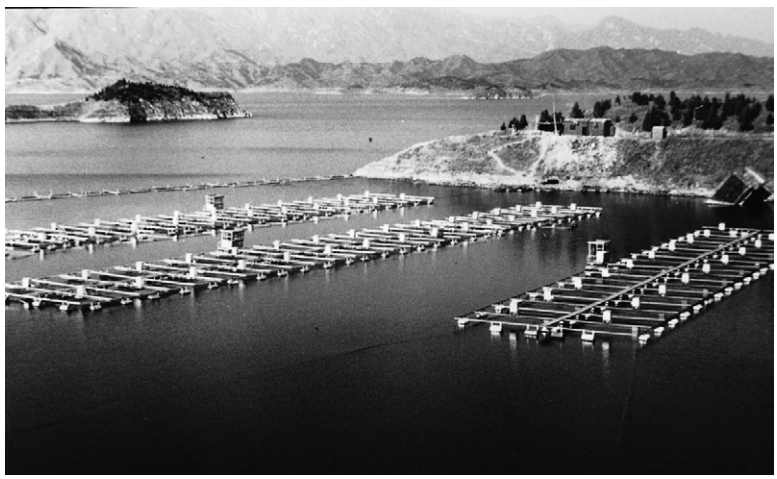


Fig. 14.5 Intensive culture of common carp in cages in a reservoir in China. Each cage is equipped with an automatic feeder.

from ensuring that most of the food resources in the system are efficiently used, offers other advantages, including higher yields, reduced susceptibility to disease and better growth rates of some species than in monoculture.

Polyculture maximises the synergistic fish–fish and fish–environment relationships and minimises antagonistic relationships. Milstein (1992) dealt in detail with synergistic interactions in polyculture systems and pointed out that synergism among fish species can be explained on the basis of two inter-related processes: increase in food resources and improvement of environmental conditions. However, antagonistic interactions occur between incompatible species and when stocking rates are unbalanced.

A concerted experimental effort occurred in India to develop suitable polyculture systems using both Chinese and Indian carps. This concept was termed composite fish culture. The basic species combination in Indian composite polyculture was catla, rohu, mrigal, silver carp, bighead carp and common carp. When stocked at a density of 5000/ha (120–250 kg/ha) the yield was nearly 9 t/ha/year when fertilised and provided with simple supplemental feed, such as a mixture of rice bran and oilcake.

Despite various experimental findings in both China and India, farming activities tend to depend on the indigenous species of each country. This trend is primarily influenced by the preferred consumer acceptance of indigenous species.

In China and India, where carp culture is the predominant form of fish culture, two or three species of either Chinese or Indian carps are polycultured. In these polyculture systems, the dominant species in China is silver carp, and in India it is rohu. The actual culture practices vary from region to region and country to country. The primary variables are the size at stocking, stocking density, fertilisation regimes, and the nature and quantity of supplementary feeds. In Chinese systems, for example, fingerlings are generally stocked at a size of about 15–20 g (>10–12 cm total length). In Andhra Pradesh, India, rohu are stocked in grow-out ponds when they are more than 2 years old (between 80 and 100 g), as it is believed that this is when they approach their maximum growth rate.

Over the past two decades, the main carp culture countries have developed their own culture protocols. This is best exemplified in Andhra Pradesh, a coastal state in south-east India. In this state, only two species of Indian major carps, catla and rohu (the latter being the dominant species at 80%), are cultured. The ponds often exceed 1 ha and are stocked at a density of 5000 fish/ha with 6- to 12-month-old (100–150 g) juveniles. Ponds are generally fertilised with poultry manure and inorganic fertiliser, and are provided with supplementary feed, often consisting of simple mixtures of rice bran (de-oiled) and oilcake (mustard, peanut). The feed mixture is suspended in perforated polythene bags from bamboo poles at several locations in the ponds (20–25 poles/ha), from which the fish soon learn to feed. In this region, production averages about 8000 kg/ha with a range of 5300–14 620 kg/ha. Fish are harvested when they are over 1.5 kg.

In China, on the other hand, polyculture is practised with Chinese carps in conjunction with common carp. There are also significant differences in regional culture practices within China. The most important difference is the dominant species in polyculture systems. For example, grass carp is the main species used in southern China, whereas silver and bighead carp dominate in central China. Li (1987) pointed out that in the recent years there has been a trend towards increasing the proportion of grazing fish such as grass carp, black carp and blunt snout bream (*Megalobrama amblycephala*), and a corresponding decrease in filtering fish such as bighead and silver carp.

More importantly, carp pond culture in China and, more often than not, in other East Asian nations, is integrated with other forms of animal husbandry, such as swine, duck and poultry. Li (1987) considered the energy structure and efficiency of a typical Chinese fish farm integrated with animal husbandry, consisting of a comprehensively managed system of aquaculture and agriculture. The structure of such a system is schematically depicted in Fig. 14.6. Accordingly, the crop subsystem produced feed for the animal husbandry subsystem (e.g. a 2.0-ha dairy farm

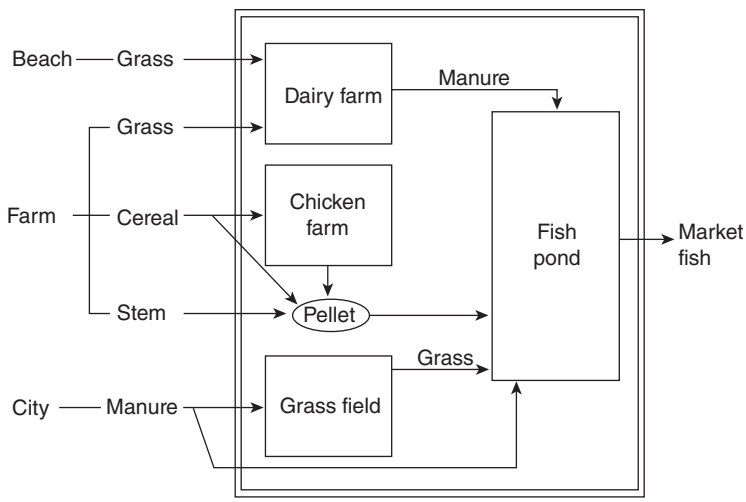


Fig. 14.6 A schematic representation of the interactions in an integrated carp pond culture and terrestrial animal rearing system. Reprinted from Li (1987) with permission from Elsevier Science.

with 182 cattle and 2.0-ha brooder chicken farm) and ingredients for the preparation of pelleted fish feed. The aquaculture subsystem consisted of 18.3 ha of grow-out ponds, 5.5 ha of nursery ponds and 3.5 ha of ponds for cultivating pearl mussels. Manure from the dairy farm was used as fertiliser for the fish ponds and chicken manure as a component of the pelleted feed. The net yield from this system was 142 070 kg (168 151–26 081 kg), equal to 7760 kg/ha/year (Table 14.8). More importantly, such systems are energy saving and sustainable over the long term.

Table 14.8 Initial number and weight of fingerlings stocked, and gross yield, from 38 ponds at Nanhui Fish Farm, China. Reproduced from Li (1987) with permission from Elsevier Science.

Species	Number	Weight (kg)	Gross yield (kg)
Silver carp	116 870	8 766	77 858
Bighead carp	20 620	1 684	15 631
Crucian carp	68 570	1 719	14 551
Tilapia	25 600	26	227
Grass carp	15 950	4 167	16 101
Common carp	55 560	4 167	21 355
Bream	56 570	3 337	9 540
Others		250	12 888
Total		26 081	168 151

The wide range of culture practices adopted in carp culture, within and between regions, makes it almost impossible to assess the potential yield from any one practice. For example, Chen *et al.* (1995), in their study of 1013 ponds on 101 farms in eight provinces in China, classified the farms into three productivity classes based on net yield. The mean yield in low-, medium- and high-productivity farms was 3321, 4981 and 7958 kg/ha/year respectively. A detailed study on carp farming systems in Andhra Pradesh, India, was conducted by Veerina *et al.* (1999), who observed two operational systems that used two species (rohu and catla) or three species (rohu, catla and mrigal). There was a very wide range in the yields among farms, and the gross yield ranged from 1730 to 14 830 kg/ha/year. However, there was no evidence to indicate that two- or three-species systems performed better than the other (Table 14.9).

14.5.3 Food and feeding

14.5.3.1 Natural food availability

In view of the fact that the great bulk of carp culture is semi-intensive, increasing the availability of natural food types in the culture systems plays a crucial role in enhancing yields. As pointed out earlier, the commonest method used for increasing natural food supply in carp ponds is through the application of common fertilisers and/or organic manures. The commonly used organic manures include cow dung, poultry litter and pig dung, and the inorganic fertilisers are superphosphate and ammonium sulphate.

Table 14.9 Mean size (kg) at harvest and production (kg/ha/year) of carp farming systems in Andhra Pradesh, India. The ranges in harvesting sizes and production are given in parentheses.

	Two-species systems		Three-species systems	
	Size (kg)	Yield (kg/ha/year)	Size (kg)	Yield (kg/ha/year)
Gross yield	–	5 900 (1 730–11 112)	–	5 857 (1 730–14 830)
Rohu	1.8 (0.8–3.2)	4 109 (1 580–7 710)	1.7 (1.0–2.8)	3 690 (1 850–9 270)
Catla	2.7 (0.9–5.4)	1 794 (440–4 690)	2.4 (1.2–5.1)	1 612 (400–3 710)
Mrigal	–	–	1.7 (1.0–3.0)	550 (60–1 660)
Net yield		5 378 (1 360–10 380)	–	5 296 (1360–14 620)
Rohu	–	3 835 (1 380–7 120)	–	3 352 (1580–9 120)
Catla	–	1 544 (440–4 690)	–	1 520 (370–3 420)
Mrigal	–	–	–	468 (60–1 410)

Modified from Veerina *et al.* (1999).

There have been many studies conducted on the effects of fertilisation and manuring in carp polyculture practices. However, apart from the fact that such practices result in increased algal production, it is impossible and impractical to make a set of general conclusions from the findings. This is for several reasons, the foremost among these being the following:

- differences in the fertilisation and/or manuring regimes used in the different studies;
- different stocking densities and species combinations used;
- climatic differences;
- management differences used (such as the time of fertilisation and/or manuring, length of trials, etc.).

Hepher (1988) presented the most comprehensive review of the influences of manuring and fertilisation of fish ponds on the production of herbivorous and omnivorous fish. Further studies have specifically considered manuring and fertilisation of ponds with polycultured carp. For example, the efficacy of fertilisers, such as rock phosphate, has been considered in polyculture systems with common carp and Indian major carps. There was also manipulation of the ratio of surface to bottom grazing species (Sahu and Jana, 1996). A ratio of 1 : 3 between surface feeders (catla, silver carp and rohu) and bottom grazers (mrigal, common carp and *Puntius sarana*) yielded the best results in ponds that received 100 kg/ha of rock phosphate, twice monthly. Sahu and Jana (1996) suggested that the bioturbation activity due to dominance of bottom grazers induced a greater release of phosphorus from bottom sediment, which resulted in higher phytoplankton production. This was used by surface feeders and resulted in better growth and production of them.

Carp culture may also benefit from the provision of suitable substrates for periphyton growth in rearing ponds.

14.5.3.2 Supplementary feeds

The supplementary feeds used in carp culture are diverse. Most supplementary feeds are simple mixes of agricultural by-products, which are readily available at a relatively low cost. The most common of these are brans of rice and wheat, often mixed with cakes or meals of various oilseeds such as mustard, canola and soybean (Table 14.10). Most farmers tend to use some sort of supplementary feed, which could be either a single ingredient or a mixture of two or three at most. The quantity of feed as well as the amount of individual ingredients used in the feed mixes may vary greatly (Table 14.10). Obviously, this is an area that needs further research, which in the long run could reduce feed use and thereby increase profitability. In addition, it could also lead to improved water quality in the ponds and cleaner pond effluent. This trend is indicative of a potential constraint to expansion of culture activities due to increasing competing demands for the same food ingredients from other animal husbandry activities and from other users (Veerina *et al.*, 1999). In Chinese polyculture, a wide range of ingredient mixes is also used as feed, the type and quantity often being dictated by availability and price. Soybean meal, sesame cake, silkworm pupae powder and canola meal are more commonly used in major carp farming systems in China.

Table 14.10 Supplementary feed combinations used in Andhra Pradesh (India) carp farming systems.

Ingredient	Percentage of farmers	Input (kg/ha/year)	
		Range	Mean (SD)
DOB	8	5 000–33 000	2 030 (7091)
DOB + CSM	4	19 000–40 000	25 280 (8187)
DOB + DOC	3	10 000–30 000	24 830 (5548)
DOB + PNC	75	5 000–50 000	27 650 (6849)
DOB + RB	1	22 000– 22 260	–
DOB + SM	1	34 000–59 000	46 800 (2293)
RB + DOB	8	20 000–39 000	29 790 (7 148)
No feed	1	0	0

The data are based on a survey of 189 farms, and modified after Veerina *et al.* (1999).

DOB, de-oiled rice bran; CSM, cotton seed meal; PNC, peanut cake; RB, rice bran; SM, soybean meal.

The 'nutritional dynamics' of semi-intensive systems are complex and incompletely understood. For example, there is very little information on the extent to which the supplementary feeds provide direct nutrition to the target species. A schematic representation of the qualitative changes that are expected to occur in a semi-intensive culture pond and the potential utility of supplementary feeding strategies is provided in Fig. 14.7.

On the other hand, because the great bulk of carp farming depends on simple supplementary feeds, when compared with other cultured fish species, there have been relatively few attempts to develop pellet feeds, except for common carp. Barlow (2000) predicted that, in 2010, 50% of carp culture (predominantly common carp) will be based on commercial feeds and will require 675 000 t of

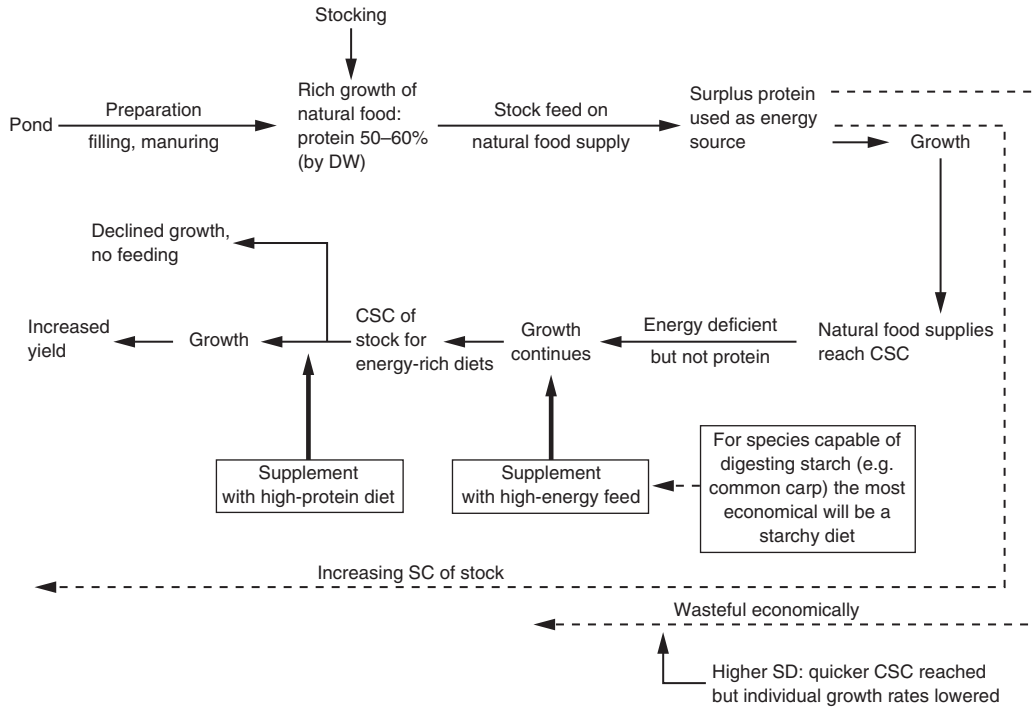


Fig. 14.7 A schematic representation of the qualitative changes that are expected to occur in a semi-intensive culture pond, and the potential utility of supplementary feed strategies. CSC, critical standing crop; SC, standing crop; SD, stocking density. Reprinted from De Silva (1995) with permission from the FAO.

fish meal. That is, the fish meal requirement for carp culture will amount to approximately 24% of the total fish meal requirement for the aquaculture industry in 2010.

Despite the estimated fish meal requirement for carp culture, and that required for culture of carnivorous fish species, research into the potential of alternative protein sources in feeds for fish is relatively limited (section 8.7.3). Substituting fish meal protein in feeds with plant protein has so far been unsuccessful.

14.5.3.3 Feeding

In general, feed management is relatively poor in carp culture. The main reason for this is that most practices depend on supplementary feeds, which are simple mixes of agricultural by-products. Perhaps the only exception is the feeding practice adopted in common carp farming in eastern Europe and in some parts of Asia. A wide range of feeding practices are used by carp farmers, from simple hand broadcasting to tying perforated bags containing food to sticks in the pond and allowing the stock to obtain feed through the perforations. It is almost impossible to assess the food lost in the latter practice, and indeed it is possible that the food has a greater effect as a fertiliser than providing direct nutrition to the fish.

Mixed feeding schedules, namely use of different feeds at different feeding times, have been found to have beneficial effects on growth and food cost reduction. However, there is little information on its use in farm practices.

14.5.4 Harvesting

Pond-cultured carps are harvested when individual fish reach a weight of about 1–1.5 kg unless they are destined for a specialised market. Harvesting in most instances is done by seining, and is labour intensive. Major carp culture ponds are rarely flow-through and are rarely completely drainable either. These factors together with the large size of ponds make harvesting by seining almost an imperative. Major carps are generally marketed fresh. It is common to retain portions of the catch alive in temporary net pens to minimise market saturation within a short



Fig. 14.8 Temporary net pens used for retaining the harvest for a few days to minimise an oversupply of fish in rural China.

period of time (Fig. 14.8). This practice is followed in most rural areas when the distances to population centres are high and the total production in an area does not justify transportation to such centres.

14.6 DISEASES

It has to be conceded that, in the past decade or so, disease-related research in aquaculture has focused mainly on shrimp culture, because of its economic importance (Chapters 10 and 19). However, carps, like most fish, are susceptible to infectious (viral, bacterial, fungal and parasitic) as well as non-infectious diseases such as carcinomas, injuries, swim-bladder disorders, etc. (section 10.5.3). There are also environmentally induced disorders caused by excessively high or low pH, nitrogenous wastes, heavy-metal pollution, etc. A very comprehensive monograph on carp diseases, diagnostic procedures and treatments was provided by Hoole *et al.* (2001), and readers are recommended to refer to this rather extensive work.

14.7 GENETIC IMPROVEMENT

As described in Chapter 7, apart from the genetic improvement of salmonid stocks and, more recently, the Nile tilapia (*Oreochromis niloticus*) and channel catfish, in general genetic improvement of cultured fish and shellfish species has lagged far behind that of farmed terrestrial animals.

Some of the cyprinid species, in particular common carp and crucian carp (*Carassius auratus*), have been domesticated for centuries. This domestication and consequent selection have resulted in the development of several strains, generally selected for aesthetic purposes rather than to augment food fish production. Examples of this are discussed in section 7.2 in relation to selection for scale type in common carp.

The lack of genetic selection of cultured stocks for favourable traits, and inbreeding of hatchery stocks, have come to light since the early 1990s. Eknath and Doyle (1990), in their study on 18 hatcheries in the south-west state of Karnataka, India, showed that the rate of inbreeding of the Indian major carps ranged from 2% to 17% per year. They suggested ways of reducing further accumulation of inbreeding in cultured broodstock of these species. The genetic resources of Indian major carps were reviewed recently (Reddy, 1999). This synthesis included biochemical and molecular genetic studies, hybridisation among Indian major carp and common carp, chromosomal manipulations and selective breeding. A concerted effort is currently being made on rohu and catla from founder populations in different river systems. A cooperative research programme for selection in five cultured carp species in Asia is ongoing, and a synopsis of the traits to be selected and the methods of selection adopted are given in Table 14.11 (Anon., 2000).

14.8 ECONOMIC VIABILITY

As carp culture is the largest and most widespread practice of animal aquaculture in the world, and since carps contribute most of the world's inland aquaculture production, it is implicit that carp culture is economically viable. If this were not the case, it would not have developed and continued to develop to the extent it has, despite the relatively low market price of the product. On the other hand, there have been very few studies on the economics of carp culture practices. In a broad study encompassing 101 farms in eight provinces of China, Chen *et al.* (1995) recognised that the stocking model used was related to the economic status in the region. Generally, farms in poorer areas tended to stock predominantly filter feeders, as

Table 14.11 A summary of the breeding programmes that are ongoing for cultured carp species in Asia, under the auspices of the International Network of Genetics in Aquaculture. Reproduced from Anon. (2000) with permission from WorldFish Center.

Country	Species	Traits	Selection method
Bangladesh	Silver barb (*Barbodes gonionotus*)	Size at harvest	Mass
	Catla	Size at harvest	To be decided
China	Common carp	Size at harvest	Mass
	Blunt snout bream (*Megalobrama amblycephala*)	Size 1 (3 cm)	Mass
		Size 2 (10–15 cm)	Two separate lines
		Size at harvest (ca. 300 g)	
India	Rohu	Size at harvest	Combined mass
	Common carp	Size at harvest/late maturity	To be decided
Indonesia	Common carp	Size at harvest	To be decided
Thailand	Silver barb	Size at harvest	Mass
	Common carp		Mass
Vietnam	Common carp	Size at harvest (early survival)	Family
	Silver barb	Size at harvest	Mass

opposed to 'feeding fish', such as grass carp and black carp (*Mylophryngodon piceus*) in the more well-to-do areas. A balance sheet of the production systems (Table 14.12) indicates that the mean profitability in low-, medium- and high-production systems was 4188, 2650 and 8068 yuan/ha/year respectively. Interestingly, the medium-production systems were less profitable than the low-production systems, and this possibly reflects relatively high labour inputs in the former.

Veerina *et al.* (1999) conducted an analysis of production factors of the carp farming systems in Andhra Pradesh, India, to assess the inter-relationships among farm inputs and fish yield. The analysis showed a strong relationship of fish yield to stocking density, buffalo manure and poultry manure inputs, and the amount of peanut oilcake (a supplementary feed) used. In addition, other supplementary feeds and additives, such as salt and mineral mix, were found to have a positive influence on fish yield. Management inputs, such as the rate of water exchange, frequency of medication and the mean weight at stocking, also had a positive influence on fish yield. Older ponds were more productive, whereas sociological variables such as the age and literacy level of the farmer had less influence on fish productivity.

14.9 CULTURE-BASED FISHERIES

Culture-based fisheries fall within the realm of aquaculture, with regard to the intervention in the life cycle and the defined ownership, either singly or collectively, of the stock. Culture-based fisheries are considered to have very high potential, particularly in the light of increasing demand for primary resources such as land and water. They are often recognised as an important avenue for increasing inland fish production, particularly in developing countries (De Silva, 2001). Culture-based fisheries use existing water resources, which may be natural oxbow lakes or water resources created for other purposes, such as reservoirs and farm dams. As such, they compete minimally, if at all, with other uses. Moreover, culture-based fisheries often do not involve external inputs, such as feed, and therefore are more environmentally 'friendly' than traditional aquaculture practices.

Apart from these factors, culture-based fisheries have significant relevance for carp culture, because the majority of such fisheries are based on Chinese and Indian major carps, occasionally augmented with tilapia and other minor species.

The culture-based fisheries in China are the most developed in the world. This fishery is confined to small- and medium-sized reservoirs throughout the country. It is estimated that the fishery yielded 815 100 t in 1995 from 1 515 600 ha of reservoirs (Ceng and Zhang, 1998), approximating 538 kg/ha/year. Song (1999), who considered the culture-based fishery in reservoirs as a separate entity, estimated the total production from this activity in 1997 as 1 165 075 t (from a total area of 1 567 971 ha),

Table 14.12 Balance sheet of the integrated carp farming systems in China. All weights and monetary values are in kg/ha/year and yuan/ha/year. Standard deviations are given in parentheses. Low, medium and high refer to a classification based on level of productivity. Reproduced from Chen *et al.* (1995) with permission of John Wiley & Sons.

	Low	Medium	High	Overall
Fish yields				
Grass carp	999	1468	2444	1865
Filter feeders	2125	3032	3716	3168
Black carp	3	53	513	282
Omnivores	638	1119	2766	1865
Gross yield	3765	5672	9439	7180
Stocking weight	444	690	1481	1045
Net yield	3321 (1498)	4982 (2006)	7958 (3113)	6135 (3250)
Revenues				
Grass carp	3514	4245	8721	6416
Filter feeders	4500	4761	6626	5677
Black carp	6	134	2808	1508
Omnivores	2057	2926	7901	5325
Animal production	101	591	30	175
Other income	16	221	121	118
Total income	10194 (5852)	12878 (5142)	26207 (13117)	19219 (12638)
Costs				
Fish stocking	1597	1903	4768	3332
Animal stocking	27	179	11	53
Feed	1889	2112	6388	4303
Fertiliser	359	520	298	364
Fuel	218	195	516	369
Miscellaneous	664	1468	2154	1630
Total (non-labour)	4754 (4397)	6377 (2881)	14135 (7903)	10051 (7432)
Net income	5440 (4397)	6501 (3769)	12072 (7586)	9168 (6905)
Labour	1252 (697)	3851 (3021)	4004 (3887)	3289 (3390)
Profit	4188 (4482)	2650 (3871)	8068 (7323)	5879 (6488)

approximating a production of 743 kg/ha/year. It was also pointed out by Song (1999) that the culture-based fishery recorded an annual growth rate of 52% between 1979 and 1997.

The culture-based fishery practices in China are based primarily on the major Chinese carps, grass carp, bighead carp, silver carp and common carp. In addition, species such as the Wuchang fish (*Megalobrama amblycephala*), black bream (*Megalobrama terminalis*) and mud carp (*Cirrhina molitorella*) may be used. In southern Asia, culture-based fisheries are primarily based on a combination of Chinese and Indian carps, the latter being predominant. De Silva (2001) recognised several features that are responsible for the immense success of the culture-based fishery practices in China. Foremost of these are the following:

- consideration (at the planning stage of reservoir construction) of those factors that enhance fishery production;
- relatively large and uniform size of fish at stocking;
- minimising the number of escapees;
- a staggered but complete harvesting of the stock;
- adoption of marketing strategies that minimise an oversupply of fish within a narrow time-frame.

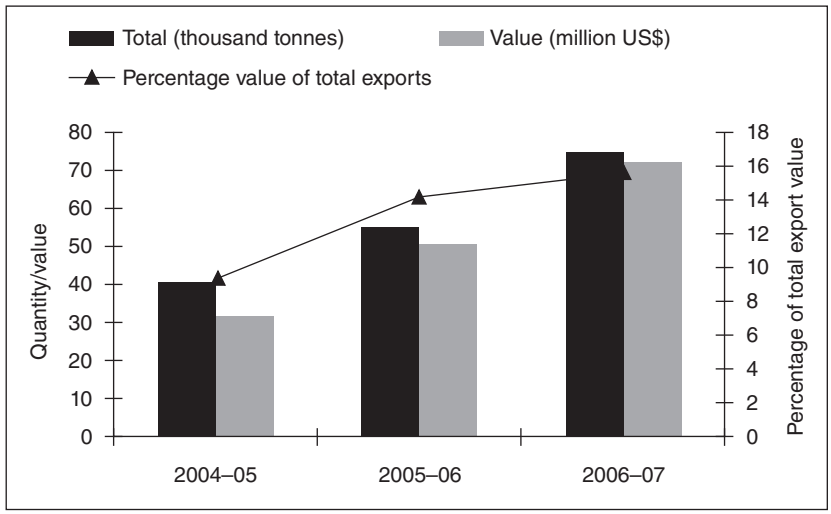


Fig. 14.9 Production and export value of rohu in Myanmar and its percentage contribution to fishery exports from Myanmar (note that the bulk of Myanmar fishery exports are based on marine fisheries).

14.10 RECENT DEVELOPMENTS IN CARP CULTURE

Cultured cyprinids are considered to be a relatively low valued group, often commanding a farm gate price less than US$2.00. Until recently they were consumed locally, barring export to a few neighbouring countries (De Silva, 2008). This situation is changing rapidly for at least one farmed cyprinid species, the Indian major carp, rohu, through an initiative that originated in Myanmar.

Rohu culture in Myanmar until recently catered exclusively for local markets with a small quantity exported, whole fish in ice, to Bangladesh. However, over the past five years rohu exports from Myanmar have increased up to 60 000 t, valued at approximately US$53 million (Fig. 14.9). The current rohu culture practices, which are semi-intensive, have been described by Ng *et al.* (2007) and Aye *et al.* (2007). They essentially consist of large earthen ponds, stocked at a size of 50 to 60 g and harvested at 1 to 2 kg size after 8 to 10 months growth (Fig. 14.10). The development of an export market for rohu has essentially exploited a 'niche market' for this species within expatriate Indian and Bangladeshi communities, at first in Middle East and later in Europe as well. Most importantly, the developments in the export markets for rohu, which is normally gutted, cleaned, glazed and frozen whole, has created many livelihood opportunities for rural women in the processing plants (Fig. 14.11), with significant impacts on the socio-economic conditions of poor communities.



Fig. 14.10 A harvest of rohu being brought by boat to be loaded into lorries for transport to processing plants.



Fig. 14.11 Processing of rohu for export in Myanmar.

14.11 CONCLUSIONS

This chapter has highlighted the importance of carp culture, some of the key features of carp culture practices and the potential of carp culture as a food source. Living standards are generally increasing throughout the world, and it is often suggested that the demand for carp species will gradually decline as a result. However, production trends do not support such a contention. On the other hand, in the light of increasing environmental concerns related to the culture of carnivorous fish species, it may be that forms of carp culture could become even more important in the coming years. One of the major constraints to further intensification of carp culture is increasing competition for supplementary food sources, which are primarily agricultural by-products. As such, a concerted effort may be required to develop suitable feeds and to develop more prudent strategies of feed management. Apart from yield increases through intensification and better pond culture practices, popularisation and development of culture-based fisheries using carp species appear to have the greatest potential to augment inland fish production. This is particularly important to developing countries, thereby making available a good-quality source of animal protein, at an affordable price, to the poorer sectors of the community.

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15
Salmonids

John Purser and Nigel Forteath[1]

15.1 INTRODUCTION

Worldwide, there are numerous species and strains of salmon, trout and charr within the family Salmonidae. Many of the important commercial species belong to the genera *Salmo*, *Oncorhynchus* and *Salvelinus*, examples of which are listed in Table 15.1. They are all either anadromous (ascending rivers from the sea to breed) or undertake their entire life cycle in freshwater. No species is solely marine. One tends to associate salmonids with aquaculture, but fishieries are also a substantial component of their production (Table 15.2). Atlantic salmon is the most important species in aquaculture, followed by rainbow trout, which is very largely produced by aquaculture (Table 15.3). Together they constitute the bulk of aquaculture production of salmonids. Pink salmon, sockeye salmon and chum (Pacific salmon species) are also three important species in terms of global production, but they are essentially derived from fisheries. More recently, there has been a significant research focus on Arctic charr.

There has been a large increase in salmonid production from both aquaculture and fisheries, especially the former. Unlike many of the major products from aquaculture, there is substantially more aquaculture production of salmonids in developed countries than in developing countries. Even more, China is only a minor contributor. An outstanding rate of increase of 10.2% over the two decades from 1987 to 2007 is largely due to increased production in the developed countries. It contrasts with the overall increase in aquaculture production of 1.7% in the developed countries over this period (Table 1.1).

Freshwater facilities are usually used to rear salmonids to the fingerling or smolt stage for transfer to marine farms (seafarms), although some farms specialise in plate size (300–400 g) or larger fish reared solely in freshwater. In contrast, seafarms grow-out small fish to market size (2+ kg). Cage culture has proved to be the most successful strategy for marine production. Where there is a nearby source of spring water of appropriate quantity and quality, the fish may be reared onshore in raceways (section 2.2.5). The alternative pump-ashore facilities, whereby water is pumped into land-based culture facilities, are largely uneconomical for the production of market-size fish, but they are used to support a few broodstock for breeding programmes. In recent years, large recirculating shore-based marine systems have been proposed as a way of controlling the culture environment and avoiding threats such as predators, algal blooms and pollution experienced in cage culture (see, for example, Fig. 5.4). Similarly, cages based on solid bag net configurations offer protection from threats experienced by conventional mesh nets, but they rely on close monitoring and low-head pumping to circulate water. The solid bag excludes algae, predators and jellyfish, and allows control of lighting using covers and temperature by altering the depth of seawater intake.

In this chapter, general husbandry practices and techniques used for rearing salmonids are outlined. It is acknowledged that many variations of these exist and that

[1]The authors dedicate this chapter to Dr Lindsay Laird (1949–2001), who worked closely with the salmon industry in Europe and who made a significant contribution to salmonid research, teaching and training.

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Table 15.1 Major salmonid species that are fished and cultured.

Common name	Scientific name
Arctic charr	*Salvelinus alpinus*
Atlantic salmon	*Salmo salar*
Brook trout/charr	*Salvelinus fontinalis*
Brown trout (sea trout)	*Salmo trutta*
Chum salmon (dog, sake)	*Oncorhynchus keta*
Chinook salmon (king, quinnat, spring, masunosuka)	*Oncorhynchus tshawytscha*
Coho salmon (silver, gin-maru)	*Oncorhynchus kisutch*
Masu (= cherry) salmon	*Oncorhynchus masou*
Pink (= humpback) salmon	*Oncorhynchus gorbuscha*
Rainbow trout (ocean, steelhead trout)	*Oncorhynchus mykiss*
Sea trout	*Salvelinus fontinalis*
Sockeye salmon (red, beni-masu)	*Oncorhynchus nerka*

Table 15.2 Global salmon production (t) from aquaculture and fisheries in 2007 showing percentages of totals in brackets.

Species	Aquaculture	Wild harvest	Total (t)
Atlantic salmon[a]	1 433 708 (99.8%)	2 989 (0.2%)	1 436 697
Other salmonids	822 153[b] (44.5%)	1 025 636[c] (55.5%)	1 847 789
Total	255 861 (68.7%)	1 028 625 (31.3%)	3 284 486

[a]Data only for the Atlantic salmon.

[b]Data based on 11 species, especially rainbow trout.

[c]Data based on 12 species, especially chum, pink salmon and sokeye salmon.

Table 15.3 Global production data of major salmonid species from aquaculture and fisheries in 2007.

Species	Production (t)
Atlantic salmon	1 436 697
Rainbow trout	608 787
Pink salmon	495 986
Sockeye salmon	164 222
Coho salmon	132 576
Chum salmon	48 638
Sea trout (*S. trutta*)	25 554
Chinook salmon	20 448

Table 15.4 Countries producing 20 000 or more t/year of Atlantic salmon and/or Rainbow trout by aquaculture amd fisheries in 2007.

	Atlantic salmon (t)	Rainbow trout (t)
Australia	24 988	
Canada	117 306	
Chile	379 466	175 139
Denmark		27 657
France		32 288
Germany		23 174
Italy		39 800
Norway	736 168	77 578
Spain		25 195
UK	130 104	
USA		23 900
Total world production	1 433 708	608 787

strategies differ between farms, countries and species cultured. The basic life cycle and production techniques for salmonids as a group are, however, very similar. The life-history characteristics of three commercial species are used to illustrate these similarities and differences. This section is followed by a discussion of freshwater and marine husbandry techniques for salmonids using Atlantic salmon production in Tasmania, Australia, as the prime example.

Approximately 60 countries are involved in culturing salmonids. The major producing countries of Atlantic salmon and rainbow trout, the two species that together constitute almost 90% of salmonid production, are shown in Table 15.4. International salmonid production and general culture techniques are covered comprehensively in the excellent texts of Laird and Needham (1988), Heen *et al.* (1993), Sedgwick (1995), Pennell and Barton (1996) and Willoughby (1999).

15.2 BIOLOGY

15.2.1 Distribution of species

The natural range of the Salmonidae covers freshwater lakes, rivers and marine coastal regions of the Northern hemisphere temperate zone. However, their range has been greatly extended as a result of a demand from recreational fisheries and aquaculture. The major species and their native regions are as follows:

- rainbow trout (*Oncorhynchus mykiss*) – Pacific coast of North America;
- Atlantic salmon (*Salmo salar*) – Atlantic Ocean from the latitude of northern Spain to Iceland;
- Pacific salmon species (*Oncorhynchus* species) – Pacific basin between 35 °N and 65 °N;
- brown trout (*Salmo trutta*) – Eurasia and North Africa;
- brook trout (*Salvelinus fontinalis*) – north-eastern North America;
- Arctic charr (*Salvelinus alpinus*) – highlands of central Europe, northern Britain, Scandinavia and the Arctic basin.

15.2.2 Life cycles of three farmed species

A generalised Atlantic salmon production cycle is illustrated in Fig. 15.1, which shows an outline of the life history and terminology used to describe the various stages.

15.2.2.1 Atlantic salmon (Salmo salar)

The life cycle of Atlantic salmon is typically anadromous, although there are some landlocked strains. Mature fish return to freshwater during the 12 months before spawning, which usually takes place in late autumn and early winter.

1. The female uses body movement to make a 'redd' (a hollow) in silt-free gravel. The redd varies between 2.5 and 15 cm in diameter and the redd is positioned to receive a relatively rapid water flow of at least 0.5 m/s. Male fish (Fig. 15.2) fertilise the eggs as they are released by the female into the redd, by releasing milt into the water column. Female salmon produce about 1500 eggs/kg body weight. The ova are buried to a depth of 30–40 cm by the female's body movements.
2. On hatching, the alevins remain within the redd, using their substantial yolk reserves and showing a negative phototaxis. Their movement within the redd is probably negligible and little, if any, energy is spent maintaining their position. This allows maximum growth on yolk reserves, an important factor for subsequent survival.
3. Emergence of fry from the gravel occurs in the spring about 800 degree–days after fertilisation (e.g. 80 days at 10 °C or 100 days at 8 °C). The fry are carnivorous and territorial. As they grow, fry develop 'thumb-print' lateral markings that are characteristic of the parr stage.
4. The parr stage may last for up to 8 years in cold environments, such as northern Norway, or as little as 1 year towards the southern part of the natural range of the species. During the parr stage, some males may mature early as '0+ fish' ('precocious' male parr) and may play an important role in fertilising ova from adult females.
5. The parr metamorphose into the smolt stage during smoltification or the parr–smolt transformation. The process is initiated several months before the seaward migration, usually at an age of 1–3 years. The smolts, which may weigh up to 60 g or more, enter the sea in spring and summer, depending on latitude. The smolts enter the sea in spring at the more southerly distributions in the Northern hemisphere. Once at sea, the fish migrate to rich feeding grounds, although feeding occurs throughout the migration. Several of these feeding grounds have been discovered off western Greenland and the Faeroe Isles.
6. Some fish will return to their natal streams and rivers after 18 months in the sea. These fish are known as 'grilse' (one sea winter fish) and weigh between 1 and 7 kg. The remaining fish may spend two to five winters at sea, returning as 'salmon' of 4–30 kg in weight.
7. Once it has spawned, the salmon is known as a 'kelt'. Females may spawn two or three times, but few males live to spawn more than once. Survival after spawning is generally low, but river conditions may play an important role in subsequent survival.

15.2.2.2 Rainbow trout (Oncorhynchus mykiss)

1. Rainbow trout spawn between autumn and spring, with females producing about 2000 mature ova/kg body weight. These hatch some 4–7 weeks after fertilisation, depending on water temperature, for example eggs will hatch within 30 days at 10 °C. Considerable egg mortality may occur at higher temperatures around 15 °C. Eggs are deposited in gravel (redds), usually in riffles.
2. Optimum temperatures for growth after hatching are between 12 °C and 18 °C. Males mature as 2-year-olds, although precocious males mature at 1 year of age, and most females mature as 3-year-olds. Age at maturity depends on growth rates. Two-year-old mature females are quite common in some strains and environments.
3. Spawning survival is low, with few individuals spawning more than once or twice in the wild. Some strains

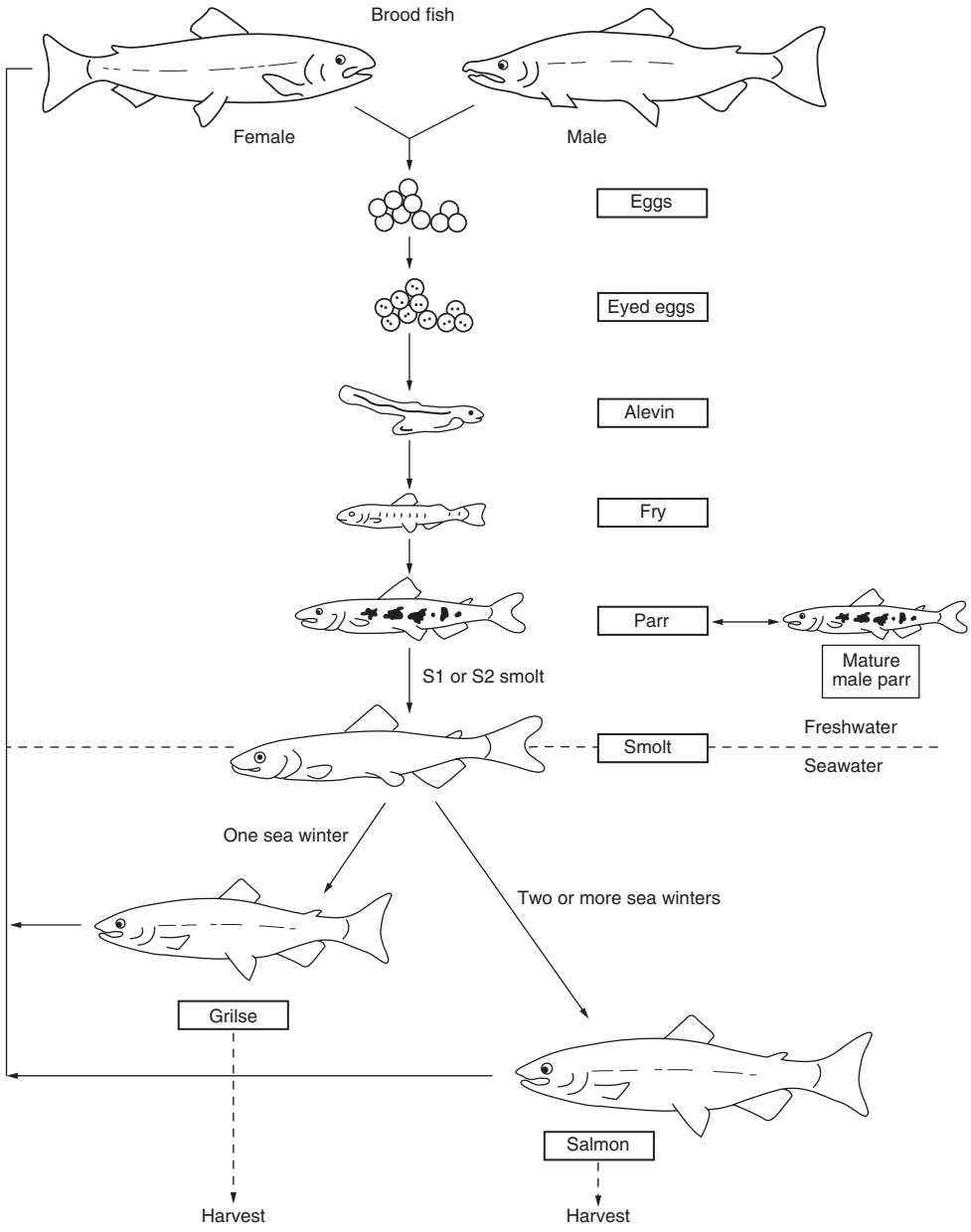


Fig. 15.1 Production cycle of Atlantic salmon, *Salmo salar*, showing specific life stages. Most anadromous salmonid species display this generalised cycle although the duration of stages is species and temperature specific (not to scale). Reproduced from Forteath *et al.* (1996) with permission from the Fishing Industry Board of Tasmania.



Fig. 15.2 Male Atlantic salmon brood fish in spawning condition.

are anadromous ('steelheads') and undertake extensive oceanic migrations, whereas others remain in freshwater throughout their lives. It is not known if anadromy is a truly genetic adaptation or simply opportunistic behaviour that is based on a genetic predisposition (Gall and Crandell, 1992).

15.2.2.3 Chinook salmon (Oncorhynchus tshawytscha)

This species has several common names, such as chinook, king, spring, tyee, masunosuka, chavycha and quinnat. The species is the largest of the Pacific salmon species and the least abundant. The name 'spring' salmon arises from the early season run of the fish entering the Columbia River in Canada. In fact, adult fish enter rivers over most of the spring, summer and autumn periods, but distinct runs are often noted.

1. Adult fish are inhibited from spawning until the temperature falls below 12 °C. An average female weighs 10 kg, although 40–50 kg specimens have been recorded. They lay between 3000 and 12 000 ova, depending on size of the female. Eggs are deposited in a redd.
2. Chinook salmon are able to migrate to sea as small fingerlings, 4 months after hatching, but some stocks remain in freshwater for 1 or 2 years as parr. Also, there are precocious males that spawn and then migrate to sea.
3. The chinook salmon is a coastal species and remains at sea for 1–7 years; the duration of the sea-going life stage is determined by geographical distribution. Female fish of the northern-most rivers remain at sea for the longest period. However, many males return prematurely as precocious adults after only one winter at sea. These are called 'jacks' (grilse). They are rarely more than 30 cm in length and weigh less than 1.5 kg. All adults die after spawning.

15.3 FRESHWATER FARMING

15.3.1 Establishing a freshwater salmonid farm

Establishing a salmonid farm requires a host of factors to be taken into account. Some of the principal considerations are the following:

- water flow and volume availability;
- water quality;
- existence of other farms upstream;
- land use upstream;
- access and location of utilities;
- topography of site;
- soil permeability;
- slope of land;
- legislation concerning water rights.

Temperature is of great importance as a controlling factor in salmonid aquaculture, and it is essential that accurate water temperature profiles be obtained throughout the year. If possible, data over several years should be available before a site is decided upon. The same must be said of water quality and flow rates. Water can be pumped or gravity fed from a bore, river, spring or lake. Water quality criteria for salmonid fish have received considerable attention, and typical data are given in Table 15.5.

Trout farmers may concentrate specifically on the production and sale of ova, fry or table-fish, but many design farms for all three. The types of building, culture system design and feeding equipment are dependent on the objective. Most salmon farms produce smolt for growing-out on seafarms, although some facilities produce large grow-out fish, eyed eggs or caviar.

15.3.2 The hatchery

15.3.2.1 General considerations

General considerations about hatchery design and operation are described in Chapter 2 (section 2.7). Water quality control in the hatchery is of paramount importance. Heavy metals, suspended solids and poor levels of dissolved oxygen (DO) are avoided at all times. Cadmium, copper, lead and zinc are all toxic to salmonid eggs, although the toxicity of these ions can be reduced by increasing the hardness of the water. In Europe and Scandinavia, adding lime to the hatchery water supply has been used to counteract pollutants such as acid rain and associated release

Table 15.5 Water composition for salmonid rearing in freshwater. Reproduced from Laird and Needham (1988) with permission from Taylor & Francis.

Parameter	Suitable range
Temperature	–0.5 °C to 24 °C (range), 4–18 °C (optimum)
Dissolved oxygen (DO)	Minimum at outflow 6 mg/L, >70% saturation
Carbon dioxide (CO_2)	Maximum 10 mg/L
Ammonia (NH_3)	<0.0125 mg/L
Nitrate (NO_3^-)	<0.1 mg/L (soft water)
	<0.2 mg/L (hard water)
NO_2-N	0.03 mg/L (soft water)
	0.05 mg/L (hard water)
Nitrogen (gas pressure)	<110% saturation
Hydrogen sulphide (H_2S)	<0.002 mg/L
Suspended solids	<50 mg/L
Chlorine	<0.03 mg/L
pH	5.5–8.5
Minerals	
Aluminium	0.1 mg/L (low pH or low calcium)
Cadmium	<0.004 mg/L (<100 mg/L alkalinity)
	<0.003 mg/L (>100 mg/L alkalinity)
Copper	<0.006 mg/L (soft water)
	<0.3 mg/L (hard water)
Iron (total)	ca. 0.15 mg/L
Iron (ferrous)	0
Iron (ferric)	<0.5 mg/L
Zinc	ca. <0.04 mg/L

of heavy metals (Needham, 1988). Waters in contact with mine tailings, etc., are avoided at all costs.

Suspended solids accumulate rapidly on incubating salmonid eggs, effectively smothering them, and some degree of filtration or settlement is usually necessary for the influent water. There is an increasing demand for oxygen as eggs develop. The influent water must be at oxygen saturation and effluent water must contain at least 6 mg/L DO. It is not unusual to experience a significant increase in biological oxygen demand (BOD) during hatch, resulting from the release of vitelline fluids and discarded egg shells. The hatching troughs generally become difficult to clean during hatch and while maintaining alevins. However, every effort is made to avoid disturbing newly hatched fish. It is not uncommon to warm hatchery water to enhance the developmental rate of fertilised ova, alevins and first feeding fry.

An increase in the level of dissolved gaseous nitrogen to above saturation is dangerous as it causes gas bubble trauma. This is characterised in its later stages by the presence of gas emboli associated particularly with the head and gills. The problem often is caused by the following:

- air leaks in intake pipes;
- air leaks in water pumps, i.e. submersible pumps sucking up air when placed in a shallow sump;
- rapid heating of the water (solubility of nitrogen decreases as temperature increases).

In the circumstances of gas bubble trauma, water must be de-gassed before entering the hatchery. The problem may arise with groundwater, which may be high in dissolved carbon dioxide or nitrogen gas. Elevated levels of carbon dioxide occur in recirculation systems and must be maintained below 10 ppm through de-gassing processes. Pure oxygen may also be added to the water before delivery to the fish, especially where high stocking densities or water recycle/recirculating conditions are used or where DO levels fall below 80% saturation, thus influencing growth.

15.3.3 Broodstock and spawning

15.3.3.1 Broodstock

Broodstock should ideally be cultured as part of a selective breeding programme based on family lines involving desirable traits. In Tasmania, such traits include amoebic gill disease (AGD) resistance, growth, flesh colouration and body shape. Atlantic salmon broodstock are maintained in freshwater for security and biosecurity purposes with the movement of fish restricted to freshwater to seafarms, minimising the risk of disease spread from seawater production to freshwater hatcheries.

On other sites, fish are simply selected from the current production run and conditioned before spawning. Where sea-grown fish are used, they are normally conditioned in freshwater for a few months before stripping (Atlantic salmon in Tasmania from February to March). Mature fish remaining in seawater will usually die, although increased survival and reconditioning may be undertaken by strategies such as photomanipulation. Male and female broodstock develop asynchronously under some environmental conditions in freshwater, and hormone preparations such

as 'Ovaprim' are used to synchronise gamete production. During the conditioning period, males and females may be kept separately or together in culture tanks or raceways (some of which are designed as continuous raceways) at low densities and in high water quality. Chemicals (pheromones) released by fish into the water may assist spawning synchrony and so some systems use water recycling through fish tanks rather than flow-through. Elevated holding temperatures in salmonid broodstock tanks can be detrimental to the quality of the developing oocytes, producing atresia pre-ovulation or poor fertilisation rates of ovulated eggs (Pankhurst *et al.*, 1996). To optimise egg viability it may be necessary to cool the water for brood fish.

Broodstock are valuable fish and must be handled carefully when checking for egg release. Stock are normally checked every few days and eggs can be strip-spawned for up to ca. 4 days after ovulation. Fish may be stripped over several days to remove all eggs from the cavity to avoid contamination by egg shells of the following year's eggs.

After stripping, many species can be reconditioned for the next spawning season. The exceptions are old fish or species of Pacific salmon, which may be euthanised before egg removal as they die after spawning.

15.3.3.2 Spawning and egg fertilisation

Spawning of Atlantic salmon in Tasmania occurs in May (autumn), whereas rainbow trout are spawned from the end of May to July (autumn–winter). Most brood fish are used over one to two seasons at 3–5 years of age, with salmon reaching 3–5 kg as first spawners and 7–10 kg as second year spawners. Although hand stripping after anaesthesia (Fig. 15.3) is the most common method, ova may also be expressed by injecting air into the body cavity using a needle (air stripping) and through dissection after euthanasia. About 1200–1500 eggs/kg of fish at a diameter of 4–7 mm are removed from the female stock of 4–14 kg. Fish are moist-towelled before stripping to minimise water contamination of eggs and premature activation of milt. Sperm cells are inactive in expressed milt, and it is important that activation through contact with water is avoided before egg fertilisation as sperm motility, once activated, occurs over a short duration of only 0.5–2 min. Consequently, to avoid sperm activation, milt may be stored during the day in syringes or small sealed bottles until it is needed or in Cortland's extender in an oxygenated environment in the refrigerator for a few days.

Under standard fertilisation techniques, ova and ovarian fluid are stripped into a dry bowl and milt is expressed onto the eggs at a ratio of ca. 1 mL: 1500–10 000 eggs and mixed before freshwater is added a few moments later. Variable fertilisation rates may occur using this technique, and some farms remove the ovarian fluid from the ova, wash with a bicarbonate solution to minimise the effects from ruptured eggs and replace the solution with an artificial ovarian fluid (saline diluent) to maintain consistency between batches before the addition of the milt. Although it should be avoided, contamination by small amounts of blood, faeces and anaesthetic does not significantly affect egg fertilisation, unlike ruptured eggs, which may impact negatively on fertilisation rates. A level of ca. 1–3% ruptured eggs in a batch caused by excessive pressure during hand stripping may reduce the fertilisation rate by as much as 80–90%. The presence of ruptured eggs is indicated by a vivid white coloration when water is added to the egg/milt mixture, indicative of the yolk precipitation which prevents spermatozoa from entering the micropyle of the egg.

Milt and ova are mixed gently by hand and allowed to stand undisturbed for 2–3 min. After fertilisation, eggs are



Fig. 15.3 Hand stripping ova into a dry bowl after anaesthesia.

washed gently with water and left undisturbed for 45–60 min to water harden. During this stage, the eggs increase in size (by about 30%) and become hard to the touch. While taking up water they become slightly 'sticky' for a short period of time. The water-hardened eggs may be counted using a variety of techniques based on egg volume or weight and laid down in the egg incubators. Salmon have larger eggs than trout numbering ca. 7000/L compared with ca. 9000/L. Eggs may be handled gently for a few hours after water hardening, but then not until the eyed-stage that occurs around 23 days for Atlantic salmon and around 17 days for rainbow trout at 10 °C. Egg fertilisation rate and survival rate may be checked at 2 days or around 7 days by the detection of the blastodisc or the neural streak (respectively) after addition of a preservative (1 : 1 : 1, acetic acid : methanol : water) to a sample of eggs.

15.3.4 Incubator systems

15.3.4.1 Factors in choice

Choice of incubator depends on the raison d'être of the hatchery. Upwellers (vertical cylinders) are preferred when large numbers of ova are being produced for sale in the 'eyed' state. Upwellers require minimal floor space and can hold up to 100 L of eggs. Water is forced up through the eggs (but without moving the eggs) to overflow over the lip at the top into a drain. Eggs may also be hatched in trays and trough systems. Trays such as the Heath system (Fig. 15.4) can be stacked in banks of up to 20 per unit and are space efficient. Water enters at the top of the stack, where it is first forced up through a perforated base supporting about 1 L of eggs spread two layers in thickness. It is then collected and gravity fed to the tray below and so on. Oxygen levels are maintained by water turbulence at the point of entry to each tray.

Troughs containing eggs baskets are often the preferred method of hatching, but these take up a considerable amount of space. Furthermore, sluggish water flows will cause detritus to accumulate on the eggs, and this may reduce oxygen availability. The Californian trough system contains up to seven baskets each holding about 1 L of eggs, i.e. 6000–8000 Atlantic salmon or 10 000–20 000 rainbow trout eggs, though higher numbers are often used. The eggs lie on a perforated base and water is forced up through them by a flange on each basket that is flush with the base of the trough.

Saprolegnia species fungus is a constant problem in hatcheries: it spreads through spores in the water from dead eggs to viable eggs. It has in the past been controlled by the bi-weekly application of zinc-free malachite green at a concentration of 2 mg/L for 1 h. However, this drug has been banned for use in many countries, being replaced by alternative treatments such as formalin, hydrogen peroxide or ozonised water.



Fig. 15.4 Vertical tray egg incubation system. Water flows from the top to the bottom of the rack through each tray.

Egg picking is possible in Californian troughs, reducing the use of chemicals, but care must be taken to create as little disturbance as possible before the eyed stage, as eggs are susceptible to handling shock and bright light (especially fluorescent lights) during this time. Also manual egg picking is very labour-intensive. Once the eggs are eyed stage, egg picking is quite safe. Mechanical pickers are used to sort healthy eggs from non-viable (opaque) ones at rates in the order of 100 000–200 000/h. Eyed eggs may first be subjected to physical shock, e.g. trays of eggs are 'banged' on the side of the troughs or poured into a bucket to rupture the shell, which turns the eggs white. This, in

conjunction with sorting, aids the hatching process, avoiding a prolonged hatch period.

15.3.4.2 Water flow

Eggs have a high requirement for DO, which increases through eyeing and hatching, and which must be supplied through adequate water flow in the incubators.

Water flow rates through the incubator systems require constant monitoring. Upwellers require considerably less water than trays or troughs. Indeed, only 25 L/min water may be sufficient in an upweller holding up to 100 L of eggs, but this will only be adequate if water quality is particularly good. Most hatcheries use 3–4 L/min water flow per 4–5 L of eggs. Water demand in a seven-basket trough system or a 16-tray incubator is approximately 15–20 L/min. Water flow in some incubators may be impaired by the accumulation of egg shells on screens requiring frequent cleaning.

15.3.4.3 Temperature control

It is becoming increasingly popular to manipulate water temperature in hatcheries to modify spawning time or to accelerate or delay embryonic development in fertilised eggs. Egg development and hatching times are based on the concept of degree-days or the number of days after fertilisation multiplied by the temperature. Bromage and Cumaranatunga (1988) described a protocol for delaying development of rainbow trout eggs, but temperature manipulation must be avoided for the first 10–12 days. Subjecting the eggs to water temperatures of 2 °C or 3 °C between days 13 and 70 results in a hatching period of about 80 days. By comparison, eggs will hatch in 30–35 days at a constant 10 °C.

Egg development in Atlantic salmon can be accelerated by increasing water temperatures within acceptable limits during egg incubation. A typical regime is 8 °C for eggs to eyed stage, and 10 °C for eyed eggs to hatch. Hatching will then take place in about 45 days. In contrast, at 3 °C, hatching will take place in about 145 days, spreading production over a longer period. Great care is taken to avoid accelerating development too rapidly as this may result in increased egg loss or poor-quality alevins.

15.3.4.4 Light

As salmonid eggs are buried in gravel in the wild, care is taken to ensure that lighting in the hatchery is limited to a few dim sources or to red lights. Ultraviolet light is harmful and daylight bulbs are avoided. Consequently, egg trays and troughs are covered and exposed to low light levels only during periods of inspection.

15.3.5 Alevins and first feeding fry

Under natural conditions, salmonid eggs are deposited in gravel and fry do not emerge from the redd until the yolk sac is all but depleted. Alevins are stressed by bright light and frantically seek sanctuary beneath each other. As with eggs, very dim or red lights are used. It is important to ensure that maximum growth is achieved during the alevin stage. First feeding fry are much easier to culture if they are about 0.2–0.7 g. This weight is best achieved by providing a suitable substrate, permitting alevins to lie quietly for long periods. Various materials have been trialled as substrates, including corrugated sheeting, meshes, artificial turf, plastic grid, gravel and plastic biomedia.

Significant differences in weight are achieved between fish supported in this manner and those forced to lie on a smooth substrate. In addition, artificial substrates may protect the alevins from suffocation caused by overcrowding (Fig. 15.5), and reduce the incidence of yolk sac constrictions. Fish are transferred from substrate in trays to larger tanks (with substrate) at about 0.16 g.

Unlike Atlantic salmon, fry of rainbow trout and Pacific salmon species swim to the water surface to inflate their swim bladder (‘swim-up’) when ready to start feeding. Water depth at this time must be shallow and not exceed 10 cm. As a rule of thumb, small quantities of feed need to be delivered at frequent intervals once 50% of the fry have ‘swum up’. A light with a 25-W red bulb suspended over the trough is often used. Once the fry are feeding, starter feed must be spread over the water surface in numerous meals to satiation or at a rate of ca. 8% body weight per day. Furthermore, feeding over a 20–22-h period with 2–4 h of darkness is beneficial as it maximises the time available for food intake and hence growth, while maintaining light–dark (LD) synchronicity. The penalty of such an intensive feeding regime may be the deterioration of water quality. Low water velocities do not allow waste to be deposited in the drain at this early feeding stage and gentle brushing of the tank bottom is necessary to remove waste materials. The fry can be damaged very easily; therefore, violent brushing or sudden increases in water flow are avoided. Extreme care is required to prevent disease outbreaks and low DO (hypoxia). Cleaning is necessary twice a day to prevent rapid increases in biological oxygen demand (BOD) and reduce bacterial loadings.

Atlantic salmon need to be weaned onto artificial diets before their yolk sacs are absorbed. It is quite difficult to

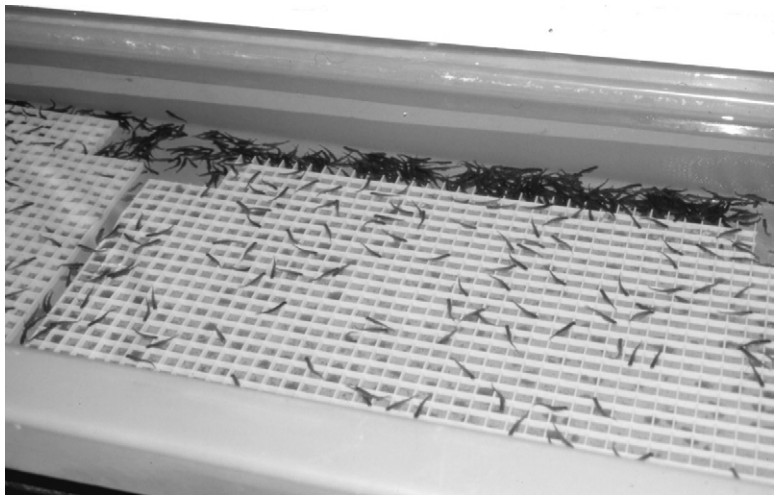


Fig. 15.5 Artificial substrate supports and spaces alevins across the trough floor compared with fish crowding induced by a smooth floor evident to the rear of this trough.

determine the best time to start artificial feeding, but useful indicators are the onset of normal dorso-ventral swimming attitude (alevins rest on their sides) and reduction of the yolk-sac to about 20% of its original size. Needham (1988) recommended regular sampling of the alevins to determine the developmental stages and to observe food particles in the gut. Moribund fry close to the water surface are often a sign of starvation. Starving fish are obvious, having large heads, emaciated bodies and black body colour. At a water temperature of 12 °C, healthy fish will start stacking in the water column by day 10 of feeding.

First feeding may be established in troughs or fry tanks. Fry tanks are favoured by large Atlantic salmon hatcheries. These tanks are usually circular, but rounded-corner square (Rathbun) tanks have been used extensively. Fry on the point of taking artificial food are carefully immersed in the tank and allowed to swim out of the shallow net or tray used for transfer. Recent designs have incorporated hatching trays and alevin incubation substrate within the alevin/fry tanks to reduce the frequency of handling and transfers.

Water temperatures between 12 °C and 15 °C are ideal for growth, but difficult to achieve unless artificial heating is available. Groundwater at 12 °C may be available in some areas, making heating unnecessary. However, this water may need to be aerated to remove high levels of nitrogen, carbon dioxide or dissolved iron and to improve oxygen levels. The farmer must strive to achieve maximum growth from a very early stage to maximise economic production in the grow-out phase.

Water temperature should ideally remain constant during the first 2–3 weeks of feeding. Light levels remain critical and must be <50 lux at the water surface. Lights not only serve to allow feeding, but also ensure a relatively even distribution of the fish over the tank bottom. In complete darkness, fry tend to form dense congregations, which can result in damage to fins and opercula.

Stocking levels used in Rathbun tanks are between 15 000 and 20 000 fry per tank or around 250 000 in larger 6-m diameter tanks. First feeding fry benefit from moderate crowding, which appears to encourage feeding in these early stages. The water flow rate initially is about 25 L/min. Stocks of rainbow trout and Atlantic salmon fry may need to be graded 4–5 weeks after first feeding.

At this point there is a major divergence in farming aims and objectives for the grow-out phase of the two species. The rainbow trout farmer will aim to grow fry to market size according to a plan designed to satisfy weekly production demands or to produce fish of 300–800 g for grow-out in seacages. The Atlantic salmon farmer will endeavour to ensure that as many fish as possible will become smolts. In both cases, husbandry of stocks in the first 2–3 months after feeding is of great importance if subsequent goals are to be achieved.

15.3.6 Culture systems for juveniles

15.3.6.1 Vessels

Many 'low-cost' trout farms keep their stock in pond systems, which are usually based on the Danish earthen pond system. Construction is relatively cheap, but little attention is sometimes paid to design efficiency. Current flow can be irregular, uneaten food and faeces accumulate on the bottom, and the sidewalls are susceptible to erosion. The recommended design dimensions for ponds are 30 m × 10 m × 1 m sloping to 1.5–2 m depth at the outflow. A Herrguth-monk is commonly used at the outlet, although wide-bore pipes are sometimes installed. The latter increase the area of dead spots in the pond. Stocking rates in earthen ponds are less than in raceways or tanks.

A typical raceway design (Fig. 2.4) is in the order of 30 m × 2.5 m × 0.7 m and is used for on-growing trout parr, Pacific salmon and, to a lesser extent, Atlantic salmon. One raceway often empties into another, making water quality control difficult. Fish densities must reflect the changing water quality towards the distal end of each raceway and any subsequent raceway. Fish tend to congregate in the upper portion of the raceway and fin damage may occur due to nipping at feeding time or rubbing on the concrete walls. Fish must be graded more frequently in raceway systems than in ponds as the aggregations of fish tend to hinder even food distribution, which results in growth divergence. Water exchange is more easily controlled than in ponds and build-up of organic material can be avoided by increasing flow velocities using baffles. Hygiene in general is better in raceways than in earthen ponds. Although concrete is commonly used for raceway construction (Fig. 2.4), earthen races are also used.

Tank farms, which can be based on 'modular' units, are expensive to purchase but upkeep is less costly relative to other systems. Furthermore, well-designed tanks contain water that is better mixed and shows a more homogeneous water quality and flow profile than raceways, enabling the fish to use the entire water volume, allowing efficiencies in water use and food distribution. A further advantage is better disease control than in either pond or raceway systems. Although raceways and ponds are used predominantly to rear trout, tanks are the preferred system for salmon (Fig. 15.6).

Water exchange in all three systems is an important consideration. In tanks and raceways it is usually sufficient to ensure 100–200% turnover per hour. The farmer must strive to ensure that the culture system is 'self-cleaning' and this will require a flow of at least 2 cm/s in a raceway. Tanks normally require a lower flow to ensure self-cleaning; flow dynamics may be optimised using combinations of inlets/outlets configurations and sloping tank floors. Only carefully designed ponds will avoid the need for regular mechanical removal of organic mud. Indeed, it is good management practice to leave some ponds fallow

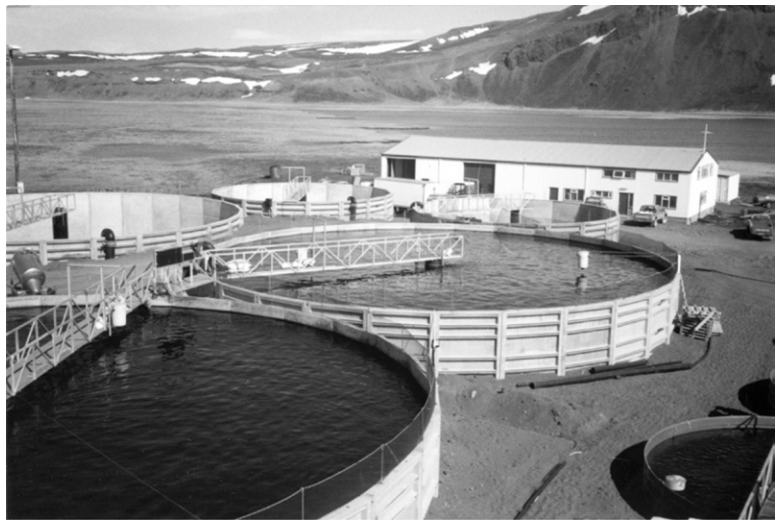


Fig. 15.6 Tank farm for Atlantic salmon, Silfurstjarnan, western Iceland (Photograph by Valdimar Gunnarsson.)

for several weeks each year to allow cleaning and facilitate disease control.

Recirculation aquaculture systems (RAS) have become popular because they may be sited closer to customers than traditional flow-through systems, and afford a greater control over water quality and fish production. Recirculation systems include components undertaking solids filtration, biofiltration, ozonation, ultraviolet treatment, pH control, degassing, oxygenation and temperature and light control (section 2.4.4). Egg and fish culture tanks may be serviced within an RAS at densities up to ca. 100 kg/m^3 during juvenile production that is supported by oxygen injection. Temperature control assists with optimal oocyte development in broodstock; controlled hatching in eggs; and regulated growth in fry and fingerlings. Photoperiod manipulation regulates out-of-season smolt production. The remaining RAS components contribute to water quality treatment of faecal and waste food loads, ammonia, carbon dioxide, pH, bacterial loads, ozone residues, dissolved proteins, tannins and other dissolved components.

15.3.6.2 Grading

An experienced farmer knows when to grade stock for optimum growth efficiency and, most importantly, to provide marketable fish throughout the year. Fry are usually size graded before grow-out in ponds, raceways or tanks. Subsequent grading will depend on the market plan. However, handling stresses fish and increases oxygen demand and is generally avoided during hot weather, low water flows, immediately before transport or while treating disease. Grading may also impair growth for a period through handling stress and the need to starve fish for 1–2 days before handling. Fish pumps and elevators may be used to deliver fish more efficiently to the graders and to reduce the handling stress associated with hand-net delivery. Compact, manoeuvrable graders are available and these handle fish from 1 to 5 g upwards. They usually rely on the variation in fish width (girth) to separate fish, using tapered or set spaces between bars or belts.

15.3.6.3 Feeding

Every grower is mindful of the high cost of food in intensive salmonid culture. Surprisingly, wastage still occurs as a result of the following:

- overzealous feeding;
- inefficient grading leading to inability of small fish to consume the pellets;
- poorly designed feeders;
- poor feeding regimes.

However, slight overfeeding can be used to maximise growth during the early phases when relatively low volumes of food usage results in low additional costs. Underfeeding may also occur, resulting in poor food conversion ratios (FCRs) low growth and possible hierarchy formation, which in turn can lead to more variable fish size distribution. It is essential that a sampling programme to measure fish size be established on the farm to determine the FCR. Samples can be taken monthly during the colder months, but in high summer once every 2 weeks is prudent. In practice, however, sampling may be more infrequent. FCR figures vary between species but should be ca. 1 : 1, with trout usually producing less efficient ratios than salmon. The use of poor-quality food increases the level of solid and soluble waste and the FCR. Quality (and high-energy) diets are available and are very favourable from growth, environmental and economic standpoints (section 4.2.1).

Most feeding strategies are based around high frequency delivery/satiation feeding, with feeding tables acting as feeding guides (section 9.11). Although automatic feeding systems (auger, spinning disc, rotating plate, pulsed air and belt feeders) can distribute feed at pre-set intervals throughout the day, they are supplemented by hand-feeding to assess feeding response, with subsequent amounts being adjusted accordingly. Some operations use self-feeders where fish activate a wire, bead or rod to release food into the water. Such devices may be simple in design or quite sophisticated computer-based monitoring systems that record the number of actuations over time to describe feeding patterns. Pellet sizes used in hatcheries range from 300 µm as starter feed to about 3–4 mm for juveniles.

15.3.7 Production of Atlantic salmon smolt

Smoltification is a process in which juvenile salmonids in freshwater undergo several morphological, physiological and behavioural changes that enable them to migrate to, and then survive and grow in, marine conditions (Langdon, 1985). This process is complex and occurs over several months before the obvious morphological and behavioural changes in the fish. These changes include the following:

- obscured lateral parr marks;
- darkened fin margins;
- silvering and easy removal of scales;
- decline in condition index;

- a preference to swim with rather than against the current;
- tolerance of high salinities.

All of these changes occur in freshwater.

Physiologically, in the final stages of smoltification, there are increases in cortisol, growth hormone and Na^+, K^+-ATPase, which aid osmoregulatory and ionic regulatory processes in seawater, and a decrease in prolactin, which is involved in freshwater osmoregulation.

Smoltification is not evident in some landlocked strains of salmon or trout. For example, rainbow trout stocks used for marine farming in Tasmania to produce ocean trout are a non-smolting strain and are transferred at a relatively large size of 300+ g. They are grown in brackish waters or are acclimated to marine conditions.

Under a natural thermal regime, Atlantic salmon parr must attain a threshold size by the start of their first winter to undergo smoltification successfully in spring (Thorpe, 1977). Hatcheries often observe a bimodality in length/frequency distribution in parr populations, the upper mode completing smolting during the first spring (S1) and the lower mode becoming smolt a year later (S2). In general, S1 smolts at the commencement of winter are approximately 10–15 cm in length, whereas S2 fish are small in the first spring, but much larger the following spring, at the time of transfer. In contrast, Pacific salmon species have a less defined smolt window or windows, are usually much smaller in size at transfer, cannot always tolerate direct transfer to seawater and may require an acclimation period in brackish water.

The aim of most Atlantic salmon hatcheries is to produce yearling smolts (S1). Unfortunately, S1 and S2 are similar in size until summer, when S2 fish become anorexic (Huntingford and Metcalfe, 1993). However, S1 smolt production can be promoted by manipulating light and particularly temperature (and feeding) to optimise growth during the early stages of development. These two environmental variables play an important role in the smolting process. Seasonally changing temperature regulates metabolism, which affects appetite, consumption of food and growth rate (Saunders and Duston, 1992), whereas photoperiod signals seasonal cycles. If the light and temperature are held constant in the early stages to optimise growth, they must be resynchronised with natural photoperiod a few months before the smolt window (i.e. in time to enter the sea when salinity tolerance is at a peak) or resynchronised to an artificial representation of a natural cycle. Both incorrect photoperiod and high water temperatures will adversely affect salinity tolerance in potential smolts.

One strategy to allow year-round harvests involves the use of out-of-season smolts. Although spring smolts are produced largely under the influence of natural light, out-of-season smolts are produced through photoperiod manipulation, effectively advancing the autumn–winter–spring light signal artificially. The natural photoperiod cycle in Tasmania ranges from a summer period of 15 : 9 LD to a winter 9 : 15 LD, whereas the photo-manipulated fish are exposed to a constant 22 : 2 LD until summer, followed by 12 : 12 LD for 8 weeks then returned to 22 : 2 LD for 6 weeks before transfer to the sea. Transfer to marine conditions of out-of-season smolt in March–May, followed by winter marine pre-smolt in June–August, before the spring smolt transfer in September–November, spreads the smolt intake period during the year, thus allowing a corresponding spread of production on the marine farms.

In Tasmania, the most popular smolt types produced are as follows:

- all-female diploid and all-female triploid out-of-season smolts;
- all-female diploid and mixed-sex diploid marine pre-smolts;
- mixed-sex and all-female triploid spring smolts.

Indoor recirculation systems using controls over water quality and temperature and altered photoperiod regimes are being used to accelerate growth and manipulate egg development and smolting.

15.3.8 Grow-out of rainbow trout

As with any species, planning for grow-out of rainbow trout must be initiated before the spawning season. The farmer needs to draw up a production schedule ensuring a continuous supply of marketable fish. Pivotal in preparing the production strategy is knowledge of the following:

- consumer demand and preferences;
- peak demand periods;
- seasonal price fluctuations;
- biological strengths and weaknesses in the stock;
- knowledge of market competitors.

European farmers are able to extend egg production over at least a 9-month period. Photoperiod manipulation of broodstock permits about 6 months supply of eggs. Temperature control of the hatchery environment may add an additional 2 or 3 weeks, and the importation of ova from Australia or other Southern hemisphere countries

adds a further 2 or 3 months supply. It is quite feasible to operate a hatchery for 12 months in Europe if desired.

Australian trout producers have fewer techniques at their disposal to ensure continuous market supply than their European or North American counterparts. Australian hatcheries are prohibited by law from importing ova, and few hatcheries employ photoperiod manipulation of broodstock. Delaying or advancing egg development by temperature control can extend the hatching period by a few weeks. However, Australian farmers have fish that spawn naturally between late May and mid- to late July. Ingram (1988) pointed out that careful selection of the offspring of early and late spawners as future broodstock will ensure a natural spread (genetically determined) of stock. At least 30 fish in each group should be used to reduce the possibility of inbreeding (section 7.5).

Although there is some flexibility to manipulate availability of rainbow trout fry to ensure an extended supply of marketable fish, the Australian farmer relies predominantly on two strategies: first, compensatory growth, whereby growth is slowed then increased to coincide with market demand; and, second, taking advantage of variable growth between fish stocks. Although the latter may be due to genetic factors, there are several parameters, acting alone or in combination, which also control growth including the following:

- water temperature;
- oxygen levels;
- tank or pond design;
- flow rates;
- density;
- feed;
- grading (efficiency and interval);
- health maintenance.

There is an inverse relationship between water temperature and DO. As water temperature increases, less oxygen is available to the fish to metabolise ingested food (section 3.2.7). Overfeeding during periods of low DO may result in death or chronic stress which often results in non-infectious disease or poor growth. The optimum temperature for growing rainbow trout is ca. 15 °C; they will, however, survive at 25 °C for several days as long as there is sufficient DO. The farmer must have facilities available to supply additional oxygen when water temperatures are above 17 °C. A plethora of commercial oxygenation systems and aerators are available to supplement low DO levels, which not only result from increasing temperatures, but occur with factors such as high stocking densities and reduced water flows.

Determining the required level of DO is very important for meeting an annual production schedule. Strategies for supplying oxygen range from gravity aerators (e.g. spray bars, water falls) between raceways to mechanical aerators (e.g. surface agitators, aspirators) and oxygen injection systems (e.g. low-head oxygenators, packed columns, U-tubes). At 15 °C rainbow trout will survive at 3 mg/L DO for a while, but die if this level persists. This so-called 'level of oxygen resistance' (i.e. 3 mg/L) rises with temperature, and therefore 5 mg/L may prove fatal at 22 °C. An oxygen meter is essential to determine DO levels throughout the warm summer months.

The most commonly used data to estimate mean daily oxygen requirements are those derived by Liao (1971). It has been calculated that 200–300 g of oxygen must be supplied for each kilogram of food. Because of the low solubility of oxygen in water, this amount of oxygen requires 100 000 L of water if the fish are extracting 3 mg/L DO through respiration without supplemental aeration. This clearly demonstrates the desirability of, or essential need for, aeration. However, knowledge of mean oxygen requirements is not sufficient when quantifying the amount of aeration to be provided (Petit, 1990). This is because instantaneous oxygen requirements of the fish may increase dramatically over periods of 20 min to several hours. Above 18 °C, the amount of food must be reduced to avoid hypoxia and stress, as oxygen requirements of the fish rise sharply after feeding (post-prandial demand). In situations of suspected low oxygen levels, the dissolved oxygen concentrations must be measured before feeding to avoid any subsequent oxygen deficits and resultant fish losses.

Timmons and Youngs (1991) gave an array of stocking densities for trout ranging from 60 to 234 kg/m^3, but such high densities rely on the ability to provide an adequate level of oxygen (Stevenson, 1980) together with experience and attention to the behaviour of the fish. At 15 °C, 25 kg/m^3 is sensible, increasing to 35 kg/m^3 with aeration or with oxygenation at higher densities. In reality, it is the environment of the tank or pond that determines the stocking density that in turn permits maximum growth. Flow rate and water exchange are tools used to ameliorate the environment, but tank and pond design will determine the ease with which this is achieved. Other water quality factors also impact fish growth. Ammonia and carbon dioxide as fish excretory products may build up in systems to sub-lethal and lethal levels. Ammonia and oxygen are the major determinants of hydraulic loading of the

system, while carbon dioxide is removed by degassing techniques.

15.4 MARINE FARMING

Great advances have been made over recent years in the technology and husbandry techniques required for farming salmonids in cages at sea. Cage structures have been developed to withstand more exposed sites (e.g. open ocean aquaculture), and they have generally increased in size and carrying capacity. Feeding, grading and pumping systems have improved fish performance and farm efficiencies. Losses due to disease and parasite infestation have decreased and growth rates improved by the following strategies:

- adoption of site rotation and fallowing practices;
- species and age separation on leases;
- reduction of stocking densities;
- development of vaccines and alternative treatments (Chapter 19).

The following sections discuss the general issues involved in salmonid farming in seacages. It is primarily based on the Tasmanian salmonid industries, but has broader applicability to salmonid industries.

15.4.1 Site characteristics

Most salmon seafarms are located inshore in high-salinity waters (32–35‰). Leases used as nursery areas or to inhibit amoebic gill disease (AGD), however, are located in estuaries influenced by freshwater. In contrast, trout are grown most successfully in estuarine areas. The positioning of low-cost cages in sheltered coastal waters (in bays, fjords and lochs) worldwide is coming under increasing pressure from recreational users and groups concerned by organic and inorganic enrichment from the farms (section 4.2.1). Alternative offshore farming technologies are being developed to overcome these concerns, but they are expensive and they create additional problems in the areas of security, navigational hazard, predator attack and servicing. On inshore farms in Tasmania waters, there is regular monitoring of quality factors, benthic changes and phytoplankton assemblages as part of the government regulations pertaining to lease permits.

Sites may be used for different purposes in coastal areas. For example, specific age classes may be sited to match the requirements of the fish (e.g. nursery site as smolt) or to minimise the impacts of algal blooms, predator attack and the spread of disease between established grow-out fish and newly introduced smolt.

The site characteristics may also govern the layout of the farm, cage size, net depth and management strategies used. For example, the current speed, water exchange, site depth and tidal range may determine the mooring configuration, cage linkage format and net configuration. These, in turn, determine the management strategy (e.g. cage servicing from boats or directly from shore), the fish density used, the type of feeding system and net changing regime.

In general, desirable site characteristics include the following:

- adequate depth (minimum 5 m beneath the net);
- minimum average current flow of about 3 cm/s (up to 40 cm/s);
- a high oxygen concentration (>80% saturation);
- temperature <18 °C;
- relatively clear water to observe fish;
- a good water exchange to flush nutrients from the area and supply DO;
- a sandy substrate;
- sufficient distance from predator colonies (e.g. seals).

Water temperature is of particular importance in Tasmania. Sites experience sea temperatures of 8–20+ °C that promote high growth rates but in summer provide conditions on the edge of the species' thermal tolerance. Warm summers combined with concerns about temperature increases associated with climate change have made this one of the major topics in the industry, particularly in relation to AGD prevalence, maturation, impact on other environmental changes such as dissolved oxygen, and food formulations relative to high temperatures, all leading to a physiological impact on growth.

In addition to the physical features of the site, socio-economic factors are important. Many sea sites are located in regions remote from large populations. Important site features that affect the efficiency and economic viability of the operation include the following:

- access to the site to deliver equipment, fingerlings or smolt and to remove harvested product;
- availability of workers and their accommodation;
- availability of utilities;
- proximity of processing facilities and airports;
- availability of servicing and maintenance facilities.

Although these requirements are related to the site requirements for the marine farming phase of salmonid

culture, they include several of the features for site selection that are discussed in Chapter 2.

15.4.2 Site rotation and fallowing

Prolonged use of sites, accompanied by poor husbandry practices (e.g. overfeeding) and suboptimal environmental conditions (e.g. poor water movement), may cause 'souring', a condition that reduces the productivity of the site and interferes with the performance of the fish. Consequently, growth and survival may decrease and the incidence of disease may increase. It is of utmost importance to the grower that sites are carefully chosen and cages are carefully managed as it is the farm that will bear the brunt of any negative impacts. Solid waste from uneaten food and faecal material may accumulate on the seafloor under the cages. Initially this may increase the benthic invertebrate productivity, but, over time, the BOD will increase and the substrate will become anoxic, with a resultant decline in the diversity of invertebrates. In extreme cases, the accumulation of organic matter and anoxic conditions lead to the production of gases (hydrogen sulphide, methane and ammonia), which may rise through the water column as bubbles.

Cages may periodically be moved around the lease area or rotated between different leases to allow for periodic recovery of the substrate, even when careful management is used. The time required for recovery ranges from 6 months to several years and is dependent upon site characteristics. Several sites may be used and each site may be used for specific age classes of fish. This production strategy enables sites to be fallowed between production cycles. Site rotation and fallowing are strategies that can be used in conjunction with, not instead of, sound husbandry practices to manage sites. Good farming sites are uncommon and growers know that these must be farmed sustainably.

Independent monitoring by government authorities of sediment condition under cages is now standard practice in several countries. Visual assessment using underwater video, chemical assessment (e.g. redox potential) and biological assessment (benthic infauna) are components of such a monitoring programme. In addition, growers closely monitor water quality parameters such as the following:

- DO;
- current speed and direction;
- tidal cycles;
- temperature;
- salinity;
- turbidity;
- microalgal concentration and type.

Measurements are taken both manually and telemetrically.

15.4.3 Transfer to seafarm from freshwater

Smolt or fingerlings are transferred to the seawater facilities from freshwater at a particular size and at suitable times of the year. Smolting species or strains are transferred during their 'smoltification window', which is usually in spring for Atlantic salmon, but variable for Pacific salmon species. In Tasmania, Atlantic salmon smolt are transferred at 50–150 g (out of season S½ and S1) or 150+ g (S1½). In the non-smolting groups (e.g. many rainbow trout stocks), the success of transfer is size-related: larger fish are more adaptable to higher salinities and, as such, yearling 300+ g fish are transferred in autumn. Some farmers do not culture rainbow trout in full salinities, preferring more brackish conditions. The timing and length of the 'smolt window' varies slightly from year to year in relation to environmental conditions such as temperature. Smolt window duration is negatively correlated with temperate.

Smolts are introduced directly into seawater from freshwater in most cases, although acclimation regimes or slightly brackish water sites are used when pre-smolt or large parr are introduced outside the smolting window. The inclusion of salt (5–10%) in the diet of smolting and non-smolting fish a few weeks before transfer has been used to aid osmoregulatory conditioning (Salman and Eddy, 1990; King, 1992), but this has been discontinued in Tasmania.

The fish are normally graded to produce a uniform stock before transfer to seafarms. A variety of methods are used to assess the actual timing for transfer. Although the fish may display a wide array of smolt-like characteristics, the best indicators are the 24 h (up to 72 h), 35‰ salinity adaptability test and the 40‰ challenge test. The first method assesses the ability of the fish to osmoregulate successfully after direct transfer from freshwater to seawater. This approach uses osmolality or sodium readings of the blood, plasma or serum and the 24 or 72 h readings compared with baseline data of fish in freshwater (usually ca. 280–310 mOsm/kg). The second test assesses survival of fish after a 24-h exposure to 40‰ as large parr may survive exposure to 35‰ over a short period. The 40‰ challenge test has been discontinued on ethical grounds.

Fish are moved to sea sites in transport tankers on trucks and are released via pipes into cages after pens are towed

inshore. Alternatively, the tankers may be barged out to the pens or the fish are released into small tankers and moved to pens by forklift or the small tankers are airlifted from shore to pen by helicopter. Choice of operation depends on cost, site characteristics and site access. Several tanker characteristics are important when fish are transported over lengthy periods. These are as follows:

- stocking density;
- oxygen concentration and distribution;
- tank ventilation;
- carbon dioxide de-gassing capability;
- temperature fluctuations.

Stocking densities up to ca. 90 kg/m^3 are supported by the use of oxygen diffused into the water, the concentration being monitored by in-tank probes in combination with read-outs and alarms in the truck cabins. The water may be de-gassed of carbon dioxide using pumps and spray-bars, aeration and tank ventilation. Trips to seafarms typically take 3–6 h.

Fish are normally deprived of food for a few days before transport to reduce oxygen demand and excretion of carbon dioxide, ammonia and faecal solids. Fish are counted or weighed during the loading process, with grading taking place sometime beforehand. It is important that, in relation to transport biomass and seafarm itinerary, an accurate count is achieved. To avoid additional handling, the fish are not usually recounted at the seafarm on arrival. Average sizes and the range of sizes are also of significance as feed sizes and net mesh sizes need to be selected for any particular batch of fish. These parameters are normally determined in relation to average size and a large size range in the batch can lead to fish being unable to accept pellets or escaping through net meshes. In Tasmania the average size of out-of-season and spring smolts are in the order of 50–150 g.

15.4.4 Cage systems

Most cages are similar in basic structure: a bag net suspended inside a floating collar, which is moored to the sea floor (section 2.2.6). There are many cage shapes, construction materials, linkage configurations and sizes, their form determining the management strategies and servicing practices used (Beveridge, 1996). Some industries use individually moored cages because of size or to better use low water-flow conditions. Many areas link cages together into platforms with service walkways between pens. Some are moored offshore using boat access (Fig. 15.7) and some extend from the shore with direct access by personnel and vehicles. Individual cage sizes vary enormously from small 1–2 t units to large 100–150+ t units. Advances in cage technology show trends to larger cages or cage systems, moored further offshore and possessing several features that enable the cages to withstand offshore conditions (e.g. flexibility, semi-submersible).



Fig. 15.7 Circular polyethylene cages are popular within the salmonid industry worldwide. There are many variations of the design involving configurations of flotation rings and stanchions (Photograph by John S. Lucas.)

In Tasmania, the three cage styles have been used:

1. Polyethylene rings (polar circle type, 60, 80, 120, 170 m circumference).
2. Square steel platforms (24 m × 24 m).
3. Solid bag nets (have been discontinued).

Net bags are generally 5–20 m in depth, the shape being maintained by the use of metal rings, weights (ca. 50–200 kg each), lead-core ropes or positive displacement of water (in the case of solid bag net systems). Maintaining the shape of open mesh nets is particularly important in preventing 'bagging' or loss of shape and volume, which occur through the resistance of biofouled nets against water currents. Net meshes on seacages range in size from about 10 mm to 40 mm (bar length), depending on fish size. They are generally constructed of a woven, knotless (e.g. raschel) and synthetic material such as nylon or polyethylene (treated for resistance against ultraviolet light), but some farms have installed steel or brass nets (with associated anodes) to overcome predator attack and biofouling. In Tasmania, these nets are stocked with fish to a level of about 5–15 kg/m^3, producing an overall biomass at harvest of 20–100 t of fish/cage, depending on cage design.

15.4.5 Biofouling and net changing

Biofouling occurs on nets, ropes and cages, and creates problems such as increased drag, increased weight, reduced water flow (exchange) and increased BOD (Hodson and Burke, 1994). Groups such as bacteria, protists and diatoms are early colonisers, with the form and orientation of the net mesh playing major roles in this colonisation process. These microscopic groups are usually followed through successive colonisation by the macroscopic algae, hydrozoans and other invertebrates such as amphipods.

Fouled nets are removed and exchanged for clean nets on cages more frequently in summer than in winter owing to the more rapid biofouling processes. For example, nets are exchanged every 7–25 days in summer and every 50–60 days in winter in Tasmania. The biofouling may then be removed from the net in a washing machine or by high pressure hoses. The nets are then checked for holes, repaired and folded ready for further use. Lifting equipment positioned on barges has become essential when servicing nets from large cages.

The net exchange process can be labour intensive. Consequently, alternative prevention and servicing methods have been developed to combat the biofouling problem. Rather than removing biofouled nets from cages, some companies have used a dual net system on each cage: one net in and one net out of the water. Periodically, the nets are alternated so that the biofouled net is air-dried on the cage. In other areas, nets emptied of fish are raised out of the water onto the handrails and are cleaned with high-pressure jets of water, or rotating brushes are used to dislodge fouling on nets *in situ*.

Biocidal and non-biocidal antifoulant paints applied to nets by dipping have been trailed as a means of inhibiting growth on netting. Biocidal paints containing tributyltin have been banned because of the deleterious effects on larval crustaceans and molluscs in the surrounding waters. Copper-based paints are relatively effective in slowing fouling and, like some of the non-biocidal paints, stiffen the net after application. Stiffened netting is helpful in deterring seals, but it makes the nets more difficult to handle when they are changed after a few months.

As an alternative, non-toxic wax- and emulsion-based paints, and paints based on some newly developed natural products, can be used on nets. As some of these measures are not necessarily successful or cost-effective, many farms still favour simple net changing.

15.4.6 Predation

The type and number of predators on seafarms vary considerably, and are site and region specific. In Tasmania, fur seals can be a major threat, removing or damaging fish through the nets or creating holes in the mesh, which allow stock to escape. Many farms use physical deterrents such as anti-predator nets of 10 to 15 cm bar mesh around individual pens or lease areas, whereas others use traps (the seals are relocated), electronic acoustic deterrents, electric fences on each cage or steel/brass nets. As double nets reduce water flows and can be labour intensive and awkward to handle, some farmers have developed stiffened heavy-ply fish nets that are weighted taut to prevent seals from entering the nets. Seals that are relocated to other regions after trapping will sometimes return to the farms within a few days, and can be trapped and relocated repeatedly. Seals have also developed behavioural strategies to overcome the deterrents:

- they swim with their heads above the water to avoid the acoustic deterrents;
- they jump over handrails to enter the cages;
- they gain entry to the space between the predator and fish nets;
- they establish territories on cage walkways and sometimes attack workers and divers.

Even when deterrents are successful in preventing losses of fish, the presence of the seals can depress the feed intake and subsequent growth potential of the fish.

Seal nets around individual cages also assist in excluding diving birds, such as cormorants, which cause damage by attacking fish through the nets. Bird nets are used over cages, particularly smolt pens, to prevent bird attack. Gulls are normally present around farms in large numbers, scavenging any dead fish or feed, which increases production costs. Droppings from birds may be a means of parasite and disease transfer; however, this is area specific. Droppings also cause work surfaces to become slippery, a potential occupational health and safety issue.

Some farms are troubled by scavengers, such as dogfish, sharks and eels, which are attracted to any dead fish in the base of the net. Such attacks can result in net damage and fish escape. Poaching and vandalism appear to be universal problems, being tackled by growers through the use of dogs, night patrols, radar or electronic surveillance techniques. Perceived problems from dolphins and large whales do not appear to be a justified risk, although their short-term presence, like seals, can stress the fish, in turn reducing feed intake and growth.

15.4.7 Fish transfer on and between seafarms

Some companies use fishing boats to tow cages of fish between leases for grow-out and for harvesting. This procedure is slow, the speed dependent upon the swimming ability of the fish, currents, tides and bagging of the net. To overcome these problems some companies use well boats.

In other situations, fish are transferred from small to larger cages during grow-out and from one cage to another because of structural damage or when splitting batches of fish. In these 'swim-throughs' the nets are connected and the fish encouraged to swim between cages as the nets are raised.

Although small farms may still use hand-operated dip nets to move fish, they are impractical on larger operations or when transferring large numbers of fish. Instead, large canvas-lined brail nets, which are lifted by cranes, fish pumps or moving devices are used to move fish during grading, harvesting or freshwater bathing for control of amoebic gill disease.

Pressure-vacuum, venturi-driven or air-lift pumps are the most commonly used methods for fish pumping; in most cases they are capable of handling fish up to ca. 5 kg in size and have a pumping rate of about 20–30 t fish per hour. Problems encountered with eye, skin and scale damage have been overcome by system automation, operator experience or new designs that do not contain closing valves in contact with the fish. Many growers also favour continuous-flow pumps over ones on a stop–start cycle. One innovative grower designed and built a fish moving device based on corral and fish lifting systems; the system being capable of rapidly moving grow-out fish of any size at a rate of 80 t/h.

15.5 FEEDS

15.5.1 Characteristics

As feed costs constitute ca. 40–50% of the production costs, much attention has been paid to feed composition, feed distribution, feeding behaviour, waste levels and feed ingestion.

'Moist' and 'wet' feeds (e.g. trash fish) were initially used during the early development of salmonid seafarming. With the development of formulated 'dry' diets (pelleted feeds), the problems of water quality deterioration, nutritional deficiencies and feed distribution associated with trash fish diets have largely been resolved. Further refinement of dry diets through the extrusion process (section 9.10.2) has provided benefits over steam-pressed pellets:

- lower dust content;
- consistent pellet size;
- high lipid content;
- higher digestibility;
- prolonged pellet integrity in water;
- variable sinking rate.

Much of the research being undertaken by commercial feed companies concentrates on the nutritional content of the pellet, particularly the level of lipid and protein, and its digestibility. Proximate composition of salmon diets varies in relation to the phase of the life cycle, size of fish, water temperature and pellet form, but a typical breakdown of energy sources is 38–50% protein, 15–35% lipid and 8–13% carbohydrate. Trout diets tend to have lower energy contents. Digestibility of carbohydrates, optimisation of lipid level, replacement of fish meal and oils with plant proteins and oils, and the formulation of diets more suited to salmon grown in warmer waters are some fields of current research.

Feed pellet diameter sizes range from 300 µm to 9 mm, corresponding to increasing fish size from first feed to grow-out. Feed rates per day depend on fish stock, water temperature, location, feed strategy, usable energy content and feed type, but they typically range from ca. 7% or

more of body weight per day for 1 g fry to ca. 1% of body weight per day for grow-out (3–4 kg) fish.

With the development of more digestible feeds and technologies to maximise feed distribution to fish, the production FCR has steadily improved to figures such as 0.8 : 1 to 0.9 : 1 for initial smolt growth and 1.2 : 1 to 1.4 : 1 for overall grow-out in seawater.

Specific feed additives may also be used:

- attractants/stimulants to improve feed intake and digestibility;
- astaxanthin for flesh pigmentation in grow-out fish and egg requirements in broodstock.

The pigment astaxanthin is included in transfer diets at 60–75 mg/kg down to 40 mg/kg for 3+ kg fish to produce flesh pigmentation levels of 7–10 mg/kg for salmon and 18–24 mg/kg for trout. Although laboratory-based techniques may be used to quantify flesh pigment levels, they are usually assessed visually with the aid of colour cards (e.g. Salmo Fan). The deposition of pigment in the flesh is affected by fish size with small fish having limited capacity compared to grow-out sizes, whereas there is loss of pigment due to redistribution to egg development during maturation.

Although the use of antibiotics has been reduced drastically in many countries in recent years, these drugs, when required, are incorporated into the pellet during manufacture or added later using oil or gelatin-based coating. Many Tasmanian seafarms are antibiotic free, using management techniques and vaccines to prevent or minimise the impact of diseases.

15.5.2 Feed distribution

Farms have developed various feeding patterns and these predominantly govern the means of distributing feed to fish. Some farms feed once or twice a day, others three to six times and yet others on a 'continuous' basis. Generally, smaller fish require more meals per day than larger fish. Pelleted feeds are distributed mechanically or by hand (section 9.11.2). Hand feeding, with the assistance of water cannons (Fig. 9.13) or air-blowers, enables personnel to assess the feeding response of the fish and other aspects of husbandry, such as the presence of dead or diseased fish, general behaviour and net or cage damage. It is, however, costly because it is labour intensive and this limits the number of meals per day or simultaneous distribution of meals to all cages. Fish are normally fed two to four times per day on a rotational basis using this method, although smolt may be fed more frequently.



Fig. 15.8 The adaptive feeding system is one of several feed-monitoring devices being used to minimise pellet wastage. This system has been connected to a feed hopper with a spinning disc pellet distributor; it is able to self-regulate feed distribution and record fish feeding patterns.

Mechanical feeders take several forms. They may consist of storage hoppers (from 50 kg to 6 t) with associated spinning discs (Fig. 15.8), vibrating plates, demand feeder pendulums or augers to distribute the pellets to the fish. Alternatively, pellets may be air blown through pipes from land-based or barge-based hoppers to cages. Preprogramme timers or computerised controls usually determine the activation of the feeders. Many of these delivery systems operate intermittently throughout the day, irrespective of fish requirements. Some farms combine hand feeding with mechanical feeders so that the advantages of both are used: observation by staff of feeding behaviour and numerous feeds throughout the day.

Inherent in all of these methods is the potential to overfeed or underfeed; however, technologies have been developed to overcome these problems:

- adaptive feeding systems (Blyth *et al.*, 1993) (Fig. 15.8);
- acoustic detection systems (Bjordal *et al.*, 1993; Dunn and Dalland, 1993);

- air-lift;
- underwater camera systems;
- hydroacoustic.

These are now being used to optimise feed distribution to the fish by monitoring pellet wastage, fish distribution in the water column or consumption of pellets.

More specifically, adaptive feeders are self-regulating and record the amount and time of feed delivery through the use of computerised controls. They also are being used to determine, in more detail, the diurnal and seasonal feeding cycles of the fish and feeding patterns in relation to changes in environmental conditions and presence of predators. This improved understanding of the feeding requirements of the fish growing through their cycle and being exposed to different environmental conditions can be used in turn to refine automatic feeder and hand-feeding delivery strategies. All of these approaches aim to minimise feed wastage and optimise FCR, thereby reducing costs and the impact of the farm on the environment.

15.6 GRADING AND STOCKING DENSITIES

15.6.1 Grading

Grading is undertaken in both fresh and marine facilities and is needed for stages from fry (section 15.3.5) to maturity. It aims to reduce size variability and feeding hierarchies in cohorts of fish or to separate maturing from non-maturing fish. Low size variability also directly aids other management tasks such as food pellet size and net mesh size selection.

Usually, the decision to grade fish is made after the size variability of a sample of fish is determined or at a particular time of the year when maturing fish can be identified. Mechanical graders, in conjunction with fish pumps (Fig. 15.9) are used during size grading, whereas hand grading combined with visual assessment is used to separate the maturing grilse or jacks from the immature fish. It is widely accepted that these mechanical operations inhibit fish growth for days or weeks and, instead, some operators either avoid grading or use swim-through bar graders to separate size groups.

As the task of weight and length sampling can inhibit growth, and add to the risk of stress and damage, techniques have been developed for measuring fish in their cages. This equipment uses the processes of acoustics, incorporating a measurement of the swim bladder (Dunn and Dalland, 1993), stereo-camera imaging or infra-red fish imaging to construct, through computer technology, size–frequency histograms. Thus, the decision to grade the fish can then be made without handling the fish. The technology can be used frequently with little effect on the fish and is useful for large cage systems; however, the equipment needs to be positioned and calibrated correctly to be accurate.

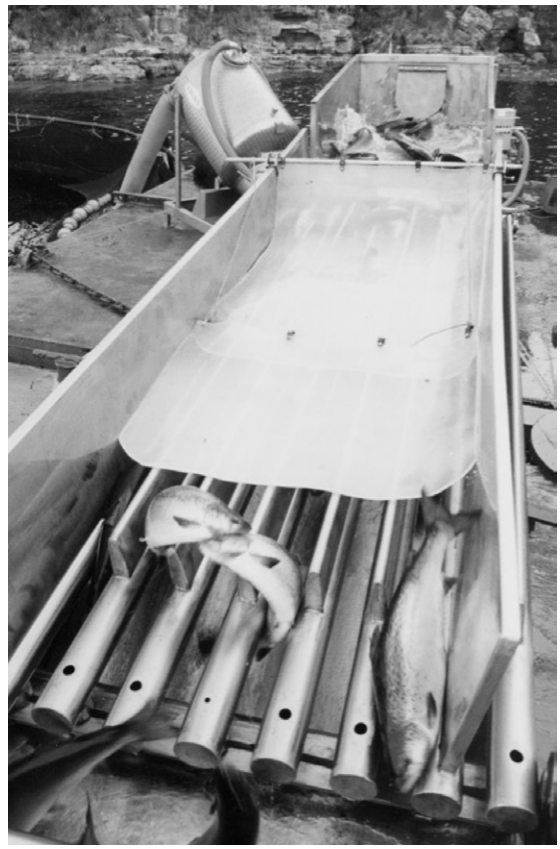


Fig. 15.9 Adjustable bar graders are used in conjunction with fish moving devices, such as fish pumps, and are used to obtain more even size distribution of fish in pens.

15.6.2 Stocking densities

Stocking densities are governed by the species of salmonid reared (e.g. Arctic charr preferentially grow at much higher densities than other species), site characteristics and environmental conditions. The trend in recent years appears to favour lower densities in seacages with a reduction from around 30–40 kg/m^3 to 10–20 kg/m^3 maximum. The advantages of this strategy include the following:

- better growth;
- lower incidence of disease;

- better utilisation of feed;
- lower concentrations of waste;
- better survival.

Initial stocking of smolt into cages is much lower at around 0.5–1 kg/m^3, although very low densities can be detrimental as these cause poor feeding responses and growth.

15.7 MATURATION, SEX REVERSAL AND TRIPLOIDY

15.7.1 Maturation

Salmonids begin to mature during seawater grow-out from spring/summer and they develop gonads ready for spawning in autumn/winter. Energy from tissue reserves and from feed intake is directed toward gonad production to the detriment of somatic or flesh growth. Survival of maturing fish in seawater becomes increasingly more difficult approaching spawning and many fish die. In addition, development of undesirable characteristics, such as the following, reduce the fish's marketability:

- hooked jaw (kype) development;
- milt and egg development;
- change of body shape;
- mucus production;
- darkening of the skin;
- loss of flesh colour;
- softening of flesh.

The harvest period, therefore, is determined by growth to an adequate market size and the onset of maturation. Normally, all fish are removed from the water and sold while in good condition. The age of maturity varies between species and stocks.

In some regions where higher water temperatures promote faster growth, fish may mature after one sea-winter. This is referred to as 'grilsing' in Atlantic salmon. The strategies to deal with these fish vary between countries. Where there is a significant proportion of maturing fish, these fish may be graded from the cages and sold before deterioration of the quality. In other areas (e.g. Australia) fast growth to an average of 3–5 kg is achieved, but is also associated with almost complete maturation after one sea-winter. In this situation, all fish are harvested. At the other extreme, almost no grilsing occurs in colder regions of the Northern hemisphere and the fish remain on the farms for up to 3 years.

Restricted feeding at specific times of the year and photo-manipulation using underwater lights are two strategies to reduce maturation. Restricted feeding in spring may provide a physiological signal to delay the maturation process through its effect on growth which in itself may not be desirable. Underwater lights are the preferred method and are positioned in cages to provide a constant light (LL) regimen to inhibit maturation (principally in out of season fish), potentially to increase growth and to recondition maturing fish. Despite the increased daylength provided by artificial lighting, feed is not offered to fish during the 'night' phase.

The production of all-female stock and sterile triploid fish (particularly all-female) are strategies that can be used to extend the availability of market fish year-round, overcoming the limitations caused by maturing fish (Chapter 7). The declining quality of maturing fish dictates the completion of harvests of diploid fish by early March (early autumn in the Southern hemisphere), in some cases leaving a shortage in the supply of market fish until the smolt of the previous year reach a suitable size in June (midwinter). Such a gap in diploid supplies is partly filled by triploids. Triploidy rates of 95–100% are currently being achieved in hatcheries, with all-female stock being used to avoid pseudo-maturation characters seen in triploid males. Triploidy has declined in popularity in many countries, but it is still used in some industries. Improved culture strategies, such as out-of-season smolt combined with photoperiod manipulation, have given growers some of the advantages gained previously from triploidy.

15.7.2 Sex reversal

Sex reversal of fish is discussed in section 7.7.3. In salmonid culture, removing male fish that potentially mature earlier is a technique used to produce all-female stock. It is a multistep procedure that initially produces all-male fish through the addition of 17α-methyltestosterone in the feed for 70 days (at 10 °C) at the first-feeding fry stage. Male fish (having XY karyotype) develop normally, but the female fish (XX) develop to produce sperm also and are termed 'neo-males'. These fish must be dissected at maturity (usually after 2–3 years) to remove the testes and milt, as the neo-males do not possess sperm ducts. The resultant XX milt is used to fertilise eggs, resulting in all-female (XX) fish which are grown to market size. It is essential that the hormone used in this procedure is not offered to market fish, only to the broodstock. It is for this reason, and the need to use the fish for this specific task, that neo-male stock must be identifiable and maintained separately during grow-out. Neomales may also be produced by bathing pre-feeding fry in a testosterone-based bath, but this technique results in neomales that express

milt; a characteristic that may cause some confusion with male fish.

15.7.3 Triploidy

The triploidy-inducing process occurs at the fertilised egg stage and is outlined in section 7.7.2. It may be used on eggs in conjunction with sex reversal techniques to produce all-female triploids or separately to produce mixed-sex triploids. As it induces sterility it can be used to overcome problems normally associated with maturation – male precocity, grilsing and early maturation in seacaged fish – which temporally restricts harvesting.

Eggs at approximately 30 min post-fertilisation (at 10 °C) are subjected to heat or hydrostatic pressure shock for a specific period of time. Details are specific to individual species, sizes of eggs and method of shock treatment, but as an example thermal shock occurs at 28.5 °C for 10 min and pressure shock at ca. 10 000 psi for 5 min, both followed by re-exposure to 10 °C water. After this treatment, normal rearing conditions are used. Although sterility is a characteristic of triploid fish, some male fish display pseudo-maturation features. This may be overcome by producing all-female triploids; the eggs are fertilised by sperm from neo-males and then subjected to the triploidy process.

Triploid fish sometimes suffer from deformities (e.g. jaw deformities or 'drop-jaw') and may possess different physical and behavioural characteristics (e.g. flesh characteristics and body form). Alternative strategies are being developed to combat the maturation problem and the associated restricted harvests. These strategies include the use of out-of-season smolt, controlled feeding strategies, use of lights to modify photoperiod and selective breeding programmes.

15.8 FISH HEALTH

Several disease pathogens are prevalent in the culture of salmonids worldwide; many are widespread, whereas some are specific to certain regions. An overview of the major diseases affecting salmonids is given in section 10.5.3. Relatively isolated regions, such as Tasmania, are largely 'disease free' in terms of the incidence of serious bacterial and viral conditions. In general, the incidence of disease is decreasing. Preventative management strategies, many of which minimise fish stress, are being favoured over antibiotic treatments. The use of embayment or coastal management strategies with restricted stock movement between sites and reduced on-site stocking densities, together with site fallowing strategies and the development of vaccines (Chapter 19), have all improved fish survival and condition. Some of the more serious diseases that have been encountered worldwide include the following:

- sea-lice (crustaceans) (*Lepeopthierus* and *Caligus* species);
- furunculosis (*Aeromonas salmonicida*);
- *Vibrio* species;
- infectious salmonid anaemia (ISA);
- bacterial kidney disease (BKD);
- infectious pancreatic necrosis (IPN);
- amoebic gill disease (AGD);
- piscirikettsiosis (salmonid rikettsial septicaemia);
- enteric redmouth.

AGD has a major impact on the Tasmanian industry, contributing up to 20% of production costs. It is caused by *Neoparamoeba perurans* (Young *et al.*, 2007). The increase in production costs is primarily due to the high labour costs that are associated with frequent bathing of the fish in freshwater, which is used to reduce the incidence of the disease and its resultant mortalities. Freshwater bathing uses tarpaulin liners positioned in new cages and filled with freshwater. Fish are pumped from the seawater cages through a de-watering apparatus to the freshwater bath in the tarpaulins, where they remain for a few hours before the tarpaulins are removed. Originally experienced only in summer, AGD is now prevalent throughout the year, mainly at full seawater sites rather than brackish water localities. Bathing frequency depends on yearly environmental conditions, but has generally increased to 8–10 times per cycle in recent years.

Salmon in Tasmania also suffer in seawater from a wasting condition called 'pinheading' (Fig. 15.10), evident

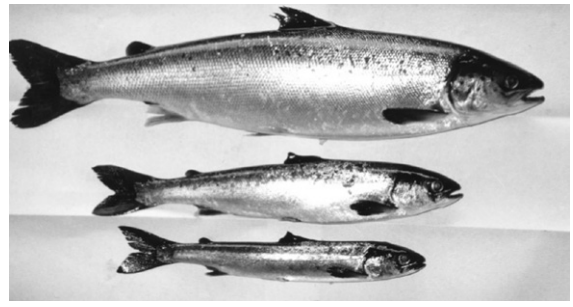


Fig. 15.10 Representative salmon from a seacage cohort showing normal growth (top), runting (middle) and pinheading (bottom).

a few weeks or months after transfer. The cause is unclear, but it is probably due to reduced appetite or poor feed intake. The level of pinheading varies between batches of fish transferred to the sea, usually <5% but on occasion it has been 30% and it appears to be different to the runting condition seen in many countries.

The pathogenic bacterium *Vibrio anguillarum* has been controlled by the development of a vaccine that is administered to the fish as a bath at the freshwater stage. Outbreaks of *Yersinia ruckeri* (a strain of enteric redmouth) occur in freshwater and after transfer to seawater, but these are not usually serious. They are currently controlled by management techniques, a vaccine and limited antibiotic treatments.

A *Rickettsia*-like organism (RLO) has been detected in some areas of Tasmania, restricting the movement of stock from these sites to hatcheries although little is known about this condition. Other health conditions occur occasionally as a consequence of algal blooms, periodic influxes of jellyfish and nutritional problems such as those associated with vitamin deficiency and food spoilage.

15.9 HARVESTING AND PRODUCTS

The average size of salmonids at harvest is determined largely by market demand balanced with the cost of production. As examples, plate-sized freshwater trout may be harvested at 200–400 g, whereas sea-grown trout may occupy a different market niche at 2–4 kg. Atlantic salmon may be harvested as one-winter fish or 'grilse', which may vary considerably in quality and size, depending on country, and range from 1.5 kg to 6 kg. Fish that are two winters or older or 'salmon' are usually 3 kg and above. In Tasmania, trout of 3–4 kg are produced in around 10 months in sea-cages whereas salmon are harvested at 4+ kg between 12 and 18 months after transfer.

Before the harvest, samples may be taken to determine the quality of the fish in terms of fat content, flesh colour, average size, size range, shape and occurrence of marks. During the harvest process, fish are removed from cages using fish pumps or brail nets, then stunned, bled and chilled (Fig. 11.3), lowering the core temperature to preserve carcass quality. The ice slurry/carbon dioxide combination once commonly used in industry to stun the fish produced elevated lactic acid and lowered pH levels in the flesh, leading to issues of gaping and softness. Consequently, carbon dioxide has been replaced by anaesthetic baths using AQUI-S or by stun guns. Once harvested, the fish are placed in ice slurry to cool their core temperature until they are processed.

Salmonid products that are sold around the world include the following:

- fresh fish;
- whole head-on, gilled and gutted fish (HOGG);
- fresh fillets and cutlets;
- frozen HOGG fish;
- smoked sides, portions or whole fish;
- gravalax (lightly sugar cured);
- pâté and rillette;
- caviar.

This is an excellent range of products from an aquaculture species and it explains some of the attraction of salmonids for aquaculturists over many decades.

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16
Tilapias

Victor Suresh and Ram C. Bhujel

16.1 INTRODUCTION

Fish resembling tilapias were featured in paintings in the Egyptian tombs of 2500 BC. Some believe that it shows that tilapias were cultivated in ancient Egypt. It is also claimed that the fish caught by Jesus' disciples in the Sea of Galilee and fed to the multitudes were tilapias. Such claims may be disputed because of the lack of hard evidence, but there can be no disputing that tilapias are one of the most successfully cultured fish of modern times. Current global production of tilapias exceeds 2.5 million t/year (Fig. 16.1). China alone contributes nearly half of the global production (Table 16.1). Other leading countries include Egypt, Indonesia, the Philippines and Thailand. Most tilapias produced in these countries through aquaculture are used for local consumption, but there is also a substantial international trade in chilled and frozen tilapia products. The largest importer of tilapias is the USA, where consumption of tilapias has increased more than five times in the past decade (Fig. 16.2) and has exceeded that of trout since 1995. Central American countries, particularly Costa Rica and Honduras, produce relatively small volumes of tilapias, but most of it is exported as high-value, fresh-chilled fillets to the USA.

The remarkable success of tilapias as a farmed fish can be attributed to two main factors:

1. Their desirable qualities as a food fish: white flesh, neutral taste and firm texture. As a result, tilapias have gained acceptance in a wide variety of human cultures with differing tastes and food preferences.
2. They are an easily farmed fish: tilapias are easy to hold and breed in a captive environment. They tolerate crowding, relatively poor water quality and other stress factors, and are less susceptible to disease than many other cultured fish. They can be grown in a wide variety of aquaculture systems (Table 16.2). They eat algae and detritus naturally produced in culture systems as well as manufactured feeds containing ingredients derived from plants. They reach typical market size (500–800 g) in about 6–8 months under optimum water temperature conditions for growth, 28–30 °C.

Based on these characteristics, Pullin (1984) termed tilapias 'aquatic chicken': an animal that can be farmed as easily and economically, and with the same broad market appeal, as chickens.

The most important problem in producing tilapias to uniform and acceptable market size (>300 g) is their early and uncontrolled reproduction in culture systems, especially earthen ponds. Their early maturation and frequent reproduction directs a significant amount of energy towards reproductive development and activities, and thereby reduces the energy available for growth. Furthermore, uncontrolled recruitment of offspring in the culture system increases demand for food and other resources, resulting in stunted growth. Thus, several solutions to this problem have been developed by researchers and tilapia farmers. Most solutions rely on the production and stocking of all-male tilapias. Male tilapias grow faster than females (Fig. 16.3) and, therefore, all-male tilapia populations provide

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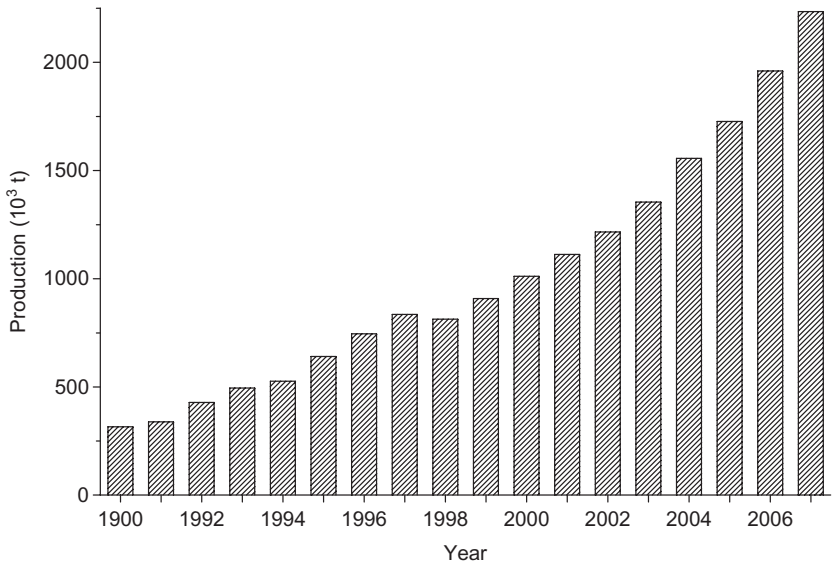


Fig. 16.1 Global production of tilapias in aquaculture, 1990–2007.

Table 16.1 Global tilapia production by countries in 2007 (Global Aquaculture Alliance 2008).

Country	Total volume (t)	Share (%)
China	1 165 000	45.6
Egypt	280 000	11.0
Indonesia	200 000	7.8
Philippines	190 000	7.4
Thailand	180 000	7.0
Mexico	106 250	4.2
Brazil	90 000	3.5
Vietnam	75 000	2.9
Taiwan	73 000	2.8
Others	196 908	7.7
	2 556 158	

an added growth factor, besides controlling unwanted recruitment.

Subsequent sections of this chapter provide a more detailed account of tilapia biology and culture. Tilapias are better understood than most aquaculture species because they have been a focus for researchers for over 50 years. In addition, a considerable amount of fundamental research has been done on tilapias. This is because of the ease with which they can be maintained in the laboratory and their short production cycle. Much of tilapia culture technology has resulted from understanding specific biological characteristics of tilapias. These are summarised in detail in several publications (Beveridge and McAndrew, 2000; El-Sayed, 2006; Lim and Webster, 2006).

16.2 FAMILY, SPECIES AND GENETIC VARIATION

16.2.1 Family

Tilapias belong to the family Cichlidae, a large family of tropical freshwater fish that have a bilaterally compressed body and exhibit parental care. Cichlid fish have a wide natural distribution throughout the tropics, but the tribe Tilapini, to which tilapias belong, occurred only in Africa and Palestine until translocations began. There are about 10 genera and over 100 species within Tilapini. The common term 'tilapias' refers to pure species as well as hybrids belonging to the genera *Tilapia*, *Sarotherodon* and *Oreochromis*, especially the larger species that are commercially exploited. These three genera are differentiated by the way they brood their eggs and larvae:

1. Tilapias of the genus *Tilapia* lay their eggs on a substrate, which may be a depression on the pond bottom or tree roots or submerged vegetation. Both parents care for the eggs until they hatch. Females fan and

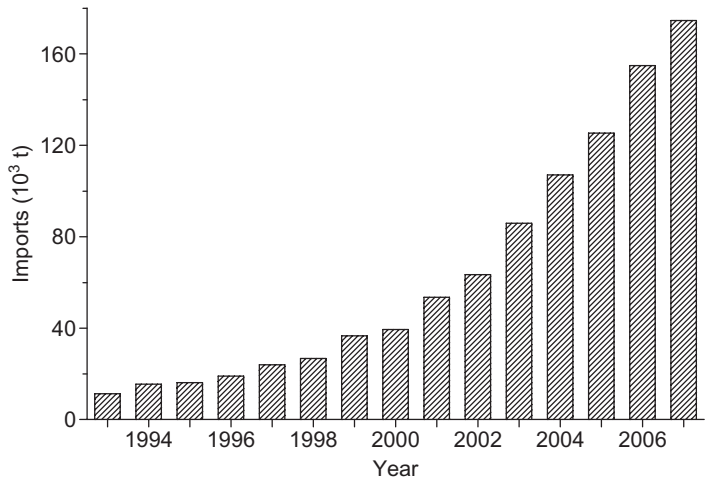


Fig. 16.2 Tilapia imports into the USA, 1993–2007, based on data provided by the National Marine Fisheries Service, USA.

clean the eggs with their fins while males guard the territory.

2. Tilapias of the genus *Oreochromis* lay their eggs in a pit or nest prepared by the males. After the eggs are laid, the female parent incubates the eggs in her mouth. Parental care continues for several days even after the eggs hatch and the fry are free-swimming.
3. Tilapias of the genus *Sarotherodon* are quite similar to *Oreochromis* in their reproductive behaviour, but both parents, or just the male parent, perform mouth brooding of their eggs and fry.

Most farmed species of tilapias belong to the genus *Oreochromis* and further details of reproduction in *Oreochromis* species are provided elsewhere in this chapter.

16.2.2 Species

Among the tilapias, members of the genus *Oreochromis*, such as *O. niloticus* and *O. aureus*, are favoured in aquaculture because of their performance under culture conditions (Table 16.3). Among the pure species, *O. niloticus* (common name, Nile tilapia) is favoured. The exceptional growth of this species in tropical freshwater conditions is the reason for its success. However, it has poor cold tolerance and does not perform well in high-saline waters. So, in subtropical waters, the more cold-tolerant *O. aureus* (common name, blue tilapia) is the species of choice. Species such as *O. spilurus*, which tolerate and grow at high salinity, are considered for farming in seawater, particularly in the Middle East, where freshwater resources are limited. *O. mossambicus* (common name, Mozambique tilapia) was the first tilapia species introduced into various parts of Asia and beyond. It has, however, lost favour owing to its excessive reproduction, which has created a bad reputation as a fish that stunts early in culture and rarely reaches its genetic potential for growth and ultimate size.

16.2.3 Strains

The wide geographic presence of species such as *O. niloticus* in Africa has meant that there has been significant genetic divergence, resulting in distinct subspecies and strains. Their subsequent transfers outside Africa have also provided opportunities for further development of distinct strains. For example, about 200 individuals of *O. niloticus* were sent from Cairo to Japan in 1962. Out of these, 120 individuals survived and formed the basis of the Japanese stock that exists today. About 50 individuals of this Japanese stock were presented to the King of Thailand in 1965 and were placed in a pond at Chitralada Royal Palace. This stock formed the basis of the Chitralada strain, which has performed exceptionally well under Thai farming conditions. Similarly, several other strains of *O. niloticus* exist in Asia and elsewhere. These strains are known by their origin in Africa (such as Ugandan strain or Ghanaian strain), the intermediate locations from which they were transferred (such as Israeli strain or Taiwanese

Table 16.2 Characteristics of tilapia culture systems.

	Extensive	Semi-intensive	Intensive	Super intensive
System type	Ditches, rice fields, backyard ponds, community ponds, reservoirs and tanks for irrigation	Ponds built specifically for fish farming	Small ponds, tanks, cages, raceways	Tanks, raceways
Stocking density (number of animals per per square metre or cubic metre)	<1	2–3	>5	>20
Source of seed (fingerlings) for stocking	Wild fish, by-product of culture	Own or commercial hatcheries	Own or commercial hatcheries	Own or commercial hatcheries
Reproductive control	None	All-male stock may be used	All-male stock	All-male stock
Fertilisation	None except incidental, run-off fertilisation	Manure and inorganic fertilisers applied	None	None
Feeds	None except occasional farm by-products and household wastes	Farm by-products such as rice bran, oilseed cakes or supplementary compound feeds	Complete, compounded feeds	Complete, compounded feeds
Aeration/water exchange	None	Limited, occasional water exchange	Used frequently	High
Culture duration	Seasonal	6–9 months	4–6 months	4–6 months
Yield (mt/ha/year)	<1	1–5	5–20	>20
Market	Producers' own consumption and local, rural markets	Local and national, export markets	Urban, high-value, export markets	Urban, high-value, export markets



Fig. 16.3 Sexual dimorphism in growth: male (top) and female (bottom) of the same age. (Photograph by Dr C. Kwei Lin.)

strain) or the destination in which they were further domesticated (such as the Philippines strain or Chitralada strain). Eknath *et al.* (1993) conducted strain evaluations in the Philippines, comparing growth performance of farmed Asian strains of *O. niloticus* and wild *O. niloticus* from across the natural range of the species in Africa. They showed that wild strains, in general, grew better than domesticated strains and that wild strains from East Africa (Egypt and Kenya) were better than those from West Africa (Ghana and Senegal). Among the domesticated strains, the Chitralada strain, further domesticated in the Philippines, fared best. The worst performance was shown by the most common strain in the Philippines, originally imported from Israel.

Table 16.3 Characteristics of *Oreochromis* species suitable for aquaculture.

Species	Growth	Critical environmental tolerance factors	Suitability
O. niloticus	Fastest growing species in many countries. Maximum size 2 kg	Lower lethal temperature = 12 °C; does not tolerate high salinity	Highly suitable for farming in tropical, freshwater and brackish water systems
O. aureus	Fast growing species. Maximum size 2 kg	Tolerates cold temperatures relatively better than most species (lower lethal temperature = 8 °C)	Best candidate for farming in subtropical freshwater and brackish water systems
O. mossambicus	Fast growth and large maximum size (ca. 1.5 kg) observed in wild, but stunting common in culture	Lower lethal temperature = 10 °C. Grows well and reproduces under salinities as high as 35‰	Suitability as a pure species is questionable. A good candidate for hybridisation if salinity tolerance is desired in the offspring generation
O. spilurus	Grows fast when young but slows down in adulthood. Maximum size ca. 1 kg	Low tolerance of cold temperatures like most other *Oreochromis* species, but performs extremely well at high salinities	A good candidate for mariculture

16.2.4 Hybrids

There are several naturally occurring tilapia hybrids arising from two or more species sharing a geographic location. Some of these natural hybrids are good candidates for aquaculture. Intentional hybridisation for aquaculture purposes started with the accidental discovery that crossing female *O. mossambicus* with male *Oreochromis urolepis hornorum* resulted in all-male offspring (Hickling, 1960). Subsequent research showed that several other crosses also produce all-male or predominantly male offspring (Table 16.4). This was attributed to the differences in sex determination mechanisms between different tilapia species. Hybrids from female Nile tilapia and male blue tilapia are widely used in China (Lai and Yi, 2004). The cross yields high male percentages (85–90%), and results in fast growth, large size, and tolerance of cold temperature and a wide range of salinities.

Table 16.4 Tilapia crosses that result in all-male or predominantly male offspring.

Female	Male	Males in offspring generation (%)
O. mossambicus	*O. urolepis hornorum*	100 in pure strains
O. niloticus	*O. urolepis hornorum*	100 in pure strains
O. niloticus	*O. nyasalapia macrochir*	100 in selected strains
O. niloticus	*O. variabilis*	100 in pure strains
O. niloticus	*O. aureus*	100 in selected strains and 70–80 in mass spawning
O. spilurus niger	*O. nyasalapia macrochir*	100 in pure strains
T. zilli	*O. andersonii*	100 in pure strains

16.2.5 Red tilapias

One of the significant advances in tilapia farming was the development of ‘red tilapias’ in the 1980s. Most tilapias, particularly *O. mossambicus*, which was then a widely cultivated species in Asia, have a dark grey-black skin

colour. Their peritoneal cavity is also black in colour. This coloration was deemed unattractive in several markets, resulting in poor acceptance of the fish. Mutants that possessed red skin were observed in *O. mossambicus*, first in Taiwan, and later in the USA and Israel (Lovshin, 2000). These mutants were developed into red *O. mossambicus* strains, which evoked strong commercial interest in the culture of tilapias. Red tilapias not only lacked the stigma associated with the coloration of the wild-type tilapias, but also resembled premium marine species such as sea bream and red snapper. They had the potential to achieve wider consumer acceptance and higher prices in many markets. Because pure *O. mossambicus* red strains had poor growth characteristics, they were hybridised with faster-growing tilapias, such as *O. niloticus*, *O. aureus* or their hybrids. As a result, a large number of red tilapia strains are available for fish culturists. There are differences between the strains in the following:

- growth rate;
- tolerance of low temperature;
- tolerance of high salinity;
- other perfomance characterisitics.

Because many red tilapia strains were derived from *O. mossambicus*, they perform well in saline environments but may have low tolerance of cold temperatures.

16.2.6 Genetically improved tilapias

Despite the impressive growth rates of some strains of tilapias, such as the Chitralada strain of *O. niloticus*, the genetic base of tilapias in many countries used to be rather poor. Genetic improvement by means of selection or cross-breeding or both has been applied in developing strains of tilapias targeting a specific trait (mainly growth rate). A well-documented effort in tilapia genetic improvement is the development of the genetically improved farmed tilapias (GIFT) line of tilapias in Asia by the World Fish Center (Ponzoni, 2008). The line was developed by crossing eight strains of *O. niloticus* followed by combined family and mass selection for body weight. Field trials demonstrated that the first generation GIFT line yielded 18–58% larger fish compared with local strains of *O. niloticus*.

GIFT tilapia lines have been transferred to many countries in Asia and subjected to further genetic improvement through selective breeding. For example, the ninth generation of GIFT strain referred to as GIFT-strain Super Tilapia, or GenoMar Supreme Tilapia™, was introduced into China in 2001 (Lai and Yi, 2004). A commercial venture called GenoMar Supreme Hatchery to undertake further development of the strain and distribution of fingerlings to the farmers has been established in the Hainan province in China.

At least two lines of true-breeding transgenic tilapias have been established (Mair, 2001). One is an *O. urolepis hornorum*-based hybrid that expresses a transgenic tilapia growth hormone gene and grows 55% faster than its non-transgenic counterpart. This was developed in Cuba and has been approved for use in commercial production in that country. The other line is an *O. niloticus* that is transgenic for a salmon growth hormone and grows three to four times faster than its non-transgenic counterpart. This line was developed in the UK and is yet to be approved for commercial use.

16.3 ECOLOGY AND DISTRIBUTION

16.3.1 General

Africa, excluding Madagascar but including parts of the Middle East, is the original home of all tilapias. In general, *Oreochromis* species are endemic to the central and eastern parts of Africa, whereas *Tilapia* and *Sarotherodon* species are more common in the western parts. However, species such as *Tilapia zillii*, *Sarotherodon galilaeus* and *O. niloticus* have a much larger native range. Another well-known species, *O. aureus*, is native to the Nile delta and the Middle East.

Tilapias live in a wide variety of natural ecosystems:

- slow-moving parts of rivers;
- floodplain pools;
- swamps;
- lakes;
- coastal lagoons.

They are considered to have evolved as riverine fish that eventually colonised lakes. They are strictly warmwater species. They stop growing at temperatures below 16 °C and do not survive below 10 °C. They survive and grow in brackish waters, and many species can tolerate and grow in seawater. Adult tilapias primarily eat plant materials (phytoplankton, benthic algae, macrophytes, etc.) and detritus derived from plant materials. They are also highly opportunistic feeders that are capable of changing their choice of food items or their feeding habits.

16.3.2 Translocations

The ability of tilapias to adapt to a wide variety of environmental conditions has led to the successful translocation of many tilapias within and outside Africa. The first

translocation outside Africa occurred in the early 1930s, when *O. mossambicus* was introduced into Java (part of present-day Indonesia). From there it was introduced into much of tropical Asia and eventually into the Americas. Most of the introductions were purposeful, mainly for controlling aquatic weeds and insect pests, farming, or enhancing fisheries. But several accidental and undesirable introductions have also occurred. For example, *O. mossambicus* was introduced into Australia (Queensland) in the 1970s as an aquarium fish, which then escaped into natural waters and became established as a feral population. Currently, it is regarded as a pest species that threatens the natural ecosystem it occupies. Other tilapias that have been deliberately introduced for farming purpose have also established their natural populations over a wide geographic range. They include *O. niloticus*, *O. aureus*, *O. urolepis hornorum*, *Tilapia rendalli* and *T. zillii*.

16.4 SEX DETERMINATION AND REPRODUCTION

16.4.1 Sex determination

Sex determination in tilapias is highly complex, with gender being determined by genetic, environmental and hormonal factors. Genetic determination is primarily through sex chromosomes and two different mechanisms for sex determination through sex chromosomes have been proposed:

1. Species such as *O. mossambicus* and *O. niloticus* possess a system similar to humans in which females are homogametic (XX) and males are heterogametic (XY).
2. Species such as *O. aureus* and *O. urolepis hornorum* possess a system in which females are heterogametic (WZ) and males are homogametic (ZZ). A cross of the species with different systems results in all-male or predominantly male progenies, as shown in Table 16.5.

Apparently, the male-determining gene (Z) in the WZ system dominates the female-determining gene (X) in the XY system, whereas the male-determining gene (Y) in the latter dominates the female-determining gene (W) in the former.

A number of studies have shown that sex chromosomes are not alone in determining sex in tilapias:

1. Genes in autosomes (general chromosomes) are suspected of playing a role in determining sex in *O. niloticus* and *O. aureus*. This may explain the inconsistent results obtained in the production of all-male *O. niloticus* × *O. aureus* progeny.
2. Some observations have been made with respect to the effect of temperature on tilapia sex determination. Exposure of *O. niloticus* fry to high temperature (34–35 °C) results in significant sex reversal in both directions, whereas that of *O. aureus* results in masculinisation. Another case is *O. niloticus* × *O. aureus*, a commercially produced hybrid in Israel. Because of its cold tolerance and, therefore, culture at relatively low temperatures, significant declines in the proportion of males have been observed in successive generations (Wohlfarth, 1994). The speculation that temperature dictates the sex of tilapia, however, remains controversial.
3. Steroid hormones have perhaps the most significant effect on sex determination in tilapias as well as in many other fish. Apparently there is an age (10–15 days after hatching) in the development of these species during which the sex is determined by the level of androgenic and oestrogenic hormones circulating in the body. Exogenous administration of a specific hormone or its analogue during this labile period will therefore result in the production of monosex stocks (section 7.7.3).

Table 16.5 Sex determination systems in tilapias that result in all-male or predominantly male progenies.

O. mossambicus, female (XX) × *O. urolepis hornorum*, male (ZZ)
↓
XZ (all-male hybrid)
O. urolepis hornorum, female (WZ) × *O. mossambicus*, male (XY)
↓
WX (25% female hybrids)
WY, XZ, YZ (75% male hybrids)

16.4.2 Reproductive biology

All tilapia species mature early (4–6 months) and reproduce year-round under suitable environmental conditions. Tilapias are perhaps the only major group of fish in aquaculture that breed in captivity without any special inducement or modification to their environment (Little and Hulata, 2000). The most common tilapia species, *O. niloticus*, reaches sexual maturity at a size of 30–40 g. When

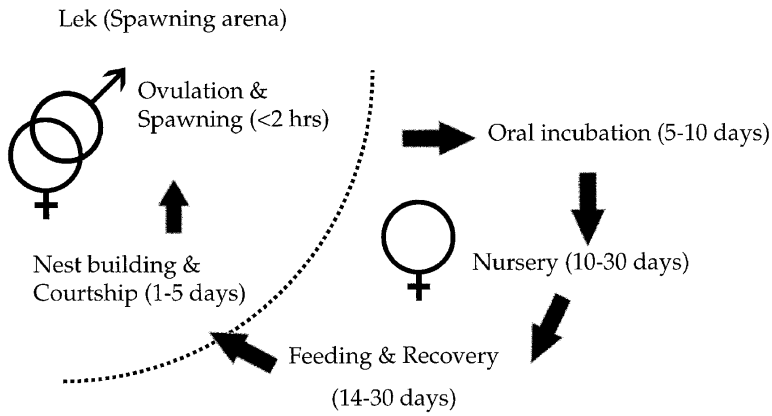


Fig. 16.4 Reproductive cycle of *Oreochromis* species.

mature, tilapias can spawn year-round if the water temperature stays between 24° and 35 °C. Typical breeding behaviour of *Oreochromis* species is shown in Fig. 16.4.

When a mature *Oreochromis* female is ready to spawn, she visits the breeding arena or 'lek'. The breeding arena consists of several males that form well-defended, individual nests. After brief courtship, the female lays her eggs while the male simultaneously fertilises the eggs. The female then picks up the fertilised eggs in her mouth for brooding and leaves the arena. Intensive parental care continues until the fry are large enough to be on their own. The female stands guard over the free-swimming fry. If the fry are threatened, they return to their mother's mouth until the threat passes. Mouth brooding lasts for 3 weeks, during which time the females eat little. Finally, the hatched fry are released in shallow waters. The female then resumes active feeding, which allows maturation of her ovaries. After a further 2–4 weeks, she is ready to spawn again.

Female tilapias lay their eggs in multiple batches. Typically, a female lays 8–12 batches/year under favourable temperature conditions. In the case of *Oreochromis* species, each batch contains about 2000 eggs. The eggs are large (3–5 mm) and contain sufficient yolk to supply nutrients to newly hatched fry for up to 5 days.

To boost broodstock productivity, the time between two successive spawnings may be considerably shortened by removing the fertilised eggs from a female's mouth. Once the clutch of eggs is removed, the female assumes that the eggs are lost and quickly returns to reproductive mode. This is similar to removing eggs from a chicken to encourage it to lay more eggs. The eggs removed from the females are incubated and hatched in down- or upwelling jars or trays with clean flowing water (Fig. 16.5) under controlled conditions that ensure high survival of eggs and fry making mass production of uniform seed possible.

16.5 CONTROL OF REPRODUCTION

As outlined in section 16.1, early maturation and prolific spawning of tilapias in grow-out systems present two major problems for tilapia farmers:

1. Energy resources are directed to the processes of sexual maturation and reproduction and become unavailable for somatic growth.
2. Continuous recruitment of young tilapias into the grow-out systems means increasing competition for resources such as space and food (Fig. 16.6).

In the past, several methods have been developed to control tilapia reproduction and recruitment. The most successful methods involve production of all male stocks because males grow faster than females in almost all tilapia species (Fig. 16.3).

16.5.1 Monosex tilapia production

16.5.1.1 Hand-sexing

Tilapias display distinct sexual dimorphism as they become juveniles. The males and females are differentiated by means of their genital morphology (Fig. 16.7):

- Males have a single longer and pointed opening which serves as an urogenital pore.



Fig. 16.5 Clutch removal: removing fertilised eggs from a female *O. niloticus* mouth in a hapa-based hatchery. (Photograph by Dr C. Kwei Lin.)



Fig. 16.6 Presence of male and female tilapias in culture systems results in reproduction before market size. (Photograph by Dr C. Kwei Lin.)

- Females have two round openings, one for urinary excretion and another for expulsion of eggs in the urogenital papillae.

This differentiation becomes more obvious when the fish are 10 g and larger. The typical practice is to sex tilapia individually before stocking for grow-out and select only the males for stocking. As this practice is very labour-intensive and wastes nearly 50% of the seed stock (females) it is rarely practised these days.

16.5.1.2 Hybridisation

The use of hybridisation to produce all-male populations was considered in section 16.4.1.

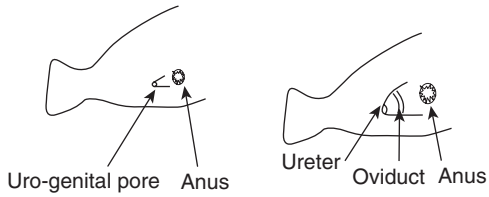


Fig. 16.7 Male (left) and female (right) tilapia genital papillae.

16.5.1.3 Hormonal sex reversal

General techniques for hormonal sex reversal in fish were outlined in section 7.7.3. Most commercial all-male tilapia stocks are produced by treating tilapia fry with synthetic androgens, particularly 17α-methyltestosterone (Phelps and Popma, 2000). The most commonly used protocol is to incorporate 17α-methyltestosterone into the fry diet at 40–60 ppm and feed it to the fry from the first feeding stage for 21–30 days. Seed stock with 99–100% males can be obtained if the protocol is strictly adhered to. A single hatchery can produce up to 20 million sex-reversed fry per month. Synthetic androgens such as fluoxymesterone and trenbolone acetate are also highly effective in producing all-male tilapia stocks. Instead of adding to the diet, the hormones could also be applied as a dip. Use of ultrasound at the time of androgen dip application has shown improvements in masculinisation success, but this method produces highly variable results and is still under development. Use of hormones in sex reversal evokes environmental and food safety concerns because of the potential for hormones to enter water bodies and the human food chain. On this basis, genetic manipulation methods that do not require hormone use for masculinisation have been tried.

Tilapias can be feminised using oestrogens. Non-steroidal oestrogens such as ethynyloestradiol and diethylstilboestrol are commonly used in feminising tilapias. The purpose of feminisation is to use feminised genetic males in breeding programmes to produce all-male stock (section 7.7.3).

16.5.1.4 Genetic manipulation

Genetic manipulation of tilapias to produce either monosex or sterile stocks has nearly a 25-year history. The method has been tried as an alternative to hormonal sex-reveral. Triploidy, gynogenesis and androgenesis are commonly used techniques that have been applied in the commercial production of tilapias. These techniques and their benefits and uses are described in detail in section 7.7.3. Chromosome manipulation techniques are not appropriate for routine production of sterile or monosex stocks because it is difficult to induce 100% triploidy, gynogenesis or androgenesis on a commercial scale. Furthermore, growth and survival of triploid, gynogenic and androgenic fish are generally inferior to those of normal diploids during early life stages.

16.5.1.5 Genetically male (YY) tilapias

Production of genetically male tilapias eliminates the need for using hormones to mass produce tilapia seed. The method, however, requires the use of hormonal sex reversal at the initial stages of broodstock development. It is a laborious and time-consuming method because of the extensive progeny testing involved. However, once a broodstock population is founded, it can be used in any hatchery system by replacing normal males with YY super-males. Hatcheries should then exercise sound broodstock management practices to prevent genetic contamination of the YY stock. Commercial stocks of genetically male tilapias are now available, including the pioneering stock produced by Fishgen Co. Ltd. together with University of Wales, Swansea. All-male tilapia are sold commercially as 'genetically male tilapia' (GMT®) to distinguish them from sex-reversed male tilapia. This stock has been shown to outperform equivalent mixed-sex tilapias and in some cases hormonally sex-reversed tilapias (Beardmore *et al.*, 2001). However, results are variable from country to country.

16.5.2 Recruitment control

Many resource-poor farmers choose culturing mixed-sex populations of tilapias because of lack of access to monosex fry. They may also prefer to raise mixed sex tilapias to produce their own seed stock for future stocking and also sell to others.

To prevent overpopulation during culture, predators that eat newly recruited tilapia fry and fingerlings may be used. Several predator species have been tested:

- seabass (*Lates calcarifer*);
- walking catfish (*Clarias* spp.);
- snakehead fish (*Channa* spp.);
- Nile perch (*Lates niloticus*);
- mahseer (*Tor* spp.);
- some South American cichlid species.

To be used reliably, this method requires optimisation of predator–prey ratios, timing of predator release,

population and size of the predator at release. It has not, however, achieved much success and has not been practised by commercial tilapia farmers.

16.6 SEED PRODUCTION

16.6.1 Harvesting from mixed-sex stock

Systems to produce seed stock for tilapia culture vary from location to location based on the following:

- local demand for seed;
- geographic conditions;
- environmental conditions;
- economic factors.

The simplest form is to use fingerlings that result as the by-product of tilapia grow-out in ponds or tanks. Any system that uses both sexes of a tilapia species in grow-out will inevitably produce tilapia seed as a result of natural reproduction (Fig. 16.6). Although reliable technologies are available to control reproduction through the production of all-male stock, many small farmers still use mixed-sex stocks in tilapia grow-out. For them, the fingerlings produced in their ponds are cost-free stocking material for further grow-out. This system, however, has many drawbacks, mainly inefficiency and unreliable supply of uniform-size seed. Hormonal sex reversal, the most common technique used in the production of all-male tilapias, has very low success rate with the fry collected from the ponds.

The result of this inefficient system is the development of dedicated seed production systems. These systems may use open ponds, tanks or net cages (hapas) in ponds or large water bodies (Table 16.6).

16.6.2 Pond systems

Earthen ponds are widely used in the production of tilapia fry or fingerlings (Fig. 16.8). The ponds are typically small (0.01–0.1 ha) and well managed by means of fertilisation, water control, etc. Brood fish are stocked at low densities (0.5–1 tilapias/m^2) with a male : female ratio of 1 : 2 or 3, and they are fed. Fry and fingerlings are netted out on a periodic basis (daily, weekly or bi-weekly). This type of system may yield fry at a rate of 0.1–3/m^2/day. The following factors influence fry yield in pond systems (Little and Hulata, 2000):

1. Pond size. The smaller the pond, the better it can be managed and harvested, and therefore this increases its productivity. Ponds with an area less than 1000 m^2 are preferable.
2. Harvest interval. More frequent harvests result in removal of the larger seed, thereby reducing cannibalism on younger fry.
3. Stocking density. Lower stocking density typically improves broodstock efficiency and produces larger seed.

Table 16.6 Comparison of various representative hatchery systems to produce tilapia fry.

	Pond	Tank	Hapa
Species	*O. niloticus*	Florida red tilapias	*O. niloticus*
Location	Philippines	Bahamas	Philippines
System size (m^2)	300–500	34	1
Stocking density (number per square metre)	1	7	2
Size (g)	Male and female 50–100	Male and female 143	Female 86, male 50–200
Sex ratio (F : M)	3 : 1	3 : 1	1 : 1
Harvest strategy	Harvest of fry, twice per day	Clutch removal every 15–16 days and artificial incubation	Clutch removal every 4 days and artificial incubation
Fry output (number per square metre per day)	0.7	91.7	20
Female productivity (fry per kilogram per female per month)	82.9	3021	6990
Source	Guerrero (1986)	Watanabe *et al.* (1992)	Mair *et al.* (1993)



Fig. 16.8 Collection of tilapia fry from ponds using a scoop net.

However frequently and efficiently fry are harvested from breeding ponds, it is practically impossible to remove all fry from a pond. The pond is soon overpopulated with recruits from early spawns. This results in increased competition for food and space, which diminishes seed output. To overcome this problem, the breeding ponds have to be periodically drained to remove all fish. An alternative to this practice is to seine the pond and remove all broodstock, which are then transferred to another pond. The fry left in the pond are further nursed to a size suitable for stocking. Another alternative, practised in Israel, is to construct breeding ponds with two compartments: a spawning compartment and a fry-collecting compartment that is built at a lower level relative to the spawning compartment (Little and Hulata, 2000). The two compartments are separated by a sluice gate that retains the broodstock and allows collection of fry with minimal handling.

16.6.3 Tank systems

Concrete, plastic or fibreglass tanks may be used in tilapia seed production. A higher degree of control over the broodstock and seed, as well as the spawning environment, is a major advantage in the use of tanks as seed production systems (section 16.5.1). Practices such as clutch removal and broodstock reconditioning, which improve hatchery efficiency, can be implemented in tank-based systems with relative ease. Tank systems are typically used when water or land resources are limited. This is because of their high seed output per unit of water and land area. The main disadvantage of tank systems is the high cost of initial investment to construct them.

16.6.4 Hapa systems

Hapa-based hatchery systems provide some of the advantages of tank-based systems at a lower cost. A hapa is a cage made of netting that can be suspended in ponds or large water bodies such as lakes and reservoirs (Fig. 16.9). Brood fish are stocked inside the hapa. Eggs and larvae are regularly collected by clutch removal. The hapa can also be designed to have two nested compartments. The inner compartment has a mesh that retains the broodstock but allows the fry to swim to the outer compartment, which is composed of fine-mesh netting. The inner cage, with brood fish, is removed every 10–15 days and the fry collected in the outer cage are allowed to grow to a size suitable for stocking. The pioneering hapa-based system developed at the Asian Institute of Technology, Thailand (Fig. 16.9), has been described in Bhujel (2000). The system uses 40–120 m^2 hapas in which male and female tilapia are stocked at 1 : 1 or 2 ratio and 5 or 6 fish/m^2 density. Seed production in the hapas is over 100 fry/m^2/day. Several private hatcheries around the world in



Fig. 16.9 Net cages (hapa) used for tilapia fry production.

Bangladesh, Brazil, China, Thailand, the Philippines and Vietnam currently use this system. A major disadvantage of the hapa system is that the mesh openings of the hapas are easily fouled. This limits water exchange and supply of natural food organisms. So, hapas must be pulled out of the water periodically (every 15 days is recommended) and washed or sun-dried. This is a labour-intensive practice, but it provides opportunities to rest and recondition the broodstock, thereby improving their productivity.

16.7 NUTRITION, FEEDS AND FEEDING

16.7.1 Diet and feeding habits

Although tilapias in general are opportunistic omnivores, there is considerable specialisation in diet in some species. Young tilapias are carnivorous and prefer zooplankton. As they become juveniles, their diets shift to plant material or detritus of plant origin or both food sources. Common foods of tilapias in nature are phytoplankton, benthic algae, macrophytes and periphyton (the community of microscopic and small organisms on the surfaces of benthic structures). *Oreochromis* species feed primarily on microscopic plant materials, whereas *Tilapia* species prefer large plants.

Tilapias use a wide variety of feeding methods:

- visual feeding;
- suction feeding;
- biting;
- grazing.

The primary method of feeding in adult tilapias, particularly in *Oreochromis* species, is continuous suction feeding, in which food particles are entrapped by filtration as water is routinely passed over the gills. The food particles are crushed by the pharyngeal bones and then passed into the alimentary tract. Tilapias possess a stomach that can reach extremely acidic conditions (pH < 1) in proportion to the stomach fullness. This acidic condition lyses plant cells and prepares food material for further digestion in the intestine. Tilapias have a very long, coiled intestine. Intestinal pH is 6.8–8.8 and conducive to the action of digestive enzymes such as trypsin, chymotrypsin and amylase. Anaerobic fermentation may also occur in the hindgut. Crude protein digestibility and assimilation efficiency of plant matter (filamentous and planktonic green algae, and blue-green algae) ranges from 50 to 80%.

16.7.2 Nutrient requirements

16.7.2.1 Protein

Like most fish species, tilapias require a high concentration of proteins in their diets when they are young. Various assessments (30–56%) of the protein requirements of young tilapias have been reported. What is clear is that the protein level required for fry is greater than for adults.

Field studies show that adult tilapias grow well when feeds containing 25–32% protein are used and that feeds containing higher protein levels do not add any substantial value in terms of growth. The recommended levels of protein in the diet range from 40 to 45% for fry to 25–30% for grow-out, with brood fish requiring a slightly higher level (25–35%) than fish intended for grow-out. There may be considerable differences in nutrient requirements and utilisation among different strains of tilapia, as Ng *et al.* (2008) demonstrated for crude protein in red tilapia hybrids and GIFT tilapia strain in Malaysia. The GIFT tilapia strain have a higher potential for growth, feed intake and feed efficiency, and are better able to utilise a diet containing higher protein than the red hybrids.

16.7.2.2 Energy

Although a feed that meets the protein requirements of tilapias is likely also to meet the energy requirements, balancing protein and energy is important in optimising feed cost relative to growth and other production parameters. The optimal protein–energy ratio in tilapias is 110–120 mg protein/kcal of energy when the fish are young. This decreases to about 100 mg/kcal of energy as they grow and reach adulthood.

Lipids and carbohydrates are cheaper sources of energy than protein. In general, warmwater omnivores such as tilapias utilise carbohydrates better than lipids. Lipid levels above 12% in feeds cause reduced growth in tilapias, probably because of reduced feed intake, whereas digestible carbohydrates as high as 40% are well utilised. Among carbohydrates, starch and dextrin are better utilised than glucose, whereas cellulose and other fibre components are not digestible. Inclusion of fibre above 5% causes depressed growth in tilapias.

16.7.2.3 Lipids and fatty acids

As noted, tilapias do not appear to use lipids effectively as an energy source. Suggested maximum lipid levels in the diet for tilapias range from 5 to 12%. Excess lipids also result in substantial carcass and visceral deposition of fats. Vegetable oils such as corn oil and soybean oil are superior sources of lipids compared with animal fats, including fish oil. It appears that tilapias require low levels (= 1% or less) of linoleic acid (18:2*n*–6) and linolenic acid (18:3*n*–3) in their diet (section 8.3.3).

16.7.2.4 Minerals

In the typical hard water used in aquaculture, there is sufficient calcium to meet the calcium requirements of tilapias. In soft waters, dietary calcium is required. Similarly, a dietary supply of magnesium is important in waters that are low in this ion. Recommended dietary levels of minerals have been based on the requirements of other freshwater fish, especially channel catfish (section 17.7.1).

16.7.2.5 Vitamins

The need for vitamin supplementation in semi-intensive pond culture of tilapias is questionable, because natural foods are rich in many vitamins. However, complete feeds for tilapias in intensive systems, particularly indoor tank systems, require comprehensive vitamin supplementation of the kind that is used in complete feeds for other fish in intensive culture (section 8.3.4).

16.7.3 Feeds and feeding

16.7.3.1 Feedstuffs

Tilapias are capable of utilising a wide variety of feedstuffs either as a single feed or as a part of compounded feed. Feedstuffs that are fed to tilapias in extensive grow-out systems include the following:

- rice bran and various other grain by-products;
- oil seed residues;
- aquatic and terrestrial plants;
- kitchen waste.

Numerous feedstuffs, including some of those above plus various animal by-products, tubers and fermentation by-products, are used in compounded feeds. Protein and energy digestibility for these feedstuffs ranges between 30 and 90% (Hanley, 2000) and, from this range, clearly some feed ingredients are to be preferred. Other factors that influence the choice of components used in feed formulations are as follows:

- cost;
- fibre content;
- amino-acid profile;
- palatability;
- toxins;
- digestive enzyme inhibitors.

Table 16.7 shows examples of some feed formulations for tilapias. Once considered essential, fish meal has declined in its importance in tilapia feeds. Today, tilapia feeds can be formulated without any fish meal and still perform optimally in most culture systems. This is a major advantage of tilapias for aquaculture.

Table 16.7 Model tilapia feed formulations based on ingredient availability and costs in the USA. Modified from Lovell (1998) with permission from Springer.

Ingredient	Semi-intensive ponds (26% protein)	Intensive ponds (32% protein)	Intensive tanks (36% protein)
Soybean meal	38.3	48.5	50.8
Wheat middlings	4	20	18
Fish meal	4	6	12
Corn	50.8	22.6	16.5
Dicalcium phosphate	1	1	0.8
Vegetable oil	1.5	1.5	1.5
Vitamin mix	0.2	0.2	0.2
Mineral mix	0.2	0.2	0.2

16.7.3.2 Feed forms and sizes

Tilapias accept feeds as dry meal, moist meal and pellets. Although dry meal is suitable for feeding fry, neither dry nor moist meals are appropriate for feeding growers in intensive culture systems. A considerable portion of meal-type feeds is wasted. Complete feeds that incorporate high-quality ingredients must be pelleted or extruded to minimise waste. Such processing also enables easy handling, storage and distribution of the feed by the farmer.

Particle size is an important consideration in selecting feeds for tilapias, which prefer smaller feed particles than many other cultured fish species. Unlike other species that swallow whole feeds, tilapias tend to chew large particles. These are repeatedly taken into the mouth and ejected until they are reduced to an appropriate size. This results in leaching of the nutrients and feed wastage. Table 16.8 presents the appropriate particle forms and sizes recommended for feeding tilapias of different body sizes.

16.7.3.3 Feed input

The extent to which natural foods are available to tilapias influences the amount and quality of feed input. In ponds, the amount of natural foods available to individual fish depends on several factors such as soil fertility, type and amount of fertilisers added, and the number and weight of fish stocked. Tilapias stocked at small size (<10 g) and low density (1–2/m^2) typically grow very well on natural foods, and pond fertilisation may be sufficient to meet the nutrient requirements for growth. However, once the fish reach 40–80 g, satisfactory growth cannot occur on natural foods alone. Supplementary feeds are needed at this stage to attain good growth rates. As natural foods are high in protein, feeds rich in energy, such as rice bran, are ideal supplementary feeds. As the fish grow further to a larger size, for example >300 g, natural foods and supplementary feeds are not sufficient to sustain growth, so complete feeds are required for further growth. As stocking densities increase, growth plateaux occurs with smaller sizes of fish, so that complete feeds must be introduced earlier in the culture cycle. The potential of complete feeds to increase growth of tilapias in semi-intensive pond culture was demonstrated by Edwards *et al.* (2000) (Fig. 16.10). *O. niloticus* juveniles of ca. 25 g initial weight were stocked at 4/m^2 in 200-m^2 fertilised ponds for 4 months. The ponds were subjected to one of the following treatments:

- (F): fertilisation (4 kg N urea, +2 kg P triple superphosphate, Na_3PO_4);
- (F + E): fertilisation + energy (pelleted cassava starch + lipid);
- (F + E + P): fertilisation + energy + protein (fish meal and soybean meal);
- (F + E + P + P): fertilisation + energy + protein + phosphorus (as dicalcium phosphate);
- (F + E + P + P + V): fertilisation + energy + protein + phosphorus + vitamins.

Growth was significantly improved as each nutrient type was added into the feed, except for the vitamins. The

Table 16.8 Feed forms and particle sizes recommended for tilapias. Modified from Luquet (1991) with permission.

Body size (g)	Particle size diameter (mm)	Recommended form
<1	0.5–1	Meal
1–2	1–1.5	Crumbles
2–30	1–2	Crumbles
30–100	2.4	Pellets/extruded particles
100–250	3.2	Pellets/extruded particles
250 to market size	4.8	Pellets/extruded particles

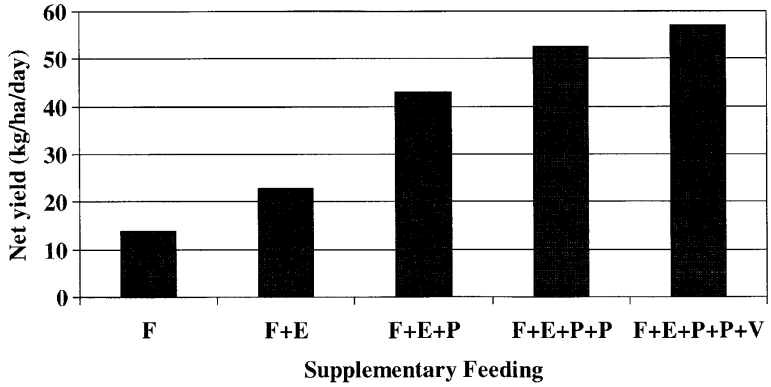


Fig. 16.10 Effect of supplementary nutrients on tilapia yield in semi-intensive ponds (see section 16.7.3 for more details). Modified from Edwards *et al.* (2000) with permission from Kluwer Academic Publishers.

addition of the energy source approximately doubled the net yield. Similarly, the addition of the protein meals approximately doubled the net yield. Addition of phosphate increased net yield by ca. 9 kg/ha/day. The increase in net yield with addition of vitamins, however, was not statistically significant, supporting the view that the natural feeds available to the animals in semi-intensive culture should provide sufficient vitamins.

16.7.3.4 Feeding allowance

There are two options for practical feeding of fish. One is to feed the fish to satiation and the other is to feed a restricted ration (section 15.5.2). Best growth is normally achieved by feeding to satiation. However, satiation levels are not necessarily the most economic feeding levels, because food conversion at satiation levels is often poor. Also, it is difficult to determine satiation levels in fish because food consumption occurs in the water medium. This may lead to overfeeding, which is wasteful and deleterious to water quality. As a result, restricted rations are recommended for feeding fish. The choice between satiation and restricted feeding should be based on the protein and energy density of the feed. Although restricted feeding of high-protein, high-energy diets is beneficial, low-protein, low-energy diets have to be fed at satiation levels to meet the nutritional requirements of the species.

As discussed in the previous section, natural availability of foods also determines feed allowance. Trials in Thailand showed that feeding at 50% of satiation rates in fertilised ponds gave similar growth and yield as full satiation feeding (Diana, 1997). Other factors that must be considered when determining feeding allowance include the following:

Table 16.9 Feeding allowance and frequency for tilapias at different body sizes. Modified from Lovell (1998) with permission from Springer.

Body size (g)	Daily feeding allowance (% of body weight)	Feeding frequency (number of meals per day)
<1	30–10	8–12
1–5	10–6	6
5–20	6–4	4
20–100	4–3	3–4
>100	3–2	2–3

1. Body size. As a fish increases in size, it is obvious that the absolute amount of feed it can consume increases. However, the amount of feed it can consume per unit of body weight decreases. Recommended feeding allowances for different body sizes are provided in Table 16.9.
2. Temperature and other water quality factors. Feeding allowances presented in Table 16.8 are for tilapias grown at optimum temperature conditions (26–32 °C). Feed consumption in tilapias decreases with decreasing temperature owing to declining metabolic rate and ceases at 16 °C. Appropriate reductions in feeding allowance will be required under such conditions

Table 16.10 Feeding allowance as modified by water temperature.

Temperature (°C)	Percentage of normal daily feeding allowance
>32	80
24–32	100
22–24	70
22–20	50
20–18	30
18–16	20
<16	No feeding

Table 16.11 Desirable water quality for tilapia culture.

Water quality parameter	Desirable level
Temperature	26–32 °C
Dissolved oxygen	>3 ppm
Total ammonia	<1 ppm
pH	6.5–8.5
Alkalinity	>20 ppm
Hardness	>50 ppm
Salinity	0–20‰

(Table 16.10). Reductions in feeding allowance are also required when temperature increases above optimum levels. At such conditions, dissolved oxygen (DO) levels decrease and the toxicity of ammonia increases in culture systems. So, feeding levels have to be lowered at high temperatures too.

Reduced feeding levels are recommended whenever water quality problems are anticipated or encountered. Adverse weather conditions such as high winds and heavy rainstorms cause changes in water quality in pond systems. Heavy algal blooms in pond systems, which may be caused by high feeding rates, can cause severe oxygen depletion. In such events, feeding is suspended until conditions improve.

High stocking densities mean high feed input to the culture system. This leads to DO depletion and high ammonia levels. Stress due to poor water quality leads to poor feed consumption and conversion. So culturists using high stocking densities must use devices such as aerators and mixers or water exchange to maintain water quality at or above acceptable levels (Table 16.11). Pond trials in Honduras showed that maintaining DO in excess of 10% saturation produces more rapid growth (Diana, 1997). In static ponds, feed application must not exceed 120–130 kg/ha/day.

16.7.3.5 Feeding frequency and time

Tilapias are known to eat continuously through the day in their natural habitat, so multiple feedings may be beneficial. Fry, which eat up to 16% of their body weight every day, need to be fed 8–12 times a day. This frequency is reduced as they grow (Table 16.9). At the grow-out stage, two or three meals per day are sufficient for optimal growth. Daytime is the best time to feed tilapias. In their natural habitat, they eat during the day, with little or no feeding activity at night.

16.7.3.6 Feeding method

Hand feeding, although labour intensive, is considered to be the best method to feed tilapias because it allows the farmer to observe the animals and their feeding response. It is essential that the feed is distributed evenly over the water surface to allow all the fish to feed. Tilapias are socially aggressive and tend to develop social hierarchies in which one individual dominates others and appropriates more feed. Uneven distribution of feed will result in dominant individuals occupying places that receive the most feed and eventually lead to large variation in fish size at harvest.

Automatic feeders and demand feeders are useful for feeding fish in cage culture, especially when access to cages is difficult or time-consuming. They are also useful in large farms that would require extensive manpower to feed the fish or when manpower is prohibitively expensive. Automatic feeders are becoming widely used in many parts of the world, including in pond culture of tilapia in China (Fig. 16.11). The various methods for feed distribution in aquaculture systems are outlined in section 15.5.2.

16.8 GROW-OUT SYSTEMS

16.8.1 Extensive systems

Extensive systems for tilapias include a broad range of culture units:

- backyard ponds;
- roadside ditches;
- irrigation tanks;
- reservoirs;
- rice fields;
- wastewater treatment ponds (Table 16.2).



Fig. 16.11 Automatic feeder used in tilapia farming in China.

These systems are located in the tropics, operated by poor rural farmers for subsistence. They use basic aquaculture technology. Stocking is irregular and may consist of small tilapias harvested from the wild. No intentional fertilisation or feeding is carried out and yields are <1 t/ha/year. Although such systems are quite underproductive compared with the yield potential of tilapias, they are important to the farmers because they provide inexpensive animal protein to the farmer's family and some supplemental cash income.

16.8.2 Semi-intensive pond systems

Semi-intensive pond systems represent a vast improvement over extensive systems of production. The ponds are intentionally built for aquaculture. Stocking is planned. Seed is produced on the farm or bought from a hatchery. Methods to control reproduction in grow-out are applied. As in other semi-intensive pond culture, fertilisation of ponds is performed. Feeds, prepared on-farm or at a feed mill, are used. Yields are typically 3 t/ha/year, but some well-managed systems may yield up to 10 t/ha/year. Most tilapia production in the world originates from semi-intensive pond systems.

Semi-intensive tilapia culture systems can be used effectively to satisfy the needs of subsistence farmers as well as the increasing desire of small-scale farmers to intensify production to generate cash income. Considerable research has been directed towards understanding the scientific basis of productivity in semi-intensive tilapia pond systems and has resulted in a vast improvement of our knowledge of optimising pond inputs such as fertilisers and feeds.

16.8.2.1 Fertilisation of ponds

The ideal levels of N and P in pond water to sustain production of phytoplankton are difficult to estimate. In general, a N : P ratio of 10 : 1 is recommended because this is the ratio between nitrogen and phosphorus in most phytoplankton. A N level of 1.3 ppm and a P level of 0.15 ppm are recommended by some. Others favour a higher level of N (15–30 N to 1 P) to discourage the growth of nitrogen-fixing blue-green algae, which are low in nutritive value to fish, and to encourage the growth of green algae and diatoms, which have a high nutritive value. Loading rates of 4 kg N/ha/day and 1 kg P/ha/day are optimum for semi-intensive tilapia ponds. N loading rates in excess of 4 kg/ha/day, however, may be counterproductive as ammonia may increase to toxic levels. Lin *et al.* (1997) recommend 1.4–2 kg P/ha/day for new ponds because P is easily precipitated into insoluble forms by cations and is strongly adsorbed by pond soils. The high level of P fertilisation is also applicable to ponds in acid sulphate soils because of the soil's sequestering action.

The recommended levels of N and P may be applied in the form of organic fertilisers, such as livestock manures or crop residues, or as inorganic fertilisers. Livestock manures have traditionally been used in Asian aquaculture as fertilisers. In addition to supplying N, P and C, they may contribute trace elements. Furthermore, they may also play a role in the detrital food chain. However, the levels of N, P and the N : P ratio of most livestock manures are below optimum. Excessive application of manures to compensate for their low nutrient density results in severe oxygen depletion in the water and accumulation of organic matter in the pond bottom, which ultimately reduces yields. So, it is recommended that ponds are fertilised with livestock manures at moderate rates together with inorganic fertilisers to compensate for the nutrient deficiency. One particular strategy that has proved effective is to apply chicken manure at a rate of 200–250 kg /ha/week (dry-matter basis) and to supplement it with urea and triple superphosphate (Na_3PO_4) at the rates of 28 kg N/ha/week and 7 kg P/ha/week respectively. At a stocking density of three tilapias/m^2, this fertilisation regime provided an extrapolated net yield of 8–11 t/ha/year.

16.8.2.2 Supplementary feeding

The rationale and principles of supplementary feeding have been covered in section 16.7.3. Supplementary feeding is important in semi-intensive pond culture of

tilapias, and most nutrients required in complete feeds are also required in supplementary feeds (Edwards *et al.*, 2000). In practice, two options exist for supplementary feeding in semi-intensive pond culture of tilapias:

1. Provision of feeds that complement the natural productivity of the ponds. In this approach, the animals are provided with feeds throughout the grow-out period.
2. Start feeding once fish growth plateau on natural foods alone. Beginning feeding at a moderate size may be more economical than at smaller sizes (Diana *et al.*, 1996).

16.8.3 Polyculture

Polyculture of tilapias with other fish species is practised in many countries. Traditional polyculture is based on the premise that various species stocked together utilise different trophic niches that exist in a pond and therefore produce more biomass than if they were stocked alone in monoculture (section 2.3.5). Tilapias have been grown with carps in the Asian polyculture systems (extensive and semi-intensive ponds) for many decades. Similarly, tilapias have been grown along with common carp in semi-intensive pond systems in Israel (section 5.5.1). A recent trend in South and Central America is to grow tilapias with shrimp in brackish water ponds, as shrimp monoculture has been affected by several viral disease problems (section 21.3.1).

The suitability of a given species in polyculture depends on its compatibility with other species, and tilapias have been shown to be compatible with some carp species and other fish. However, in polyculture, tilapias and other fish species are stocked at low densities: 5000–10 000 fish/ha compared with 30 000–40 000 fish/ha as stocked in semi-intensive monoculture of tilapias. Limited studies and field experience suggest that tilapias are not appropriate species for polyculture at high densities. Tilapias are typically more aggressive in their feeding, and tolerate crowding and poor water quality conditions better than most tropical aquaculture species. When tilapias fetch attractive prices in the local or export markets, it is probably more profitable to grow them at high densities in monoculture than at low densities in polyculture. In many countries, such as Taiwan and Israel, tilapia farming began as polyculture, but has eventually developed into intensive monoculture. Tilapia polyculture is more desirable, however, when a farmer requires production of a variety of species for the market and where the other species used in polyculture also fetch an attractive price. In rural south-east Asia, tilapias are polycultured with carps and silver barbs because the last two also fetch an attractive price in the local markets.

16.8.4 Integrated farming

Tilapias play an important role in integrated agriculture/livestock-aquaculture systems in Asia. Integration of tilapia farming with pig, chicken and duck production systems is practised mostly in China and south-east Asia. Typically, the housings for the livestock are constructed adjacent to or above the tilapia ponds (section 2.3.6 and Fig. 2.11). The pond, therefore, receives manure and uneaten feed from the livestock system on a regular basis. An extrapolated net fish yield of 10 t/ha/year was reported in a tilapia–duck integrated system in Thailand and much of the production was attributed to the inefficient feeding of ducks that resulted in feed wastage. In rural Nigeria, small farmers fertilise their tilapia ponds with excreta from their chicken farms. The ponds serve an important purpose in being the source of water for the farm crops and for poultry in the dry season (Njoku and Ejiogu, 1999). Ponds integrated at 1000 chickens/ha received an excreta load of 3600 kg/ha/month (dry-matter basis), which resulted in an extrapolated net yield of 18.25 t/ha of an African catfish species and 14.9 t/ha of *O. niloticus*. Integrated aquaculture practices are further discussed in sections 2.3.6 and 4.7.1.

16.8.5 Intensive pond systems

Semi-intensive pond systems in the tropics are typically stocked at a rate of two or three tilapias/m^2, and they yield an average of 3 t/ha per crop, as previously indicated (Table 16.2). In areas where land or water or both are limited, or climatic conditions restrict growing season to less than 1 year, it is desirable to achieve very high productivity by using high stocking densities. Experimental culture of tilapias in earthen ponds at stocking densities of 5–10/m^2 has demonstrated that intensive tilapia farming is feasible in earthen ponds. Such intensive pond systems, however, must be managed to maintain adequate water quality, otherwise significant retardation in growth and food conversion will occur. For management purposes, the ponds must be small (<0.5 ha) and designed to drain and fill effectively within a short period. Aerators are used to maintain desirable DO levels, especially during the critical late night and early morning hours (Fig. 3.6). Adequate circulation of the water must be provided to minimise accumulation of organic waste in the pond bottom. There may also be daily exchange of a part of the water to remove organic debris accumulating on the pond bottom. In Israel and Taiwan, where intensive pond systems were



Fig. 16.12 Red tilapias reared in cages in Colombia. (Photograph by Dr George Chamberlain.)

developed, the production systems are connected to a large reservoir that serves as a water treatment body (section 5.2). Wastes accumulating on the bottom of ponds are flushed to the reservoir periodically by means of water exchange. Tilapias and carps are stocked in the reservoir at low densities to harvest the algae and thereby reduce nutrient load in the water. Yields ranging from 10 to 30 t/ha per crop are possible in intensive pond systems.

16.8.6 Cages

Cages provide the opportunity to grow fish in manageable units in large bodies of water such as lakes, reservoirs and the open ocean, as well as running water bodies such as rivers and irrigation canals (Fig. 16.12). An additional advantage of using cages for tilapia grow-out is that it minimises unwanted recruitment as most eggs drop through the bottom of the cages (although the stock still engage in energy-wasting reproduction). There is commercial cage culture of tilapias in Colombia, Brazil, Indonesia, the Philippines, Thailand, Vietnam and China. Reservoirs built for irrigation and hydroelectric power generation are used in many locations to grow tilapias in cages. The cages may be made of locally available bamboo, netting, etc., or constructed from commercial-grade materials such as PVC and steel. A wide range of cage sizes are used. Large cages (>1000 m^3) are used, but are hard to manage. Such cages are typically stocked at 20–25 tilapias/m^3 and yield about >12 kg fish/m^3/year. For intensive production, smaller cages (>500 m^3) are preferred and stocking densities are usually around 100 tilapias/m^3. The typical yield in such systems exceeds 24 kg fish/m^3/year. Popma and Rodriquez (2000) reported that cages in two major reservoirs in Colombia produced ca. 2225 t tilapias/year. Productivity ranged from 67 kg/m^3/year to 116 kg/m^3/year.

16.8.7 Raceways, tanks and water recycle systems

Intensive culture of tilapias in raceways is practised when there is an abundant supply of gravity-fed running water. There are a handful of commercial projects in Central America that grow tilapias in raceways. The source of water includes rivers, irrigation canals and reservoirs for hydroelectricity generation. The raceways are typical of this culture system, being generally long and narrow, lined with concrete and having water exchange rates in the range of 300–2400% per day. High stocking density (>50 tilapias/m^3) and biomass (>20 kg/m^3) are used. Typical yields of more than 40 kg/m^3 per 6 months are possible in raceway systems.

Rectangular, square, octagonal or circular tanks, lined with concrete or plastic, are also used in intensive tilapia farming (Figs 16.13 and 16.14). Stocking densities and yields are as high as those in raceway systems, but the tank systems are commonly used in conjunction with a water recirculation system to minimise water use. The recirculation system may involve exchange with an extensive pond for waste removal as described in section 16.8.5.

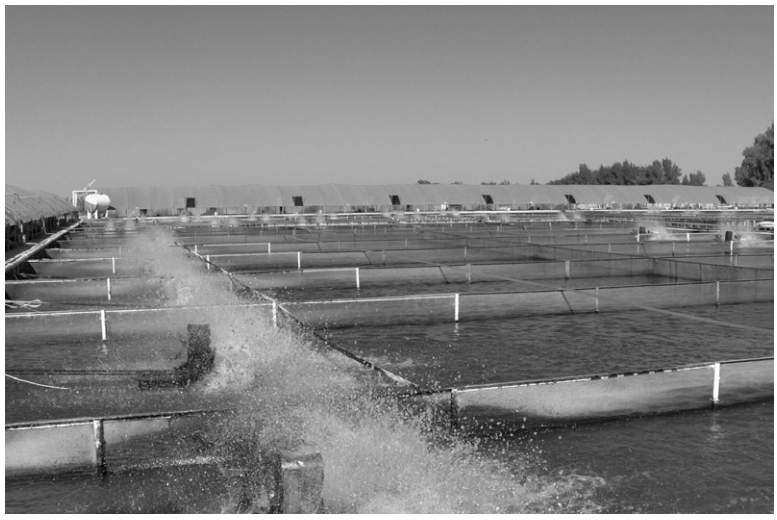


Fig. 16.13 Net-partitioned raceways to raise tilapia fingerlings. (Photograph by Al Ballaa Est., Saudi Arabia.)



Fig. 16.14 Concrete tanks for growing tilapias intensively in Taiwan. (Photograph by Dr C. Kwei Lin.)

Alternatively, it may involve a sophisticated recirculating system with components for removal of solid waste, soluble nitrogenous compounds, addition of oxygen and disinfection of the water (section 2.4.4). These systems are generally used in temperate regions of the world, particularly the USA, for commercial production of tilapias. In many cases, the water for these systems is derived from either geothermal springs or power plant effluents. The systems are generally housed within a greenhouse or a similar enclosure to conserve heat (Fig. 16.15). The systems may also be coupled with hydroponic production of vegetables, such as lettuce or tomatoes, which takes advantage of the nutrient-rich effluents from the tilapia culture units.

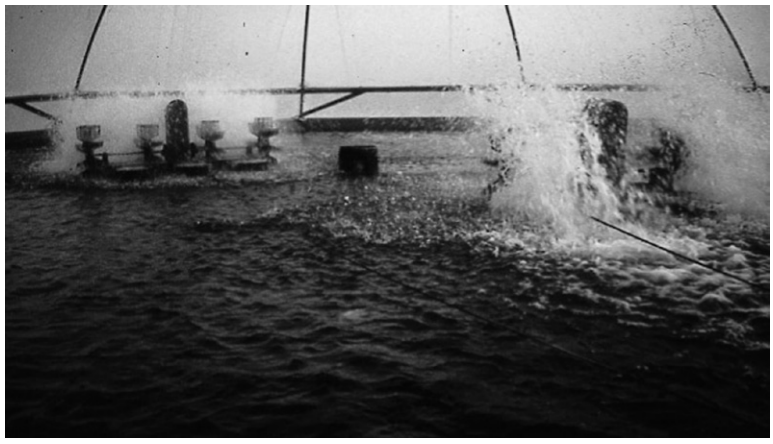


Fig. 16.15 Careful water quality management in an intensive tilapia culture system in southern California, USA. (Photograph by Dr C. Kwei Lin.)

16.8.8 Growing tilapias in saline waters

The ability of tilapias to tolerate and grow in saltwater has resulted in the development of commercial tilapia grow-out systems that utilise brackish or seawater (Suresh and Lin, 1992). Although semi-intensive culture of tilapias in brackish water ponds is now widely practised in Egypt, Ecuador and the Middle East, there are only a handful of experimental projects involving intensive tilapia culture in seawater in the Caribbean islands and the Middle East. Tilapias cultured in these systems have achieved growth rates and yields that are similar to tilapia cultured in equivalent freshwater systems. For example, Florida red tilapias grown in 0.2 ha brackish water (20–27‰ salinity) ponds at three fish per square metre and fed a 25% protein diet reached an average weight of 452 g in 160 days and yielded a crop of 11.5 t/ha in 220 days (Watanabe *et al.*, 1997). In Ecuador, tilapia replaced or supplemented shrimp stocked in brackishwater ponds when shrimp monoculture was affected by various viral diseases. Tilapia production in shrimp ponds grew rapidly in the late 1990s so that Ecuador became the largest supplier of fresh tilapia fillets to the US market in 2000 (Velasco and Freire, 2008). Average tilapia yield in brackishwater ponds is 7 t/ha achieved in 370–408 days.

16.9 DISEASE MANAGEMENT

Tilapias are far more tolerant of adverse water quality conditions and other stress factors, and are less prone to diseases than most other cultured fish. However, there has been a steady increase in the incidence and severity of diseases in intensive tilapia culture systems during the past few years. Common infectious and parasitic diseases of tilapias are presented in detail by Conroy and Conroy (2008).

16.9.1 Common diseases

16.9.1.1 Bacterial diseases

Streptococcosis due to *Streptococcus* species, particularly *S. iniae* and *S. agalactiae*, has emereged as a serious threat to intensive farming of tilapias worldwide (Klesius *et al.* 2006). Infection results in septicemia and neurotropic disease and causes cumulative mortality ranging from 30 to 50%. Early clinical signs are anorexia, lethargy, loss of orientation and erratic swimming. Later, the fish exhibit exophthalmia, a deformed back, and hemorrhages in the periorbital, intraocular area and base of fins and perianal region. Adverse changes in water temperature are usually associated with the onset of disease. Parasitic infections predispose fish to streptococcal infections.

Motile Aeromonas septicaemia due to *Aeromonas hydrophila* and related species is another common disease of tilapias. Clinical signs include the following:

- frayed fins;
- haemorrhaged skin and fins;
- inflamed skin and fins;
- scale loss;
- ulcerations on the body, head and mouth;
- liver pale, with small red spots;
- dark-red spleen.

Aeromonas infections occur in freshwater. Poor water quality, cold temperature and skin injury may facilitate infections. The mortality is typically chronic with low daily losses.

Other bacterial infections include vibriosis (due to *Vibrio* species), edwardsiellosis (due to *Edwardsiella tarda*) and columnaris (due to *Flavobacterium columnare*). Recently, a rickettsia-like organism identified as *Francisella* species has also been associated with high tilapia mortalities in Central America (Soto *et al.*, 2009). Non-specific clinical signs, such as erratic swimming, anorexia, anaemia, exophthalmia and high mortality, are displayed. Infected fish have an enlarged spleen and kidney that contain white nodules. The disease causes mortality of about 50%.

Clinical signs of most bacterial infections in tilapias are quite similar, so it is necessary to isolate, culture and identify the pathogen for diagnosis and treatment purposes.

16.9.1.2 Viral diseases

Lymphocystis and infectious pancreatic necrosis-like virus have been known to occur in tilapias, but neither poses a major disease threat. There are a few isolated reports of diseases due to identified or unidentified viral pathogens, but, so far, no viral disease has been widespread in the tilapia industry.

16.9.1.3 Fungal diseases

Secondary infections due to the water mould *Saprolegnia parasitica* are common in many fish species, including tilapias. Infections occur after injury and particularly when water temperature drops below the optimum. It is a common problem in hatcheries, affecting eggs and reducing hatch rates.

16.9.1.4 Parasite problems

The protozoan parasite *Ichthyophthirius multifiliis* (common name, ich), can cause severe mortalities in tilapias and other freshwater fish (section 17.7.4). This parasite is most lethal at water temperatures between 20 °C and 23 °C, so it is not a problem when tilapias are farmed in their normal warmwater temperatures. Other ciliated protozoans that affect tilapias are *Trichodina*, *Trichodinella*, *Chilodonella*, *Apiosoma*, *Ambiphrya* and *Epistylis* species. Common monogenetic trematodes such as *Dactylogyrus* and *Gyrodactylus* species, and parasitic crustaceans such as *Lernaea*, *Ergasilus* and *Argulus* species, have been observed in tilapias. Tilapias grown in seawater are highly susceptible to an ectoparasitic marine monogenean flatworm, *Neobenedenia melleni* (Watanabe *et al.*, 1997). Although most parasites do not cause mortality, they predispose the fish to other serious infections such as streptococcosis, because they damage the scale, skin, fins or gills.

16.9.2 Control of diseases

Table 16.12 shows some of the chemotherapeutic treatments for common infectious diseases in tilapias. Although prophylactic therapies are recommended as part of a disease prevention programme, treatment therapies with antibiotics have been limited in success. Relapse of diseases after antibiotic therapy is common.

Considerable success has been achieved in vaccinating tilapia against *Streptococcus* species (Klesius *et al.* 2006). The vaccine is prepared by culturing the infectious organisms and killing them in formalin (section 16.9.2). Researchers have found that the most effective vaccine incorporates not just the killed cells, but also the culture medium in which the infectious organisms are grown. The vaccine is called an extracellular products (ECP) vaccine. Optimum body size for vaccinating tilapia is 1–5 g. An immersion bath in the vaccine is used for primary vaccination. A booster delivery of the vaccine is provided through feed for 21–30 days after the bath. Tilapia vaccinated in this manner are protected against infections for 9–12 months and typically have 85–95% lower mortality than unvaccinated tilapia.

Plumb (1999) recommended the following environmental and stress management practices to reduce the incidence of diseases in tilapia culture:

- maintain the highest possible water quality;
- maintain prudent stocking densities and standing crops;
- disinfect the water supply and equipment used in the culture facility;
- expedite removal of dead and moribund fish;
- handle tilapias gently during stocking, sampling, etc.;
- use prophylaxis during and after handling to aid wound healing.

Diseases are also better managed by understanding the requirements of the disease agent. Tilapia hatcheries use saltwater at 5–10‰ to control ciliate protozoan parasites. Marine flatworm may be controlled by the use of low-salinity water. Although this type of treatment is possible in a land-based system, it is impossible or very difficult for seacages, which must be towed into low-salinity water, if this is feasible. So, a novel method of using tropical cleaner fish such as the cleaning goby (*Gobisoma genie*)

Table 16.12 Chemotherapeutic treatments for common infectious diseases of cultured tilapias. Modified from Plumb (1999) with permission from the World Aquaculture Society.

Drug/chemical	Concentration	Duration	Treatment purpose
Potassium permanganate	2–4 ppm	Indefinite immersion	Prophylaxis, external bacterial infections such as septicaemia and columnaris, and parasites
Formalin	20–25 ppb	Indefinite immersion	
Copper sulphate	0.5–3 ppm	Indefinite immersion	
Potassium permanganate	4–10 ppm	1-h immersion	
Formalin	167 ppb	1-h immersion	
Chloramine-T	10–20 ppm	1-h immersions for 3 days	External bacterial infections
Terramycin	50 mg/kg/day	In feed for 12–14 days; 21-day withdrawal period	Systemic bacterial infections such as vibriosis, edwardsiellosis and streptococcosis
Romet 30	50 mg/kg/day	In feed for 5 days; 42-day withdrawal period	
Erythromycin	50 mg/kg/day	In feed for 12 days	
Amoxycillin	50–80 mg/kg/day	In feed for 10 days	
Hydrogen peroxide	500 ppb	1-h immersion	Fungi

and the neon goby (*Gobisoma oceanops*) has been found effective in reducing the parasite load in such conditions.

16.10 HARVEST, PROCESSING AND MARKETING

Harvest size of tilapias varies from 150 g to over 600 g, depending upon the market requirements. Some markets, such as in the Philippines, prefer smaller size tilapias because the typical portion size is a whole fish per person. So, a family of four or five members prefers to buy four or five fish weighing a total of ca. 1 kg for a meal. Larger harvest sizes are required for filleting. Tilapias have relatively low dressing yields: only 50–55% of the fish is available as dressed carcass and 25–30% as fillets. So a tilapia weighing more than 500 g is required to produce a fillet weighing 100 g.

Seining is the most common method used to harvest tilapias from ponds. It is difficult, however, to harvest tilapias by seining alone because they tend to jump over the seine or escape underneath the seine. Partial or complete draining of the ponds is required for a complete harvest. Tilapias reared in more intensive systems are relatively easier to harvest because of the smaller size of the system and the high density of fish.

Tilapias reared in ponds often develop an off-flavour problem due to metabolites of bacteria and blue-green algae that thrive in nutrient-rich ponds. This problem is solved by stopping feeding and flushing water through ponds 3–7 days ahead of harvest. Alternatively, the fish could be harvested and stocked in tanks with a flow-through water supply.

Harvested tilapias may be sent directly to the market or to a processor for further processing and packing. When market conditions dictate that the tilapias are sold alive, extreme care must be exercised in harvesting and transporting the fish. Typically, the fish are transported in metal or plastic holding tanks with adequate aeration. Fish for further processing may also be transported alive or in ice. Tilapias are processed in several different ways depending upon market requirements. The whole fish may be cleaned and frozen whole or frozen as fillets. However, fresh-chilled products fetch higher prices than frozen products in most markets. For chilling, the fish may be gutted and de-headed before refrigerated packing, or converted into fillets. Fresh-chilled products have a shelf-life of 10–15 days after slaughter.

Market value of tilapias ranges widely. Although whole tilapias in a local market in a developing country may cost less than US$1/kg, the retail price of fresh whole tilapia in a developed country may exceed US$10/kg (Fig. 16.16). This enormous range of prices provides opportunities for producers as well as processors to maximise their returns by keeping their operations flexible, which in turn is beneficial from an economic perspective (section 12.5). Producers may serve the entire spectrum of the market or



Fig. 16.16 Seafood stand, with tilapia, at the Pike Market, Seattle, Washington State, USA. The sign says 'FRESH WHOLE TILAPIA'.

target a specific market niche and optimise their resources to meet the needs of the chosen niche.

Nutritive value of tilapia to humans is regarded as low by some nutritionists based on data that the fish has low levels of *n*–3 fatty acids and high levels of *n*–6 fatty acids, especially arachidonic acid (Weaver *et al.*, 2008). This assessment is not widely accepted by other nutritionists who point out that the overall fat content of tilapia is low, and therefore its fatty acid levels cannot be compared with oily fish such as salmon (Harris, 2008). Further, it is pointed out that the association between arachidonic acid intake and poor heart health has not been unequivocally established. Table 16.13 presents comparative nutritive values of tilapia, salmon and chicken breast meat to show that tilapia has lower *n*–3 fatty acids than salmon, but lower fat, higher *n*–3 fatty acids and lower *n*–6 fatty acids than chicken breast. Recently, it has been shown that tilapia reared in semi-intensive pond systems have higher levels of *n*–3 fatty acids than tilapia intensively reared in tanks (Karapanagiotidis *et al.*, 2006). Overall, tilapia presents a food option that is high in protein, and low in fat and cholesterol, even though it does not have a high level of *n*–3 fatty acids like oily fish such as salmon.

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Table 16.13 Selected nutrients provided by 100-g raw portions of tilapia, farmed Atlantic salmon, and chicken breast meat (source: Nutrient Data Laboratory of the United States Department of Agriculture).

Nutrient	Tilapia	Farmed Atlantic salmon	Chicken breast meat
Energy (kcal)	96	208	263
Protein (g)	20.08	20.42	14.7
Total lipid (g)	1.70	13.42	15.75
Total saturated fatty acids (g)	0.77	3.05	3.26
Total monounsaturated fatty acids (g)	0.65	3.77	6.48
Polyunsaturated fatty acids (g)			
1. Linoleic acid (*n*–6)	0.21	0.9	3.2
2. Linolenic acid (*n*–3)	0.043	0.167	0.14
3. Arachidonic acid (*n*–6)	0.003	0.06	0
4. Eicosapentaenoic acid (*n*–3)	0.007	0.862	0
5. Docosahexaenoic acid (*n*–3)	0.113	1.104	0
Cholesterol (mg)	50	55	41

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17
Channel Catfish

Craig Tucker

17.1 INTRODUCTION

> Sometimes we vary our diet with fish – wall-eyed pike, ugly, slimy catfish, and other uncouth finny things
>
> *(Theodore Roosevelt, twenty-sixth President of the USA, 1858–1919).*

President Roosevelt's sentiment, although perhaps overstated in the quotation above, illustrates a major obstacle that was faced by farmers trying to market catfish in the USA. Quite simply, catfish had a public relations problem – until recently. Through effective advertising and promotional campaigns, farmers have managed to change the public's perception of catfish to be more in line with the sentiments of America's foremost humorist and author, Mark Twain. Against all odds, farm-raised channel catfish (*Ictalurus punctatus*) is now the most important aquaculture species in the USA. Although annual production has decreased in the past few years – from a high of 300 000 t in 2003 to 255 000 t in 2007[1] – catfish farming still represents about half the total USA aquaculture production.

Until about 2000, nearly all channel catfish aquaculture took place in the USA where fish are grown primarily for domestic markets. Since that time channel catfish aquaculture has rapidly developed in China, with annual production increasing from ca. 45 000 t in 2003 to 205 000 t in 2007. Unlike in the USA – where almost all catfish are grown in ponds – a large proportion (perhaps half) of China's channel catfish are grown in cages or pens placed in large reservoirs or rivers. Most channel catfish grown in China are exported and have become serious competitors of domestically produced catfish in the USA. There is little reliable information on culture techniques for channel catfish grown in China, so this chapter will focus exclusively on conditions in the USA.

[1]Data in this chapter on culture production are from FAO Fishstat Plus (2009).

17.2 BIOLOGY

Channel catfish are members of the family Ictaluridae (the North American catfishes), comprising seven genera with at least 45 species. Many ictalurids are fine table fish, but channel catfish account for nearly all commercial foodfish production. Channel catfish are native to central North America between the Rocky and Appalachian mountains, from the Gulf of Mexico north to the Hudson Bay drainage. They are popular sport fish and have been widely introduced throughout North America.

The fish is slender, scaleless, and normally pigmented fish are white to silvery on the undersides, shading to greyish blue or olivaceous to nearly black dorsally. Albino channel catfish are a beautiful peach colour (Fig. 17.1). Albinism is rare in wild fish but is not uncommon in certain domesticated strains. Young fish have irregular dark spots on their sides, but spots are absent in albino fish and are usually lost in normally pigmented fish over ca. 0.5 kg. Eight barbels, four dorsal and four ventral, are located around a sub-terminal mouth. Channel catfish have soft-rayed fins; however, the pectoral and dorsal fins

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Fig. 17.1 Normally pigmented and albino channel catfish fingerlings weighing ca. 50 g.

contain sharp spines. The anal fin is rounded and contains 24–30 rays. Another distinguishing characteristic is the deeply forked tail, although the depth of the fork may be much reduced in older fish, especially in large breeding males.

Channel catfish thrive in a variety of habitats – from clear, swiftly flowing streams to sluggish rivers, lakes and ponds (Jackson, 2004). They are bottom dwellers preferring substrates of sand and gravel. In nature, channel catfish are opportunistic omnivores and use food items in proportion to availability. Young fish feed primarily on detritus, aquatic insects and zooplankton; adults feed primarily on aquatic insects, freshwater crayfish and small fish.

Channel catfish tolerate a wide range of environmental conditions (Hubert 1999; Hargreaves and Tomasso, 2004). They evolved in a temperate climate and are adapted to grow and survive over a wide temperature range. Optimum water temperature for growth is 25–30 °C, but fish can survive at temperatures from just above freezing to ca. 40 °C. Growth is slow at temperatures less than 20 °C and feeding activity essentially stops at temperatures below ca. 10 °C. Channel catfish are stenohaline and their salinity tolerance varies with life stage. Eggs tolerate salinities as high as 16‰, but tolerance decreases to 8‰ at hatching and then increases to an upper salinity tolerance for adults of ca. 12‰. Growth of adults is slowed at salinities above ca. 6‰.

Age at sexual maturity varies from 2 to 12 years, depending on fish strain and the length of the growing season (fish generally mature faster in warmer climates). In nature, 2–4 years may be required to reach a weight of 0.5 kg, although growth rate depends on temperature and food availability. Channel catfish may live for over 20 years and attain weights in excess of 20 kg.

17.3 COMMERCIAL CULTURE

17.3.1 Aquaculture attributes of channel catfish

Regardless of potential market demand, no aquaculture industry can develop past infancy if the animal is difficult to produce. Taken as a whole, channel catfish possess a combination of biological and cultural attributes that make them excellent fish for commercial aquaculture. Channel catfish do not reproduce in culture ponds (owing to the absence of nesting sites), so the producer has control over pond populations. This is important because uncontrolled reproduction can lead to overabundance of fish, which may reduce the yield of marketable fish. Channel catfish are easy to spawn, and large numbers of fry are readily obtained using simple hatchery methods. Fry accept manufactured feeds at first feeding and fish do not require special food at any life stage. Channel catfish are hardy, tolerate a wide range of temperatures and environmental conditions, and adapt well to all commonly used culture systems. The flesh of channel catfish grown in aquaculture is firm and white, with a mild flavour that is highly esteemed by American consumers.

Although various strains of channel catfish account for almost all commercial production, there is considerable interest in interspecific hybrid females and × male blue catfish. Blue catfish have several desirable traits for aquaculture and the hybrid possesses many of the best features of both parents. In particular, the hybrid grows rapidly and is resistant to enteric septicaemia of catfish, the most important bacterial disease of channel catfish. The fish is also much easier to harvest than channel catfish. The major obstacle to the commercial use of this hybrid is the low hybridisation rate, which makes it difficult to obtain adequate numbers of fry for large-scale commercial use. Currently, there are problems in synchronising egg and sperm maturation, and damage of sperm or eggs during handling. Also, males must be killed to obtain blue catfish sperm, which is wasteful, especially if genetically superior individuals are identified. Other problems initially included inadequate knowledge of fertilisation protocols, incubation procedures and broodstock conditioning – all resulting in poor hatching and fry production rates. These problems are being addressed through research and the number of hybrid fry produced for culture increased from fewer than 4 million in 2002 to more than 30 million in 2007.

17.3.2 Industry development

Channel catfish have always been a popular foodfish in the south-eastern USA, and traditional markets were initially supplied by harvest from local rivers and lakes. The regional popularity of the fish stimulated interest in pond culture in the 1950s and 1960s. By 1965, ca. 5000 ha of ponds were devoted to channel catfish culture, and production totalled ca. 7500 t. Local markets remained the outlet for all production.

The industry began to expand at a rapid rate in about 1975. Expansion was stimulated, in part, by declining profits from traditional agriculture (mostly cotton and soybeans), and a desire to diversify agricultural production. Cooperation among farmers, particularly in the development of large feed mills and fish processing plants, and an effective national advertising campaign facilitated rapid growth of the industry. In 1980, ca. 15 000 ha of ponds produced 35 000 t of channel catfish; by 2003, ca. 100 000 ha of ponds were in production and over 300 000 t of fish were processed.

Development and growth of catfish farming in the USA before 2003 resulted from complex interactions of biology, physical geography, sociology, and economics. Hargreaves (2002) described catfish industry growth as a holistic, yet step-wise process, where complex factors interacted in often unplanned ways to allow small local enterprises to develop into a large aquaculture industry. He recognised four phases of industry growth:

1. development of culture technologies (1900 to the mid-1950s);
2. entry of independent farmers selling mainly to local markets (mid-1950s to about 1970);
3. vertical integration of farming, processing, and feed manufacture (mid-1960s to the early 1980s);
4. a period of industry intensification (1980–2000).

Since Hargreaves' review in 2002, global economic forces have caused significant reduction in US catfish production (section 17.10) and the future of US catfish farming is impossible to predict.

17.4 CULTURE FACILITIES

Over 98% of the channel catfish produced in the USA are grown in earthen ponds because production costs are generally lower for catfish grown in ponds than for those grown in any other culture system. The remainder of this chapter focuses on facilities and production practices used in pond culture of channel catfish. Detailed information on channel catfish aquaculture is provided by Tucker and Robinson (1990) and Tucker and Hargreaves (2004a).

Channel catfish are grown commercially in at least 35 states, although more than 90% of all US production occurs in three states (Mississippi, Alabama and Arkansas) located in the south-eastern USA. Within those states, production occurs in two well-defined geographic regions:

1. the Mississippi River floodplain in northwest Mississippi and southeast Arkansas;
2. the Blackland Prairie region of west-central Alabama and east-central Mississippi.

These two catfish-growing regions are separated by about 250 km and have similar climates, but pond hydrology and water use differ considerably between the two regions.

About 50 000 ha of ponds are used for catfish aquaculture in the Mississippi River floodplain. Nearly all the catfish fingerlings used in catfish farming are grown in this region. The land is flat and fish are grown in embankment ponds ranging in size from 2 to 6 ha and 1 to 2 m depth. Embankment ponds can be built in large contiguous tracts (Fig. 17.2) with little watershed to supply water by runoff. Groundwater pumped from a shallow alluvial aquifer is used to fill ponds and maintain water levels during dry periods.

An additional 25 000 ha of catfish ponds are located in west-central Alabama and east-central Mississippi, mostly in the western portion of the Blackland Prairie soil edaphic region. The land is nearly flat to moderately hilly. High-yielding aquifers in the Blackland Prairie are considerably



Fig. 17.2 A large channel catfish farm in northwest Mississippi.

deeper than along the Mississippi River, and this is reflected in water-use patterns and pond types in the prairie region. Whereas all catfish ponds in the Mississippi River floodplain are embankment ponds filled with groundwater, ca. 75% of the commercial catfish ponds in the Blackland Prairie are watershed ponds that use mainly rainfall and storm runoff for filling and maintaining water levels. The remaining 25% are embankment ponds similar to those used in the Mississippi River floodplain. These ponds are filled with pumped water from wells or surface water from nearby creeks.

17.5 PRODUCTION PRACTICES

Channel catfish farming begins with spawning of brood fish. Spawning begins in the spring when water temperatures increase to above 20 °C. At that time, brood fish held in ponds randomly mate and the fertilised eggs are collected from spawning containers and moved to a hatchery. Eggs hatch after 5–8 days of incubation at 25–28 °C and fry are reared in the hatchery for an additional 4–10 days. Fry are then transferred to a nursery pond, fed daily through the summer, and harvested in autumn or winter as fingerlings weighing 20–40 g each. Fingerlings are then stocked into foodfish grow-out ponds, fed daily, and harvested when they reach a size desired for processing (0.4–0.8 kg). In the catfish-producing areas of the south-eastern USA, where water temperatures are below 20 °C for about 5 months out of the year, roughly 18–24 months are required to produce a food-sized channel catfish from an egg.

This succession of events implies that there is a clearly defined production cycle, which, like terrestrial crops, is determined by seasonal temperature variation. Although seasonal changes in water temperature regulate fish reproduction and growth, market demands are such that there is no well-defined schedule for producing food-sized fish. Brood fish spawn and fry are produced in spring, but fingerlings are stocked into foodfish ponds throughout the cooler months of the year and foodfish are harvested year-around to meet the needs of processing plants. On most farms, ponds contain fish at various stages of grow-out throughout the year.

Farming practices are also complicated by management decisions made to optimise the production strategy for each farm. Some farmers specialise in producing fingerlings which are then sold to farmers specialising in production of food-sized fish. Other farmers combine all aspects of production. Those farms will have brood fish ponds, a hatchery, fry nursery ponds, and foodfish grow-out ponds. Specific management practices also vary among farms. This is particularly evident in the variety of management schemes used in foodfish grow-out ponds.

17.5.1 Reproduction and breeding

An important consideration in choosing a fish species for aquaculture is the ease with which the reproductive phase can be controlled or manipulated. In that respect, channel catfish are ideal for aquaculture. Channel catfish broodstock are easy to maintain in pond culture, and spawning efficiency (percentage of female brood fish spawning in a year) is reasonably good without any special manipulation of environmental conditions or the need for hormone treatments to induce ovulation. However, the farmer has control over reproduction because sexually mature channel catfish will not reproduce in ponds unless they are provided with a nesting container in which to mate. Eggs and fry of channel catfish are relatively large and easy to handle. They develop rapidly at the proper temperature and are hardy. Excellent egg hatchability and fry survival can be obtained in simple, inexpensive hatcheries. Fry accept relatively simple manufactured feeds at first feeding, which is important because it precludes the need for expensive special feeds during the early life stages.

Although channel catfish may mature sexually at 2 years, when weighing as little as 0.3 kg, they must be at least 3 years old and weigh at least 1.5 kg for reliable spawning. Fish of 4–6 years old, weighing between 2 kg and 4 kg, are considered prime spawners. Older fish produce fewer eggs per unit body weight, and larger fish may have difficulty entering the containers commonly used as nesting sites. During brood fish selection, sex of fish is determined so that females and males can be stocked into brood ponds in the desired ratio. Adjusting sex ratios is important because mostly males (which grow faster than females) may be selected if only the largest fish from a population are chosen as broodstock. The sex of mature channel catfish can be accurately determined by examining external urogenital features and secondary sex characteristics, which include a wide muscular head in the male and a narrower head and a soft, full abdomen in the female.

Broodstock are maintained at relatively low standing crops (less than 2500 kg fish/ha) to provide good environmental conditions and minimise suppression of spawning by overcrowding. Brood fish are seined from ponds and inspected every year or two. Large fish, which may be poor spawners, are culled and replaced with smaller younger brood fish. Broodstock replacement ensures a vigorous brood population and re-establishes proper fish standing crops.

Periodic inspection of broodstock also provides an opportunity for adjusting the sex ratios within brood populations. Female channel catfish spawn once a year, but males can spawn two or more times. Therefore, stocking more females than males make more efficient use of pond space. A good sex ratio in brood ponds is about two males for every three females.

Broodstock nutrition is important because a poor diet may result in poor egg quality or reduced spawning success. Limited ration can affect female brood fish in particular because males are more aggressive feeders and may out-compete females for scarce food. When water temperatures are consistently above 20 °C, broodstock are fed a nutritionally complete manufactured feed of at least 28% crude protein daily at ca. 1–2% of body weight. At water temperatures of ca. 13–20 °C, less feed is offered and feeding frequency is reduced to every other day. Channel catfish do not actively feed at water temperatures below 10–13 °C. Some producers stock forage fish, such as fathead minnows (*Pimephales promelas*), into brood ponds to provide food in addition to manufactured feeds.

Channel catfish reproduction is controlled by seasonal changes in water temperature. Exposure to relatively cool water temperatures (less than ca. 15 °C) for 1 month or more stimulates gametogenesis. A subsequent slow rise in average water temperature to 20–25 °C usually initiates spawning in the spring. Water temperatures of around 25–27 °C are considered optimum for spawning.

Channel catfish must be provided an enclosed nesting site for spawning. Various containers have been used successfully as artificial nesting sites (Fig. 17.3). Most containers have an internal volume of ca. 75 L and an opening of 15–25 cm across. Containers of that size are adequate for brood fish up to ca. 5 kg. Containers are placed in the brood pond shortly before water temperature is expected to rise into the range for spawning.



Fig. 17.3 Containers used as artificial nesting sites for channel catfish.

When water temperature reaches the spawning range, the male chooses a container and cleans the inside of debris and sediment. The female is attracted to the container by chemical signals and mating begins. Spawning occurs over a period of several hours as several layers of adhesive eggs are deposited. Females of 2–5 kg typically lay 6500–9000 eggs/kg body weight. Once spawning is complete, the male chases the female from the nest and guards the egg mass. The eggs are ca. 4.5 mm in diameter and initially are light yellow, becoming brownish yellow with age. Spawning success (percentage of females spawning) ranges from 40 to 80% each year, and depends mainly on the condition and age of the female brood fish and water temperatures during the spawning season.

Nesting containers are checked every 2 or 3 days for eggs. The container is gently brought to the surface and drained of water. If the male remains in the container, he is allowed to swim out before the egg mass is retrieved (Fig. 17.4). The egg mass usually sticks to the floor of the container and is gently removed by scraping it from the container. Eggs collected from the brood pond are placed in an insulated, aerated container and transported to the hatchery.

17.5.2 Hatchery practices

Channel catfish hatcheries are simple facilities that use single-pass, flow-through tanks for egg incubation and fry rearing. Desirable characteristics of channel catfish hatchery water are summarised in Table 17.1. Water temperature is perhaps the most important characteristic. If water temperature is below 25 °C, egg hatching and fry development are prolonged and fungi may invade the egg masses. If the water temperature is above 30 °C, embryos may develop too fast and there will be a high incidence of malformed or non-viable fry. Also, bacterial diseases of eggs and fry, and channel catfish virus disease of fry (section 17.8.1) are more common at higher water temperatures. Heating or cooling water is expensive, so it is desirable to use a source that supplies water with a stable temperature near optimum (27 °C). Most hatcheries use deep wells (300–400 m deep) to obtain water that is warmed by the internal heat of the Earth, making it unnecessary to heat or cool the water.

Shallow tanks holding ca. 400 L of water are used to incubate eggs and to rear fry. Water flows through the tank at 10–15 L/min. Egg hatching tanks are equipped with a series of paddles attached to a shaft running the length of

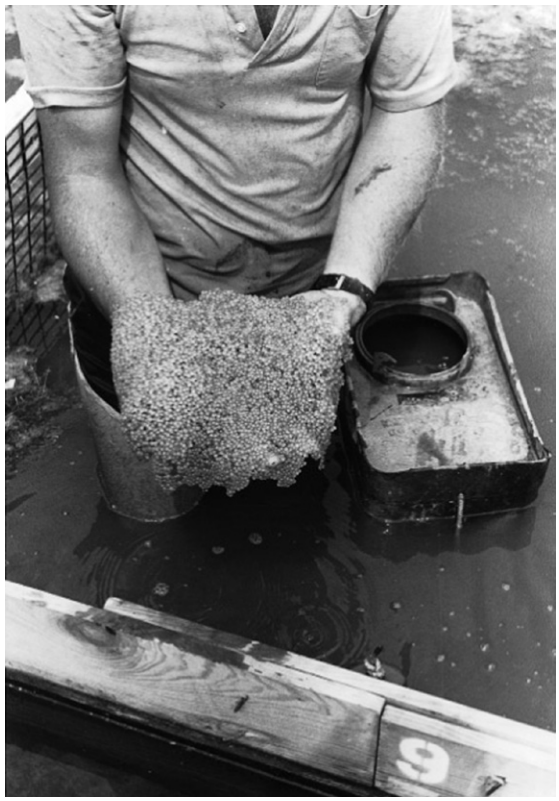


Fig. 17.4 A typical channel catfish egg mass retrieved from the spawning container.

Table 17.1 Desired water quality for channel catfish hatchery water supplies.

Variable	Desired level
Temperature	26–28 °C
Total dissolved gases	<105% total gas pressure
Dissolved oxygen (DO)	6 mg/L to saturation
Carbon dioxide	<10 mg/L
Ammonia (un-ionised)	<0.05 mg/L
Total iron	<0.05 mg/L
Hydrogen sulphide	<0.005 mg/L

the tank; the paddles are spaced along the length of the tank to allow wire-mesh baskets to fit between them. One or two egg masses are placed in each basket and paddles gently rotate through the water to provide water circulation and aeration. The motion of the paddles simulates the action of the male parent, who, under natural conditions,

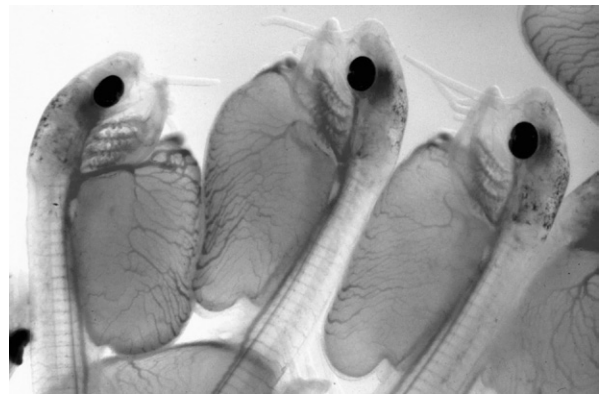


Fig. 17.5 Channel catfish sac-fry ca. 2 days after hatching. (Photograph by Dr Les Torrans.)

circulates water over the egg mass by fanning the water with his tail.

Incubation time for channel catfish eggs varies from 5 to 8 days, depending upon water temperature. At hatching, the fry (called sac-fry at this point) fall or swim through the wire-mesh basket and school in the corners of the tank. Sac-fry are easily siphoned into a bucket and transferred to a fry-rearing tank. Fry-rearing tanks are usually the same dimensions as egg-hatching tanks but are not equipped with paddles. Aeration in fry-rearing tanks is provided by surface agitators or by air or oxygen bubbled through air-stones.

Sac-fry (also called red fry; Fig. 17.5) are initially reddish-gold in colour and are not fed because they derive nourishment from the attached yolk sac. Over a 3- to 5-day period after hatching, they absorb the yolk sac and turn black. At that time, fry (now called swim-up, or black, fry; Fig. 17.6) swim to the water surface seeking food. Swim-up fry must be fed 6–12 times per day for good survival and growth. Fry feeds are generally dry and finely ground. They are nutritionally complete feeds containing at least 50% crude protein. Most of the protein should be supplied as fish meal. Fry are fed in the hatchery for 2–10 days before they are transferred to a nursery pond.

17.5.3 Nursery pond management

After a brief stay in a hatchery, fry are moved to a nursery pond for further growth. Fry stocking density determines the average size of fingerlings harvested from nursery ponds after one season of growth (150–180 days). To produce a 20–40 g fingerling, fry are stocked at 200 000–300 000 fry/ha.



Fig. 17.6 Channel catfish swim-up fry ca. 7 days after hatching. (Photograph by Dr Les Torrans.)



Fig. 17.7 Large channel catfish fingerlings (ca. 40 g/fish) ready for transfer to a foodfish grow-out pond. (Photograph by Dr Les Torrans.)

Nursery ponds are fertilised to provide abundant natural foods because fry are difficult to feed the first several weeks after stocking. Fertilisation practices vary with soil type, but a common programme involves weekly application of a high-nitrogen inorganic fertiliser, sometimes in combination with an organic fertiliser, such as cottonseed meal, rice bran or alfalfa pellets. Fertilisation begins 2–3 weeks before fry are added and continues until fish are actively accepting manufactured feed. Although fry may not be seen feeding on manufactured feed for several weeks after they are added to the nursery ponds, a finely ground, high-protein feed (40–50% crude protein) is offered once or twice daily to train fish to accept the feed. As fish grow, feed particle size is increased and feed protein level is decreased. One month or so after stocking, the fish (now called fingerlings) are fed once or twice daily to satiation, using a small floating pellet with 32–35% crude protein.

Fingerlings 5–9 months of age are harvested from nursery ponds in the autumn, winter and early spring for transfer to foodfish grow-out ponds (Fig. 17.7). Fry survival to the fingerling stage exceeding 75% is considered very good. Fingerlings from nursery ponds vary considerably in size and are usually graded to obtain a more uniform population for stocking. Grading is accomplished by using a seine with a mesh size that selectively retains fish of a certain minimum size.

After all fingerlings are harvested, the pond is drained and allowed to dry. Removing all fish from the nursery ponds is important to prevent cannibalism of fry stocked in the subsequent cycle of fingerling production. Allowing the pond bottom to dry helps reduce populations of predacious aquatic insects that prey on catfish fry.

17.5.4 Grow-out

In contrast with cultural practices used in hatcheries and nursery ponds, which are relatively standardised across the industry, management of foodfish grow-out ponds varies greatly from farm to farm. Wide variation in cultural practices is due, in part, to differences in production goals between farms and to the lack of information on the economics associated with different production strategies. Individual farmers have developed and used various production schemes based on experience, personal preference, and perceived productivity and profitability.

In the early years of the catfish industry, most farmers used some variation of the ‘single-batch’ cropping system,

wherein the goal was to have only one year-class of fish in the pond at a given time. Fingerlings were stocked, grown to a desired harvest size (usually 0.4–0.8 kg) and then completely harvested (usually in late autumn) before the pond was restocked with new fingerlings to initiate the next cropping cycle. Fish were either harvested at one time or harvested in two to four separate seinings, spaced over several months. After as much of the crop as possible had been harvested by seining, the pond was either drained and refilled, or restocked without draining to conserve water and decrease lost production time between crops.

The single-batch cropping system is still used by some farmers and remains common in areas where watershed ponds are used. Some watershed ponds are too deep to allow harvest without draining the pond, making it necessary to raise single crops of fish interrupted by pond drawdown and draining. The single-batch system is also used as the final stage of a three-phase production system wherein fingerlings are grown to 'stockers' weighing 100–200 g/fish in the second year, and stockers are grown to food-sized fish in the third year.

In the mid-1970s, the catfish industry was expanding past the point at which sales were primarily to local markets, and a new cropping system was developed to provide a year-round supply of food-sized fish to meet the demand of the new regional and national markets. This cropping system has become known as 'understocking', 'topping' or 'multiple-batch' (Tucker *et al.*, 1994). In the multiple-batch system, several different year classes of fish are present after the first year of production. Initially, a single cohort of fingerlings is stocked. The faster-growing individuals are selectively harvested using a large-mesh seine and fingerlings are added ('understocked') to replace the fish that are removed plus any losses incurred during grow-out. The process of selective harvest and understocking continues for years without draining the pond. After a few cycles of harvest and understocking, the pond contains a continuum of fish sizes ranging from recently stocked fingerlings (20–40 g) to fish over 1 kg.

Oddly, the single-batch system has several advantages over the multiple-batch, even though the multiple-batch system is more commonly used. Fish harvested from single-batch ponds tend to be more uniform in size than those from understocked ponds (Fig. 17.8), and uniform fish size is highly desired by fish processors. It is also easier to maintain accurate inventory records when using the single-batch cropping system because fish populations are 'zeroed out' after each round of grow-out. Feed conversion efficiencies also tend to be better for fish in single-batch ponds, probably because there is little or no year-to-year carryover of big fish, which convert feed to flesh less efficiently than small fish.



Fig. 17.8 Food-sized channel catfish harvested from a pond under the multiple-batch cropping system. Note the range of fish sizes (ca. 400–1000 g/fish).

On the other hand, when all ponds on a farm are managed as multiple-batches, more ponds will contain fish of marketable size at any one time than if the single-batch system is used. This is important because pond-raised catfish are often temporarily unacceptable for processing because of algae-related off-flavours (section 17.6.3). So, if timely harvest of fish from a particular pond is constrained by the presence of off-flavours (or other factors, such as ongoing losses to an infectious disease), there is a greater probability of having acceptable fish to sell from another pond when ponds are managed as multiple-batches. Economic analyses indicate that single-batch cropping systems generate greater net revenues than multiple-batch ponds when fish can be harvested and sold without constraint. But, in the real world, where the presence of off-flavours and other market constraints often prevent timely fish harvest, the multiple-batch system is the economically preferred cropping system (Engle and Pounds, 1994). This is probably why the multiple-batch system is the most commonly use cropping system and is likely to remain the cropping system of choice until reliable solutions to off-flavour problems are developed.

The other important management decision that farmers must make, besides choice of cropping system, is fish stocking density. There is no consensus on the best stocking density, and this is evident from the wide range of stocking densities used in the industry, which varies from less than 10 000 fish/ha to more than 35 000 fish/ha. Lack

of consensus on optimal stocking densities is, in part, related to the counterintuitive fact that profit-maximising management practices are seldom the same as yield-maximising practices.

High stocking densities and corresponding high feeding rates produce the highest fish yields, although fish grow slowly and environmental conditions in degraded ponds may put fish at greater risk of loss to infectious diseases. In the absence of information to the contrary, most farmers tend to manage for maximum yield, believing that yield and profits are simultaneously maximised. However, in two studies that examined the relationship between stocking density and economic return (Losinger *et al.*, 2000; Tucker *et al.*, 1994), profits were maximised at stocking densities well below yield-maximising stocking densities. For example, Losinger *et al.* (2000) used econometric modelling and farm survey data to identify profit- and yield-maximising stocking densities for catfish farming in the south-eastern USA. Maximum yield occurred at a stocking density of ca. 30 000 fish/ha, whereas profit-maximising stocking densities ranged from 17 000 to 21 000 fish/ha. This study did not address the effect of fingerling size at stocking, which is the third important management decision for foodfish grow-out (cropping system and stocking density being the first two). Obviously, much additional work needs to be conducted to determine the best grow-out procedures for food-sized catfish.

Despite considerable variation in fundamental production variables, daily management of foodfish grow-out ponds is similar from farm to farm. When water temperatures are above ca. 15 °C, fish are offered an extruded floating feed of 26–32% crude protein. Feed is dispensed from mechanical feeders that are mounted on, or pulled by, vehicles. Feed is scattered over a wide area of the pond; usual practice is to feed fish once daily to near satiation based on visual assessment of fish feeding activity. Because fish densities are high in commercial ponds, relatively large amounts of feed are offered daily. Feed allowances in commercial grow-out ponds average between 75 and 125 kg feed/ha/day during the late spring to early summer period of maximum feeding activity. Feeding activity declines as water temperatures drop in late autumn and feeding rates average less than 30 kg feed/ha/day during midwinter, although feed allowances may be considerably higher during abnormally mild winters. Of course, feed allowances vary depending on fish biomass and fish health. In fact, sudden changes in feeding activity are an important indicator of the general health of fish and reduced feeding activity is often the first sign of an infectious disease outbreak.

Foodfish grow-out ponds managed with the multiple-batch cropping system are drained only for purposes of pond renovation. Most commercial ponds remain in production for 3–20 years between pond renovations, with an average lifespan of about 7 years. Renovation usually involves partial drying of the bottom and scraping to smooth the bottom profile and restore the drainage slope. Accumulated sediment on pond bottoms is derived from erosion of levee slopes by waves. Thus, material scraped from the bottom is used to rebuild the levee and restore the proper slope. Other than measures needed to restore proper pond morphology, no treatment of the bottom soils is undertaken during renovation.

17.6 WATER QUALITY MANAGEMENT

High fish densities and large additions of manufactured feed are necessary for profitable culture of channel catfish in ponds. These practices result in large quantities of fish metabolic waste entering the water. Because channel catfish ponds are operated with no water exchange during the culture period, waste accumulation directly or indirectly causes deterioration of environmental conditions, which ultimately limits the production of fish. Availability of dissolved oxygen (DO), accumulation of nitrogenous waste products (primarily ammonia) and development of environmentally derived ‘off-flavours’ in fish are the primary factors that limit the intensification of channel catfish culture in ponds (Hargreaves and Tucker, 2003). Reviews of water quality management in channel catfish ponds are provided by Boyd and Tucker (1998), Tucker (1996) and Tucker and Hargreaves (2004b), and in ponds in general in Chapter 3.

17.6.1 Dissolved oxygen (DO)

Phytoplankton metabolism is the most important variable affecting DO budgets of channel catfish ponds (section 3.3.4). In ponds with dense phytoplankton blooms, DO concentrations often fall to critically low levels at night during the warm months of the year. The DO budget differs from pond to pond because ponds contain different standing crops of fish, different plankton densities and different densities of benthic organisms. The key to successful DO management in channel catfish ponds is early identification of those ponds that may require supplemental mechanical aeration to keep fish alive. Aeration is initiated (during darkness) when DO concentrations fall to a level considered critical by the individual farmer (usually around 3–4 mg/L) and continues until measurements indicate that DO concentrations are increasing as a result of photosynthesis during daylight (Fig. 3.6).



Fig. 17.9 Two 7.5-kW paddlewheel aerators in a catfish pond. (Photograph by Dr Les Torrans.)

Rates of photosynthesis and respiration decrease as water temperatures decrease; consequently, the amplitude of daily changes in dissolved oxygen decreases during the cool season. Problems with low dissolved oxygen are rare in channel catfish ponds when water temperatures fall below 15 °C, and most producers discontinue DO monitoring when water temperatures are expected to remain below that value.

Paddlewheel aerators (Fig. 17.9) are the most common type of aerator used in channel catfish ponds. Most paddlewheel aerators are powered by an electric motor (typically 7.5 kW) and are mounted on floats and anchored to the pond bank. Current practice is to provide aeration at ca. 2–4 kW/ha; for instance, two 7.5-kW aerators may be placed in a 6-ha pond.

17.6.2 Ammonia and nitrite

The use of mechanical aeration allows farmers to use high fish stocking densities and feeding rates without fish kills attributable to DO depletion. Nevertheless, aeration does not allow unlimited fish stocking densities and feeding rates because accumulation of potentially toxic nitrogenous waste products affects fish health and decreases fish growth rates as the intensity of the culture increases.

Virtually all the combined inorganic nitrogen in channel catfish ponds originates from nitrogen in feed protein and is excreted by fish as ammonia. The maximum nitrogen loading rate (in the form of fish feed) possible without unreasonable accumulation of ammonia in the water is governed in the long term by the rate at which natural physical and microbiological processes remove nitrogen from the pond (sections 3.2.12 and 3.2.13). Practical experience shows that feeding rates up to ca. 75–125 kg feed/ha/day are possible during the summer growing season without unreasonable accumulation of ammonia. This is generally accepted as the upper range for feeding rates in channel catfish culture ponds managed with no water exchange.

Nitrite occasionally accumulates in channel catfish pond waters and poses a threat to fish health (section 3.4.2). Nitrite from the water enters the bloodstream of channel catfish and oxidises haemoglobin to methaemoglobin. Methaemoglobin is a brown-coloured pigment incapable of binding oxygen. Fish with high levels of methaemoglobin in their blood may suffocate even when dissolved oxygen concentrations are high.

In south-eastern USA, episodes of elevated nitrite levels are most common in the spring and autumn when environmental conditions are rapidly changing. Fortunately, nitrite toxicosis is easily prevented by assuring that adequate chloride is present in the water. Chloride competes with nitrite for uptake at the gills and maintaining a ratio of 10 mg/L of chloride for every 1 mg/L of nitrite-nitrogen prevents nitrite toxicosis in channel catfish. Chloride is

inexpensively added to channel catfish ponds as common salt, NaCl.

17.6.3 Off-flavours

Objectionable flavours ('off-flavours') in processed channel catfish may be caused by feed ingredients, post-mortem rancidity or odorous compounds absorbed from the environment (section 11.11). The most common off-flavours described from pond-cultured channel catfish are attributed to geosmin and 2-methylisoborneol, which are two earthy–musty smelling metabolites produced naturally by certain species of cyanobacteria (blue-green algae). Species of the cyanobacterial genera *Oscillatoria* (= *Planktothrix*) and *Anabaena* are most commonly implicated in producing odorous compounds in channel catfish ponds (Tucker, 2000).

Off-flavours are common in pond-raised catfish and all fish are 'taste-tested' before scheduling pond harvest. Testing is conducted by trained personnel at the fish processing plants. Typically, fish samples are submitted a week or two before the desired harvest date, a day or two before harvest, and immediately before unloading live fish from transport trucks at the plant. The entire pond population of fish is rejected for processing if off-flavours are detected in any sample. The development of off-flavour is a severe economic burden for catfish farmers because it prevents the timely harvest of fish, increases production costs, disrupts cash flows and interrupts the orderly flow of fish from farm to processor. Furthermore, if off-flavoured fish are inadvertently marketed, the negative reaction of consumers may adversely affect market demand, with the overall effect of reducing profits for all segments of the industry.

The occurrence of earthy–musty off-flavours in channel catfish is episodic and coincides with the appearance and eventual disappearance of the cyanobacteria responsible for synthesis of the odorous compounds. Management of off-flavours in fish relies upon natural elimination of the odorous compound from the flesh to improve flavour quality once the fish is no longer in the presence of the organism producing the compound. Fish with off-flavours often are simply left in the culture pond until the odour-producing cyanobacteria disappear from the plankton community. Elimination of geosmin or 2-methylisoborneol from fish flesh is rapid; however, an unpredictable period (weeks to months) may be required for disappearance of the odour-producing algae from the community. More commonly farmers treat ponds with approved algicides to eliminate the door-producing algae. Fish flavour quality rapidly improves after the source of the odorous compound is eliminated.

17.7 NUTRITION, FEEDING AND FEED FORMULATION

Feed cost is the largest cost of producing channel catfish, accounting for about half of the total cost input. Efficient and economic feeds, together with effective feeding practices, are therefore essential in catfish farming. Knowledge of fish nutrition is the most advanced area of channel catfish culture. The general nutrient requirements of channel catfish have been established, and practical diets can be formulated from relatively few ingredients to meet those requirements. Feeding practices are less well standardised than nutrient requirements or feed manufacture and some consider feeding channel catfish to be much more of an art than a science. Feeding practices for each stage of channel catfish production were summarised above. Reviews of nutrition and feeding of channel catfish are provided by Li *et al.* (2004), Robinson *et al.* (2004) and Robinson and Li (2007).

17.7.1 Nutritional requirements

Forty nutrients have been identified as essential for normal physiological function of channel catfish. The qualitative nutritional requirements of channel catfish are similar to those of other animals, but the absolute and relative amounts of those nutrients needed by catfish may differ from those needed by other animals. Also, nutrient requirements for channel catfish may vary with fish size, growth rate, stage of sexual maturity, water temperature, diet formulation, feeding rate and other factors. Many of those interrelationships are poorly understood. Nutrient levels recommended for channel catfish grow-out feeds are shown in Table 17.2.

The range of dietary protein levels in Table 17.2 has been found adequate for grow-out of fingerlings to food-sized fish. Generally, the dietary protein level is higher in feeds used for smaller fish: recommended levels are 40–50% of diet for fry and 32–35% for small fingerlings. Also, the vitamin and mineral requirements for channel catfish were determined in aquarium studies using small, rapidly growing fish and may not accurately reflect the requirement for older, slower-growing fish. Furthermore, catfish ponds are fertile ecosystems with abundant natural food organisms that contribute protein and other essential nutrients to the diet of catfish. Nutrients in natural foods – particularly vitamins, minerals and essential fatty acids – offset part of the requirement in manufactured feeds, although the magnitude of that contribution is unknown

Table 17.2 Recommended nutrient levels for extrusion-processed channel catfish grow-out feeds (adapted from Robinson and Li, 2007).

Nutrient	Recommend level
Protein	26–32% of diet
Lysine	5.1% of dietary protein
Methionine[a]	2.3% of dietary protein
Digestible energy	8.5–9.5 kcal/g protein
Lipid	4–6% of diet
Carbohydrate	25–35% of diet
Vitamins	
Thiamine	2.5 mg/kg of diet
Riboflavin	6 mg/kg of diet
Pyridoxine	5 mg/kg of diet
Pantothenic acid	15 mg/kg of diet
Nicotenic acid	None
Folic acid	2.2 mg/kg of diet
B_{12}	0.01 mg/kg of diet
Ascorbic acid	50–100 mg/kg of diet
A	2200 IU/kg diet
D_3	1100 IU/kg diet
E	30 mg/kg of diet
K	4.4 mg/kg of diet
Minerals	
Available phosphorus	0.3–0.35% of diet
Cobalt	0.05 mg/kg of diet
Zinc	200 mg/kg of diet
Selenium	0.1 mg/kg of diet
Manganese	25 mg/kg of diet
Iodine	2.4 mg/kg of diet
Iron	30 mg/kg of diet
Copper	5 mg/kg of diet

[a]Cystine can replace 60% of the methionine requirement.

and likely to vary among ponds. Mineral requirements are particularly difficult to quantify because part or all of the requirement for some minerals may be met by absorption of dissolved minerals from the water.

17.7.2 Feed formulation and manufacture

Feeds used in intensive pond culture of channel catfish are formulated to provide all required nutrients in the proper proportions. No single foodstuff can supply the optimum mix of nutrients, so a combination of feed ingredients is used to meet nutritional needs. Fortunately, the number of different foodstuffs needed to formulate affective channel catfish feeds is small. Examples of formulations for practical channel catfish feeds are shown in Table 17.3.

Feeds for fry are prepared as meals or flours of small particle size (<0.5 mm). They are formulated to contain high dietary protein levels and high levels of fish meal. Most feeds for fingerlings and foodfish grow-out are prepared by extrusion cooking and drying (section 9.10.2). That process produces a hard expanded pellet that floats in water. Extruded feeds must contain ca. 25% corn or other high-carbohydrate grains for proper gelatinisation and expansion of pellets during extrusion. Soybean meal provides most of the protein in fingerling and foodfish feeds. Small amounts of fish meal or other animal protein may be added to improve amino acid balance and enhance palatability. Commercial trace mineral and vitamin premixes are added to all channel catfish feeds to ensure nutritional adequacy.

17.8 INFECTIOUS DISEASES

Infectious diseases played a minor role in fish losses on catfish farms before 1980 because low-intensity management practices generally resulted in good pond water quality and low disease incidence. Lower fish densities also made transmission of disease organisms among fish populations less efficient. Over the years, stocking and feeding rates steadily increased and producers adopted a multiple-batch cropping system wherein new populations of fingerlings were stocked into ponds with existing populations of larger fish and ponds were seldom drained. These practices lead to the emergence of infectious diseases as the primary limiting factor in catfish production, and disease outbreaks are not uncommon even on well-managed facilities.

Overall losses of catfish from the fry stage through harvest as foodfish has not been well documented but results of formal surveys (USDA/APHIS 1997a,b; 2003a,b) and anecdotal evidence indicates that overall survival is relatively poor and highly variable among pond populations. According to USDA/APHIS (2003a), survival during the nursery stage (fry to fingerling) averages about 70% across the industry. Anecdotal evidence indicates that survival is lower than indicated in this government survey, and may lie in the range of 50–60%. Survival varies greatly among individual pond populations, and may range from less than 10% to more than 90% on individual farms. Of the fry and fingerlings lost, approximately

Table 17.3 Examples of practical catfish feed formulations. Two examples of foodfish grow-out feeds with different crude protein and fish meal levels are shown. Values in parentheses are feed crude protein levels (adapted from Robinson and Li, 2007).

	Feed type			
	Fry	Fingerling	Foodfish	Foodfish
Ingredient	(50%)	(35%)	(28%)	(32%)
Fish meal (menhaden)	75	8	–	4
Soybean meal	–	44	35.5	42
Cottonseed meal	–	10	10	10
Corn grain	–	28	32	32
Wheat middlings	20	7.5	20	10
Dicalcium phosphate	–	0.5	1.0	0.5
Oil or fat	5	2.0	1.5	1.5
Mineral/vitamin premix	Include	Include	Include	Include

65% are believed lost to infectious diseases (USDA/APHIS 2003a). The bacterial diseases enteric septicaemia of catfish (ESC) and columnaris accounted for more than 50% of the reported loss (or nearly 80% of all infectious disease losses). Bird depredations accounted for 6% of the total loss of fry or fingerlings, while nearly 20% of the total losses were unexplained.

Survival from fingerling to foodfish has not been reported in government surveys, although experience indicates that overall survival may average between 70 and 80%. As with fingerlings, survival among individual pond populations is highly variable. Farmers reported that infectious diseases accounted for the largest percentage of foodfish losses, followed by bird depredations and dissolved oxygen depletions (USDA/APHIS 1997a).

Assuming 70% survival of fry to fingerlings and 75% survival from fingerling to foodfish, survival over the entire production cycle (discounting losses from egg to swim-up fry in the hatchery) averages just over 50%. Infectious diseases are by far the major causes of fish loss Infectious diseases of channel catfish are reviewed by Hawke and Khoo (2004) and Wise *et al.* (2004).

Economic losses resulting from infectious diseases are difficult to quantify because recordkeeping varies among farmers and many diseases go unreported. Nevertheless, infectious diseases cost producers many millions of dollars in direct fish losses each year. In addition, infectious diseases influence profitability by increasing treatment costs, reducing food consumption by fish, increasing feed conversion ratios, and causing harvesting delays. Fish-eating birds may also be attracted to ponds with sick and dying fish, causing further losses.

17.8.1 Viral diseases

There are two known viral diseases of channel catfish: channel catfish virus and channel catfish aquareovirus. Channel catfish virus disease (CCVD) is an important infectious disease of young catfish and can cause large losses in hatcheries or fry nursery ponds. Channel catfish aquareovirus appears to be of low pathogenicity and is not considered economically important.

CCVD is caused by a highly virulent herpes virus and channel catfish is the only species known to sustain natural epizootics. Fish with CCVD usually have a distended abdomen and exophthalmia due to accumulation of fluid in the body cavity. Fish feed poorly and swim erratically, often in a spiralling fashion. The severity of the disease is strongly affected by fish size and water temperature. Fish less than 1 month old are considered very susceptible to the disease. Older fish are more resistant and fish over 20 cm long (typically ca. 9 months old) are considered resistant to CCVD. Fish do not develop CCVD at water temperatures below 15 °C; disease occurrence is irregular and mortalities are usually low at temperatures between 20 and 25 °C. Losses can be devastating at temperatures above 30 °C.

There is no cure for CCVD, but losses can be reduced by minimizing stress in susceptible fry or fingerling

populations. Disease incidence can be reduced by using hygienic hatchery practices to reduce the opportunity for spread of the disease.

17.8.2 Bacterial diseases

ESC and columnaris are the most significant bacterial diseases in channel catfish aquaculture. Both are especially important in the nursery phase, and can cause large losses of fingerlings.

Enteric septicaemia of catfish is caused by the Gram-negative bacterium *Edwardsiella ictaluri*. In practice, the bacterium is host specific for channel catfish, although infections can be established in several other fish species. The disease may manifest as an acute gastrointestinal septicaemia with rapid mortality or as a chronic form characterised by an ulcerative lesion on the top of the head, which may proceed to septicaemia and death. Fish with ESC are listless and may swim in slow erratic spirals at the surface. Infected fish often have a rash of pinpoint haemorrhages around fins and on the ventral surface.

The occurrence of ESC is highly temperature dependent. In south-eastern USA, epizootics occur only in the late spring and early autumn when water temperatures are between 22 and 28 °C. Channel catfish of all sizes and ages are susceptible to ESC, but fingerlings account for most of the fish lost to the disease. Vaccine development has been an active area of research and shows some promise of reducing losses to ESC. However, the only practical method of vaccinating channel catfish is by mass immersion of fry in the vaccine just before they are transferred from the hatchery to a nursery pond. This is a major constraint to the use of vaccines in channel catfish because immunocompetency may not be fully developed in very young fish. Until effective vaccines and vaccine delivery systems are perfected, prompt diagnosis and antibiotic therapy with medicated feed will remain critical to successful treatment of ESC.

Columnaris is an acute to chronic disease of channel catfish and many other freshwater fish. The disease is caused by the Gram-negative bacterium *Flavobacterium columnare*. The bacterium is ubiquitous in aquatic environments, and outbreaks of the disease are usually associated with some predisposing stress factor, such as poor environmental conditions. Epizootics occur throughout the warmer months of the year, particularly in spring and autumn, when water temperatures are between 20 and 25 °C. Most outbreaks of columnaris in pond-raised channel catfish involve mixed infections with other bacteria, especially *Edwardsiella ictaluri*. Columnaris usually begins as an external infection of the gills or body, which progresses to an internal, systemic bacteraemia. Gross lesions on the gills are characterised by yellow-brown areas of necrosis at the distal end of the gill filaments. Skin lesions appear as areas of depigmentation, which progress to large necrotic ulcers. Infections of *Flavobacterium columnare* can be controlled by antibiotic therapy using medicated feed. Long-term successful treatment of columnaris is, however, difficult unless the predisposing stressor is identified and corrected.

17.8.3 Fungal diseases

Saprolegniasis is an imprecise, yet widely accepted term describing infections by fungi of the genera *Saprolegnia*, *Achyla* and *Dictyuchus*. Of these, species of *Saprolegnia* and *Achyla* most commonly affect channel catfish either in the hatchery (as eggs) or in ponds (as fingerlings or foodfish). In ponds, the disease is often referred to as winter fungus because it occurs during those months when temperatures are below ca. 15 °C. These fungi belong to the class Oomycetes, or the water moulds, which are ubiquitous and widely distributed fungi that are common, saprophytic micro-organisms that usually cause problems only when environmental conditions are less than optimal. Nearly all freshwater fish are susceptible to infection.

Problems with fungal infections of eggs are most common in hatcheries using water of less than 25 °C. Fungal growth begins on infertile or dead eggs and may spread to and kill healthy eggs. Infected eggs can be treated in a 15-min bath of 100 ppm formalin to kill the fungus. Proper water temperature for egg incubation is, however, the best solution to the problem.

External fungal infections of channel catfish are common in the winter months when water temperatures are below 15 °C. Infections are easily diagnosed by the presence of brownish, cottony patches on the external surfaces. Areas of de-pigmentation and loss of the mucous layer may be present before masses of fungal mycelia are obvious. It appears that the disease results from the combination of two factors: suppression of the catfish immune system by low water temperatures and coincident high concentrations of *Saprolegnia* zoospores in the pond water. When conditions are conducive for the disease, mortality rates may be high. There is no cost-effective treatment for fungal infections of pond-raised channel catfish.

17.8.4 Myxozoan and protozoan parasites

Proliferative gill disease is a common and often severe disease of pond-raised catfish caused by the myxozoan parasite *Henneguya ictaluri*. The common benthic oligochaete worm *Dero digitata* is the intermediate host for the

parasite in ponds. Spores of the myxozoan are released from the oligochaete, infect catfish and encyst in the gills. The gills may then become severely inflamed, with breakage of the filamental cartilage and varying degrees of necrosis. The gill lesions reduce respiratory efficiency and death is presumably caused by tissue hypoxia.

Outbreaks of proliferative gill disease occur most commonly in spring and autumn, when water temperatures are between 15 and 20 °C, although parasites may be found in fish throughout the year. There is no treatment for the disease once it occurs, although losses can be reduced by maintaining high dissolved oxygen levels during an epizootic.

'Ich' (*Ichthyophthirius multifilis*) is a common protozoan parasite of freshwater fish. Epizootics are rare in channel catfish aquaculture, but losses may approach 100% of the affected population when conditions are optimal for spread of the disease. Ich is a ciliated protozoan with a biphasic life cycle. The adults (called trophozoites) reach ca. 1 mm in diameter, are covered with cilia and have a C-shaped nucleus. Trophozoites cause great tissue damage as they migrate through the epidermis, feeding on tissue fluids and cell debris. The trophozoite leaves the fish after maturing and settles to the pond bottom, where it encysts and divides to produce up to 2000 infective cells, called tomites. Ich is an obligate fish parasite, and tomites will die if they do not locate a fish host within a few days.

Epizootics of Ich are most common when water temperatures are between 20 and 25 °C; fingerlings are particularly susceptible because they are held at high densities that enhance the spread of the disease. The most obvious sign of Ich is the presence of many raised, white, pinhead-sized spots on the skin.

Ich is difficult to control because the parasite resides beneath fish skin. Treatment consists of breaking the life cycle by killing trophozoites after leaving the fish or killing the tomites before they infect the fish. Five to seven treatments of copper sulphate applied to the water every other day usually controls the disease if treatment is initiated early in the epizootic.

Species of *Trichodina*, *Trichophrya*, *Ambiphrya* and *Ichthyobodo* are commonly found in low numbers on the external surfaces (particularly the gill filaments) of channel catfish and cause insignificant damage to the fish. Under certain conditions, however, the abundance of protozoans increases dramatically, possibly killing the host fish. Factors regulating fish parasitism by these protozoans are poorly understood, but epizootics are commonly associated with overcrowding and poor environmental conditions. Most of these infestations can be treated with chemicals, such as copper sulphate or potassium permanganate, applied to the water. Such treatments must be carefully considered, however, because the chemicals are toxic to fish at concentrations only slightly higher than the therapeutic dosage. Prevention of disease outbreaks by maintaining good environmental conditions is the best management practice for these protozoans.

17.8.5 Metazoan parasites

A variety of metazoan parasites may occasionally infest the gills, skin, gut or internal tissues of channel catfish. These include various species of monogenetic nematodes (gill and skin flukes), digenetic trematodes ('grubs'), nematodes (roundworms) and cestodes (tapeworms). Metazoan parasites usually cause few problems, although there is an important exception. In the late 1990s, infestations of channel catfish with the digenetic trematode *Bolbophorus damnificus* caused significant mortality and morbidity on several catfish farms in extreme southern Louisiana. Losses associated with the parasite have subsequently been confirmed outside southern Louisiana, including north-west Mississippi and southern Arkansas – the region with the highest concentration of catfish farms in the USA.

The life cycle of *B. damnificus* involves three hosts. Adult trematodes develop in American white pelicans (*Pelecanus erythrorhynchos*). Eggs contained in the faeces of infected pelicans may be released into ponds or lakes, where they hatch into free-swimming miracidia. Miracidia infect ram's horn snails (*Planorbella trivolvis*), which are common in the littoral of freshwaters in the south-eastern USA. If ram's horn snails are not present in the body of water, further development of the trematode cannot take place and fish cannot become infected. If, however, ram's horn snails are present and they become infected, the miracidia eventually develop into free-swimming larvae called cercariae, which leave the snail and infect channel catfish. The parasite's life cycle is complete when infected fish are eaten by pelicans.

When *B. damnificus* cercariae penetrate and migrate into tissues of fish, they cause mechanical damage, haemorrhage and inflammation. Extent of damage depends on level of infection and size of the fish. Cercariae eventually encyst in the tissues of fish. The encysted stage is called a metacercaria. Cysts are often subdermal, and small raised bumps may be particularly noticeable in the caudal region. If levels of infection are low, there is little, if any, effect on the host after encystment; however, if great numbers of metacerciae are present, considerable damage can be done to affected tissues. Infections produce a range of outcomes,

including death, reduced feeding activity and increased susceptibility to secondary bacterial infections.

Economic impacts are related to within-population infection rates, with the most serious effects seen in severely infected populations of smaller fish. Severely infected populations are usually recognised by farmers based on poor feed consumption or by dead or morbid fish that are diagnosed with the disease. Moderate or light infections may, however, represent a greater long-term risk to farm profitability because they can go undetected for long periods. Moderate infections are usually not associated with high mortality rates and, at any particular time, fish feeding activity may appear normal.

There is no effective therapy for fish after they have been infected. Management of the disease therefore depends on breaking the parasite's life cycle. The most effective approach involves a combination of activities, including discouraging pelicans from areas near ponds, reducing stands of aquatic vegetation around the pond perimeter to reduce snail habitat, and treating pond margins with lime or copper sulphate to kill snails.

17.9 HARVESTING AND PROCESSING

After fish reach the desired market size, they are harvested, transported to the processing plant and processed. These practices are relatively standardised across the channel catfish industry.

17.9.1 Harvesting

Harvesting fish from levee ponds is simple because most ponds have regular shapes (usually rectangular) and are shallow with smooth bottoms devoid of snags. Seines used for harvest are purchased from commercial sources. A typical seine is ca. 2.75 m deep and ca. 1.5 times as long as the maximum width of the pond. Seines are usually made of knotted polyethylene and have a float line at the top and a weighted lead line on the bottom. The lead line is fitted with rollers or covered with a 'mud line' to help the bottom line slide over the mud bottom.

Seines are built with a tapered tunnel ca. 50 m from one end. The tunnel is fabricated with a closure that can be connected to the metal frame of a 'live car'. The netting used to construct seines and live cars is available in a variety of mesh sizes. The mesh is sized to capture the smallest fish size desired. Seines for foodfish commonly use a square mesh size of ca. 4 cm, which retains fish greater than 0.35 kg and allows smaller fish to escape and remain in the pond for further growth.



Fig. 17.10 A seine being hauled in on a seine reel. As the seine is reeled in, fish swim into the attached live car.

Two tractors are used to pull the seine through the pond. One tractor pulls a hydraulically operated seine reel that serves as a seine storage unit. Once the two tractors have moved the length of the pond, the ends of the seine are brought together and the seine is gathered onto the seine reel. As the seine is reeled in, the fish become increasingly crowded in the remaining area (Fig. 17.10). At this point, the live car is attached to the seine. The live car is an open-topped bag made of netting. One end of the live car is equipped with a metal frame that is attached to the drawstring closure on the seine. As the seine is spooled further onto the seine reel, the crowded fish flow into the live car (Fig. 17.11). The live car is detached from the seine and closed after it is filled to the recommended capacity. Another live car may then be attached to receive additional fish if the harvest is large. Fish are allowed to grade in the detached live car for several hours.

After grading is complete, fish in the live car are loaded onto transport trucks. The live car is positioned near the bank and fish are scooped into a loading net attached to a hydraulic boom (Fig. 17.12). In-line scales record the harvest weight.

17.9.2 Processing

Fish are transported alive to processing plants. Transport trucks carry four to nine aerated tanks, each ca. 3 m^3 in volume. Tanks are filled with ca. 1500 L of water and each tank can hold ca. 1000 kg of fish. At the processing plant,

a sample of fish from the transport truck is tested for flavour quality. If fish are of acceptable quality, they are unloaded into large concrete tanks containing flowing, aerated water. When needed in the processing plant, fish are removed from the tank, weighed and stunned with alternating current electricity. Stunning renders the fish immobile and makes them easier to handle in the processing line.



Fig. 17.11 Catfish crowded in the seine flow into the live car, where they are held until loaded onto transport trucks.

Much processing is done by hand, but there is a trend towards increased automation to reduce processing costs. A variety of products are marketed, with common products being fillets, fillet strips, nuggets (the 'belly flap' section removed from the fillet), steaks and whole dressed fish. Overall, about half of a live catfish is converted into saleable products after processing. Processed catfish are available either as fresh, ice-packed product or as various frozen products. Frozen fillets account for the largest portion (ca. 55%) of total sales.

17.10 THE FUTURE OF CHANNEL CATFISH FARMING

Channel catfish aquaculture expanded at a remarkable rate in the period from 1980 to 2000. Industry growth was facilitated by a stable US economy, low feed prices, aggressive marketing and a general trend towards more fish in the diet of American consumers. Since 2003, however, negative economic forces have caused a significant reduction in US catfish production. Beginning in the late 1990s, market growth slowed owing to competition from the following:

- imported pangasid catfishes from Vietnam (section 1.8.1);
- imported channel catfish from China;



Fig. 17.12 Catfish are scooped from the live car and loaded onto live-haul trucks for transport to the processing plant. (Photograph by Dr Les Torrans.)

- imported tilapia;
- general competition within the seafood marketplace – especially from farm-raised tilapia.

More recently, increased energy costs and higher grain prices combined to cause dramatic increases in feed costs. Interactions among increased feed costs, increased operating expenses due to high energy costs, and competition from imports will certainly have long-term impacts on US catfish farming, although the nature of those impacts is impossible to predict.

Annual yields of catfish average ca. 4000 kg/ha under commercial conditions, which is about half of that attainable under controlled, experimental conditions. Losses to infectious diseases and bird depredation, and postponement of harvests by frequent episodes of off-flavour, account for the difference between potential and actual farm productivity (Hargreaves and Tucker 2003). Clearly, technological improvements in management of those problems will improve farm economic performance. Advances in other areas also offer considerable potential for improving farm economic performance. Better yields and more efficient production may soon be realised, as improved germplasm becomes widely available and production systems are improved. In addition, improvements in feeding practices can dramatically impact profitability because feed represents the largest single cost of production.

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18
Marine Fish

John Tucker

18.1 INTRODUCTION

Successful marine fish farming depends on the requirements of the cultured species being well known and then provided at reasonable cost on an economically viable scale. Ideally, none of the stages would be unusually stressed by captivity and handling. Cultured fish are much more crowded than wild ones, face artificial conditions and barriers, and have a restricted diet compared with those in the wild.

This chapter comprehensively considers the general techniques of marine fish culture and then the particular techniques used for a broad range of species. Because there is not a clearly defined division between marine species and those that inhabit brackish but not ocean water, some of the latter also are included in this chapter. Some of the latter species are called diadromous species and include anadromous (spawning in freshwater but juveniles and adults usually live in salt- or brackish water) and catadromous (spawning in salt water but juveniles and adults usually live in fresh- or brackish water) species.

18.2 EARLY DEVELOPMENT

Although they have similar basic requirements, there is a wide range of robustness, resilience and survival ability among marine fish larvae. Most of them need to be treated gently at first. Tolerance to turbulence and toxins varies among species, with smaller larvae usually more sensitive. Demersal (bottom-dwelling) larvae are usually more capable than planktonic (drifting) larvae, and inshore larvae more capable than offshore larvae. Small larvae that normally live in deep water tend to survive better in larger tanks, probably because they are not adapted to barriers like tank walls and bottoms. Hatchery systems need to maintain the highest water quality possible while keeping larvae and their food optimally dispersed and allowing gentle adjustment of water level, water exchange, circulation, aeration, light, etc., as the larvae grow. In well-managed tank systems, it should be possible to maintain excellent fish health without the use of drugs.

Depending on the species, natural spawning can occur as infrequently as once a lifetime to as often as every day. High-quality eggs of many species are easy to obtain with standardised conditioning and/or hormone treatment (Table 18.1). Ideally, a hatchery would maintain families of domesticated broodstock bred for adaptability to captivity, rapid and efficient growth, and high survival. They also would spawn voluntarily and predictably after managers put them through a proven environmental cycle. However, most marine fish are raised from eggs obtained by more primitive methods, and some only from those captured in the wild as juveniles.

Most marine fish produce externally fertilised eggs (oviparous). Some release small numbers of advanced young that are not from eggs (viviparous), and others hold their eggs internally until hatching, then release the larvae (ovoviviparous). Some species deposit masses of eggs in rocks, weeds, holes or on free-floating rafts, but most produce individual eggs. Demersal eggs sink and planktonic eggs drift (Fig. 18.1). The main parts of a teleost egg are the chorion (shell), yolk, single cell developing into an

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Table 18.1 Best spawning techniques, approximate hatchery period and level of difficulty, common market size and approximate total time to reach it, and most common types of grow-out units for representative marine fish raised commercially. The given hatchery period is close to the length of the larval stage or a little longer. Best spawning technique: CV, conditioned voluntary spawning; CS, conditioned and strip-spawned; HT, hormone-induced tank spawning; HS, hormone-induced and strip-spawned. Level of hatchery techniques: R, routine; M, moderate; D, difficult; V, very difficult.

Fish	Best spawning technique	Hatchery period (d)	Hatchery level of difficulty	Market size (g)	Total time (months)	Grow-out mostly is in:
Chanidae						
Milkfish	CV, HT	ca. 21	R	450	12	ponds, pens, cages, tanks
Gadidae						
Atlantic cod	CV	ca. 50–80	M	3000	30	cages, tanks
Centropomidae						
Barramundi	CV, HT	ca. 25	R	3000	21	cages, ponds, tanks
Moronidae						
European sea bass	CV, HT	ca. 45	R	375	15	cages, ponds, tanks
Sunshine bass	HS	ca. 30	R	1300	19	ponds, tanks, cages
Serranidae						
Orangespotted grouper	CV	ca. 40	D	1000	18	cages, ponds
Rachycentridae						
Cobia	CV	ca. 30	R	5000	12	cages
Carangidae						
Japanese yellowtail	CV, HT	ca. 55	D	5000	24	cages, pens, ponds
Lutjanidae						
Asian red snapper	CV	≥35	M	1000	16	cages, ponds
Sparidae						
Gilthead sea bream	V, HT	ca. 40	R	450	14	cages, ponds, pens, tanks
Red sea bream	CV	≥30	R	1000	24	cages, pens, ponds
Sciaenidae						
Red drum	CV	ca. 35	R	3000	19	ponds, cages, tanks
Mugilidae						
Striped mullet	HT	ca. 28	R	1000	13	ponds, pens, tanks
Polynemidae						
Sixfinger threadfin	CV	≥26	R	500	8	cages
Siganidae						
Yellowblotch spinefoot	CV	ca. 45	R	250	9	ponds, cages, pens
Scombridae						
Pacific northern bluefin tuna	CV	≥30	V	2700	12	cages
Bothidae						
Hirame	CV	ca. 50	R	1000	17	tanks, cages
Scophthalmidae						
Turbot	CV,CS	ca. 50	R	1000	14	tanks, ponds, cages
Pleuronectidae						
Atlantic halibut	CV,CS	ca. 100	M	4000	36	tanks, cages
Soleidae						
Common sole	CV	ca. 56	R	250	24	tanks
Tetraodontidae						
Tiger puffer	CV	ca. 30	M	1000	20	cages, tanks

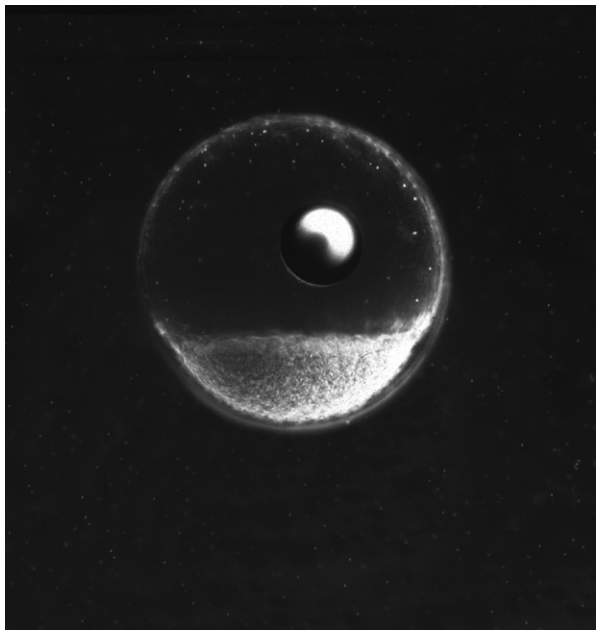


Fig. 18.1 Spotted seatrout egg 2 h after spawning (ca. 0.77 mm diameter). The almost clear yolk at the top mostly supplies the early growth process (until 2–3 days after hatching), and the oil globule inside it especially provides energy. The dividing cells at the bottom are heavier and make the egg drift or float in this position. (Reproduced from Tucker (1998) with kind permission from Springer Science+Business Media B.V.)

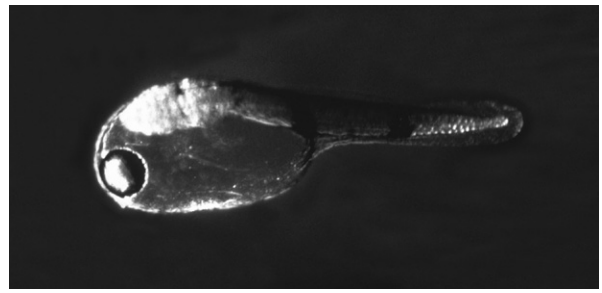


Fig. 18.2 Common snook larva just after hatching (ca. 1.5 mm). It will take about 36 h for vision and feeding ability to develop. (Reproduced from Tucker (2003a) with permission of *World Aquaculture Magazine*.)

embryo, and sometimes one or more oil globules. The perivitelline space separates the chorion from the yolk, oil and embryo, and contains fluid. The micropyle is a pore through which a sperm enters to fertilise the egg. The yolk consists mostly of protein and is used for growth. The oil is a concentrated source of energy and essential fatty acids (EFAs, see section 8.3.3), but free amino acids in the yolk also contribute energy. After fertilisation of a typical planktonic egg, the egg absorbs water, the perivitelline space forms, the micropyle closes and the chorion hardens. Most marine teleost fish have egg diameters in the range 0.3–15 mm and hatchling lengths of 0.7–45 mm. Most actual and potential mariculture species have egg diameters of 0.6–3.4 mm and hatchling lengths of 1.3–9 mm. All fish lengths given in this chapter are total lengths.

The smaller the hatchlings, usually the more difficult they are to raise. Just after hatching, larvae of most mariculture species are fragile and cannot see, swim well, or catch food (Fig. 18.2). Eyes usually become pigmented and functional just before first feeding. The senses of taste and smell develop between hatching and 16 days after hatching in several species. The ability of larvae to detect pressure changes in the water around them develops before eye pigmentation in many species. This functions in predator avoidance, but also allows the larvae to be stressed by unnatural vibrations. In species with small hatchlings, gas exchange occurs through the skin at first, but gill filaments are necessary in larger fish and usually are present by the late larval or early juvenile stage.

Most marine fish larvae transform into juveniles, which have the major adult characteristics (including bones, fin rays, teeth and scales) except for size and sexually maturity. Only a few types (mainly eels and flatfishes) also metamorphose (go through large morphological changes) in order to become juveniles. Juveniles have greater swimming and feeding abilities, and are more tolerant of handling.

When larvae have used all their yolk and oil, they are completely dependent on food. Exhaustion of the oil globule often is a day or two later than exhaustion of the yolksac (EYS). Species with a long interval between first feeding and exhaustion of yolk and oil have a survival advantage, which often can make rearing them easier.

Most species, including those that are herbivorous as adults, have carnivorous larvae. Larval marine fish in their natural environment eat the following:

- diatoms, dinoflagellates, flagellates (phytoplankton);
- tintinnids and other ciliated protozoans;
- rotifers, cladocerans, copepods, appendicularians;

- eggs of bivalves, cladocerans, copepods, chaetognaths and fish;
- larvae of polychaetes, bivalves, gastropods, barnacles, cladocerans, copepods, decapods and fish.

If something is the correct size, not repulsive and has the right movement, it probably will be considered as potential food, but digestibility and nutritional value vary greatly, and the larvae do look for certain cues. For many marine fish larvae, copepods are the primary prey; however, smaller or more easily caught zooplankters often are preferred from first feeding until the fish are more skilled at catching the larger, faster copepods. Methods for raising and providing live foods in the hatchery are fairly standardised, with emphasis and scheduling of prey depending on fish species and facility preferences (see also section 9.5.1).

The digestive system does not have all the necessary organs and enzymes at first. The early larval stages of most marine fish will not accept artificial foods or survive and grow well on them. Many species can be weaned to artificial food just before or during transformation. Increasing feed digestibility and attractiveness will help accomplish earlier weaning. Very acceptable, nearly optimal, feed formulas are available for juveniles and adults of most types of fish; all or nearly all fish meal can be eliminated as an ingredient, even for strict carnivores.

Good survival of larvae depends on much more than good physical conditions and water quality. For most cultured marine fish that have small larvae, the time from first feeding until just after yolk and oil exhaustion is often called the 'critical period'. Important factors include the following:

- High-quality gametes, plus correct temperature, salinity, timing and handling are needed at fertilisation.
- Strong embryos, correct temperature, dissipation of hatching enzymes, lack of toxic substances and correct light intensity (for some species) are needed at hatching.
- Strong normal larvae, correct size, availability, quality, etc., of food, appropriate lighting and correct timing are needed at first feeding.
- Adequate yolk and oil until food intake is energy positive, correct temperature and sufficient uptake of nutrients from exogenous sources is needed near the time of yolk and oil globule exhaustion.
- Strength of larvae, light not too intense, appropriate circulation and lack of surface films are needed at the time of gas bladder filling.
- Digestive capability, correct size, availability and quality of food, correct timing, and an adequate transition period are needed when the diet is changed.
- Good overall health of larvae is needed during the transition to gill gas exchange.
- For some species, cannibalism (usually among late larvae and early juveniles) can be a problem to overcome (Kestemont *et al.*, 2003).

18.3 ENVIRONMENTAL CONDITIONS FOR CULTURE

Juveniles being grown out to market size are usually not very sensitive, but every effort should be made to provide an optimal habitat for broodfish and larvae. Juveniles and adults of estuarine and coastal species tend to be more tolerant of environmental extremes, turbidity, pollutants and other stressors than those of offshore (especially reef) species. Larvae that are stressed (e.g. by fright, strong current, toxins, pathogens, malnourishment) might appear exhausted or stunned, swim erratically, drift with the current, and/or not feed well.

18.3.1 Temperature

Temperature is the most important environmental factor influencing fish growth. Fish can be classified as coldwater (<15 °C), coolwater (15–25 °C) and warmwater (>25 °C). Temperature ranges for spawning, egg survival and hatching are usually narrower than those for other life phases. Furthermore, some eggs and larvae can be harmed by a change of more than 2–3 °C in a day.

The range for growth is narrower than that for survival. At sub-optimal temperatures, feeding and growth are reduced. Up to the optimum, increasing temperature enhances growth by increasing food intake and food utilization. Above that level, increasing metabolic requirements for food processing, locomotion and other functions reduce the proportion of food energy available for growth. Thus, the optimal temperature for growth often is higher than that for food utilization efficiency.

Fish are more susceptible to handling injury and disease at temperatures lower or higher than normal. If fish must be caught or transferred during cold or hot weather, they must be protected from temperature stress and given adequate aeration. When fish are transferred from one body of water to another, if temperature differs by more than 2 °C (1 °C for eggs and larvae), they first need to be acclimated, or tempered, by gradually changing the temperature of the water they are in to that of their new environment. A change of 5–10 °C in one day can be too much

for juveniles or adults of a sensitive species. If a species suitable for the temperature range of the available water source cannot be chosen, economical temperature control will be necessary. Solar energy can be manipulated to collect heat in winter and exclude heat in summer.

18.3.2 Salinity

Typical open ocean salinity usually is in the range 33–37‰, but coastal and polar waters vary much more (section 3.2). An appropriate salinity range is important for fish and their prey to maintain salt balance of internal body fluids, and to facilitate gas exchange and maintain balanced external water chemistry. Ionic concentration of internal fluids in most marine fish is similar to that of 10–12‰ seawater. Theoretically, energy required for osmoregulation should be minimal in one-third seawater for species that are not stressed at that level. About 28‰ or more is usually needed for planktonic eggs to be buoyant and not sink to the bottom. Once they reach the late larval stage, many coastal species can tolerate less than 15‰, but offshore species usually require at least 20‰. Hybrid striped bass should be grown out in fresh or slightly brackish water. Barramundi, snooks, striped bass, some snappers, several sea breams, several drums and mullets can be grown out in fresh, brackish, or salt water. Groupers can be grown out in brackish water (≥15‰ for many). Typical pelagic species (e.g. dolphinfish, tunas) require higher salinity for all stages (≥20–25‰). Providing extra minerals in the feed can reduce the minimum salinity requirement to some degree.

18.3.3 Solids

Total solids (TS) include total suspended solids (TSS) and total dissolved solids (TDS) (section 3.2.1). When water is passed through a 2-μm filter, TSS are collected on the filter and TDS pass through. Settleable solids (SS) are suspended solids that settle out after a specified period. Solids are important in fish culture because high levels of organic solids can decrease oxygen and increase levels of toxins like ammonia and hydrogen sulphide. Also, high levels of suspended abrasive solids can injure fish gills, causing hypoxia and infection. High suspended solids can also lower visibility and reduce growth of needed algae. TSS might need to be kept at <5 mg/L (ppm) for sensitive larvae. Juveniles and adults of estuarine and coastal species seem to tolerate suspended solids better than offshore (especially reef) species, which seem to suffer from gill irritation more easily.

18.3.4 Light

Wavelength distribution (spectrum), intensity (illuminance) and duration (daylength) of light can be critical, especially for early stages. The light spectrum can affect visibility of prey. As in nature, filtered sunlight probably would be best for cultured marine fish. In the ocean, most ultraviolet (UV) and infrared radiation is filtered out in the upper 1 m of water, and most fish eggs and larvae do not float right at the surface but are suspended from a few metres deep to 100 m or more. Adaptations for protection of eggs and larvae from UV damage include shading of vital organs with pigment, vertical migration governed by light intensity, and daily or seasonal restriction of spawning to times when the sun is not high in the sky. Shading from natural turbidity and water movement are also protective. Ponds and tanks receiving much light usually have a good bloom of algae, which provides shade.

Illuminance is usually measured by the unit lux, which equals 1 lumen/m^2. Up to a point, raising illuminance increases activity and feeding success, but too much light will stress the fish, keep them near the bottom of the tank, hinder feeding and possibly cause skin diseases. Illuminances used successfully for larval rearing are shown in Table 18.2.

For some species (e.g. Atlantic cod), decreasing the illuminance (e.g. to 300 lux) seems better for late larvae and juveniles, which naturally live deeper in the water than early larvae. The range 1000–3000 lux is probably suitable for larvae of most warmwater species. Larvae of high-latitude species and larvae that naturally live under low-

Table 18.2 Illuminances used successfully for larval rearing of fish.

Species	Illuminance (lux)
Atlantic cod	1–2400 lux (1200–2400 might be best)
Turbot	1000–2000 (or 4000)
European plaice	At least 100
European sea bass	800–1000
Striped mullet	600–1400
Gilthead sea bream	600–1500
Atlantic halibut	1000–1500
Groupers	1000–3000
Black sea bream	3000
Red sea bream	Up to 5000

light conditions (e.g. well below the surface) seem to need less light. Light should be distributed as evenly as possible across hatchery tanks, but reduced near walls and pipes (especially surface drains) where bright spots can attract larvae and cause mortality. If natural light is used, larval, juvenile and adult tanks need to be deep enough so that, if the light becomes too intense, the fish can retreat towards the bottom.

The best daylength varies among species and stages. Light stimulates hatching in some species (e.g. zebrafish) and inhibits it in others (e.g. Atlantic halibut). For larvae, good approaches are to use either the same (or slightly longer) daylength as during the natural spawning season, or a daylength known to be good for related species, or 14–16 h/day as a first approximation. Less-developed larvae can benefit from extended or continuous light. Larvae of some species (Atlantic cod, barramundi, groupers, yellowblotch spinefoot, gilthead sea bream, turbot, common sole) grow and survive well with continuous light; however, inhibition of gas bladder inflation (e.g. in European sea bass) and other stresses can occur in some. Even for light-sensitive species, survival may be improved by a low intensity nightlight (10–100 lux), at least to help with orientation (e.g. to stay off the bottom). Juveniles and adults seem to benefit from a daily dark period (to rest). If total or supplemental artificial light is used for grow-out, a daylength between the annual average and the longest day would be appropriate for frequent feeders to maximise food intake. A shorter daylength might be better for infrequent feeders to reduce activity and improve feed conversion. Continuous light delays maturation in some species (e.g. turbot, Atlantic halibut). This shortens grow-out time by maximising growth because food energy is not diverted to reproductive development.

18.3.5 Tank habitat

Tank depth and colour influence light enough to affect behaviour and survival of many species with planktonic larvae. Interior tank surfaces, especially at the waterline, should be non-glossy to minimise distracting reflected light. Dark colours are best, especially for the walls. Dull black is best for the waterline to minimise aggregation of prey and larvae. Lower walls can be a little lighter, but not too light. It seems easier for larvae to locate and capture prey with the high contrast of dark walls and more even distribution of light. Vertical surfaces of other solid structures (e.g. standpipes, water inlet pipes, surface film skimmers) in the water also should be as dark and non-glossy as possible. The bottom can be striped or have lighter patches (e.g. blue and black, dull white and black) to increase visibility of fish, their food, algae and sediment to the culturist (Fig. 18.3). If the bottom is too bright, it can cause enough disorientation of live food organisms that they migrate downward and are less available to the larvae. The larvae themselves might be attracted so much that they are found grovelling on the bottom. For many fish, dark seems best for larvae and lighter (but not white) for juveniles.

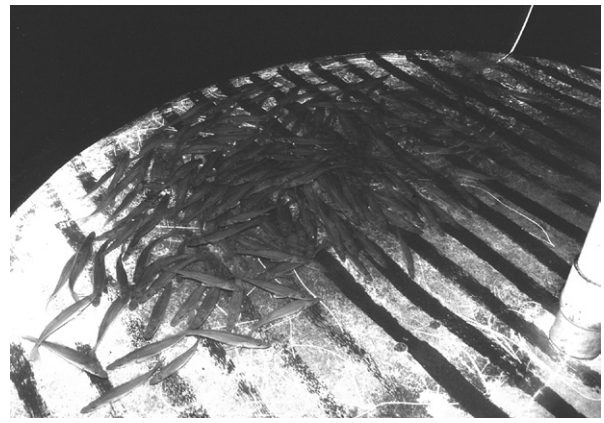


Fig. 18.3 Common snook (average ca. 25 cm) in a 3.5–m^3 tank with black walls and a black and white bottom. (Reproduced from Tucker (2003a) with permission of *World Aquaculture Magazine*.)

Although planktonic marine fish larvae do not require structured habitats, some demersal larvae might benefit from material on the tank bottom. Sand can benefit juveniles of some flatfish, and shelves can increase carrying capacity for different benthic larvae or juveniles. Plastic milk crates will accelerate transformation of grouper larvae. The presence of hiding places is more natural and reduces stress for juveniles adapted to hiding. However, they make the tanks harder to keep clean, can increase territoriality or ambush types of cannibalism (thus decreasing rather than increasing the carrying capacity) and possibly interfere with harvesting.

18.3.6 Noise

Larvae of some species can be killed by tapping on the tank, especially if they are drifting near the surface after hatching. If very buoyant, they can become trapped in the surface tension and suffocate. Older fish might seem less sensitive to constant noise, but still can eat and grow less. Vibrations and noise from pumps and other machinery can

be conducted through walls, floors and pipes to the tanks. Air and water pumps must be separate from the fish tanks and at a reasonable distance. Keeping motors away from walls and padding their bases can help. Air pumps that seem quiet to humans can send very loud sound through airlines into the tanks. An in-line muffler after the pump can help. Where appropriate, soft couplings will dampen the noise.

Anything that can conduct noise to the tanks should be isolated and/or padded. Fish that naturally live in quiet habitats might be more sensitive to noise. If a hydrophone is available, sound levels in rearing units holding water can be checked with all machinery off, then on, to locate and isolate sources.

18.3.7 Aeration and water circulation

Water should be thoroughly aerated before or as it enters fish tanks. Water movement is important for the following:

- to prevent thermal stratification;
- to maintain oxygenation (at least 5 mg/L dissolved oxygen (DO) for most species) (section 3.3.4);
- to disperse live food;
- to disperse compound feeds;
- to help keep larvae from aggregating too much.

With too little turbulence, zooplankton and fish can become concentrated in bright areas of the tank, resulting in localised oxygen depletion, frequent collisions and feeding difficulty for the fish. With too much turbulence, the fish are battered. Strong bubble streams or currents hitting larvae might disorient, stun or injure them. For sensitive early larvae, only very gentle circulation of tank water should be used for the first few days. Aeration and circulation can be provided without direct aeration in the tank by adding water and air together through a diffuser pipe. Then, when the larvae can swim better and are feeding well, the circulation can be increased, but not to the point that larvae are swept helplessly into the stream of bubbles and battered by them or violently pushed against tank walls. One arrangement for late larvae is a single airstone (or small ring) near the centre and a ring of airstones next to the wall, with air flow increasing over time just enough to limit aggregation and maintain DO. The most precise valves available should be used for exact control of air flow for larvae. Currents usually should not be strong enough to keep fish from maintaining at least their feeding positions, which can lead to energy loss, exhaustion, disease and mortality. Dolphinfish and cobia larvae need stronger circulation to keep them from settling to the bottom.

Water exchange rate can be increased gradually, for example, from 10% per day for eggs and early larvae to at least 300% for small juveniles and 500% or more for larger fish, depending on tolerance of the fish, their density and the need for flushing of wastes.

18.3.8 Water chemistry

18.3.8.1 pH

Moderate changes in pH can alter water chemistry, increase or decrease toxicity of dissolved substances, and affect gas exchange between fish and water (section 3.2.9). Extreme pH can interfere with electrolytes, gas exchange or other physiological processes. It can damage skin, fins, gills or bones, and cause mortality. Low or high pH can influence blood pH and cause ammonia retention. Many fish appear to tolerate pH of 6.5–8.4, but offshore fish (especially as larvae) are likely to have a narrower range. Ideally, the pH of full strength seawater should be kept at 8.0–8.4. The pH of brackish water normally will be between 7.0 and 8.4, depending on salinity. Buffering capacity is proportional to salinity, with seawater better than freshwater, but its capacity tends to decrease with age of the water.

Except in certain cases (e.g. eutrophic ponds in the summer, acid well-water, or re-circulating systems), pH is not likely to become a problem. When algal growth is heavy, pH rises in the light while photosynthesis depletes carbon dioxide and produces oxygen, but both pH and oxygen decrease in the dark while photosynthesis stops producing oxygen and respiration continues to convert oxygen into carbon dioxide (section 3.2.10 and Fig. 3.6). In recirculating systems, as ammonia is oxidised by nitrifying bacteria, pH buffering capacity can be reduced. If pH varies too much, there are possible solutions: increased water exchange, increased aeration, foam fractionation or addition of sodium bicarbonate. Buffering agents like bivalve shells, aragonite, calcite, dolomite, marble chips, lime or calcium carbonate can be added to closed systems to help keep pH from going below 7.5.

18.3.8.2 Minerals

Hardness of seawater is high (section 3.2.11) and usually not a consideration in mariculture. However, when marine fish are raised in fresh or nearly freshwater, measurement and adjustment of hardness might be required, because the fish need magnesium and calcium. Alternatively, some species will grow as well in nearly pure water (5 mg/L hardness) as in saline water, if their feed contains enough minerals. In freshwater, 200–250 mg/L $CaCO_3$ equivalent

is suitable for young striped bass and hybrids. Good hardness and chlorinity (chlorine content) for juvenile red drum are >100–400 mg/L $CaCO_3$ equivalent and >150–500 mg/L Cl^-, possibly depending on dietary minerals. Without specific data or proven dietary mineral supplementation, hardness probably should be at least 250 mg/L $CaCO_3$ equivalent. Alkalinity usually is not a concern in mariculture except in closed systems, where ion concentrations can change. In freshwater, if hardness is acceptable for marine fish, alkalinity probably is too. Acidity can be important in some freshwater or industrially polluted seawater sources and can increase in recirculating systems. Redox potential is of most concern in recirculating systems, and near 300 mV is likely to be appropriate.

18.3.8.3 Nitrogenous compounds

Ammonia is the main waste product excreted by fish from protein and nucleic acid metabolism. Three main mechanisms for ammonia disposal in aquaculture are water exchange, bacterial nitrification and uptake by algae. Fish that have died from acute ammonia toxicity typically have gaping mouths, extended gill covers and rigid U-shaped bodies. If poisoning is low to moderate, fish might stay at the surface, either still or swimming slowly and occasionally gulping for air. Chronic sublethal exposure usually causes gill injury and dysfunction (environmental gill disease). Bacteria can invade, causing bacterial gill disease, which is worse. In either case, mortality can occur within a few days. Ammonia toxicity can be higher in salt water than in freshwater because of the higher pH. Safe limits for ammonia, nitrite and nitrate are only approximately known; more definitive data will require more testing. Unionised ammonia concentrations as low as 1 µg/L NH_3-N (10 µg/L NH_4^+-N, ionised) can affect feeding, growth, or survival of sensitive species or stages. Ammonia ideally should be kept near zero, but practical goals are <1 µg/L NH_3-N for larvae and <5 µg/L NH_3-N for older fish (unless no-effect levels are known to be higher).

Nitrite (NO_2^-) interferes with oxygen transport to tissues, thus causing 'brown-blood disease'. It is less toxic in salt water than in freshwater because of other ions. Marine fish kept in freshwater are at more risk. With a mature biofilter, nitrite probably will not accumulate unless the filter is overloaded or the water flow is too slow. Likely limits for nitrite nitrogen (NO_2^--N) are 0.1 mg/L for larvae and 1 mg/L for older fish, but zero is best.

Nitrate (NO_3^-) is relatively non-toxic, but can promote growth of disease organisms. It can cause haematological and biochemical alterations, and weaken the immune response. Some marine fish can tolerate 300 mg/L but 100 mg/L can reduce growth and survival of larvae and some juveniles. Nitrate is relatively harmless but should not be permitted to accumulate. Nitrate nitrogen (NO_3-N) probably is best kept at <20 mg/L for eggs and larvae, and <50 mg/L for older fish. Fertilised ponds should be watched for problems (section 3.3.7).

18.3.8.4 Dissolved gases

A consistent and appropriate level of DO is necessary for fish to survive, feed, grow and reproduce. Chronic hypoxia can result in developmental deformities, especially skeletal and circulatory. Dissolved oxygen needs to be at least 5 mg/L for warmwater fish and higher for coldwater or very active fish (hopefully near saturation). In water with pH higher than 8, carbon dioxide (CO_2) normally is low because it is converted to bicarbonate. It can be tolerated up to at least 8 mg/L by most larvae and adults if sufficient DO is present.

Over-fertilisation, over-stocking, over-feeding and build up of organic sediment in ponds should be avoided, especially during warm weather. During the day in a healthy pond system, oxygen produced by photosynthesis will probably exceed that consumed by plant and animal respiration. At night, both plants and animals are using oxygen, but virtually none is produced (Fig. 3.6). Therefore, the most dangerous time for fish in a pond or restricted natural water body is early morning. As temperature rises, oxygen solubility decreases and fish metabolism and demand for oxygen increase. During warm weather, it is not unusual to see crowded pond fish gasping at the surface or at least staying just under the surface, where DO is highest. Two of the most common causes of sudden fish kills in ponds are low DO, sometimes with high ammonia (mainly in the morning), and high DO with high temperature leading to gas bubble trauma (mainly in the afternoon). After an oxygen crisis, fish must not be fed before they are ready and should be watched for two weeks for signs of disease.

Hydrogen sulphide (H_2S) is formed in low oxygen environments, like accumulations of fine sediment, deep unaerated water, and pipelines or filters that have sat too long without oxygenated water flowing through them. Because of higher pH, problems are less likely in seawater than in freshwater, but anaerobic areas of the system still can generate lethal amounts of H_2S, even in seawater. Hydrogen sulphide released from pond sediments (e.g. during harvest) can quickly cause total mortality. H_2S can be toxic to fish eggs at ≤12.0 µg/L and to juveniles at ≤2.0 µg/L. Sensitive species can be affected by 0.1 µg/L. This is far below the human smelling threshold. Ideally,

there should be zero hydrogen sulphide in fish culture water.

Chlorine is used in many hatcheries for disinfection, but usually not close to live fish in uncovered tanks. Because gaseous chlorine is heavier than air, it can settle in a layer near the floor, drift to a lower elevation, or travel down one standpipe and up another, thence directly into fish tanks or the air system. Chlorine at a concentration of 3 µg/L can be lethal to fish and lower levels can be harmful. There should not be any chlorine or chlorine-produced oxidants in seawater for larval fish culture, but older fish might tolerate occasional trace amounts.

Ozonation of freshwater followed by aeration is relatively safe. However, with bromide present, ozonation of seawater produces the same bromine and chlorine compounds that chlorination does, with similar toxicity. The toxicant half-lives can be short in freshwater but more than 24 h in seawater. Signs can be burned or damaged gills, gill hypertrophy, mineral imbalances, blood cell imbalances, hyperglycaemia and mortality. Larvae are especially susceptible and should not be exposed to ozone or its oxidants in seawater containing bromide. Older fish might tolerate occasional trace amounts. More than 2 µg/L of ozone-produced oxidants can be toxic. Artificial seawater without bromide can lower the risk.

18.3.8.5 Organic contaminants

Large amounts of some naturally occurring organic compounds can interfere with growth and reduce disease resistance. Some can be skimmed out by foam fractionation, adsorbed by activated carbon, or filtered out by special resins. Polycyclic aromatic hydrocarbons, polychlorinated biphenyls, petroleum products, organic solvents, surfactants, pesticides and wood preservatives have poisoned fish, and cannot be allowed to contaminate culture water. Even vegetable oils can be lethal to marine organisms if they accumulate. Hatching enzymes can be toxic to hatchlings if the fish are crowded or otherwise stressed, and they should be flushed out.

18.3.8.6 Metal contaminants

Toxic metals brought in with the water supply or introduced in the facility can cause a wide range of injury to fish. The most likely metals to cause trouble are copper, mercury, tin, lead and zinc. An increase in pH can cause iron salts to precipitate on fish gills and interfere with gas exchange. Because of low concentration, chromium, aluminium, cadmium, manganese, silver, arsenic and selenium are less likely to cause problems. Antimony, nickel, beryllium and vanadium can be toxic, but are not likely to be at dangerous levels, except in areas of high industrial pollution or if contamination occurs through misuse of metals in water systems. Boron and molybdenum can be slightly toxic to salmonids.

Direct contact of toxic metals (e.g. zinc from galvanised steel) with fish culture water or positioning them where condensation can drip into the water must be minimised. Except for high grade titanium, it is best to keep all metals away from fish culture water as much as possible. Even good stainless steel (type 316) contains molybdenum and is not corrosion-proof in seawater.

Some metals (e.g. iron, copper, manganese) are partly removed in sand filters by precipitation on the sand grains. Some can be reduced by chelating or precipitating agents. Algae will remove some, but then should not enter the food chain. Naturally occurring substances (e.g. humic acid, fulvic acid, amino acids, polypeptides, suspended solids) can remove metals by chelation, and others (e.g. clay, sand, gravel) can adsorb metals.

18.3.8.7 Contaminants from culture facilities

Especially in cold weather, condensation inside a hatchery can release a large amount of water that falls directly into larval tanks. It can carry toxicants from the roof or ceiling, and its impact can injure larvae. The least toxic materials possible should be used above fish tanks, and, if necessary, a temporary or permanent ‘umbrella’ can be placed above the tanks. People tending marine fish larvae must be very careful to avoid poisoning the fish with residues of substances like soap, chlorine, oil, gasoline and pesticides clinging to their hands, nets or other objects.

Fresh concrete is very alkaline and might require soaking for a few months if sensitive species are to be raised in water contacting it. Plastics also should be soaked and pipelines flushed as long as is practical before use. Polyester resins and gel coats used in making fibreglass and wooden tanks contain mainly polyester resin and styrene monomer. Styrene released into water can be very toxic if the resins are not fully cured. Mold wax makes it possible to remove the hardened tank from the mold and helps seal pores in the gel coat. New tanks for sensitive fish like larvae should be soaked, scrubbed (not enough to damage them) and aged thoroughly before use. PVC cement vapour (contains methyl ethyl ketone and tetrahydrofuran) is extremely toxic to some fish and has been measured in water coming from pipes that were glued 10 years earlier. Fresh PVC cement vapour can settle into tanks of larvae and kill them. Glueing must not be done near sensitive fish, and glued pipes must be allowed to dry thoroughly before water is put into them. Without enough

ventilation, styrene vapour (from polystyrene) also can settle onto water and kill larvae. Harder plastics tend to be more inert than soft plastics, which are likely to contain more plasticisers like phthalates, which can be toxic to fish at only a few mg/L. Silicone gaskets are safer than neoprene ones. Vinyl tubing should be avoided as much as possible.

18.3.9 Biological factors

18.3.9.1 Surface death

Larvae of some fish (especially groupers, but also others such as bonito and tuna) can be trapped then die by sticking to the water surface, maybe because surface tension is high. Often, the tail sticks first, then the side of the fish, possibly because of abundant mucus in some species. The larvae seem most vulnerable during the first 10 d after hatching. Some possible partial solutions are as follows:

- moderate water flow and aeration rates;
- moderate even lighting (about 1000 lux or less at water surface);
- a tank that is not completely round to disrupt continuous swimming.

Addition of microalgae to make an oil film, or actual oil, has been used, but oil can injure the fish in other ways, especially by blocking gas bladder inflation (oil must be removed before this can occur). Chicken egg white blended with water also has been added to larval tanks for this purpose. Repelling larvae by constant movement of the surface film can reduce the rate of entrapment by adhesion.

18.3.9.2 Handling

Eggs are more resistant to handling than larvae, but some are sensitive until after gastrulation. Handling of larvae should be avoided, and gently lifting larvae in a container of water is the safest way. Nets should be considered for only the most durable larvae. Once they are juveniles, covered with scales, they can be gently netted. However, juveniles with small gill surface areas can suffocate more easily in air. Some species also seem to be frightened easily.

18.3.9.3 Gas bladder non-inflation

Many fish species have an internal gas bladder for regulating buoyancy and conserving energy. In physostomous fish (e.g. anchovies, herrings), from larvae to adults, the digestive system and gas bladder are connected by a pneumatic duct that can be used for inflation and deflation.

Physoclistous fish do not have such a duct as juveniles and adults, but they often do as larvae. They are vulnerable to experiencing non-inflation if their gas bladders cannot be filled normally before the duct permanently closes. In some batches of larvae, the non-inflation rate is high. The affected fish become highly stressed and cannot maintain neutral buoyancy or swim normally. They often develop lordosis or kyphosis (abnormal vertical spinal curvature), which usually becomes obvious in early juveniles (Fig. 8.3[1]). Initial gas bladder inflation in various marine fish from planktonic eggs often occurs at about the same age, between mouth opening and about two weeks after hatching, by gulping of air at the surface or a gas bubble below the surface. Factors contributing to gas bladder non-inflation and lordosis include surface films, high temperature, very slow current and stressfully fast current. Factors that will help maximise gas bladder inflation (and minimise lordosis) in many species includemoderate tank depth, moderate current and aeration (slowly increasing), surface film prevention and removal, water sprinkled on the surface, optimal light intensity, long daylength, low fish density (10–20 larvae/L), good egg quality and good nutrition. Shortened daylength or longer twilight periods during initial filling can help some species, especially if daytime light is very intense.

18.3.9.4 Gas bubble trauma

This can occur after an abrupt diet change, stressful currents, gas supersaturation, or bacterial infection of the gas bladder. Gas bubble trauma is like decompression sickness (the bends), which occasionally occurs in humans diving with compressed air. It happens in fish when culture water becomes supersaturated with a gas or gases, which then induces supersaturation of internal fluids. Bubbles formed in blood vessels and other tissues become large enough to interrupt circulation and oxygen transport (embolisms), thus causing hypoxia and tissue disruption. Possible causes are heating of water, high-pressure aeration, sudden drop in water pressure, high photosynthesis, and bacterial production of large amounts of hydrogen, oxygen, nitrogen or methane. Contributing factors can be increased salinity or handling, but malnutrition, crowding, hypoxia, toxicants and other stresses also are possible. Acute gas bubble trauma can cause high mortality, often from cerebral anoxia and haemorrhages. In bad cases, gas bubbles can form in the skin of fish within minutes. Embolisms can occur almost anywhere and block blood flow. Signs of chronic gas bubble trauma include bubbles in the gut and

[1]Although the curvature in this case arises from a vitamin deficiency.

buccal cavity, and an overinflated gas bladder, which often causes the fish to float at the surface and die. Low level mortality can last for weeks or months after an incident of gas bubble trauma.

18.3.9.5 Treatments with drugs

Many drugs given to fish also can harm them. Overdoses can cause anaemia, damage to liver, kidneys and nerves, deformities, sores, reduced growth, and mortality. Antibiotic overuse can give a competitive advantage to resistant strains of pathogenic bacteria, allowing their populations to increase. Copper treatments can be toxic, especially in salt water. Many substances used to treat fish for diseases (e.g. copper, zinc, iron, methylene blue, neomycin, erythromycin and nifurpirinol) can harm or inhibit beneficial microorganisms like probiotic and nitrifying bacteria.

18.3.9.6 Predators

Predatory invertebrates (e.g. hydroids, jellyfish, anemones, isopods, copepods) can be introduced with eggs, water or live food, and then suddenly appear by the thousands and kill fish larvae very quickly. Eggs should be washed well, water filtered well and food cultures kept clean (and sieved and rinsed before feeding). Many aquatic insects, while obvious in ponds, can also fly into indoor rearing units and are especially attracted to electric lights at night. Wading and diving birds can eat juveniles in ponds, and skimming birds can eat larvae. Steeply sloping pond edges inhibit wading birds, and bird nets help keep divers and skimmers out. Reptiles (e.g. crocodiles, alligators) and mammals (e.g. otters and rats) can be excluded with good fences.

18.3.9.7 Beneficial microorganisms

These include viruses, algae, protozoans, bacteria and fungi. All groups except algae also include pathogens, and some algae can produce toxins. Many bacteria, algae and protozoans have positive effects. Heterotrophic bacteria usually obtain their nutrition from dissolved organic matter. Nitrifying bacteria convert ammonia and nitrite into nitrate (section 3.3.7). Denitrifying bacteria convert nitrate into nitrogen gas. Probiotic bacteria can protect aquatic organisms from pathogenic bacteria and/or release growth-promoting substances. Algae function as food for zooplankton and can also remove ammonia and nitrate. Protozoans can have a positive effect as food for larval fish or their zooplanktonic prey, or they can have a negative effect by competing with fish or zooplankton (e.g. for oxygen, food).

18.3.9.8 Harmful microorganisms

More than 300 species of dinoflagellates, chloromonads, chrysophytes, prymnesioids, diatoms and cyanophytes have been identified as toxic. Toxins produced by red (or brown) tide organisms in harmful algal blooms can cause heavy mortality or contamination of fish and shellfish (section 23.5.3) . Damage to fish gills is a common sign. Nutrient enrichment combined with favourable physical conditions promotes these blooms. Caged fish are most vulnerable, but fish in ponds and tanks are not totally safe. Precautions include good site choice, monitoring, deep cages, barriers around cages, pumping clean water into cages, oxygenation of sediment in ponds or below cages, low fish density to reduce stress, and ability to move fish to a safe place. Actions directed against the algae include skimming, ultrasound, flocculation and algicides.

18.4 REARING SYSTEMS

18.4.1 Selection

Choice of unit type, size and system depends on several factors:

- temperature;
- water quality;
- feeding requirements of the fish;
- growth characteristics of the fish;
- expected production;
- site characteristics;
- site and construction costs;
- labour and energy costs;
- water cost;
- access and security requirements;
- environmental, social and legal constraints.

18.4.2 Range

There is a range of water utilisation levels, from just using an existing water body, to letting new water flow through a rearing system with no treatment, to almost 100% recirculation with sophisticated treatments. The range of water use levels is outlined in section 2.4.

The great variety of structures, ponds, tanks, raceways, cages and pens/hapsas used in culturing the various stages of fish culture from larvae to grow-out are outlined in section 2.2. Distributed through the text there are various illustrations of these structures and the reader should consult the index to locate them.

18.4.3 Applications of rearing systems

18.4.3.1 Holding

Temporary holding of fish for stripping of gametes or other processing can be in tanks or cages just large enough for them to swim.

18.4.3.2 Quarantine

Quarantine tanks need to be large and quiet enough to minimise stress, but set up to facilitate observation and examination.

18.4.3.3 Reproduction

Reproduction and oogenesis in teleost fish was described by Jalabert (2005). Reproduction of captive marine fish can occur voluntarily when stress is low and conditions are correct. Broodstock mostly are kept in tanks but sometimes in ponds or cages. Traditional Japanese marine fish spawning tanks are circular, concrete, 40–100 m^3 in volume, and situated outside. Gregarious fish like sea breams easily spawn at high densities, but territorial fish might need more space or cover. Territoriality in some species decreases during the spawning season.

18.4.3.4 Egg incubation

Egg incubation for species with hatchlings that tolerate handling can be conducted in small tanks, from which the larvae then are transferred to rearing tanks sometime between hatching and first feeding. Those with sensitive hatchlings can be separately incubated but moved before hatching. Separate incubation allows a more accurate estimation of number stocked and minimises stocking of bad eggs. Demersal eggs (attached or unattached) usually need a continuous stream of well-oxygenated water and sometimes need a certain amount of movement. Planktonic eggs need good oxygenation and gentle movement to prevent them from depleting oxygen or sticking together. A large water surface area and good circulation control help keep planktonic eggs from bunching up. Usually, small tanks (e.g. 300- to 1000-L circular) or fine-mesh cages in larger tanks are used for them. Temperate marine planktonic eggs might take weeks to hatch (typically more than 2 days), but most warmwater planktonic eggs need only 0.5–5 days (typically 2 days or less). The longer incubation periods often require more elaborate systems and effort.

18.4.3.5 Larval rearing

Larval rearing usually occurs in tanks of fibreglass, concrete, etc., and sometimes in ponds (rarely in cages or bags). Tank rearing works for most larvae, and extensive rearing in fertilised ponds works for certain species if the weather is stable enough., An indoor hatchery might be necessary for fish with sensitive larvae. Recirculating water treatment systems can be used, but larvae need to be protected from seemingly minor toxicant build up. Hatchery tanks usually have diameters from 1 to 10 m, and larvae of some fish survive better in large tanks (e.g. 3–10 m^3), apparently because the walls are not encountered as often and conditions can be more stable. Larvae usually do not tolerate sudden environmental changes as well as older fish. Tanks should be deep enough to avoid confinement stress. There should not be areas where larvae can easily be trapped. Dull, dark walls with uniform lighting will help keep the prey and fish more evenly distributed. Constant or slowly changing temperature and salinity are best. Airstones resting on the tank bottom can make stressful tapping sounds and push larvae against the bottom more than airstones held off the bottom, a position that also will move the water better. A ring of airstones near the outer wall will cause a doughnut-shaped circulation. A second ring near the centre will cause a second donut. If moving at appropriate speeds, these two circulation cells will mix the water well with less chance of larvae being pushed into the walls, bottom or central standpipe. Water usually enters at or near the surface near the tank wall and flows out from the surface at the centre. Bottom and surface drains should be covered with screens with small enough mesh that larvae cannot swim through them and of large enough surface area that larvae are not pulled into them by the current. A smooth, relatively large-diameter pipe leading to a harvest pit outside the tank can allow the fish to be collected more efficiently and, hopefully, more gently than by netting.

In Asia, larval rearing tanks often are circular or rectangular, but sometimes other shapes (e.g. octagonal) are of concrete, hold 0.5–200 m^3, and are usually outside under shade or inside under skylights (Fig. 18.4). In Europe, shape usually ranges from deep cylindro-conical to shallow cylindrical, and size is 1–30 m^3 for intensive culture and as large as 250 m^3 for semi-intensive culture.

In a favourable environment, ponds are good for rearing larvae and early juveniles of some species (e.g. striped bass, red drum), but they require careful management to ensure adequate food and water quality and to limit predators (Fig. 18.5). Coastal impoundments and 50- to 300-m^3 plastic bags (containing natural zooplankton for food) have been used for Atlantic cod, turbot and Atlantic halibut larvae in Norway.

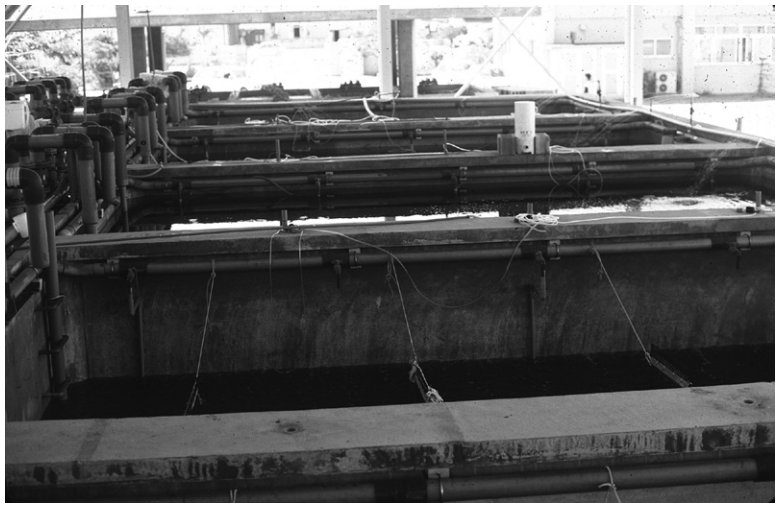


Fig. 18.4 Concrete tanks (50 m^3) for rearing larvae in Okinawa. (Reproduced from Tucker (2003a) with permission of *World Aquaculture Magazine*.)



Fig. 18.5 Concrete-walled pond supplied with water by the tides, in Taiwan.

To avoid growth inhibition, early juveniles probably need a tank volume of at least 400 L and 1 kg of fish at least 3000 L. The more active species need more room.

18.4.3.6 Grow-out

Grow-out to market size requires conditions resulting in the best combination of growth rate and feed conversion. The most appropriate type of grow-out depends on characteristics of the species and site. Tanks and raceways are feasible if water is cheap and abundant or can be recirculated. Bottom feeders like milkfish and mullet often are raised in ponds (sometimes fenced off into pens). Fish that feed from just off the bottom to mid-water, like sea breams, sea bass, groupers and snappers, are often raised in small to medium cages and sometimes ponds or coastal impoundments. Pelagic fish, like jacks, tunas and dolphinfish that swim constantly and often in straight lines, need large rounded or many-sided cages or tanks that gently guide them in a circle. To provide gradual transitions and to maintain maximum stocking density, grow-out sometimes

is separated into two or more phases, using progressively larger culture units.

18.5 FISH FOR STOCKING

18.5.1 Wild juveniles

Wild juveniles caught by seining, trawling, trapping, etc. have often been used for traditional farming, but this requires a reliable, available stock of juveniles and usually is impractical, except in special cases or until the wild stocks are depleted.

18.5.2 Hatchery juveniles

Usually, enough juveniles can be obtained only by raising them from eggs taken directly from the parents. The technology for each species requires time for development. Fish eggs can be obtained by plankton or substrate collections (usually impractical), strip-spawning of freshly caught running-ripe wild adults, strip-spawning of wild or reared adults in which ovulation has been induced by hormone treatment, spawning of wild or reared adults previously treated with hormone(s) while they are in a rearing unit, strip-spawning of untreated captive wild or reared adults, or voluntary spawning of untreated captive wild or reared adults.

18.5.3 Broodfish

Broodstock can be captured or reared. Rate of adaptation to captivity varies among families and species. Adults usually are captured as gently as possible by traps, hook and line, seines, or electroshockers (sometimes trammel nets or hand nets). Gill nets and trawls are rough and should be avoided. Depending on species and capture depth, the gas bladder might expand too much for the fish to recover on its own (e.g. it floats helplessly upside down). When deflation is necessary, a hypodermic syringe with a 20-gauge needle attached and plunger removed can be carefully used. Broodfish should be put in well-aerated or oxygenated water immediately after capture. The water should be filtered or exchanged in a manner conforming with transport time, temperature, fish density and fish sensitivity. Broodfish to be kept for more than a few days should be given plenty of room, high water quality, appropriate temperature, easy access to shade and protection from unnecessary disturbance. Sometimes captured fish are treated with parasiticides (e.g. formalin bath) and/or antibiotics. If kept for more than a few days, they should be well fed with a complete pelleted diet or a variety of high-quality freshly frozen natural foods supplemented with vitamins. Good nutrition is especially important during early vitellogenesis. With hermaphroditic species, sex control or reversal can be necessary to maintain an optimal male : female ratio for maximising egg production. Broodfish always should be handled as gently (with soft nets, plastic bags, or hands, not towels) and infrequently as possible. Some fish, like snook, yellowtail snapper and striped mullet, will not tolerate much handling; however, others like turbot and Atlantic halibut can be strip-spawned a few times a week without affecting egg production. With species that move too much and cannot be held without injuring them, anaesthesia can be used.

18.5.4 Natural ovulation

Fish are adapted to reproduce in response to environmental cues appropriate for that species or population. The culturist has to provide the cues that really matter in the right combination, degree and order. Susceptibility to conditioning varies. Milkfish, cods, groupers, jacks, dolphinfish, snappers, sea breams, drums, gobies, rabbitfishes, largetooth and righteye flounders, common sole, triggerfishes and puffers are fairly easy to condition to spawn voluntarily. Hormones sometimes are used to accelerate, synchronise or otherwise control their spawning schedule. Striped mullet have not spawned voluntarily but can be conditioned to a point at which they are hormonally induced to ovulate and then spawn in tanks. Turbot and Atlantic halibut will ovulate naturally, but usually are stripped. Barramundi are tank- and strip-spawned with and without hormones, but common snook have tank-spawned only after hormone treatment.

During the natural spawning season, broodfish can be caught running ripe or held for a short time until ovulation occurs (if they are not very sensitive to captivity stress). If caught near the beginning of the spawning season, handled gently and kept under spawning conditions, some species will quickly adapt to captivity and spawn voluntarily within days or weeks (e.g. black sea bass, dolphinfish, spotted seatrout). Some species need more time to adapt to captivity, possibly months to years. Long-term conditioning depends on holding fish under ambient conditions, simulated ambient conditions or an artificial cycle that works. Increases or decreases in temperature and daylength usually are most important. These two factors can be used to advance, delay, extend or compress spawning cycles. Other possible factors are lunar cycle, salinity, water chemistry, water level, current, habitat structure, suitable spawning substrate, sex ratio and stocking density. Behavioural interaction and pheromones can be factors. Some fish spawn over a wide temperature range and others over a narrow range. Some temperate species might respond more to daylength as a stimulus and some tropical



Fig. 18.6 Spawning rush by Nassau groupers Two males are close beside a female that is releasing eggs, while another female awaits her turn (lower right). (Reproduced from Tucker (2003b) with permission of *World Aquaculture Magazine*.)



Fig. 18.7 Sea bream spawning tank in Japan. The compartment attached to the side is used to filter the planktonic eggs out of water overflowing from the tank.

species (e.g. Nassau grouper) might respond more to temperature. European sea bass, gilthead sea bream, striped mullet and red drum are stimulated to spawn by decreasing temperature and daylength. Simulated tidal movements (also indicating lunar phases) can affect intertidal spawners (e.g. silversides, some puffers), because of their need to place eggs at the best sites, and oceanic fish (e.g. groupers), possibly for spawner synchronisation and quick dispersal of eggs. Groupers will spawn in pairs or groups (Fig. 18.6). Spotted seatrout spawn better in groups. Schooling pelagic fish like herrings, dolphinfish and tunas may spawn in pairs or small groups but do not move far from the school.

18.5.5 Egg collection

Planktonic eggs in a tank can be collected by overflowing (gently and close to the same level) surface water into a smaller tank (Fig. 18.7) with a fine screen to retain the eggs, which are then skimmed out. Eggs in ponds can be gathered in a neuston net downstream from a paddlewheel. Some fish will deposit eggs in a nest on a substrate (e.g. PVC pipes, gravel, shells). Some sinking adhesive eggs can be collected with mats on the tank bottom.

18.5.6 Induced ovulation

Final maturation and ovulation have been induced mostly by administration of specific pituitary, carp pituitary, salmon pituitary, human chorionic gonadotrophin (HCG), pregnant mare serum, and various gonadotrophin-releasing hormone-analogues (GnRHa) (Figs 6.1 and 18.8).

HCG and pregnant mare serum are usually dissolved in saline solution and injected intramuscularly. HCG dosage ranges from at least 100 to 40 000 international units (IU) HCG/kg body weight and mostly is in the range 200–1500 IU/kg. Sometimes pituitary extract plus HCG or pregnant mare serum are used. HCG is not very effective for some fish, but at least for some members of the families Clupeidae, Centropomidae, Percichthyidae, Serranidae, Carangidae, Lutjanidae, Sparidae, Sciaenidae, Siganidae and Pleuronectidae, HCG results in nearly complete ovulation, short time to ovulation, and fertile and viable eggs. However, overdoses of HCG can be more likely than GnRHa to cause abortion, over-hydration, egg retention, and female mortality or simultaneous hydration and ovulation followed by mortality.

GnRHa has been successfully used for members of several families, including Clupeidae, Acipenseridae, Chanidae, Salmonidae, Centropomidae, Percichthyidae, Serranidae, Lutjanidae, Sparidae, Sciaenidae, Mugilidae, Paralichthyidae, Pleuronectidae, Soleidae and Tetraodontidae. Given by injection or implantation, it causes the pituitary to secrete and release endogenous gonadotrophin. GnRHa is effective with some species that are resistant to HCG and seems to cause more prolonged spawning in some. It is less likely to cause an immune response after long or repeated treatments, which can result in resistance. For acute administration, it is dissolved in saline solution and injected intramuscularly at 0.1–300 µg GnRHa/kg body weight, with typical doses of 5–30 µg/kg. Usually, two injections of GnRHa work better than one.

Some species (e.g. Japanese eel, milkfish, winter flounder; frequent spawners like European sea bass, gilthead sea bream) need a long gonadotrophin surge for successful

spawning and might have to be injected frequently or given time-released hormones. GnRHa can be implanted intraperitoneally in a pellet, microcapsules, or microspheres containing 5–350 µg GnRHa per dose. These have worked well in milkfish, barramundi, European sea bass, striped bass, white bass, red sea bream, gilthead sea bream, striped mullet, southern flounder and winter flounder.

Females are selected for induced ovulation on the basis of oocyte development (diameter, colour, structure) as seen in ovarian biopsies or determined or inferred by other means (e.g. ultrasound). Taking of biopsies must be minimised because the procedure is stressful and sometimes causes infection and reduced egg viability. If stage of ovarian development can be predicted with reasonable certainty, biopsies can be avoided. A short length of surgical polyethylene tubing with an inside diameter slightly larger than ovulated egg diameter is good for taking biopsies. Germinal vesicles of translucent oocytes can be viewed with a microscope if they are flattened slightly by the weight of a cover slip on a slide or Petri dish. Opaque oocytes can be cleared by immersion for 5–20 min in a mixture of 60% ethanol, 30% formalin and 10% acetic acid. A simpler but possibly not as effective method is immersion in a series of 5-min baths of 30, 50, 70, 90 and 100% ethanol.

Most success with induced ovulation has probably occurred by injecting fish with oocytes in the range from late vitellogenesis (approximately fully yolked) to early hydration (normal fluid accumulation). Other factors are sensitivity of the fish and conditions (especially temperature). The number of injections required and the intervals between them depend on species, initial degree of ripeness and temperature. To help insure constant blood levels, injections usually are spaced at 24-h intervals, but special situations might require different intervals. If ovulation occurs after 36 h or less, only one injection might be needed.

When held under spawning conditions, males can stay running ripe for weeks or months. They also can be injected to increase the volume of milt, but that usually is temporary and the milt soon decreases. It is best to avoid injecting males, unless they can be replaced. For warmwater marine fish, one injection 12–24 h before milt is needed usually is good for a few days. Sustained-release implants will work longer. Pituitary extract, salmon gonadotrophin, HCG, pregnant mare serum, GnRHa, progestins, methyltestosterone and other androgens have been used. Typically, males are given one injection when females receive their last one, but at a lower dosage (e.g. half of the female dose of HCG).



Fig. 18.8 Hormone injection in the back of a female squaretail coral trout to induce ovulation. (Reproduced from Tucker (2003b) with permission of *World Aquaculture Magazine*.)

The injections usually are given intramuscularly in the thicker back muscles between the dorsal fin and lateral line (Fig. 18.8), but large-scaled fish can be injected under the pectoral fin to avoid dislodging scales. A 26-gauge needle is good for most species; finer needles can be used for small fish and coarser needles for thick-skinned fish. Pituitary injections and implants are usually placed in the peritoneal cavity, just inside the abdominal wall to minimise internal injury. If newly caught broodfish are used, the hormone should be administered as soon after capture as possible, to limit stress effects on oocyte development.

Hormone-treated fish should be held at natural spawning temperature and salinity. Dissolved oxygen should be adequate and disturbance minimal. Females are handled as little as possible, but are monitored closely for swollen abdomen, protruding genital papilla, stretching of the membrane holding eggs in, and spawning coloration. They are checked more often (e.g. once an hour) just before the predicted time of ovulation. The time from ovulation to over-ripeness (deterioration of eggs) might be only 1–2 h or less for warmwater fish.

With full normal ovulation, eggs flow easily and quickly after the protruding membranes are broken (Fig. 18.9). Indicators of good egg quality are as follows:

- normal number of eggs (not always);
- buoyancy at correct salinity;
- normal transparency;

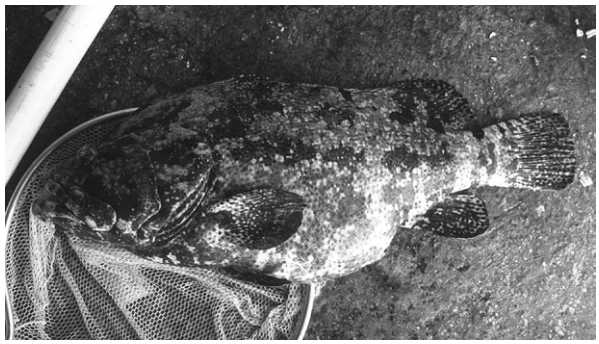


Fig. 18.9 Female brown marbled grouper that has just ovulated and is about to be strip-spawned.



Fig. 18.10 Collecting milt from a male Nassau grouper with a 3-mL syringe (needle removed). (Reproduced from Tucker (2003) with permission from Springer Science+Business Media B.V.)



Fig. 18.11 Stripping eggs from a female Nassau grouper into a graduated beaker, which allows estimation of egg number.

- normal shape;
- normal and uniform egg size;
- normal chorion uniformity and strength;
- lack of abnormal stickiness;
- normal osmotic properties;
- normal size and number of oil globules;
- lack of inclusions;
- normal perivitelline space;
- high fertilisation rate;
- normal cell division (usually symmetrical at first);
- normal chromosome pattern;
- resistance to bacterial infection;
- high hatching rate;
- long starving time;
- high first feeding rate;
- low larval deformity rate.

Fertilisation must be conducted promptly but carefully in an area free of sunlight and with constant temperature close to that of the water holding the broodfish. Ideally, the female membranes are not broken until milt has been collected in 3-mL hypodermic syringes (without needles) or other clean airtight containers, and then set aside in a safe place (Fig. 18.10).

The female is caught, anaesthetised if necessary, then held gently but firmly over a cup, beaker, pan, etc. for receiving the eggs. A strong dark plastic bag or soft net can be used to hold the front end of the fish. Light, steady pressure on the anterior abdomen should start the eggs flowing (Fig. 18.11). As the flow decreases, pressure is applied further back and on the sides of the abdomen until no more eggs can be easily expressed. If the membrane does not break easily, it might be better to tear it directly rather than apply excessive pressure to the abdomen, which can injure both fish and eggs.

In the wet method, eggs are stripped into enough clean filtered water to cushion and slightly disperse them. Milt is added immediately on top of the eggs and the mixture is swirled for 3–5 min. In the dry method, water is added after the eggs and milt. The dry method is easier and probably better. Eggs of some species become infertile in 1 min and others in a few hours. Once activated by water, sperm motility for most cultured marine fish probably is in the range 2–20 min. The safest approach is to mix the gametes as quickly as possible after stripping.

18.5.7 Eggs and sperm

18.5.7.1 Egg care

After fertilisation, the eggs are transferred to a larger container and washed very well by water exchange and/or repeated egg transfer. Stripped eggs are more easily processed than tank-spawned eggs because they are more concentrated, cleaner and do not have to be washed as thoroughly to remove potential pathogens. Eggs can be sensitive to handling until they water harden (after several hours), then resistance might improve. Small numbers of eggs can be counted individually with a pipette and large numbers estimated volumetrically in a graduated beaker or cylinder.

To allow better monitoring of egg health, they are usually incubated separately under very clean, constant, optimal conditions. Demersal eggs can be kept in jars, troughs or trays. Planktonic eggs are usually held in aquaria, clear acrylic tanks, or fibreglass tanks. Moderate egg density and water exchange are best and will vary with species and temperature. Planktonic eggs must not be allowed to pile up for more than a few minutes at a time. Dead eggs must be removed promptly. Water must be exchanged fast enough to prevent oxygen depletion, ammonia build up, cloudiness (bacteria, fungi) and foul odour. Larvae from planktonic eggs are likely to survive better if the eggs are moved to a different container before hatching. If they hatch in the incubator, disinfection before or during incubation can be helpful.

Eggs in freshwater tend to get fungal infections, whereas bacterial infections are more likely in salt water. For some species, some disinfectants are toxic at less than effective concentrations. Red drum eggs have been safely and effectively dipped in 3% hydrogen peroxide for 5 min, but yellowtail snapper and spotted seatrout eggs cannot tolerate that concentration. Atlantic halibut eggs were safely dipped in 400–800 mL/L glutaraldehyde solution at 5 °C for 5–10 min, which reduced bacteria by more than 80%, and turbot eggs can be dipped in 400–800 mL/L glutaraldehyde solution at 12 °C for 2.5 min. A 10-minute bath of 400 mL/L glutaraldehyde or 1000 mL/L chloramine-T apparently was safe for American plaice eggs and reduced bacteria by about 85%. Hydrogen peroxide seems to be the best disinfectant for planktonic eggs. Stickiness of adhesive eggs can be removed to prevent the eggs from clumping together and dying from hypoxia by using a bath of clay, urea, sodium sulphite and/or tannic acid.

Eggs are transferred to rearing tanks just before hatching, or larvae are transferred just before first feeding, if they can tolerate it. Tank stocking density ranges up to 1–30/L and occasionally higher (mainly coldwater or very robust fish). In Taiwanese concrete and earthen ponds, grouper egg stocking rate can be 600–1500 eggs/m^2.

18.5.7.2 Bad eggs

Masses of clear normal planktonic eggs might appear yellowish, greenish or with other tints, depending on species. Preliminary fertilisation rate can be determined in one to several hours, depending on the developmental rate. A few hours later, the distinction between fertilised and unfertilised eggs will be much more obvious because of further cell division. Unfertilised planktonic eggs will sink and begin to discolour soon after stripping. Aborted oocytes are infertile ones typically released before full development and usually are less than full size with an abnormal shape; some individual oocytes can be yellowish and partly opaque because yolk globules have not fused or yolk has coagulated; there can be more oil globules than normal. Over-ripe (over mature) eggs result when they have been ovulated but then not fertilised in time and are losing the ability to be fertilised and develop correctly. They might collapse and lose their normal shape, have irregular structure, transparency loss, discoloration, soft torn chorions, dimpled cytoplasm and fused cortical alveoli, but usually the normal number of oil globules.

18.5.7.3 Gamete storage

Males of certain species can be difficult to collect in some locations. A possible solution is to cryopreserve sperm with liquid nitrogen (−196 °C) when ripe males are available and store it until eggs are obtained. Fish eggs are difficult to cryopreserve, but milt is easy. Short-term storage near 0 °C also can work for milt.

18.6 NUTRITION OF LARVAE

18.6.1 Larval food criteria

For typical early marine fish larvae, being able to find the right amount of high quality food throughout the lighted period is essential for survival. The live feeds used in fish hatcheries were reviewed by Støttrup and McEvoy (2003) and are discussed in detail in Chapter 9. Once nutrient reserves have accumulated, the fish can go longer without food. Criteria for acceptable larval fish foods are as follows:

- availability;
- density;
- appearance;
- size;

- smell or taste (possibly);
- nutritional quality;
- microbial status;
- digestibility;
- stability of the food;
- ability of the fish to manage it.

Extra care is necessary during first feeding and weaning to avoid high mortality. First the larval fish must locate the food without wasting energy. Then the fish must be attracted to the food, be capable of swallowing it and be willing to swallow it. The food must contain an appropriate balance of nutrients that can be utilised by the fish. The food must not disintegrate or lose an appreciable amount of nutrients before the fish eats it. Larval survival and growth will be enhanced if feeding effort is kept low. Particular attention must be paid to size, fatty acid balance and content, amino-acid balance, and nutrient density of foods given to marine fish larvae.

Typical marine fish larvae in the wild (which have a choice of food items) tend to begin feeding on small, slow prey (e.g. dinoflagellates, ciliates, mollusc larvae, larval copepods, larval cladocerans) and progress to larger, faster prey (e.g. juvenile and adult copepods and cladocerans, barnacle larvae, and larval and juvenile shrimp, amphipods, isopods) as their ability increases. Mouth size, swimming ability and patterns, chemical and mechanical senses, and digestive capability vary greatly among species, but there also is individual variation (a major factor producing runts).

Prey density should be adjusted to be high enough so that larvae can easily eat, but not so high that prey quality or water quality can deteriorate. Water must be well mixed and light evenly distributed to avoid too much prey patchiness. Dark tank walls make prey more visible to the larvae. The best prey size for marine fish seems to be about 25% of the mouth width (or 45° gape height) at first feeding, increasing to 50% within a few days. Some fish can eat *Artemia* nauplii or large copepods at first feeding, but many need prey the size of protozoans or young rotifers and copepods. Optimal prey width at first feeding for many marine fish is in the range 35–100 µm. Preferred prey size increases as mouth size and feeding ability increase. For some larvae that need small prey, either a small strain of rotifers can be used or small ones can be sieved from the rotifer culture.

The senses of smell and taste are not fully developed in some species during the first days of feeding, when food size and movement can be primary feeding cues. This is one reason why young marine fish larvae tend to ignore formulated microfeeds. Later, when those senses develop, the fish are more likely to eat microfeeds, especially those with added olfactory and gustatory stimulants.

Important nutritional factors contributing to normal development include vitamins A, C, D and K, essential fatty acids and phospholipids. Docosahexaenoic acid (DHA, 22:6*n*–3), eicosapentaenoic acid (EPA, 20:5*n*–3), arachidonic acid (ARA, 20:4*n*–6) and possibly *n*–6 -docosapentanoic acid (*n*–6DPA, 22:5*n*–6) appear to be essential nutrients (essential fatty acids, EFAs) for the marine fish whose requirements are known (Table 8.5). A 10 : 5 : 1 ratio of the first three (e.g. 2% DHA, 1% EPA, 0.2% ARA) is good for some species, but seems to vary between species. For juveniles, equal amounts of DHA and EPA might be better. Linoleic acid (18:2*n*–6) and linolenic acid (18:3*n*–3) are essential for some fish and should be included in balanced proportions. Growth of some fish also can be promoted by linolenic acid. Mono-unsaturated (palmitic acid, 16:0) and saturated fatty acids (oleic acid, 18:1*n*–9; 20:1*n*–9; 22:1*n*–11) are better than highly unsaturated fatty acids for energy and should be included in balanced proportions.

Rotifers can be, and *Artemia* are usually, deficient in at least one EFA (DHA, EPA, ARA, *n*–6DPA). The lipid content and fatty acid composition of feeding rotifers, *Artemia*, *Daphnia pulex* and *Moina* sp. are derived from their recent foods, but for male rotifers (which do not feed) and newly hatched zooplankters like *Artemia* nauplii (which do not feed), fat content and composition depend on the composition of their mothers' diets. Deficiencies in fatty acids and maybe amino acids make *Artemia* nauplii risky to use, but after the metanauplii begin feeding, enrichment can mostly correct those (section 9.4.7). Copepods tend to maintain good fat and essential fatty acid contents but also can benefit from enrichment.

Foods for marine fish larvae should contain enough EPA, DHA and ARA (possibly with some linolenic acid). A dietary *n*–3 highly unsaturated fatty acid (HUFA) content of 3–4% of dry weight, including at least 1.0% EPA and 1.0–2.0% DHA, will probably be enough for most species. Some larvae might not need that much, but a little extra will not hurt. To reach these levels, *Artemia* must be enriched by feeding them *n*–3 HUFA contained in oil added to yeast, an emulsion or microfeeds.The nutritional value of zooplankters will decrease if they are not feeding. Adding rotifers to larval tanks at least twice a day will help keep prey nutrient density high, but greenwater is the best insurance for prolonging prey quality. Rotifers and other zooplankton can be refrigerated before or after enrichment to conserve their nutrients.

Microorganisms can affect the health and production of cultured algae, rotifers, *Artemia*, copepods and fish. Live foods must not contain pathogens, but they should contain probiotic (beneficial) bacteria as much as possible.

18.6.2 Choice and culture of foods for larvae

Larvae of most cultured marine fish can be fed a sequential diet of rotifers, brine shrimp and dry feeds. It is important to harvest both phytoplankton and zooplankton cultures often enough to avoid crowding and to maintain good production rates. Rotifers, brine shrimp and cultured cladocerans, calanoid copepods and some harpacticoid copepods are mostly planktonic. The usual prey density ranges used are as follows:

- 100 dinoflagellates/mL;
- 5–20 rotifers/mL;
- 1–10 copepod nauplii/mL;
- 0.5–6 *Artemia* nauplii/mL;
- 0.5–3 adult copepods/mL.

18.6.2.1 Microalgae

Methods for culturing microalgae are detailed in section 9.3. Microalgae are the usual food given to zooplankton that will be fed to larval fish. Stock cultures, usually in glass tubes or small flasks, are maintained under clean constant laboratory conditions and are used or replaced before they degrade (section 9.3.1). The first one or two levels of scaling up usually are done in the laboratory (Fig. 9.2). Final stages can be inside under artificial light, in a greenhouse with partial sunlight, or outside with full sunlight. Nutritional value of microalgae for zooplankton and the fish eating them is influenced by the type of culture, temperature, nutrients, other conditions and growth phase (section 9.3.3). Species commonly used include *Pavlova lutheri*, *Rhodomonas baltica*, *Nannochloropsis oculata*, Tahitian *Isochrysis* (also called T-*Isochrysis* or T-Iso) and *Tetraselmis suecica*. *Pavlova lutheri* (ca. 5 µm in diameter) can be a good source of EPA and DHA (e.g. 28% EPA, 15% DHA) and so can *Rhodomonas baltica* (ca. 8 µm, e.g. 10% EPA, 9% DHA). *Nannochloropsis oculata* (ca. 3 µm) can be good for EPA and ARA (e.g. 10–37% EPA, 4–5% ARA) and T-*Isochrysis* (ca. 5 µm) for DHA (e.g. 8–19%). *Tetraselmis suecica* (ca. 7 × 13 µm) usually has a moderate amount of EPA (e.g. 0–8%). Frozen and/or concentrated pastes of various species of microalgae including *Nannochloropsis*, *Isochrysis*, *Pavlova* or *Tetraselmis* are available commercially (section 9.3.4). They are convenient but care is necessary to disperse them well and avoid fouling the water.

18.6.2.2 Rotifers

Rotifers (Fig. 9.5) are raised in batch cultures, which are harvested all at once at short intervals, or in continuous cultures, which are run for months (with water replacement or recirculation) and partly harvested (section 9.4.2). *Brachionus* strains include (with size ranges at moderate temperatures) the following:

- large or L-strain, *B. plicatilis* (possibly four species and typically 130–340 µm long); the width of L-strain rotifers is often about 80% of their length;
- small or s-strain, *B. ibericus* (possibly four species and typically 100–230 µm long);
- super small or ss-strain, *B. rotundiformis* (possibly one species, and typically 70–120 µm long).

Local strains of *Brachionus plicatilis* can be raised. Freshwater rotifers like *Brachionus calyciflorus* are good for larvae reared at low salinity and are increasingly used for the larval culture of freshwater ornamental fish (section 25.12.7). Rotifer lifespan can be 17 days or more (depending on strain, food, temperature and water quality), but 7–12 days is more typical.

Rotifers are raised in many kinds of container, both inside and outside. Their health and nutritional quality depend on several factors, including type of culture, water quality, temperature, foods, rotifer density and age of the culture. Doubling can occur in less than a day, and densities of up to 30 000/mL (and even 190 000/mL in experimental systems) have been reported. With high densities, tank space and operating cost per rotifer are reduced, but additional equipment and attention are needed to avoid population crashes. More typical harvest densities are in the range 100–1000/mL and sometimes to 3000/mL, which can be precarious unless water is exchanged or filtered very well. Lower temperature or salinity tends to produce larger rotifers (e.g. 15 °C, L-strain longer than 400 µm). Higher temperature (e.g. 30 °C), higher salinity, or yeast as food tends to produce rotifers as much as 20% shorter. Optimal temperature and salinity vary with the strain. *B. plicatilis* can tolerate 1–97‰ salinity, but about 10–30‰ seems best. A pH of 7.5 is good, but at high density, 7.0 (sometimes adjusted with hydrochloric acid) will reduce un-ionised ammonia. There should be enough aeration to maintain circulation but not enough to batter or clump the rotifers. DO should must be at least 4.5 mg/L and oxygen might need to be added.

Rotifers can eat particles 3–17 µm wide (preferably 3–12 µm) and have been raised on many species of microalgae. Microalgae fed to rotifers should contain significant

amounts of essential fatty acids. *Nannochloropsis oculata*, *Isochrysis galbana*, *Pavlova* spp., *Rhodomonas* spp. and *Tetraselmis* spp. are often fed to rotifers. Yeasts and compound microfeeds also are used. Vitamin B_{12} is essential and usually is supplied by bacteria growing in the rotifer culture unit.

18.6.2.3 Brine shrimp

Brine shrimp (*Artemia*, Fig. 9.6) cyst hydration in fresh or low salinity water for 1–2 h at 25 °C causes the slightly shrivelled chorion to regain a spherical shape, which facilitates hatching. Usually, the hydrated cysts then are incubated in salt water in a cone- or V-shaped tank with moderately high light and turbulence until they hatch (section 9.4.4 and Fig. 9.6). Then the hatching tank is used as a separator funnel. Aeration is halted, and heavy debris sinks to be drained off and discarded. Nauplii are drained off until the surface layer of floating debris is just above the bottom. Unaerated nauplii will usually quickly sink to the bottom, but a strong light can be used to attract them to a chosen section of the hatching tank. Suitable hatching conditions are 25–30 °C, 20–25‰, pH near 8 (maybe up to 9), >2 mg/L DO, light near 2000 lux; <5 g cysts/L of water for vessels up to 20 L, or 2 g cysts/L for vessels more than 20 L. Nauplii should be rinsed very well before giving them to the fish. Decapsulation of cysts often is practised to minimise debris that can decompose or be mistakenly eaten by fish larvae, and it also increases disinfection of the cysts. After hydration, the capsules are treated with sodium or calcium hypochlorite, which is then neutralised, and the cysts are washed well before hatching.

Hatching success, nauplius size, nutritional value and contaminants in different batches of cysts can affect the value of *Artemia*. The reported size range of nauplii is 375–450 µm wide and 428–517 µm long, with compressed widths as small as 176 µm. Decapsulated cysts and newly hatched nauplii are not very digestible and should not be relied on as main foods. Cysts are also not attractive and not likely to be eaten well. Natural EFA content of *Artemia* is usually not adequate for marine fish, and they have to be enriched (section 9.4) with EFA and vitamins (C especially) if used for more than a few days.

Nauplii can be stored at 8–12 °C with light aeration for a day or two and at 0–4 °C for up to 2 days, without losing much value. Enriched metanauplii have been stored for 1–2 days at 8–12 °C. Brine shrimp can be raised to larger sizes on microalgae; a combination of two microalgae species can provide a better balance of EFAs, but some enrichment still is advised. Other foods include yeast, ciliates, other protozoans, spirulina, other dried algae, bacteria, rice bran, corn bran, soy protein, whey and microfeeds.

Many marine fish can easily digest rotifers, but digestibility of *Artemia* varies with age of the *Artemia* and with species and age of the fish. Before they become instar II metanauplii, ca. 12 h after hatching, the digestive system of *Artemia* is not open, which apparently limits entry of external digestive enzymes and makes the *Artemia* less digestible to early larvae of some fish. By 1 day after hatching, internal digestive enzymes in *Artemia* increase to about five times the initial amount.

Because they are so easy for larvae to catch and eat, even when the fish already are full, gorging on *Artemia* is sometimes a cause of mortality for mid-stage larvae. The fishes' guts can be blocked, or *Artemia* can pass through undigested. Gorging can be minimised by adding the *Artemia* in small amounts and by continuing to feed rotifers, copepods or both for at least several days after *Artemia* are started.

18.6.2.4 Copepods

Copepods are the primary natural food for many marine fish larvae. Their use in aquaculture was reviewed by Lee *et al.* (2005). They are usually nutritionally complete and are good as supplements if not as a main food (section 9.4.8).

Calanoid copepods (Fig. 9.8) that have been successfully cultured and used as fish food include *Acartia clausi*, *Acartia tonsa*, *Acartia sinjiensis*, *Centropages* spp., *Eurytemora affinis*, *Gladioferens imparipes*, *Paracalanus* sp., *Parvocalanus* sp., *Pseudocalanus* sp., *Pseudodiaptomus* spp. and *Temora* spp.

Examples of harpacticoid copepods are *Euterpina acutifrons*, *Nitokra lacustris*, *Tigriopus japonicus*, *Tigriopus californicus*, *Tigriopus brevicornis*, *Tisbe* species (usually *Tisbe furcata* or *Tisbe holothuriae*) and *Amphiascoides atopus*.

Cyclopoids in the genera *Oithona*, *Dioithona* and *Apocyclops* have been successful. Care is needed when using untested copepods, especially cyclopoids, because some species will attack fish larvae.

Tigriopus japonicus and *Acartia tsuensis* are mainly used as foods for fish larvae in Japan, but also *Paracalanus* spp. and *Pseudodiaptomus marinus*. *Pseudodiaptomus annandalei* (6–7 days for each generation) and *Apocyclops royi* (5–11 days generation time) are raised in ponds and used in Taiwan. Other examples are *Acartia tranteri* and *Tisbe* sp. in Tasmania, *Acartia tonsa*, *Oithona* sp. and *Pseudodiaptomus pelagicus* (6–9 days generation time) along the northern Gulf of Mexico and eastern Florida,

whereas *Tigriopus californicus* is used in California, and *Acartia tonsa* in Florida.

Other examples of generation times are 5–6 days for *Acartia sinjiensis* at 29 °C, 7 days for *Acartia tonsa* at 25 °C, 8 days for *Acartia tsuensis* at 28 °C, and 7 days for *Tisbe holothuriae* at 22 °C. *Acartia*, *Centropages*, *Eurytemora* and *Temora* spp. can be raised for months without restarting. Generally harpacticoids can be raised at higher densities, but *Acartia* sp. can reach 3000/L, with two-thirds as nauplii. Some calanoids (e.g. *Acartia* spp., *Gladioferens imparipes*) have reached as high as 5000/L, and *Parvocalanus* sp. nauplii up to 30 000/L.

Rearing methods are similar to those for rotifers, but copepods require more effort to produce because they cannot be crowded as much and most take 5–10 times longer than rotifers to double. Copepods have been raised in bottles, tanks, bags, ponds and impoundments. Intensive culture mostly is in tanks holding 200–2000 L, but up to more than 100 m^3 for commercial scale. Formalin can be used to disinfect copepod eggs. Foods include the following:

- microalgae: *Chlamydomonas* sp., *Dunaliella tertiolecta*, *Nannochloropsis* sp., *Tetraselmis* sp., *T. maculata*, *T. suecica*, *T. tetrathele*, *Chaetoceros danicus*, *C. gracilis*, *C. muelleri*, *Phaeodactylum tricornutum*, *Isochrysis galbana*, *Pavlova lutheri*, *P. salina*, *Nannochloropsis oculata*, *Skeletonema costatum*, *Thalassiosira pseudonana*, *Rhodomonas baltica*, *R. lens*, *Chroomonas salina*, *Cryptomonas* sp., and the dinoflagellates, *Gymnodinium* sp., *Oxyrrhis marina* and *Heterocapsa niei*;
- sea lettuce, *Ulva* sp.;
- lettuce;
- baker's yeast, baker's yeast with cuttlefish liver or other marine oils, marine yeast;
- rice bran, wheat, alcohol fermentation wastes, soy sauce cake;
- fish muscle, dried shrimp, dried mollusc, tunicates, and powdered or flake fish feed.

Copepods also eat microorganisms that have fed on the added foods. Smaller foods (4–8 µm diameter) such as *Isochrysis galbana* and *Nannochloropsis oculata* seem more useful to early stages of copepods than to adults. Feeding setae (bristles) of *Acartia clausi* are spaced at 4–6.5 µm in stage VI nauplii and 15–25 µm in adults. *Acartia tonsa* copepodites consume particles in the range 14–59 µm. Feeding two types of algae (*Nannochloropsis* and T-*Isochrysis*, or *Tetraselmis* and T-*Isochrysis*) usually is better than one because they complement each other in nutrients and cell size. *Nannochloropsis oculata* has been reported to be a bad food for some calanoid copepods, possibly because cellulose in the cell wall is difficult to digest and/or the cells are too small. *Rhodomonas salina* has been reported to promote fecundity in copepods. Some algae (e.g. *Chaetoceros* sp. and *Skeletonema* sp.) may contain factors that reduce fecundity. Fertilisation of ponds can favour rotifers at the expense of copepods, but using rice bran does not seem to do so.

Nauplii can be stored under refrigeration for several weeks (up to 1–3 g/L), then raised. Subitaneous eggs (which normally hatch in hours to days) of *Acartia tonsa* stored without oxygen at 2–3 °C for up to 1 year hatched into good quality nauplii and have been used to start new cultures. Their diapause (resting) eggs have been stored without oxygen at 25 °C for more than 1 year, but diapause eggs are difficult to obtain.

18.6.2.5 Cladocerans

Cladocerans like the freshwater *Moina* sp. and *Daphnia* sp., brackish water *Diaphanosoma aspinosum*, and saltwater *Podon* sp. raised on bacteria, microalgae, marine yeast, baker's yeast, rice bran, pig manure and poultry manure have been used as larval fish food or have potential.

18.6.2.6 Alternative live food types

Alternative types should be good for the fish larvae, have wide environmental requirements, tolerate crowding and be easy to feed and handle. Larvae with small mouths might need prey smaller than marine rotifers (width 35–200 µm for most). The marine dinoflagellate *Gymnodinium splendens*, marine ciliates like *Euplotes* sp., the young of freshwater ciliates like *Paramecium* sp. and larvae of many copepods have widths of 20–50 µm, and are good for some fish. Different ciliates have been raised with microalgae such as *Dunaliella tertiolecta*, T-*Isochrysis* and *Nannochloropsis oculata*, and with bacteria, yeast or both, but their nutritional value for larvae is not well known.

Oyster, clam or mussel trochophores (ca. 40–60 µm) can be a good supplemental starter food if given with small rotifers and then replaced by rotifers as soon as the fish are ready. Shells can form within ca. 8 h, but cryopreservation or UV light treatment can prevent shell development. Larvae of polychaetes, barnacles, sea urchins, ascidians and fish have been tried but are not usually as reliable in quantity or quality. Nematodes that are 50 µm long have been raised and used. Nutritious wild plankton (e.g. tintinnids, copepods, crab megalopa) can be collected with nets or traps. The high risk of introducing pathogens or pests

(e.g. parasitic or predaceous copepods, isopods, medusae, anemones, worms or toxic dinoflagellates) and variation in quantity and quality limit the value of wild plankton. Extensive culture of wild plankton in ponds or impoundments can reduce unwanted organisms compared with newly collected plankton, or the plankton can be sorted manually and raised in tanks.

18.6.3 Enrichment

To ensure lipid quality of zooplankton, they can be enriched (section 9.4.7), mainly with the essential fatty acids rather than non-essential ones, but with all fatty acids balanced well enough so the fish can utilise them normally. Decomposition of enrichment media is likely to result in fouling of water and high bacterial growth after 4 h, and rancidity of oils can occur after 18 h at 27 °C. The author usually relies on microalgae as the only rotifer food, but sometimes enriches rotifers for up to 3–4 h in salt water filtered to 5 µm (24–28 °C), and usually enriches *Artemia* for 4–8 h.

Wild copepods are the most natural food for typical marine fish larvae, and if the copepods are well fed they have a good balance of essential fatty acids. Unless enriched, cladocerans can be deficient in essential fatty acids.

18.7 LARVAL CULTURE TYPES

18.7.1 Larval density categories

18.7.1.1 Intensive

Intensive culture is the least natural. It is mostly is indoors, in tanks (usually less than 20 m^3 but up to 200 m^3), with a high density of larvae. Close observation and control of environmental conditions and feeding are needed, often with some automation. Rotifers (sometimes enriched) and *Artemia* (nearly always enriched) are used. Four subcategories of stocking density are as follows:

1. Semi-intensive (30–50 larvae/L).
2. Intensive (80–100 larvae/L).
3. Hyper-intensive (100–150 larvae/L).
4. Super-intensive (>150 larvae/L).

Intensive methods are the most risky. Control of conditions and food must be very good. Larvae can be stressed by crowding and there can be more deformities and cannibalism. Only fish whose culture needs are very well known (e.g. European sea bass, gilthead sea bream, white sea bream, turbot, common sole) are reared intensively. Sometimes, continuous monitoring by the hatchery staff is needed.

18.7.1.2 Extensive

Extensive culture is the most natural. It is mostly outdoors, usually in ponds or impoundments with volumes up to thousands of cubic metres, but occasionally it is done in plastic bags, cages or tanks. There is very little environmental control or none. Eggs or hatchlings can be placed in a pond that was fertilised (section 9.9.2) and stocked with microalgae and zooplankton timed to become abundant when the fish need them. The algae and zooplankton can be cultured or wild, and sometimes both kinds are used.

Outdoor larval tanks or ponds can be filled through a fine-mesh sock filter 1–2 days before stocking the fish to inoculate the water with zooplankton, but also limit their initial size and keep them from growing too large for first feeding larvae. Rotifers, copepods and other zooplankters raised in separate ponds of 0.05–0.1 ha can be pumped directly into the fish ponds or collected in nets and transferred.

Operating costs are low, fish quality usually is high, but fish density and production rates are not as high as in tanks. A food chain is set up. Stocking density is low (0.1 to 1 larva/L). A short cycle (1–2 months) produces late larvae or early juveniles for weaning and further growth, often to be under more intensive conditions. A longer cycle (2–6 months) provides larger juveniles for stocking or extensive grow-out. Extensive larval rearing in ponds or impoundments can produce larger numbers of juveniles more economically than intensive rearing in tanks, but ponds need to be managed very carefully. A good beginning is essential (it is difficult to restart the food chain). Fairly good weather is needed (it is otherwise difficult to control the pond environment). Then, larvae will tend to be less stressed and healthier than in intensive systems. Mullets, red drum and hirame often are reared this way.

18.7.1.3 Semi-extensive

Semi-extensive (mesocosm) methods are in between and are used to gain some of the benefits of both intensive and extensive culture. This method is usually practised indoors, but can be partly outdoors. Usually, 30–100 m^3 tanks are used, but sometimes bags. Stocking rate is two to eight larvae/L. Intermediate environmental control is exerted. Rotifers, *Artemia* and sometimes other zooplankters are used. Blooms can become too thick or thin. Fish reared this way include Atlantic herring, milkfish, Atlantic cod, barramundi, temperate basses, groupers, cobia, snappers, sea breams, drums, mullets, turbot, southern flounder, Atlantic halibut, European plaice and common sole.

18.7.2 Benefits of phytoplankton in the fish tanks

If not too dense, a healthy growth of phytoplankton in larval rearing water usually is beneficial. It is food for zooplanktonic animals that the fish larvae prey upon, thus maintaining health, quality, behaviour and population size of the prey. Algae remove ammonia, generate oxygen and keep pH high. They can release antibacterial or growth-promoting substances and can promote growth of beneficial bacteria in the tank or in the larval gut. Genera found to help stabilise and optimise microbiota in the culture unit and/or the fish digestive systems include *Isochrysis*, *Nannochloropsis*, *Pavlova*, *Tetraselmis*, *Chaetoceros*, *Phaeodactylum* and *Skeletonema*. *Spirulina* contains antiviral substances. Algae can stimulate behaviour, feeding, digestion, immunity or other functions and can provide direct (e.g. trace nutrients, enzymes, feeding stimulants, or immunostimulants from the algal cells) or indirect (e.g. glucose dissolved in the water) nutritional benefits. Algae can obscure the view of people or other disturbing images, resulting in less distraction and less stress for fish larvae. They can improve prey visibility by increasing background contrast. The diffusion of light through the algae suspension can lead to more even distribution of larvae and prey, and less sticking of either to the surface or each other. Rotifers remain out in open water and away from the tank walls. Larvae tend to be 1less crowded and have fewer collisions. Greenwater has been good for rearing milkfish, Atlantic cod, European sea bass, gilthead sea bream, mullets, turbot, summer flounder, hirame, Atlantic halibut, common sole and others. Microalgae are rarely intentionally added beyond the early juvenile stage. A thin layer of microalgae or macroalgae (e.g. *Chaetomorpha* sp., *Enteromorpha* sp.) and nitrifying bacteria growing on the tank walls can be a relatively stable backup mechanism for removing some ammonia.

18.7.3 Brown water

Natural water not fully filtered (to allow bacteria in) is matured (microbially conditioned with age). The water is kept in rearing units for a while before it is used, allowing a shift away from opportunistic pathological bacteria towards more neutral or beneficial bacteria.

18.7.4 Food chain management

18.7.4.1 'Natural'

With the 'natural' food chain (extensive to semi-extensive) method, the culture unit is stocked with natural seawater filtered through 250–350 µm mesh to allow inoculation with natural prey (especially tintinnids, rotifers and copepods), but also to exclude predaceous and nuisance organisms as much as possible. This causes a more complete (endogenous) food chain, but at lower densities than the greenwater method. Sometimes *Chlorella* sp. or *Nannochloropsis oculata* is added (e.g. 15 000–20 000 cells/mL) or *Tetraselmis suecica* (e.g. 500–1000 cells/mL). The water usually is fertilised (e.g. 0.4–0.5 g/L nitrogen, 0.1–0.15 g/L phosphorus).

18.7.4.1.1 Greenwater

In greenwater (extensive to semi-extensive) culture, food is also mainly endogenous (Palmer *et al.*, 2007). Continuous ('static') blooms of phytoplankton and zooplankton are started early in the larval tanks, and (when successful) sustained by environmental conditions (e.g. sufficient light) and a balance between the populations of algae, prey and larvae (more complete food chain than intensive methods and more lasting than pseudo-greenwater method detailed below). Water is not renewed. Water is first highly filtered and sometimes chlorinated and dechlorinated. It usually is fertilised (e.g. 1–2 g N/m^3 and 1–2 g P/m^3). Microalgae at 50 000–100 000 cells/mm and rotifers at 0.1–0.2 rotifers/mm are added. The food chain and environmental quality can deteriorate during 15–25 days. Densities of fish larvae, their prey and algae must be kept in balance. With too few fish larvae, rotifers can increase, then microalgae decreases, rotifers starve and DO and pH decrease. With too many larvae, rotifers can decrease, then microalgae increase, DO increases near the surface but decreases towards the bottom of the culture unit, and larvae get hypoxia and sometimes gas bubble disease. Well-controlled low-density greenwater can be successful for sensitive species that need high water quality, but large amounts of algae can be dangerous. A range of at least 30 000–700 000 cells/mL has been used, but unless water quality safeguards are very good, an upper limit of 200 000 cells/mL might be safer. A sudden decrease in culture water transparency to less than 1 m because of phytoplankton is a danger signal.

18.7.4.1.2 Pseudo-greenwater

In pseudo-greenwater (intensive to semi-extensive) culture, which is intermediate between greenwater and clearwater, food is both exogenous and endogenous. Phytoplankton and zooplankton blooms are started and maintained but not relied upon completely. Daily addition of algae and zooplankton usually is necessary, especially after a few weeks when the zooplankters are being consumed faster. Microalgae need to be added (e.g. 100 000–200 000 cells/mL) one to five times a day. Rotifers are

added as needed. Water is renewed a little near the beginning and in greater proportions later (e.g. from 2 to 20% per day as larvae grow, then more for juveniles).

18.7.4.1.3 Clearwater

In clearwater (intensive to semi-extensive) culture, food is mainly exogenous. Zooplankton, but not phytoplankton, are supplied from separate cultures outside the larval tanks. There is high water exchange (as much as >10% per hour) and no intentional algal bloom (the least complete food chain for tanks). Larvae have to be fed often. This works best with fish that can eat *Artemia* early in their development (e.g. European sea bass, jacks, tunas). Gilthead sea bream larvae do not do well with the high water exchange.

18.7.5 Compound larval feeds

Larvae of most cultured marine fish will learn to eat compound feed and will thrive on it if they are weaned during transformation. Larvae must be able to ingest and digest microfeeds, then absorb, transport and utilise their nutrients. Even with slow-sinking, highly palatable and digestible larval feeds of the right size, larvae of many marine fish might still require live foods for the first few days or weeks. The initial research goal has been early weaning, and an ultimate goal for some species is the complete elimination of live food use. Although many early marine fish larvae have difficulty in accepting and digesting experimental microfeeds (section 9.7), some success has been had with older larvae. Besides the accomplishment of early weaning for many species, another benefit of this research has been earlier co-feeding of compound feeds with live foods, which can at least reduce the overall live food requirement. The main specialised commercial microfeed types are described in section 9.6.

Compound larval feeds will deteriorate quickly in water, especially at higher temperatures. Microcapsules usually last longer than microparticles because their contents are better protected. Soluble components like vitamins leach out, and lipids become rancid. Bacteria and fungi grow on the feed, possibly producing toxins. All feeds, but especially those for larvae, need to be stored in a freezer, if possible.

18.7.6 Weaning

With most cultured marine fish, weaning from live to dry food can begin a few days before transformation and be finished by the time the fish are juveniles. Feeding can be by hand or machine, but early weaning by hand helps to minimise food wastage and water fouling. Weaning can take from 3 days to 3 weeks and the transition must be gradual for slow learners to survive. Food size should be the largest that can easily be swallowed (25–50% of mouth width for soft particles and about 25% for hard ones). Weaning is often started by offering highly attractive 'appetiser' foods to the larvae before or along with dry feed. Examples are minced fish, molluscs, or crustaceans; frozen or freeze-dried *Artemia* or krill; freeze-dried shrimp, mussel or scallop meal. Recently, weaning has been initiated by co-feeding microfeeds with the live foods.

Weaning fish too early can result in lower production and higher cost per juvenile, but early weaning of some robust species has resulted in reasonable survival and growth. Late larvae that will become herbivores, omnivores or bottom fish (e.g. sea breams, mullets) seem less particular about food and can be slightly easier to wean than more raptorial fish like snooks, sea basses and groupers. Some species at some ages seem to need enough movement of the feed for it to look alive. If comparing sciaenids, bottom-feeders like red drum are easier to wean than more raptorial species like spotted seatrout. Very active fish like jacks and tunas can be difficult to wean.

18.7.7 Cannibalism

Cannibalism can occur in early juveniles of piscivorous species like common snook even when size differences are small. Cannibalism tends to decrease after weaning, but can increase during weaning. Early weaning reduces losses, but only if the food is acceptable. Cannibalism can become a problem if the fish are not very well fed and graded (if and when necessary). Good environmental conditions and availability of food when needed must be maintained. Increasing fish density can present cannibals with too many targets, making it harder to aim at one. Decreasing fish density can spread the targets out, making it harder to find one. Increasing turbulence can also make it harder to aim. Lowering the temperature when there is a long interval with less feeding can help reduce cannibalism and improve feed conversion. Kestemont *et al.* (2003) reviewed the biotic and abiotic factors influencing size heterogeneity, cannibalism and competition in cultured predator fish larvae.

18.7.8 Diverse fish capabilities

Various methods have been used for feeding larvae of different species. Efficacy and efficiency can be attained by balancing standardisation with flexibility. The culturist must observe larvae very closely (especially when they are feeding) to help determine needs and detect problems.

Larvae of marine fish have similar nutritional requirements. However, smaller larvae tend to need smaller prey with higher nutritional density for the first few days, and live food for a longer period, than larger larvae. In larvae that have small energy reserves, the line between well-fed and malnourished (i.e. dead) is often very fine. For many early- to mid-stage marine fish larvae, copepods are the most natural and most nutritionally reliable food: they usually contain large amounts of essential fatty acids. The author prefers to feed cultured copepods through most of the hatchery phase along with rotifers and brine shrimp. Unenriched rotifers (raised on high-quality algae) are used first, and feeding of enriched rotifers is delayed until the larvae can tolerate the resulting decrease in water quality. Unenriched brine shrimp nauplii are used for only one or two days and, thereafter, most of the *Artemia* are enriched metanauplii. Survival can be enhanced by making the transition from one live food to the next over a period of at least 4–5 days.

Length, swimming ability and mouth size at first feeding are important factors in larval survival. Although mouth size is not always proportional to length, most larvae that hatch at 1–2 mm are harder to feed and need the smallest rotifers or equivalent prey. Fish approximately 2–3 mm long usually first feed well on small to medium rotifers. Some longer than 3 mm can first feed on *Artemia* nauplii and others longer than 4 mm might not need any live food.

18.8 JUVENILE AND ADULT NUTRITION

18.8.1 Feed quality and cost

Fish shape, colour, flavour, texture, nutritional quality, safety and processing quality are affected by the way they are raised and what they are fed.

The cost of feed is usually the greatest variable cost during grow-out, fish meal prices have increased, and excessive use of fish meal is environmentally irresponsible (section 8.7. The nutritional requirements of cultured fish were reviewed by Webster and Lim (2002) and are described in Chapter 8. They vary among categories of fish (pelagic carnivores, demersal carnivores, demersal omnivores, demersal herbivores, planktivores) and are affected by age, size, growth rate, developmental stage, density, food quality, water quality, temperature, salinity, pH, photoperiod, stress and health. Ingredient availability and cost varies among regions, and will influence formula choice. Starter feeds are used during and just after weaning. Grower feeds are then used until market size is reached. Complete feeds are formulated to fulfil all the species' requirements and should be used when significant feeding on natural foods does not occur. Incomplete feeds do not satisfy all known requirements and are used to supplement natural foods (e.g. in ponds). Fish can be grown on cheaper feeds until just before harvest, then given better finishing feeds to boost their quality (e.g. EFAs, pigmentation). Sometimes this results in compensatory (faster than normal) growth; on the finishing feed, they catch up and reach the same size as if they ate it all the way through grow-out.

Feed conversion ratio (FCR) is the weight of feed (as fed or dried) needed to produce a unit weight of wet fish and is used as an index of how efficiently a fish grows. Multiplying the cost/kg of feed by FCR (as fed) gives the cost of feed to produce 1 kg of fish. Gross growth efficiency is the reciprocal of FCR except that it is calculated on a dry/dry basis (dry weight gained per unit dry weight of feed eaten) or sometimes wet/wet (e.g. for wet food). High-quality starter feeds can be more economical because the fish grow faster and the amount of starter consumed is a small part of the total feed. Daily ration is the dry weight of feed eaten per day divided by fish wet weight.

18.8.2 Macronutrients

Carnivorous marine fish need relatively high levels of dietary protein, and omnivores and herbivores need less. Protein requirement (percentage) decreases with increasing size or age. As growth rate slows, less protein is needed, and older fish seem capable of using more carbohydrate. Protein ingredients are usually more expensive; balancing protein, fat and carbohydrate contents to maximise utilisation of protein for growth instead of for metabolism and activity leads to protein sparing. Greater detail on protein nutrition of fish is given in section 8.3.2.

Optimal total fat (lipid) content (range about 7–29%) varies with natural feeding habits, age, activity level and temperature. With possible exceptions, sedentary fish might tolerate less dietary fat than more active ones. A minimum dietary *n*–3 HUFA content of 2%, including at least 1.0% EPA plus 1.0% DHA for young juvenile marine fish and 0.7% EPA plus 0.7% DHA for older fish, might be a little higher than necessary for all species but seems to be a reasonable safeguard, and some fish might benefit from more. Larvae might need 3–4% *n*–3 HUFA, including 1% EPA and 1–2% DHA. Active species need more fat and possibly more EFAs. Marine fish, invertebrate and algal oils are the best sources, but usually at least half the dietary fat can be from terrestrial plants and/or mammals. Many fish can tolerate half plant fat if at least 5% marine animal (or equivalent) oil is included. Up to a 60% (of total fat) balanced mixture of plant oils can be included for some fish. Linoleic and linolenic acids are used for

energy by many fish and are essential for at least some fish (0.5% of each for Japanese and European eels); moderate amounts should be included. See section 8.3.3 for more information on lipid nutrition of fish.

Carbohydrates are much less digestible than fat and protein for most fish and can add too much fibre to the feed. Practical limits for good-quality, relatively digestible carbohydrates are about 10% for young fish, and 10–20% for strict carnivores, 15–35% for omnivores and 30–50% for herbivores. Digestible (e.g. hydrolysed) starch is preferred. Good sources of carbohydrate include soybean meal, high gluten wheat flour, wheat middlings, whole wheat flour, Irish potatoes, cassava, sago palm, sorghum, rice, rice bran and corn meal. Crushing and steam cooking improve digestibility of dietary carbohydrate. More general coverage of carbohydrate nutrition in fish is given in section 8.3.6, and digestion and absorption of dietary carbohydrates in post-larval fish is described by Krogdahl *et al.* (2005).

18.8.3 Micronutrients

Fifteen vitamins have been shown to be essential in the diet of fish (section 8.3.4): thiamin (B_1), riboflavin (B_2), niacin (B_3), pantothenic acid (B_5), pyridoxine (B_6), folacin (B_9), cyanocobalamin (B_{12}), biotin (H), choline, inositol, ascorbic acid (C), retinol (A), cholecalciferol (D), tocopherol (E) and menadione (K). Para-aminobenzoic acid, lipoic acid, citrin and unidentified growth factors might act as vitamins. Para-aminobenzoic acid is not currently considered essential. It is best to add all essential vitamins. Careful vitamin over-fortification does not have to raise feed costs excessively, and safety margins often are added to compensate for processing and storage losses, to allow for increased requirements for different species and different ages, and to stimulate growth. Minerals should not be over fortified.

Besides the main four, carbon, hydrogen, nitrogen and oxygen, the elements calcium, phosphorus, magnesium, potassium, sodium, chlorine, sulphur, probably fluorine, molybdenum, and possibly silicon, boron and aluminium are essential for fish, but are usually available from the major feed ingredients and/or rearing water. The other essential minerals zinc, manganese, iodine and chromium are usually added, and sometimes iron, copper, cobalt and. With less animal content in the feed, more mineral supplementation might be required. Arsenic, nickel, tin and vanadium are reported to be necessary for some animals, but not fish. The essentiality of bromine, cadmium, lead, strontium and titanium has not been confirmed. Arsenic, cadmium and lead are toxic to fish. Too much iron, molybdenum, strontium, aluminium, tin, lithium, lead or cadmium can cause skeletal abnormalities. Fish can sometimes substitute an abundant related mineral for a deficient one. With practical feeds, most of the mineral requirement probably will be provided by other (especially animal) feed ingredients. When fish meal is reduced for marine fish raised in freshwater (e.g. European sea bass, barramundi, red drum), replacement of the missing sodium chloride might be necessary (up to 2–5% of feed).

Pigments are needed by some fish for full colour development, and they can have a beneficial effect on reproductive efficiency and other functions. Astaxanthin (e.g. 1% of feed) seems to be the best pigment commonly used. Some dietary algae (e.g. *Chlorella*, *Ascophyllum*, *Porphyra*, *Spirulina* and *Ulva*) are good both for carnivores and herbivores. Yeasts, β-glucans (from yeast cell walls), astaxanthin, alginate, nucleotides, certain synthetic peptides, betaine and deacylated chitin have been shown to enhance non-specific immunity.

18.8.4 Non-nutrients

Fibre is not needed by fish, but a small amount can help digestion a little by increasing gut residence time. Too much fibre reduces nutrient density and can obstruct enzyme activity or absorption. Omnivores and herbivores can tolerate more fibres (e.g. 8–20%) than carnivorous fish (e.g. 4%). The percentage of ash (non-organic component) depends mainly on bone or shell content of animal ingredients. Some can be tolerated, but ash reduces nutritional density and can interfere with absorption of zinc and other nutrients. Ash content should be low (e.g. 10–15% for most species, up to 25% for some), especially for small fish that need large rations. Moisture higher than 11% can lead to vitamin loss, fat rancidity, and growth of fungi and bacteria. The standard industry limit is 10% maximum moisture in ‘dry’ feeds.

18.8.5 Additives

Natural attractants for carnivorous fish decrease as the proportion of animal ingredients in feed is reduced, but they can be replaced. Digestive enzymes might be useful for carnivores eating feeds with high plant content. Growth-promoting substances might provide benefits in fish culture, but only if the environment and human consumers are not adversely affected. Binders (e.g. high-gluten wheat flour) stabilise a feed long enough for fish to eat it. Microbial spoilage can be limited by sanitary processing and storage, and addition of preservatives (e.g. propionates, ascorbates, sorbates, sodium benzoate, ascorbic acid, benzoic acid, sorbic acid and citric acid).

Antioxidants are necessary because oxidative rancidity of lipids, even at low levels, can reduce survival and growth of fish. The natural antioxidants tocopherol, selenium, carotenoids and sometimes ascorbic acid occur in feeds, but are usually supplemented with synthetic ones (e.g. ethoxyquin, BHA, BHT, sometimes nitrites or nitrates), which unfortunately can be toxic. Aerobic microorganisms and fat oxidation can be reduced by excluding oxygen (e.g. packing under nitrogen, carbon dioxide or partial vacuum). Sometimes water activity in semi-moist feeds is controlled by sucrose, sodium chloride, glycerol or propylene glycol. Natural preservatives and antioxidants are preferred if they work.

18.8.6 Nutritional problems

Pathological conditions often related to low food quality are exopthalmia, cataracts, fin or skin haemorrhage, fin erosion, fatty liver, scoliosis and lordosis. Starvation can occur when food is inadequate, access to food is limited or fish refuse to eat.

- Starving fish are often dark, have soft flesh, slender bodies, little abdominal fat, pale gills and sometimes more parasites than normal fish.
- Amino-acid deficiency can cause darkening, growth reduction, cataracts, dorsal fin erosion and spinal deformity.
- EFA deficiency often causes enlarged pale fatty livers, with anaemia and higher mortality.
- Carbohydrate excess can result in liver cell degeneration, high liver glycogen and high visceral fat.

Correct choice, preparation, storage and testing of ingredients and feeds will help avoid problems with anti-nutritional factors and toxins. Naturally occurring contaminants include the following:

- toxins from fungi, bacteria, algae, higher plants, or protozoans;
- anti-nutritional factors I (interfere with absorption of nutrients);
- products of rancidity.

Anti-nutritional factors in soybeans include indigestible carbohydrates, phytic acid, saponins, lectins, protease inhibitors, trypsin inhibitors, phyto-oestrogens, antivitamins and allergens. Canola can contain erucic acid, phytic acid, glucosinolates, protease inhibitors and tannins. Cottonseed can contain gossypol, cyclopropenoic acid, phytic acid, phyto-oestrogens and antivitamins. Lupins can contain indigestible carbohydrates, protease inhibitors, saponins, alkaloids and phyto-oestrogens. Amounts can vary with the plant strain and processing methods, and any amount remaining can set a maximum limit on use of that ingredient (Gatlin *et al.*, 2007).

Oxidation of lipids can increase free radicals, peroxides, hydroperoxides, aldehydes and ketones, which are toxic and can degrade proteins and vitamins. Lipoid liver degeneration can occur over time. Some signs are anorexia, cataracts, pale gills, clubbed gills, darkening, exophthalmia, haemorrhages, steatitis, muscular dystrophy, skeletal myopathy, lordosis and low growth efficiency and rate.

With a natural diet, wild salmon fillets and cod livers are normally fatty. Abnormally fatty fillets and livers commonly occur in reared fish because their feeds are very available, nutritious and digestible compared with natural foods, and they exert less effort to eat, so excess fat is stored wherever possible. One solution is to reduce dietary fat. Another is to use feed that is digested more slowly. If fattiness is not severe enough to affect fish health, fish quality or costs, nothing has to be done.

18.8.7 Feed formulation

Least-cost formulas are designed to satisfy minimum requirements with the most economical combination of available ingredients. Enough high quality protein, fat, EFAs, vitamins and minerals should be included. Carbohydrate, fibre, ash and moisture should be within limits. A moderate protein to energy balance is used so that fish eat enough to grow well but do not waste protein for energy. EFA requirements will be met if about 5% fish oil (or equivalent) is included. Omnivores and herbivores usually can be given less protein and fat and more carbohydrate and fibre than carnivores. By the late 1990s, the author eliminated fish meal from his grower feeds (successfully used for the carnivorous common snook, Nassau grouper, spotted seatrout and the omnivorous striped mullet) and reduced it to 10–20% in starter feeds. Current starter feed formulas contain 0–10%. For common snook over the range 70–700 g, growth (0.9–1.0%/day) and feed conversion ratio (0.9–1.1) with 0% fish meal were similar to or better than those with 10–60%.

The diet of broodfish can affect fecundity and fertility in the parents and egg buoyancy, development, hatching success, survival, growth, gas bladder inflation success and stress resistance in the offspring. Females' dietary nutrients during oogenesis affect egg quality more than their stored nutrients. Important factors in broodstock nutrition include fatty-acid composition (e.g. about 2–3% *n*–3 HUFA in feed, 2 : 1 DHA : EPA), lipid content, protein

quality and content, vitamin E (e.g. 250 mg/kg), vitamin C (e.g. 400 or 500 mg/kg), astaxanthin (e.g. 10–30 mg/kg), other pigments, also probably vitamin A, folacin, pyridoxin. Squid (e.g. 30%), cuttlefish (e.g. 30%) and mussel meals, tuna orbital oil, krill (oil or whole, but not too much, maximum probably 10% dry meal) and paprika (e.g. 2%) contain helpful nutrients or factors.

Starter feeds usually should contain about 50–60% high quality protein. As the fish grow, protein content can be reduced at a rate depending on the species. Equal amounts of DHA and EPA seem appropriate for grower feeds. Finishing feeds can be used to increase pigmentation and essential fatty acids.

The maximum allowable percentage of most fish meal substitutes (not squid, cuttlefish) from animal sources can be limited by nutrient imbalances or shortages in them (e.g. essential amino acids or fatty acids) and other factors. Common or specific usage rates are given below. The entire animal ingredient inclusion range is good for most cultured marine fish. Roughly the lower half of the plant ingredient inclusion range is good for carnivores, and the entire plant range is good for omnivores or herbivores. Some omnivores can tolerate even higher amounts. Some carnivores can tolerate the higher part of the plant range. Levels that are too high are likely to reduce digestibility.

Poultry by-product meal and meat with bone meal can be limited by high fat and ash (e.g. 15–25%). Hydrolysed feather meal usually is limited by digestibility (e.g. 1–10%). Shrimp (e.g. 10–25%), crab (e.g. 5–10%) and krill (e.g. 10%) meals usually are limited mostly by high ash. Other possible ingredients are spray-dried blood meal (e.g. 1–10%) and whey (e.g. 5–10%).

Some plant sources also can be limited by high fat, high ash and/or low digestibility, plus low protein, high carbohydrate, high fibre and/or anti-nutritional factors. Potential plant sources are as follows:

- wheat meal (e.g. 5–35%);
- wheat gluten (e.g. 5–15%);
- wheat middlings (e.g. 5–40%);
- soybean meal with low phytate, saponins, lectins, protease inhibitors and trypsin inhibitors (e.g. 10–30%, higher amounts require methionine supplementation);
- soybean protein concentrate;
- textured vegetable protein;
- corn gluten meal (usually 10–15%, can be up to 40%, but at high levels makes fillets yellow and lysine supplementation might be needed);
- corn meal (e.g. 5–35%);
- sunflower meal (e.g. 5–20%);
- sorghum meal (e.g. 10–30%);
- alcohol-washed, fibre-reduced canola meal with low erucic acid, glucosinolate, phytate (e.g. 20–40%);
- glandless or solvent-extracted cottonseed meal with low gossypol and possibly low cyclopropenoic acid (e.g. 10–25%);
- corn distillers grains with solubles (e.g. 5–15%);
- cassava meal (e.g. 15–35%) pea meal substituted for soybean meal (e.g. 10–30%) low-alkaloid lupin meal (e.g. 10–30%), yeast (e.g. 1–10%).

Methionine deficiency of soybean meal and lysine deficiency of corn meal can be mutually compensated if both of those meals are used together.

More information on formulation of feeds to satisfy the nutritional requirements of fish is given in section 8.5. The use of sustainable plant products in aquafeeds was revived by Gatlin *et al.* (2007).

18.8.8 Feeding methods

Most cultured marine fish are not surface feeders, and some are reluctant to eat floating pellets. Fish in the families Centropomidae, Percichthyidae, Serranidae, Carangidae, Lutjanidae, Sparidae, Sciaenidae and many others will eat and often prefer slow-sinking dry pellets. Training early (at weaning) is most effective for those that do not like hard pellets. Size, shape, texture or bad-tasting ingredients can reduce meal size. The best feed amount and frequency depend on species, size and conditions. Wild fish with stomachs generally eat between one meal every two days and five meals a day. Young fish, herbivores, species with a small or no stomach, and very active fish need frequent meals. After they reach about 100 mm in length, one good meal a day is probably enough for individual fish with large stomachs that normally eat large infrequent meals (e.g. eels, barramundi, snook, groupers, plaice) and others that normally eat smaller, more frequent meals (e.g. cod, whitemouth croaker). However, two meals a day would give all fish a better chance of filling up. Many fish seem to eat best and possibly convert food energy best when they are fed late in the day.

Feeding can be by the following methods:

- manual (hand feeding);
- human-operated mechanical;
- mechanical on a timer;
- mechanical on demand.

These feeding methods are described in detail in section 9.11.2. Many kinds of fish can be trained to use demand

feeders (section 9.11.2) once they are large enough (e.g. temperate basses, sea breams, drums). Demand feeders can reduce wastage, stress and size heterogeneity, and increase growth rate. Electronically activated demand feeders can be used by smaller fish. Feeding mechanisms include vibrators, conveyer belts, augers, rotating compartments and compressed air. For large-cage farming, feed sometimes is moved by water or air from a central storage bin through pipes or hoses to the cages. Systems that release feed only while surface swimming continues or until a wastage limit is reached have been developed. Wide, even feed distribution will allow smaller or less-aggressive fish to eat better. Feeds must not be exposed to heat, oxygen or moisture long enough for vitamin loss, rancidity or fungal growth to occur.

Fish have been known to consume a daily ration from 1 to 50% of body weight, depending on species, size and conditions. Ideally, fish can be observed and all are fed to satiation, but that is not possible on a large scale. Feeding tables constructed from previous ration, growth and conversion data for different sizes of fish at different temperatures can be used by the culturist to try for maximum growth, minimum FCR or a compromise (section 9.10.1, Table 9.12). Appetites will vary from day to day, often because of factors not considered in making the predictions. With automatic feeders, overfeeding and underfeeding will occur occasionally; but, with practice, wastage can be minimised.

18.9 HEALTH

It is best to prevent disease by practicing good sanitation methods such as the following:

- stocking specific-pathogen-free juveniles,
- using vaccines if available (Chapter 19)
- not using raw trash fish for food;
- raising fish in a clean beneficial environment.

Refinement of health management techniques that lead to stable, predictable survival and growth will be an important step towards sustained, routine farming of marine fish in all suitable regions.

18.9.1 Disease types

See also section 10.5.3.

18.9.1.1 Neoplasia

A neoplasia is an excessive, uncoordinated production of cells after the irritating stimulus has gone and can result in tumours or leukaemia, which are rare in fish.

18.9.1.2 Viruses

Viruses can cause systemic infections, ulcers, haemorrhages, nerve disorders, necrosis of internal organs, hyperplasias, tumours and high mortality. They can be present in healthy fish and not cause disease until the fish are stressed too much. Many viruses will affect young fish and not the adults.

18.9.1.3 Bacteria and fungi

Possible signs of bacteria or fungi include systemic infections; lesions, erosion, haemorrhages, and ulcers of skin on sides of the body and fins and around the eyes and mouth, and of internal organs; swollen eyes, cloudy eyes, blindness; haemorrhages of eyes, mouth, gill cavities; anorexia, increased ventilation rate, lethargy, erratic swimming, anaemia, emaciation, abdominal oedema and distension, and mortality. External bacterial infections have been treated with freshwater, formalin or iodine (100 mL/L for 1 h) baths.

18.9.1.4 Protozoans

Protozoan parasites include flagellates (blood, gills, skin), coccidians (internal organs), cryptosporidians (digestive system), haemogregarines (blood), microsporidians (intracellular), myxosporidians (muscles, internal organs) and ciliates (gills, skin, eyes). External protozoans have been controlled by baths of freshwater, chloroquine diphosphate, formalin or copper salts (copper is dangerous in salt water).

18.9.1.5 'Worms'

Parasitic monogenean flatworms attach to and feed on skin and gills and have been controlled by baths of freshwater, hydrogen peroxide (e.g. 150 mL/L solution for 30 min), praziquantel, mebendazole, formalin (e.g. 30 mL/L solution for up to 24 h) or trichlorfon. Digenean trematodes damage internal organs, gills and sometimes eyes. Cestode (tapeworm) larvae migrate through internal organs, and adults infest the digestive system. Nematode larvae migrate through internal organs and muscles, and adults usually live in the digestive tract. Acanthocephalans (spiny-headed worms) encyst in visceral organs of intermediate hosts, and adults live in the intestines of final hosts.

18.9.1.6 Crustaceans

Among crustaceans, branchiurans are ectoparasites that feed on skin and subdermal tissues. Some copepods crawl around feeding on the skin or gills, whereas others firmly attach and feed on deep tissues (Fig. 18.12). Copepods

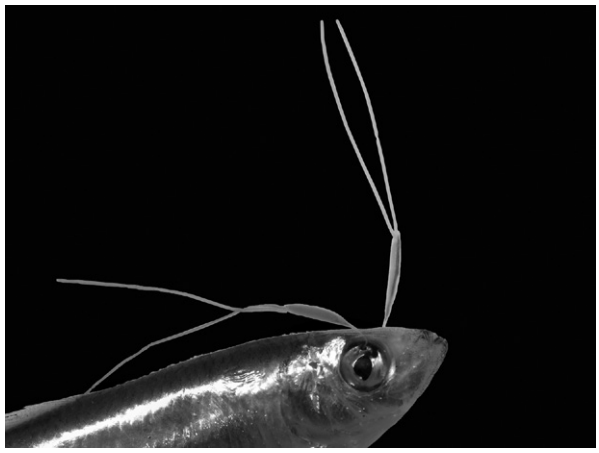


Fig. 18.12 Parasitic copepods (*Lernaeenicus sprattae*) bedded into the left eye of a sprat. (Photograph by Hans Hillewaert.)

have been treated with hydrogen peroxide (e.g. 150 mL/L for 30 min), freshwater (e.g. 30 min) or formalin (e.g. 100 mL/L for 2–3 min, 30 mL/L for up to 24 h) dips or baths. Ectoparasitic isopods feed on gills, skin and the inside of the mouth, and have been treated with formalin baths or removed with forceps.

18.9.1.7 Other ectoparasites

Leeches feed on blood, and lampreys rasp off the flesh. A 50 mL/L formalin bath for up to 1 h has been used to control leeches.

Other health problems can be genetically or environmentally induced (bad conditions, sudden pressure changes, red tides, pollution, irritants, sunburn). Developmental abnormalities can occur. Malnutrition, allergies and drug overdoses can cause sickness.

18.9.2 Stress

Healthy fish can often resist pathogens, unless stress becomes too high. If possible, raising more resistant species or strains would help. Diseases often result from system deficiencies or operator errors. Stress can decrease immunity, growth rate and growth efficiency. It can upset hormone balances and interfere with reproduction. Crowding can reduce growth through processes like aggressive behaviour, energy budget alteration, reduced food intake and hormone level changes. Enough vitamins should be included in the feed to compensate for stress depletion.

18.9.3 Prevention guidelines

The following precautions can go a long way towards preventing diseases in fish culture.

18.9.3.1 Isolation

The facility should be designed to prevent spread of pathogens by air, water, people, fish, foods and any other likely route (even insects and birds, if possible), and so that all parts can be cleaned and disinfected as easily as possible; then it should be cleaned and disinfected routinely.

Every reasonable thing must be done to be sure that broodstock, seedstock, feed, water and air are not contaminated, either from outside sources or from different parts of the facility. Live foods and fish from outside sources should be quarantined (isolated) until they are checked for diseases of concern. If possible, different batches of fish are not mixed. Separate nets, buckets and similar equipment are assigned to each tank or set of tanks that are connected or close enough to become cross-contaminated by splashed or sprayed water. This equipment is not used for other tanks or sets of tanks. All shared equipment is thoroughly disinfected before and after use. Different personnel are assigned to different areas of the facility and their movement is restricted to those areas where they work (e.g. workers do not go from the grow-out area to the hatchery unless they are completely washed and disinfected, and not on the same day, if possible). Hand and foot disinfection are used for entering and exiting sensitive areas. Visitors are managed so they do not transfer pathogens.

Indoor culture units should be drained, scrubbed, disinfected, rinsed very well and dried before the next use. Treatment systems and pipes should be disinfected regularly. Separate modules make this easier. Drying and liming of ponds is a standard method for minimising disease organisms remaining in the sediment (section 21.4.2). Whenever possible, all fish and food culture units should have a dry period between crops.

18.9.3.2 Requirements

The culturist must be aware of the needs of the fish. They should be raised under as close to optimal, constant conditions as possible, especially water temperature, salinity, light, water quality (DO, ammonia, nitrite) and nutrition. Systems to promote beneficial microbiota, and confirmed probiotic organisms can be added. Foam fractionation removes irritating substances and some pathogens, but also desirable things like live foods and maybe probiotic bacteria. The fish should be given the best possible foods

(within economic feasibility), on the best known schedule, with confirmed immunostimulants when appropriate.

Overcrowding, disturbances, excessive handling, toxicants, chemicals and drugs are to be avoided. Multiple disturbances are especially stressful and must be followed by sufficient recovery time before the next disturbance. Handling must be minimised, especially for sensitive stages. Anaesthetics should be used when necessary. Those used for fish include isoeugenol, tricaine methanesulphonate (20–150 mg/L), 2–phenoxyethanol (50–200 mL/L), quinaldine, benzocaine (e.g. 40 mL/L), metomidate and clove oil (e.g. 10–40 mL/L). Choice depends mainly on government approval, reliability and preference (e.g. for human safety, possible residues). Concentration and time for treatment depend mainly on species, age and temperature. Both anaesthesia and recovery should occur within a few minutes each.

18.9.3.3 Records

Thorough records of fish history (e.g. feeding, growth, behaviour, stresses) and water quality should be kept. If possible, larval food cultures (phytoplankton, zooplankton) and the fish should be monitored to ensure balanced beneficial microbiota. Fish must be monitored for signs of disease and, if possible, water samples and fish tested for likely or suspected diseases. Awareness of the fishes' normal behaviour (especially at feeding time) and responses to stress is needed. An observant and experienced fish culturist often will notice the early warning signs of a potential health problem (e.g. unusual behaviour, darkening, blanching, scraping against the tank, not eating, sores) and interrupt the process before it escalates.

18.9.3.4 Response

There must be preparation so that it is possible to isolate and treat the fish for any diseases they are likely to get. Emergencies require quick reaction. Sick fish should be isolated as much as possible. Current published treatment methods and government regulations must be consulted before treating fish. Many drugs still used in certain regions are dangerous to the environment, the fish and/or the consumer. If treatment with a drug is determined to be necessary, the one used must be appropriate and safe for the situation and officially approved as appropriate and safe by agencies of any government involved in culture or sale of the fish. The best drug should be chosen (e.g. by testing bacterial sensitivity to the drug in a culture). The approved treatment method and withdrawal time must be used, and no drug should be used that can cause harm by contaminating the environment or human food. Disrupting bacteria of the biofilters should be avoided. Reduce crowding and stop feeding a day before treatment if possible, maintain high DO and avoid temperature stress.

18.9.4 Disinfection

18.9.4.1 Water and equipment

High water quality is necessary, and pathogens (especially bacteria and protozoans) will build up when fish are crowded or water is dirty. Water for fish and zooplankton can be disinfected with UV radiation (section 2.7.1) following filtration. If the UV bulb is submerged, its quartz sleeve must be kept clean. There are reports that devices that do not submerge the bulb can be much (e.g. 1000 times) better. Bulbs must be replaced before they lose effectiveness. Ozonation is very effective, but risky. Ozonating or chlorinating culture water can be unsafe, unless bromine-free artificial seawater is used and oxidants are dissipated. If both ozone and UV are used, the sequence can be clarification, ozone, UV, because ozone helps clarify and UV helps remove ozone. Algal culture water can be chlorinated, but with a small risk of chlorinated hydrocarbons passing through the food chain to the fish. Autoclaving of water for algae is preferred for small quantities; UV can be good for larger amounts, but chlorination is the most practical and effective method. Even fish culture water is sometimes chlorinated (e.g. 10 mL/L for 24 h or 200 mL/L for 0.5–1 h); the concentration goal is 1–3 mL/L after chemical reactions have occurred; ca. 1.4 g sodium thiosulphate neutralises 1 L of 200 mL/L chlorine.

Tanks and everything used to handle fish should be disinfected between batches. Commonly used disinfectants include the following:

- isopropyl or ethyl alcohol, an iodophor, or quaternary ammonium compound for hands and feet;
- chlorine, an iodophor, potassium permanganate, chlorhexidine diacetate, 50% benzalkonium chloride, or other quaternary ammonium compounds for equipment, tanks and cages;
- calcium oxide, calcium hydroxide, or sodium hydroxide for ponds;
- hot water alone or with a detergent such as Alconox® just for cleaning small components.

18.9.4.2 Eggs

Eggs are contaminated with bacteria from the parents and the water. Surface disinfection of eggs is only a temporary measure, because exposure to pathogenic bacteria can

occur later from the water, air, foods, people, new environments, or other sources. Eggs have been disinfected with hydrogen peroxide (3% for 5 min), bronopol, ozone (0.4 mL/L for 2 min), acriflavine (e.g. 5 mL/L for 1 min), glutaraldehyde (400–800 mL/L for 2.5–10 min) or formalin, but tolerance varies with species.

18.9.4.3 Food

Pathogenic bacteria often occur on wild and cultured plankton. The gut flora of fish larvae are established non-selectively before first feeding. In feeding larvae, infections are usually caused by contaminated food (especially mollusc larvae, rotifers and copepods, and sometimes *Artemia*). Antibiotics are sometimes used to reduce bacteria in food cultures, but the combination of sanitation, probiotic inoculations and improved environmental management is a better strategy. Rotifer and *Artemia* cysts can be disinfected with chlorine before hatching, but must then be rinsed very well. Standard pasteurisation (63 °C for 30 min) can be used to eliminate essentially all pathogens in fresh foods. High-temperature pasteurisation (72 °C for 15 s) eliminates essentially all pathogens and reduces total bacteria.

18.9.5 Microbial management

18.9.5.1 Microbial environment

Good management of the microbial environment can protect larvae from pathogens and eliminate the need for antibiotics and other drugs. Larvae of most species have weak immune systems or none. Without management, the bacteria populations can vary from bad to good. Bacteria are found on the skin and in the gut, but not the muscle or other organs, of healthy fish.

Prebiotic substances are indigestible, and probiotic substances are digestible. Probiotic organisms combat pathogens with inhibitory substances and by competition for space, competition for nutrients (e.g. iron) or immunostimulation. Especially when combined with good sanitation, probiotic bacteria can help reduce and possibly eliminate the need for drug treatment of fish in tank culture systems. They can help fish internally by stabilising the gut microflora and pH, excluding pathogens, improving digestion and absorption, and providing beneficial compounds (e.g. enzymes, vitamins, other growth promoters). They also occur in the water and can improve and stabilise the bacterial ecology of the rearing systems. Maintaining normal bacteria in plankton cultures is good for the fish larvae directly, and for live food health and production, especially when zooplankton are given dry feeds instead of algae.

Probiotic bacteria normally found with the cultured fish species in its natural environment can be easier to raise and maintain, and can be more effective than bacteria from other sources (e.g. terrestrial). Bacteria selected from a specific environment have a far greater capacity to produce appropriate anti-pathogen and pro-host substances and to compete for ecological prevalence in that environment than bacteria from a very different source. They might also not need special treatment or have to be re-inoculated frequently. Fish and bacteria that naturally live together are more likely to have similar environmental requirements.

Especially when fish are raised for cage grows-out or stock enhancement, it is good for larvae not to be naive but to have some exposure to pathogens, so they can develop some resistance.

18.9.5.2 Immunostimulants

Immunostimulants are compounds or mechanisms that enhance non-specific or specific immunity, and the two types of action overlap. Immunostimulants include nucleotides, synthetic peptides, polysaccharides (glucans, peptidoglycans, lipopolysaccharides), lactoferrin, astaxanthin, alginates, betaine, chitin, chitosan, aluminium salts, levamisole, Bacille Calmette Guerin, *Corynebacterium parvum*, killed mycobacteria, bacterial cell wall extracts (LPS antigen), bacterial, fungal, yeast and plant extracts, most of the essential minerals and vitamins (e.g. C, B_6, E, A) and most of the essential amino acids (arginine, glutamine, L-tryptophan, histidine, leucine, isoleucine, methionine, lysine). The term ‘adjuvant’ usually applies to an immunostimulant that is mixed and injected with a vaccine to stimulate the immune response to the vaccine. Pesticides, some antibiotics and other toxins often will depress the immune system.

18.9.5.3 Vaccines

Vaccines cause the immune system to produce antibodies as if the actual disease were present (Chapter 19). Vaccinations are given by injection, hypersaline immersion (20-s dip), immersion, bath (30 min to hours), spray and ingestion (in decreasing order of effectiveness because of the resulting type of immunity). Hypersalinity increases uptake, but can be stressful. Normal immersion is less stressful and can result in sufficient uptake. Oral vaccines have been improved recently by coating the antigen (e.g. as microspheres) to protect it from digestion. Once perfected, they can induce better mucosal immunity than other methods and be less stressful and cheaper. Some vaccines are available for vibriosis, photobacteriosis

(pasteurellosis), furunculosis, flexibacteriosis, pseudomonadiasis and streptococcosis. Vaccines against nervous necrosis virus and *Cryptocaryon irritans* seem possible. A very detailed account of the development, modes of action and use of vaccines with fish is given in Chapter 19).

18.9.5.4 Drugs

Because of possible damage to the fish's immune system, the culture system (e.g. nitrifying bacteria) or outside non-target organisms, chemical control of fish diseases has to be a last resort. Because some compounds are banned for use in farmed fish and the approved lists are revised as necessary, a current list from the appropriate government agency must be consulted before using drugs for fish. In the USA, aquaculture drugs are on three lists, which change as new treatments are developed and approved:

- approved;
- unapproved/low priority;
- investigational.

The drugs used must be effective, not make the disease worse, not harm the fish or other non-target organisms, not leave long-term residues in food fish, and be stable under normal storage and use. The drug must not be used if suspected to cause resistant strain development. Drugs used for treatment of humans must be avoided if a chance of inducing resistant strains of human pathogens exists.

Drugs that are dangerous to the health of aquaculturists, consumers, or the environment include the following:

- chloramphenicol;
- nitrofurans (e.g. nitrofurazone, furacin, furanace, furazolidone, furoxone);
- malachite green;
- gentian violet;
- certain steroid hormones.

These must never be used on fish to be eaten by humans, and if considered necessary for non-food fish, must be used cautiously and only under direct veterinary guidance. In 2007, the United States Food and Drug Administration tested imported seafood for chloramphenicol, nitrofurans, fluoroquinolones, malachite green, oxolinic acid/flumequine, ivermectin and oxytetracycline (limited use).

There are six main treatment methods: fish are held in a net and briefly immersed in the treatment solution (dip); the drug is added to water flowing through the fish container (flush); fish are held in a constant concentration of the drug for a specified period (bath); the drug is added to the food, but only if fish are eating (oral administration); the drug is applied directly to the lesion on valuable fish (topical application); the drug is given by intramuscular, intraperitoneal, or (rarely) subcutaneous injection to valuable fish.

18.9.5.5 Transport precautions

Fish must be protected from infections caused by handling, and diseases must not be spread. Procedures vary with species and expected dangers. Fish to be transported more than a short distance should have a health inspection completed before departure and/or just after arrival. They should be quarantined at the destination to a degree and duration consistent with the risks. Current veterinary guidelines must be followed.

18.9.5.6 Quarantine

If fish from different stocks are to be mixed (especially if adding wild fish to captive populations, which is not recommended, except when broodfish need replenishment), the new fish should be held separately and observed for 1–4 weeks. Extra amounts of vitamins A, C and E plus 5 g/kg beta glucan can be included in the diet, for immunostimulation. Tests for suspected pathogens should be conducted. If chemical or drug treatments are chosen, they must be among those approved by the appropriate regulatory agency.

18.10 FAMILY ACCOUNTS

The following section is an account of the major marine and diadromous fish that are used in or have been studied for aquaculture. Some of the information presented below is summarised in Table 18.1 (p. 385) for some of the major culture species.

A significant omission in the account below is the family Salmonidae, which is treated in detail in Chapter 15. The annual production of salmonids is huge compared with all other families (Fig. 18.13). It is notable that country production of marine fish is dominated by countries that are major producers of Atlantic salmon: Norway, Chile, Canada and the UK (Fig. 18.14a). Considering country production of both marine and diadromous fishes, however, the order of the first three countries does not change, but then Egypt (based on tilapia and mullet production) and some south-east Asian countries become important (Fig. 18.14b). It is surprising in these data that China is not shown as producing diadromous fish.

Maximum sizes that have been reported are given in parentheses after the scientific names. In a few cases, ages and sizes given for beginning of the juvenile stage can be

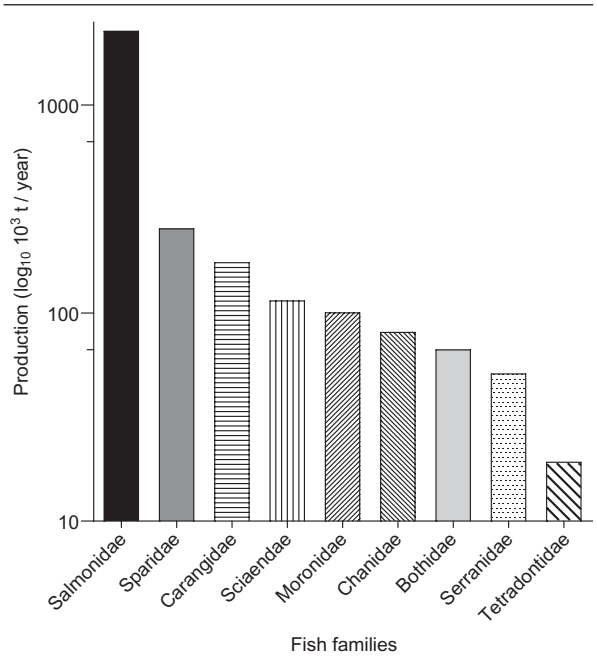


Fig. 18.13 Global production of marine fish in 2007 by family. Note that the vertical axis is exponential and that the Salmonidae are magnitudes greater than other families. Note also that annual production of the family Cyprinidae (carps, etc.), which inhabit freshwaters, and are not included, is approximately another magnitude greater than the Salmonidae. (Figure by John S. Lucas.)

slightly low because the measured fish had not fully transformed into juveniles (with scales) yet.

18.10.1 Anguillidae: 'freshwater' eels

18.10.1.1 European eel

European eels (*Anguilla anguilla*, 137 cm, 9 kg) are raised mainly in the Netherlands, Denmark, Italy, Spain, Greece, Germany, Sweden and China; market size is 100–600 g. Global production was 9183 and 8366 t in 2006 and 2007, respectively[2].

[2]Production data for 2006 and 2007 are provided to give an assessment of the status of the industry based on the species. Some are essentially at an experimental stage, others are major industries of great economic importance. Where no data are provided, there are no records of production in FAO FishStat Plus (2009), i.e. production values were less than 1 t in those years.

18.10.1.2 Japanese eel

Japanese eels (*Anguilla japonica*, 150 cm) are raised mainly in China, Taiwan, Japan, Republic of Korea and Malaysia; market size is 150 g to several kilograms. Global production was 229 656 and 265 354 t in 2006 and 2007, respectively.

18.10.1.3 Shortfinned eel

Shortfinned eels (*Anguilla australis*, 130 cm, 7.5 kg) are raised mainly in Australia. Global production was 8 and 0 t in 2006 and 2007, respectively.

Freshwater eels spend most of their lives in freshwater, but when mature they migrate to offshore ocean waters to spawn. Early eel larvae (leptocephali) drift in the oceanic plankton, become glass eels that reach the coast, then elvers as they move upstream into freshwater. That can take about 10 months for American eels and 22 months or more for European eels. Before metamorphosis, wild freshwater eels eat zooplankton, during metamorphosis they might not eat, and as elvers migrating up rivers, they successively eat detritus, zooplankton, larger crustaceans, aquatic insects, small fish and other animals.

Freshwater eels are raised from wild glass eels and elvers caught as they migrate inland from the sea. Japanese eels are caught mainly during October–May in Japan and October–March in Taiwan. European eels are caught mainly during November–April in Portugal and Spain and December–July (mostly February–May) in France and the UK. Both Japanese and European wild eels first are kept in quarantine tanks until they are weaned juveniles at about 5 g. Then they often are grown out in a series of ponds or tanks, increasing in size as the fish grow. Both species are graded every 5–7 weeks.

Japanese eels (20–22 °C), European eels (20–23 °C) and shortfinned eels (24 °C) have been induced to ovulate with various hormones. The resulting European eel eggs are 1.0–1.4 mm diameter. Routine spawning and larval rearing are not yet possible. Some larvae have been reared to juveniles, but the difficulty of spawning eels, the long larval period and uncertain early nutritional needs make sustained hatchery production a long-term goal.

Japanese eels (29 °C) hatch from planktonic eggs 1.3[3] mm diameter in 2.0 days at 2.9 mm total length (TL), reach end of yolksac (EYS) 6–7 days after hatching (DAH), first

[3]In the rest of this chapter, most single numbers and some ranges are approximations.

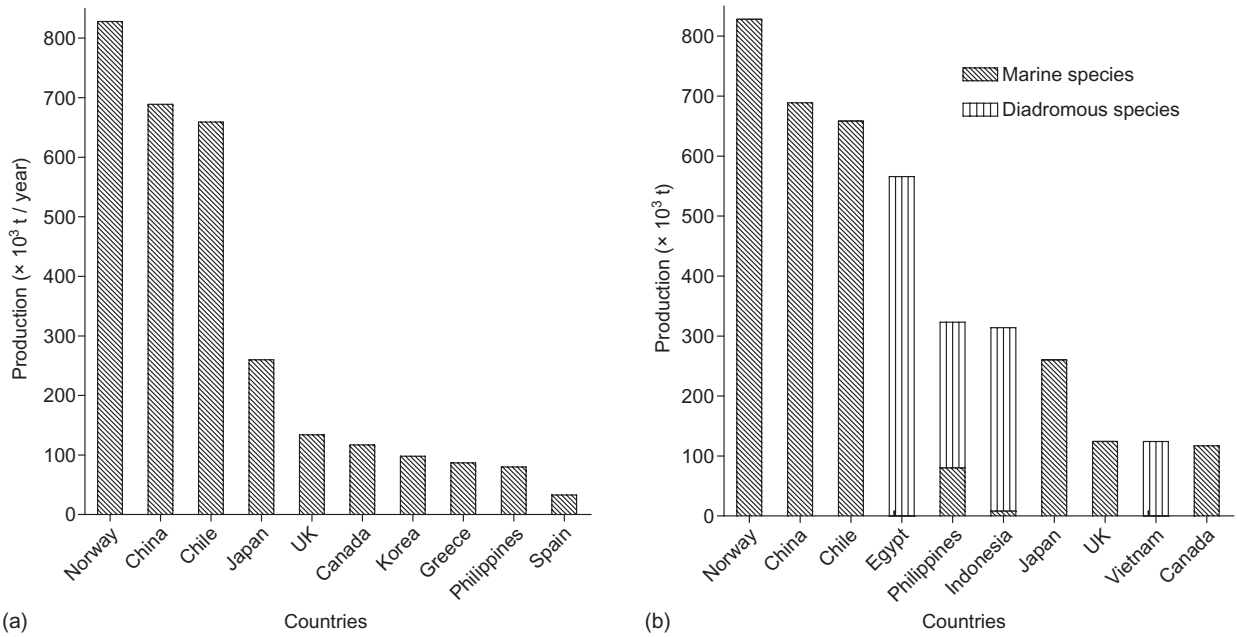


Fig. 18.14 Production of marine and diadromous fish in 2007 by the top ten countries. (a) Marine fish; (b) marine and diadromous fish. (Figure by John S. Lucas.)

feed by 7–13 DAH and transform[4] 95–210 DAH at 51–69 mm TL. Optimal temperatures are about 24–26 °C for eggs and 26–28 °C for yolksac larvae. Many attempts to raise leptocephali have ended in starvation. Leptocephali will eat solid food like squid paste. Elvers do not require live food and can be given minced bivalves, fish or worms, and compound feeds. Elvers must be fed well to minimise cannibalism.

Juvenile Japanese eels grow well at 23–30 °C and European eels at 22–26 °C. When feeds are used, mainly moist paste is fed to glass eels and extruded moist or dry pellets to older fish. Grower feeds can contain 50–60% crude protein, 11% fat, 16% carbohydrate until 20 g, then 40–50% crude protein, 15–25% fat, 25% carbohydrate. To minimise fouling of water by waste, compound feed can be given to eels in a pond on a perforated tray, and fresh food hung from a wire. During grow-out, one meal per day should be enough for European eels at 15 °C and four meals per day at 30 °C.

[4]Develop into a juvenile, which has the major adult characteristics (including bones, fin rays, teeth and scales).

Often in Japan, the early stages are reared in a series of nursery ponds (up to 1 ha) inside greenhouses, and the final stages are raised in either greenhouse ponds or outdoor ponds (sometimes concrete tanks, up to 300 m^2). Pond walls have to be steep enough to prevent elvers from climbing out. Starter foods include tubifex worms, ground beef liver, fish or clams, paste feeds and dry feeds. Grower foods include paste or pelleted feeds. Good survival is 70–90% in the nursery and 90–95% during grow-out. Growth rate varies, with 10–20% of the eels reaching 200 g in 6 months and the rest in 12–18 months. They can be stocked in ponds at 600 g/m^2 (thirty 20-g elvers) and harvested at 6 kg/m^2.

Early eel culture in Europe was by vallicoltura, blocking of coastal lagoons to trap wild larvae of different fish. Recently in Europe, eels have been raised in tanks, ponds (up to 1500 m^2), and raceways as large as 500 m^2. Cod roe, cow spleen, paste feeds and dry feeds are used. They can grow from 0.3 to 150 g in 18–21 months; density in recirculating tanks can increase from 50 kg/m^3 at stocking to 250–300 kg/m^3 at harvest.

Eels are susceptible to viruses, Gram-negative bacteria, fungi, internal protozoans, nematodes, and ectoparasitic ciliates, monogeneans, branchiurans and copepods.

18.10.2 Ophichthidae: snake eels

18.10.2.1 Rice paddy eel

Rice paddy eels (*Pisodonophis boro*, 100 mm) are raised mainly in Singapore. Global production was 35 and 35 t in 2006 and 2007, respectively.

18.10.3 Clupeidae – Herrings

18.10.3.1 Pacific herring

Pacific herring (*Clupea pallasii pallasii*, 46 cm) culture is established in Japan. They have voluntarily spawned in tanks at 5–10 °C. They hatch from sinking adhesive eggs of 1.45 mm diameter at 6.8 mm TL and reach EYS 7 DAH.

18.10.3.2 Atlantic herring

Atlantic herring (*Clupea harengus*, 50 cm, 1.05 kg) (8 °C) hatch from sinking adhesive eggs of 1.2 mm diameter in 15 d at 7 mm TL, first feed 4 DAH, reach EYS 6 DAH and transform 112–168 DAH at 30–40 mm TL. Larvae have been reared on wild zooplankton (7–12 °C).

18.10.3.3 Japanese sardine

Japanese sardines (*Sardinops sagax melanostictus*, 45 cm) (20 °C) hatch from planktonic eggs of 1.62 mm diameter at 3.0 mm TL, first feed and reach EYS 3 DAH and transform 50 DAH at 40 mm TL. Larvae have been reared on rotifers and *Artemia* in greenwater (20 °C), then fish eggs and larvae and compound feed.

Herrings are susceptible to fungi.

18.10.4 Chanidae: milkfish

18.10.4.1 Milkfish

Milkfish (*Chanos chanos*, 2.0 m, 20 kg) are raised mainly in the Philippines, Indonesia, Taiwan, Singapore and Pacific islands. Market size is mainly 250–500 g. Global production was 585 577 and 667 508 t in 2006 and 2007, respectively.

They are very popular in Asia, but grown ones are hard to catch. At 2–3 weeks after hatching (about 10 mm) wild milkfish move into swamps, lagoons, etc., where they can be caught for farming. They sometimes are polycultured with shrimp or mullet.

Milkfish have voluntarily spawned in ponds, cages and large tanks at 25–33 °C (but not always predictably) and can be induced to ovulate. At 29 °C they hatch from planktonic eggs of 1.1–1.3 mm diameter in 29 h at 3.5–3.7 mm TL, first feed (5.4 mm TL, mouth width 258 µm), and reach EYS 3–5 DAH and transform 21 DAH at 24 mm TL. Larvae are reared in ponds on mixed zooplankton or in tanks on rotifers (until 14 DAH) and *Artemia* (by 12 DAH) in greenwater. Trochophores sometimes are used as an early food and enriched *Artemia*, copepods, cladocerans and/or microfeeds can increase survival and growth. Hatchery production can be as high as 10/L or more at 21–30 DAH.

Juveniles grow well at 26–30 °C, but 23–33 °C is acceptable (tolerated range 15–40 °C). They can survive 1 mg/L DO but need at least 3 mg/L to feed well. They usually grown out in ponds or outdoor tanks, where they feed on benthic algal mats (lab-lab), but cages and pens are used, too. Ponds can be shallow with lab-lab as the primary juvenile food or deeper with plankton as the primary food. Supplemental feeds are also used and nutritional requirements are not high. Lab-lab is a combination of algae, diatoms, bacteria and small benthic animals from pond bottoms, on which milkfish at moderate density can live. Lab-lab can be dried and made with a binder (e.g. 4% wheat flour) into pellets containing about 11% protein, 1% fat, 19% carbohydrate. It also is incorporated into commercial pellets (e.g. with 30% protein, 8% fat, 40% carbohydrate) for fish at higher densities. Common supplemental foods are peanut, soybean, copra, rice bran and dried algae. Supplemental feeds with fish meal or soybean meal as the protein source can contain 45% crude protein, 7–10% fat, 29% carbohydrate for small juveniles, then 25–30% crude protein, 7–10% fat, 45% carbohydrate near market size. In fully stocked ponds, a complete ration can be given at >28 °C, three-quarter ration at 25–28 °C and half ration at 21–24 °C, twice a day when natural food is abundant and three times a day when it is not. Broodstock in cages or tanks need two meals a day.

Typical stocking densities are 2000–2500/ha at 10–100 g for shallow ponds (1–2 m), but deep ponds (2–3 m) have been stocked up to 200 000/ha. Sometimes, a series of progressively larger ponds with a mixture of different size groups in each is used. Between crops, the ponds are dried, refilled and fertilised. Pens have been stocked up to 35 000/ha and cages up to 30/m^3. Bait size (80–120 g) can be reached in 4–5 months and food size (200–500 g) in 8–12 months. Two or three crops per year can be produced from the same set of ponds, with annual production of 2000 kg/ha. Possible problems include the high costs of maintaining broodstock and spawning oysters, plus high pond temperatures and heavy rains during the hatchery season. Milkfish generally are resistant to disease, but possible pathogens include Gram-negative bacteria, internal proto-

zoans, cestodes, nematodes, and ectoparasitic protozoans, branchiurans, copepods and isopods.

18.10.5 Osmeridae: smelts

18.10.5.1 Ayu

Ayu (*Plecoglossus altivelis*, 70 cm) are raised mainly in Japan, Taiwan and the Republic of Korea. Global production was 7022 and 6371 t in 2006 and 2007, respectively.

The life cycle is about 1 year long. Wild juveniles (20–90 mm) are harvested for grow-out. They also have voluntarily spawned in tanks at 18–20 °C and can be induced to ovulate. Larvae have been reared in ponds or tanks up to 30–60 m^3. Ayu (15 °C) hatch from sinking adhesive eggs of 0.9–1.0 mm diameter in 20 days at 6–7 mm TL, first feed and reach EYS 4–5 DAH and transform in 110 days at 50–70 mm TL. Larvae can be reared on rotifers, *Artemia* and microfeeds (as early as 7 DAH), with high survival. The best grow-out temperature is 20–25 °C (range 13–30 °C). They have been grown out in outdoor and greenhouse concrete ponds. Ayu can reach at least 75–90 g in 10–11 months (maximum 200 g). From 100–300 juveniles/m^2 stocked, 10–30 kg/m^2 can be harvested. Ayu pathogens include Gram-negative bacteria, Gram-positive bacteria and internal protozoans.

18.10.6 Gadidae: cods

18.10.6.1 Atlantic cod

Atlantic cod (*Gadus morhua*, more than 2 m, 100 kg) are raised mainly in Norway and Iceland. Global production was 13 228 and 12 172 t in 2006 and 2007, respectively.

They have voluntarily spawned in tanks, cages, pens and bags at 1–10 °C. With a winter–spring photoperiod and 6–9 °C, they will spawn during any month. Atlantic cod (6 °C) hatch from planktonic eggs of 1.3–1.5 mm diameter in 14–15 days at 4.5 mm TL, first feed 4–5 DAH, reach EYS 6–9 DAH and transform at ≤106 DAH at ≤65 mm TL (with an adult type of digestive system, not just external adult features). At higher temperature, development is faster and they can transform at 50–80 DAH. Larvae have been reared in large tanks, plastic bag enclosures, or impoundments on wild zooplankton (tintinnids, copepods, cladocerans) and rotifers, usually in greenwater. *Artemia* sometimes are used, mainly as a supplement. They also have been reared on rotifers (until ≥22 DAH) and *Artemia*. Cannibalism among mid-stage larvae to early juveniles can limit the possible rearing density, and weaning can be difficult. Brown *et al.* (2003) described larviculture of this species, and Rosenlund and Halldórsson (2007) discuss the production of juveniles.

Although there is some variation among geographic stocks, eggs and yolksac larvae will survive at about 4–10 °C but do best at 6–7 °C. Older larvae and early juveniles can tolerate and grow well up to 15 °C, but 10–12 °C is best for them. A good sequence is 14 °C (12–15 °C) for 50-g cod, decreasing to 6 °C for 5000-g cod. Continuous lighting at 1200–2400 lux is good for larvae, but 300 lux for 18 h/day is better for juveniles. Atlantic cod (at 4–200 g) can be fed 50–60% crude protein, 15–20% fat and up to 15% carbohydrate; then (at 200–900 g) 50–60% crude protein, 10–20% fat and up to 15% carbohydrate. Cod are adapted to eating crustaceans and can be given feed with 15–20% fish bone or crab shell meal. As much as 20% soy protein concentrate and 20% wheat gluten can be in feed for fish weighing 100–500 g, and 50% soy protein concentrate and wheat germ combined for fish at 1600–3200 g. It takes 6–7 months to produce juveniles of 30–100 g for stocking in cages. Density can be up to 40 kg/m^3 in grow-out cages. They can reach 3 kg in 2–3 years. Atlantic cod are susceptible to viruses, Gram-negative bacteria, internal protozoans, cestodes, nematodes and ectoparasitic copepods. Juveniles sometimes are vaccinated against vibriosis.

18.10.6.2 Haddock

Haddock (*Melanogrammus aeglefinus*, 112 cm, 17 kg) are raised mainly in Iceland. Global production was 46 and 0 t in 2006 and 2007, respectively.

They (7 °C) hatch from planktonic eggs of 1.4 mm diameter in 12 d at 3.5 mm TL and reach EYS 6–7 DAH.

18.10.6.3 Pacific cod

Pacific cod (*Gadus macrocephalus*, 119 cm, 23 kg) have been raised in Japan and the Republic of Korea. They have voluntarily spawned in tanks at 6–8 °C. They hatch from sinking adhesive eggs of 1.0–1.3 mm diameter in 10 days at 3.6 mm TL and reach EYS 3–4 DAH.

18.10.7 Atherinidae: silversides

Miscellaneous silversides (Atherinidae) are raised mainly in the Ukraine. Global production was 25 and 6 t in 2006 and 2007, respectively.

18.10.7.1 Japanese silverside

Japanese silversides (*Atherion elymnus*, 5 cm) have voluntarily spawned in tanks. They (21 °C) hatch from sinking adhesive eggs of 0.95–1.0 mm diameter in 13–17 d at 4.4 mm TL, reach EYS 2–3 DAH and transform at 20 mm

TL. Silversides can first feed on *Artemia* soon after hatching, but sometimes rotifers are used.

18.10.8 Atherinopsidae: neotropical silversides

18.10.8.1 Pejerrey

Pejerreys (*Odontesthes bonariensis*, 50 cm) have been raised mainly in Peru.

18.10.8.2 Inland silversides

Inland silversides (*Menidia beryllina*, 15 cm) have voluntarily spawned in tanks.

18.10.8.3 Atlantic silversides

Atlantic silversides (*Menidia menidia*, 15 cm) (25 °C) hatch from sinking adhesive eggs of 1.1 mm diameter in 6–10 days at 5.2 mm TL.

Neotropical silversides can first feed on *Artemia* soon after hatching, but sometimes rotifers are used.

18.10.9 Scorpaenidae – Scorpionfishes

Miscellaneous scorpionfishes (Scorpaenidae) have been raised, mainly in the Republic of Korea.

18.10.9.1 Kurosoi

Kurosoi (*Sebastes schlegeli*) culture is established in Japan and the Republic of Korea. Global production was 27 517 and 36 564 t in 2006 and 2007, respectively.

They are ovoviviparous and have voluntarily reproduced in tanks at 12–17 °C. Larvae have been reared on rotifers, *Artemia*, minced sand lance, copepods, mysids and fish eggs in greenwater. Best temperature for juvenile growth is 15–18 °C. An early grower feed can contain 50% crude protein, 11% fat. They can reach 1 kg within 2 years. Kurosoi are susceptible to viruses, Gram-negative bacteria, Gram-positive bacteria and ectoparasitic flagellates.

18.10.9.2 Kasago

Kasagos (*Sebasticus marmoratus*) have voluntarily reproduced in a cage at 13–14 °C. Larvae have been reared at 13–14 °C in greenwater.

18.10.10 Synanceiidae: stonefishes

18.10.10.1 Devil stinger

Devil stinger (*Inimicus japonicus*, 29 cm, 480 g) culture is established in Japan.

18.10.11 Latidae: lates perches

18.10.11.1 Barramundi

Barramundi (*Lates calcarifer*, 200 cm, 60 kg or more) are raised mainly in Thailand, Taiwan, Malaysia, Indonesia, Australia, Singapore and the Philippines. Market size often is in the range 250–4000 g, and it is economical to raise them to 3 kg or more. Global production was 31 707 and 37 611 t in 2006 and 2007, respectively.

Barramundi have voluntarily spawned (any month) in tanks and cages at 28–30 °C (range 26–34 °C) and can be induced to ovulate. One voluntary method includes lowering the water level to simulate a falling tide at noon (31–32 °C) followed by raising the water to simulate a rising tide (27–28 °C). Hormone treatment (GnRHa) seems more necessary as distance from the equator increases, but hormone-induced tank spawning is routine.

Barramundi (27–28 °C) hatch from planktonic eggs of 0.75–0.85 mm diameter in 17 h at 1.4–1.7 mm TL, first feed 2–3 DAH at 2.6 mm TL (mouth width 224 µm), reach EYS 4–5 DAH and transform 25 DAH at 17–25 mm TL. Larvae have been reared in tanks with cultured or wild zooplankton. In tanks, they are fed rotifers (at least during 2–15 DAH) and *Artemia* (beginning 10–12 DAH), but copepods, cladocerans and enriched *Artemia* will increase survival and growth. Weaning to conventional dry crumbles can be accomplished by 26 DAH and to microfeeds several days earlier. Larvae also have been reared in fertilised ponds. In the nursery phase, they usually are weaned and raised in tanks, ponds, or cages from 20 to 75–100 mm, the minimum size for beginning grow-out. Early, complete weaning is important for minimising cannibalism, which occurs mostly between 18 mm (2–3 weeks) and 120–150 mm, especially if fish are at high density and not fed well enough. Frequent grading (at ≤7- to 10-day intervals) might be needed.

Best temperatures are about 27–28 °C for larvae (26–30 °C acceptable, range 25–31 °C, maybe to 35 °C) and about 27–30 °C for juveniles (26–32 °C acceptable; tolerance range 21–39 °C; 12–16 °C and 43 °C can be lethal. Salinity can be reduced to zero by 15 DAH. A starter feed can contain 50–55% crude protein, 16% fat, 15% carbohydrate, and a grower feed 45–50% crude protein, 14–19% fat, ≤20% carbohydrate. For barramundi 20 g or larger, half of the fish oil in feed can be replaced by soy, canola or linseed oil. Lupin meal or solvent-extracted soybean meal can be included up to 30%.

Barramundi are mostly grown out in cages in natural waters, ponds, impoundments or reservoirs, but mainly recirculating tanks are used in southern Australia. They easily reach 500–600 g in 1 year, but at high temperatures, 800 g is possible in 1 year and 3 kg in 18–24 months. Barramundi stocked in a freshwater reservoir grew from 5 g to 5.2 kg during 2–20 months after hatching, and others reached 10 kg at 3 years. Pond harvest density can be

2200–2800 kg/ha for 500 g fish. In freshwater, minimum cage density is 15 kg/m^3, maximum 40–65 kg/m^3 and optimum 25–30 kg/m^3. Up to 80–100 kg/m^3 has been reported for very intensive freshwater tanks. Barramundi are susceptible to viruses, Gram-negative bacteria, Gram-positive bacteria, fungi, epizootic ulcerative syndrome, internal protozoans, and ectoparasitic flagellates, ciliates, monogeneans and crustaceans.

18.10.11.2 Japanese lates (snook)

Japanese lates (*Lates japonicus*, 130 cm, 87 kg) wild juveniles that were fed raw fish and shrimp at 13–27 °C in a 20-m^3 concrete tank grew from 147 to 346 g in 288 days, then from 358 to 767 g in 211 days. They ate well at >25 °C but not at all at <16 °C.

18.10.12 Lateolabracidae: Asian seaperches

18.10.12.1 Japanese seaperch (seabass)

Japanese seaperch (*Lateolabrax japonicus*, 102 cm, 87 kg) have been raised mainly in Japan, China, the Republic of Korea and Taiwan, and market size is 600 g. They live in saltwater and freshwater and have been raised from wild juveniles and eggs. They have voluntarily spawned in tanks and can be induced to ovulate. They (20 °C) hatch from planktonic eggs of 1.4 mm diameter in 3 days at 3.9 mm TL, reach EYS 3–5 DAH and transform 60 DAH at 43 mm TL. Larvae can be reared on rotifers and copepods or rotifers and *Artemia* in greenwater; cladocerans also are used. A good temperature range for juveniles is 20–25 °C. An early grow-out feed can contain 45% crude protein, 13–14% fat. They can reach 600 g in 11–13 months.

Global production was 223 249 and 260 422 t in 2006 and 2007, respectively.

18.10.13 Centropomidae: snooks

18.10.13.1 Common snook

Common snook (*Centropomus undecimalis*, 140 cm, at least 24 kg) have been raised mainly in the Dominican Republic (from wild juveniles), and miscellaneous snooks (*Centropomus* spp.) in Brazil. Induced spawning of newly caught or captive wild snook is routine; fish usually are stripped. They have tank spawned only after hormone treatment, and egg quality varies. Although not well known in most of the USA, they are popular from Florida southwards, and related species are becoming better known to consumers internationally (barramundi and Nile perch). Wild juvenile common and fat snook have been grown out, mainly on trash fish in earthen ponds, and sold locally in Brazil and Venezuela. Food quality is similar to that of barramundi.

Common snook (28 °C) hatch from planktonic eggs of 0.7–0.75 mm diameter in 17 h at 1.5 mm TL, first feed 2.5 DAH, reach EYS 4–5 DAH and transform 35 DAH at 35–40 mm TL. Larvae can be reared on rotifers (until 20–28 DAH), copepods (3–30 DAH) and *Artemia* (by 12–20 DAH), but gorging on *Artemia* can cause mortality. Cannibalism is worse in early juveniles than in late larvae and is increased by higher fish density and food shortages.

Spawning in nature occurs mainly at 25–31 °C, but maybe as low as 22–23 °C. Optimal range for larvae is about 26–27 °C, but 25–29 °C is good. The optimum for juvenile growth is about 28 °C (range 26–30 °C). Lethal limits are 10–14 °C (depends on strain and rate of decrease) and 35 °C or higher. Snook can survive transfer to freshwater 15 days after hatching, and juvenile growth is at least as fast as in salt water. They can survive very low water quality (e.g. DO down to 0.9–1.0 mg/L, high turbidity). Snook are gregarious and tolerate crowding (Fig. 18.15). They have been grown out on experimental feeds in tanks and ponds to 3 kg and can reach 2 kg at 2 years. A starter feed can contain 50–55% crude protein, 13–15% fat, 15% carbohydrate, and a grower feed 45–50% crude protein, 10–13% fat, 20% carbohydrate.

18.10.14 Percichthyidae – Temperate Perches

18.10.14.1 Chinese perch (Mandarin fish)

Chinese perch (*Siniperca chuatsi*, 70 cm, 10 kg) are raised in China. They are euryhaline, but live mostly in freshwater. They spawn in nature mostly at 26–30 °C and are



Fig. 18.15 Three-month-old juvenile common snook. Reproduced from Tucker and Jory (1991), with permission of *World Aquaculture Magazine*.

induced to ovulate for farming. They (27 °C) hatch from sinking non-adhesive eggs of 1.2–1.4 mm diameter in 1 days at 4 mm TL and first feed 2–2.5 DAH. Larvae usually are reared in tanks, and their food is mainly other live fish larvae. Grow-out is in ponds, cages and pens, often in brackish water. They can grow to 500 g within 1 year and 1–1.5 kg in 2 years. Pond harvest density can be up to 7500 kg/ha. They are susceptible to Gram-negative bacteria, Gram-positive bacteria and ectoparasitic ciliates.

Global production was 176 727 and 211 713 t in 2006 and 2007, respectively.

18.10.14.2 Australian bass

Australian bass (*Macquaria novemaculeata*, 60 cm) are catadromous and have been induced to ovulate. They (20 °C) hatch from planktonic eggs of 1.1 mm diameter at 3.1 mm TL, first feed 5–7 DAH, reach EYS 8–9 DAH and transform 56–70 DAH at 20–25 mm TL. Larvae can be reared in tanks on rotifers (until 19 DAH) and *Artemia* (by 14 DAH) or in ponds on mixed plankton. Eggs and larvae are best at 16–20 °C. In ponds, they can reach at least 345 mm at 3 years.

18.10.15 Moronidae: temperate basses

18.10.15.1 European sea bass

European sea bass (*Dicentrarchus labrax*, 103 cm, 12 kg) have been raised mainly in Greece, Turkey, Italy, Spain, Egypt, France, Croatia, Portugal, Morocco, Tunisia, Cyprus, Israel, Malta and Libya. Typical market size is 350–400 g. They can be conditioned to spawn any month by controlling daylength; broodfish are kept at 8–17 °C, but spawning usually occurs at 13–15 °C. Ovulation usually is induced for tank spawning (e.g. two intraperitoneal injections of 5 and 10 µg GnRHa/kg 4–6 h apart).

Global production was 59 653 and 62 764 t in 2006 and 2007, respectively.

They (17–18 °C) hatch from planktonic eggs of 1.0–1.4 mm diameter in 2.2 d at 3.0 mm TL, first feed 4–5 DAH, reach EYS 5 DAH and transform 40–45 DAH at 20 mm TL. Hatchlings can be stocked at 100/L or more. They can be fed rotifers (until 15 DAH) and *Artemia* (by 11 DAH). At 15 °C and 13 or 26‰ salinity, first feeding can be delayed until 9 DAH without decreasing growth or survival. That allows larvae to be reared with only *Artemia*, which have to be enriched as soon as possible. Partial substitution of microfeeds for *Artemia* is possible before 20 DAH, and weaning to microfeeds can be accomplished during 20–23 DAH. Feeding of conventional dry feed has occurred as early as 36–45 DAH, but sometimes is delayed until 60–80 DAH. Because size variation can encourage cannibalism, juveniles are usually graded when necessary.

Good temperatures are 16–20 °C for larvae and 22–27 °C (tolerance limits about 2–32 °C) for juveniles. Grow-out feeds can contain 50–55% crude protein for small fish and 45–50% crude protein for larger ones, with 15% fat (range 12–18%). Up to 60% of dietary fish oil can be replaced by soybean or linseed oil. Carbohydrate should be less than 30%. At 20 °C, three meals per day are good for 60-g European sea bass. They can grow from egg to 350–400 g within 15 months and have been grown out in cages, ponds, tanks and impoundments. Semi-intensive lagoon production can be up to 700 kg/ha/year. Grow-out density can be up to 20–35 kg/m^3 in tanks and 20 kg/m^3 in cages. European sea bass are susceptible to viruses, Gram-negative bacteria, Gram-positive bacteria, internal protozoans, nematodes, and ectoparasitic dinoflagellates, ciliates, monogeneans, copepods and isopods.

18.10.15.2 Striped bass

Striped bass (*Morone saxatilis*, 200 cm, 57 kg) have been raised mainly in the USA, Israel and Italy. Market size mostly is in the range 450–1400 g. They are anadromous, have voluntarily spawned in tanks at 16–20 °C, and can be induced to ovulate. Striped bass (20 °C) hatch from sinking non-adhesive eggs of 3.6 mm diameter in 1.5–2.0 days at 3 mm TL, first feed 4–5 DAH, reach EYS 7–14 DAH and transform 37 DAH at 30 mm TL.

Global production was 3 t in 2006 and 2007, respectively.

Hybrid striped bass usually are produced from strip-spawned eggs because voluntary spawning of the (different-sized) parent species is not always reliable. Larvae of both striped bass and palmetto bass (*Morone saxatilis* × *M. chrysops*) can eat *Artemia* at first feeding and do not need rotifers, but palmetto bass are not raised as often as sunshine bass because striped bass females are more difficult to obtain than white bass females.

18.10.15.3 Sunshine bass

Hybrids (*Morone chrysops* × *M. saxatilis*) (18 °C) hatch from sinking adhesive eggs of 0.9–1.0 mm diameter in 1.8 days, first feed by 6 DAH and transform at 30 DAH. Sunshine bass need rotifer-sized food until 9–12 DAH, and can eat *Artemia* by 6–8 DAH. Larvae usually are reared in ponds. Supplemental feeding might be needed by 21 DAH, and cannibalism also can begin at that time.

Global production was 5884 and 5524 t in 2006 and 2007, respectively.

Larvae of striped bass do well at 18 °C and sunshine bass at 23–25 °C. Juveniles of these fish can tolerate at least 13–32 °C, but 24–25 °C (range 22–26 °C) is good for striped bass and 25–27 °C for hybrids; hybrids can grow faster at up to 31 °C, but FCR gets worse. Juvenile striped bass grow well in fresh or salt water, and hybrids grow best at low to intermediate salinity. Freshwater ponds need at least 100 mg/L hardness (possibly up to 250), 100 mg/L alkalinity, and 0.15–0.5 mg/L iron. DO should be at least 5–6 mL/L. A grower feed can contain 40% crude protein, 10% fat, 20% carbohydrate.

Standard culture of striped bass and its hybrids can occur in three phases: (1) 5-day-old larvae stocked at up to 500 000/ha grow to 25–60 mm in 30–60 days (25–80% survival); (2) phase I fish stocked at 25 000/ha grow to 75–250 mm in 5–9 months (80% survival); (3) phase II fish stocked at 1000–4000/ha grow to market size in about 9 months (90–100% survival). Doubling the densities for phases II and III will slow growth but can increase yield. Pond size usually ranges from 0.25–1 ha for phase I to 0.5–4 ha (minimum 1.8 m depth) for Phase III. Tanks and cages also have been used for grow-out. Striped bass can reach at least 600 g in 12 months, 1 kg in 18 months, 2.6 kg in 3 years, and 6.5 kg in 5 years. Sunshine bass can reach 1300 g at 19 months (Fig. 18.16). Striped bass are susceptible to viruses, Gram-negative bacteria, Gram-positive bacteria, fungi, digenean trematodes, cestodes, nematodes, and ectoparasitic flagellates, ciliates, crustaceans and leeches.

18.10.16 Serranidae: groupers, sea basses

Several species of grouper are farmed in cages, ponds and tanks in the Indo-Pacific and Middle Eastern regions, but they mostly are raised from wild juveniles (75–100 mm) and are fed trash fish. Typical market sizes are 400–700 g in the Philippines, 600–900 g in Singapore, and >1 kg in Kuwait. In Hong Kong, live groupers are sold at 0.5–2 kg. The usual minimum market size for groupers in the USA is 2 kg, but smaller fish can be sold in specialised markets, especially in large cities.



Fig. 18.16 Sunshine bass. They were raised by the author to 1.3 kg (average) at 19 months.

Groupers have voluntarily spawned in fibreglass, concrete or plastic tanks (1–21 200 m^3), concrete ponds (1.8–860 m^3), and cages (26–75 m^3). Larvae have been reared mainly in fibreglass or concrete tanks holding 1–100 m^3 and sometimes in concrete ponds of up to 500 m^3 or earthen ponds up to 1 ha; a minimum of 3.5 m^3 is good but 7 m^3 or larger is better. Tanks and cages up to at least 75 m^3 and larger ponds have been used for grow-out (Tucker, 2005).

Voluntary spawning has occurred mostly under ambient temperature and partial or total natural light during the natural spawning season. Daylength seems to be less important than temperature. Some species spawn near certain moon phases and others any day of the lunar month. Sometimes, groupers can be caught just before spawning and held in tanks or cages for up to a few days until they ovulate naturally. The eggs are stripped, or rarely, if running ripe males are present, the fish are left in the tank for voluntary or accidental fertilisation to occur. Many serranids are protogynous hermaphrodites.

Hormone-induced ovulation of groupers and sea basses generally is reliable. Typically, a female with fully yolked oocytes (developing eggs) will ovulate within 24–72 h (usually 36–50 h) after the first of one to three injections of 500–1000 IU HCG per kilogram body weight. Similar results have been obtained with one to three injections of 10–50 µg GnRHa per kilogram, and GnRHa implants have also worked.

Larvae of most grouper species are small and fragile and have small mouths at first feeding. Known egg diameters are in the range 0.7–1.2 mm, and total length of hatchlings 1.3–2.3 mm. Yolk and oil tend to be exhausted quickly (range 2–5.5 days). The larval period of most species is long (often about 50 days, range 35–70 days), and groupers tend to require live food longer than most cultured marine fish. Although eggs or larvae have been stocked up to 40/L or more, 5–20/L is safer.

Tanks with diameters of at least 3–5 m are better for grouper larvae than smaller ones because surface film removal is safer, temperature and water quality changes are moderated, and the fish contact the tank walls less often. The best survival rates usually occur in larger tanks or ponds (60–500 m^3) under partial sunlight. Early grouper larvae, especially when stressed, sometimes exude a large amount of mucus, which can cause them to stick to each other, to the surface film or to solid objects. Larvae of some species seem to be especially sensitive to noises,

such as bumping of their tanks, which induces rapid, frantic swimming.

Most first-feeding groupers can eat rotifers, but species with small mouths need very small rotifers, trochophores (oyster, clam or mussel larvae), copepods, or other zooplankton. Grouper larvae are usually reared in greenwater, mostly with the phytoplankton *Nannochloropsis*, *Tetraselmis* and/or *Chlorella* spp. stocked at 10–500 cells per microlitre during 0–3 DAH to 12–40 DAH. They tend to prefer copepods to rotifers, and growth and survival can be improved with copepods or mixed zooplankton. Certain ciliates (with widths of 20–80 µm) can be added to improve survival during the first few days of feeding, but some are not nutritious. Cladocerans sometimes are given to larvae. *Artemia* enriched with essential fatty acids can be a main food beginning at 10–30 DAH, but their abundance should be limited to minimise gorging and incomplete digestion. It is best to delay feeding of *Artemia* (e.g. until 20 DAH or later) if other foods are sufficient. Mysid shrimp sometimes are used for late larvae and early juveniles. Microfeeds have been used as a supplement during the first week of feeding but probably are not digested much until at least 2–4 weeks. Weaning from live food to dry crumbles or small pellets can be completed just before or during transformation. Minced seafood (e.g. fish, shrimp and scallop) is often used as an appetiser or transitional food. Cannibalism among early juveniles is a potential problem, and can be minimised by feeding the fish well, weaning them as soon as possible, and grading, sometimes as often as every 5–7 days.

When groupers are too small to stock in grow-out ponds or cages, they are raised in concrete or fibreglass tanks, ponds, or small cages called hapas inside ponds or larger cages, usually from 20–30 mm to 50–100 mm in 30–45 days. In grow-out cages, groupers are first stocked at up to 60 fish/m^3 (<1 kg/m^3), but sometimes as high as 100/m^3. Intensive pond farms have stocked juveniles at 60 000–80 000/ha and harvested 80% of the fish, for a production density as high as 30 000–40 000 kg/ha (usually 10 000–30 000 kg/ha).

In south-east Asia and other areas, groupers have been fed mainly trash fish supplemented with vitamins and minerals, secondarily moist or semi-moist pellets and, rarely, high-protein dry pellets. Nursery foods have included live or minced fish, mysid shrimp and larger zooplankton (e.g. adult *Artemia*, mysid shrimp, mosquito larvae), and then soft–moist pellets. They first are fed four to six times a day, decreasing to twice a day by the time the fish are transferred at about 75 cm TL. A suitable starter feed for groupers can contain 50–60% crude protein, 12–16% fat, ≤15% carbohydrate, <3% fibre and <16% ash. By the time they reach 500 g, groupers can be given a feed with ca. 45% crude protein, ca. 9% fat, ≤20% carbohydrate, ≤4% fibre and ≤22% ash. Lower quality feeds are likely to result in worse feed conversion and possibly slower growth. During grow-out in south-east Asia, a daily ration of 10% chopped fish or 3% pellets can be fed in two meals until fish weigh 200 g, then a daily ration of 5% fish or 2% pellets in one meal. When the fish reach 200 g, they can be fed once a day in summer and every other day in winter.

Groupers are susceptible to viruses, Gram-negative bacteria, Gram-positive bacteria, internal protozoans, digenean trematodes, cestodes, nematodes, and ectoparasitic dinoflagellates, ciliates, monogeneans, acanthocephalans, copepods and leeches. Nervous suffering disease (signs can include gill, blood, gas bladder, liver, heart, brain and nerve damage) probably is caused by rancid dietary fats. Miscellaneous groupers (*Epinephelus* spp.) have been raised mainly in China, Taiwan, Indonesia, Thailand and the Republic of Korea. Miscellaneous groupers (Serranidae) have been raised mainly in the Philippines.

In addition to the species listed below, FAO aquaculture statistics show that the bulk of global production is reported as unidentified groupers (*Epinephelus* species). This was 57 763 and 69 604 t in 2006 and 2007, respectively. Most of this production was in south-eastern Asia.

18.10.16.1 Redspotted (Hong Kong) grouper

Redspotted groupers (*Epinephelus akaara*, 53 cm, 2.5 kg) have been raised mainly in Hong Kong and Japan. They have voluntarily spawned in tanks and ponds at 20–29 °C and can be induced to ovulate. They (24 °C) hatch from planktonic eggs of 0.73–0.83 mm diameter in 24 h at 1.6 mm TL, first feed 2–4 DAH, reach EYS 4 DAH and transform 45–50 DAH at 30 mm TL. Larvae can be reared on rotifers and *Artemia* in greenwater. They can be weaned by the time they transform. Cannibalism was reported to be the main cause of mortality at 40 DAH.

18.10.16.2 Orangespotted (estuary) grouper

Orangespotted groupers (*Epinephelus coioides*, 120 cm, 15 kg) have been raised mainly in Taiwan, Malaysia, Thailand, Singapore, the Philippines and Brunei Darussalam. They have voluntarily spawned (any month) in tanks and cages at 23–32 °C and can be induced to ovulate. They (27 °C) hatch from planktonic eggs of 0.8–0.85 mm diameter in 22 h at 1.7 mm TL, first feed 3 DAH (mouth gape 180 µm), reach EYS 4 DAH and transform 35–40 DAH at 25–30 mm TL. They can be reared on rotifers (<90 µm is best to start) and *Artemia* in greenwater, but

copepods can increase survival. Orangespotted groupers can grow from egg to 600 g in 1 year or from 60 mm TL to 400–800 g after 8–10 months in cages or 10–14 months in ponds.

Global production was 92 and 87 t in 2006 and 2007, respectively.

18.10.16.3 Brownmarbled grouper

Brownmarbled groupers (*Epinephelus fuscoguttatus*, 120 cm, 11 kg) have been raised mainly in Taiwan, Indonesia, Malaysia and Singapore. They have voluntarily spawned (any month) in tanks, cages or ponds at 28–32 °C and can be induced to ovulate. They (28 °) hatch from planktonic eggs of 0.75–0.9 mm diameter in 18 h at 1.3 mm TL, first feed 2.5–4 DAH at 2.8 mm TL (mouth width 225 µm), reach EYS 4–5 DAH and transform 35–40 DAH at 31 mm TL. Larvae can be reared on rotifers (until 14–20 DAH) and *Artemia* (by 10–12 DAH) in greenwater (28–30 °C is good). When fed trash fish in cages, they can grow from 30 to 600–800 g in 8–10 months.

18.10.16.4 Malabar grouper

Malabar groupers (*Epinephelus malabaricus*, 234 cm, 150 kg) have been raised mainly in Taiwan, Thailand, the Philippines and Singapore. They have voluntarily spawned in tanks and ponds at about 30 °C and can be induced to ovulate. They (29 °C) hatch from planktonic eggs of 0.8–0.85 mm diameter in 19 h at 2.3 mm TL, first feed and reach EYS 3 DAH and transform 36–60 DAH at 35 mm TL. Larvae can be reared on rotifers (until 20–30 DAH) and *Artemia* (by 15 DAH) in greenwater, with copepods (by 10 DAH) and maybe cladocerans (by 30 DAH) very helpful. They can grow from egg to 600 g in about 1 year, with cage grow-out. A pond harvest density of 1.4 kg/m^2 for 1.25-kg fish was reported.

Global production was 84 and 57 t in 2006 and 2007, respectively.

18.10.16.5 Camouflage grouper

Camouflage groupers (*Epinephelus polyphekadion*, 109 cm) have voluntarily spawned in tanks and ponds at 25–30 °C and can be induced to ovulate. They hatch from planktonic eggs of 0.75–0.85 mm diameter in 21 h at 1.7 mm TL and transform 40 DAH at 36 mm TL. Larvae can be reared on rotifers and *Artemia* (28–29 °C is good). They can grow from 60 to 600 g within a year.

18.10.16.6 Squaretail (areolate) grouper

Squaretail groupers (*Epinephelus areolatus*, 47 cm, 1.4 kg) have been raised mainly in Hong Kong.

Global production was 41 and 52 t in 2006 and 2007, respectively.

18.10.16.7 Greasy grouper

Greasy groupers (*Epinephelus tauvina*, 107 cm) have been raised mainly in Malaysia, Hong Kong, Singapore and Taiwan. They have voluntarily spawned in tanks, but mostly are induced to ovulate. Because wild males are not caught often, it is not easy to establish captive wild broodstock, and hormone-induced sex reversal of females has been practised. They (27 °C) hatch from planktonic eggs of 0.9 mm diameter in 1.0 day at 1.7 mm TL, first feed 3–4 DAH, reach EYS 4 DAH and transform 36–50+ DAH at ≥33 mm TL. Greasy grouper larvae need small first food (e.g. trochophores). They can grow from 90 to 600–800 g in 6–8 months, when fed trash fish in cages.

Global production was 4740 and 5184 t in 2006 and 2007, respectively.

18.10.16.8 Kelp grouper

Kelp groupers (*Epinephelus moara*, 60 cm) (25 °C) hatch from planktonic eggs at 2.1 mm TL, first feed 3 DAH, reach EYS 5 DAH and transform 40–45 DAH at 37 mm TL. Larvae can be reared on small rotifers, large rotifers and *Artemia*.

18.10.16.9 Polka-dot grouper

Polka-dot groupers (*Cromileptes altivelis*, 70 cm) have been raised mainly in Indonesia. They have voluntarily spawned (any month) in tanks at 26–28 °C and can be induced to ovulate. They (27–28 °C) hatch from planktonic eggs in 19 h at 1.5–1.6 mm TL, first feed 2–3 DAH (mouth width 145 µm) and reach EYS 4 DAH. Larvae can be reared on rotifers, *Artemia* and microfeeds. After reaching 3–5 cm in 50–60 days, juveniles are raised to about 10 cm in the nursery phase.

Global production was 3 and 1 t in 2006 and 2007, respectively.

18.10.16.10 King (giant) grouper (groper, brindlebass)

King grouper (*Epinephelus lanceolatus*, 400 cm, at least 440 kg). Production in commercial numbers has been reached during the past several years by Taiwanese hatcheries. Broodfish are large and difficult to handle, but this species grows very fast and is in much demand. King groupers can grow from 75 mm to 2.4 kg in 1 year and 15 kg in 2 years.

18.10.16.11 Barred-cheek coraltrout (spotted coral grouper)

Barred-cheek coraltrout (*Plectropomus maculates*, 115 cm, 25 kg) have been raised mainly in Singapore.

Global production was 21 and 4 t in 2006 and 2007, respectively.

18.10.16.12

Other farmed Asian species include duskytail (*E. bleekeri*, 76 cm), yellow (*E. awoara*, 60 cm), sixbar (*E. sexfasciatus*, 28 cm), bluespotted (*E. trimaculatus*, 49 cm), longfin (*E. quoyanus*, 40 cm), longtooth (*E. bruneus*, 100 cm), and honeycomb (*E. merra*, 31 cm) groupers.

Spawning and larval rearing of dozens of serranids have been studied. Leopard coraltrout (*Plectropomus leopardus*, 135 cm, 24 kg) have voluntarily spawned in tanks at 23–24 °C and ponds (planktonic egg diameter 0.8–0.9 mm) and can be induced to ovulate. Culture in saltwater cages is being developed in Taiwan and Japan. Squaretail coraltrout (*Plectropomus areolatus*, 73 cm) have voluntarily spawned in tanks at 29–32 °C, and can be induced to ovulate (planktonic egg diameter 0.74 mm). Blackspotted groupers (*Epinephelus amblycephalus*, 50 cm) were induced to ovulate, and larvae were reared to transformation (40 DAH, 35 mm TL). Sevenband groupers (*Epinephelus septemfasciatus*, 155 cm, 63 kg) have been induced to ovulate, and larvae were reared past transformation (60 DAH, 30 mm TL).

18.10.16.13 Black sea bass

Black sea bass (*Centropristis striata*, 66 cm, 4.3 kg) have been raised in the eastern USA. They have spawned voluntarily in tanks at 16–20 °C and can be induced to ovulate. They (20 °C) hatch from planktonic eggs of 0.9–1.0 mm diameter in 48 h at 2.1 mm TL, first feed 3.5 DAH, reach EYS 5.5 DAH and transform by 43–52 DAH. They can be reared on rotifers (until 20 DAH) and *Artemia* (by 14 DAH) in greenwater. Larvae grew faster at 22 °C than at 18 °C. Juveniles are good at 22–28 °C. They can grow from 300 g to 1 kg in 7–8 months.

18.10.16.14 Nassau grouper

Nassau grouper (*Epinephelus striatus*, 122 cm, about 27 kg) has been a major food fish from Brazil to Bermuda and has been experimentally raised in the USA and Bahamas. They have voluntarily spawned in tanks and a cage at 23–27 °C and can be induced to ovulate. They can be conditioned to spawn during any month, mainly by temperature cycling (i.e. raising or lowering temperature to the best spawning range 24–27 °C). They (26 °C) hatch from planktonic eggs of 0.9–1.0 m diameter in 26 h at 1.9 mm TL, first feed 2.5 DAH, reach EYS 5 DAH and transform 46–70 DAH at 35–50 mm TL. The best temperature for Nassau grouper larvae is about 24–27 °C; feed intake by juveniles and adults is minimal at <18 °C, but feeding and growth are good at 22–30 °C; best growth is likely near 30 °C. Improvement of early growth rates (e.g. by better temperature control, earlier weaning) will allow production of 600-g Nassau groupers within 12 months, 1-kg groupers within 18 months and 2-kg groupers within 24 months, with FCR rising from 0.9 to 1.8.

18.10.16.15 Goliath grouper

Goliath grouper (*Epinephelus itajara*, 250 cm, 455 kg) wild juveniles fed fresh fish in floating cages grew from 787 to 1788 g in 90 days. In circular tanks, 3- to 6-kg juveniles reached 12–13 kg in 480 days. Similar studies have been conducted with another Western Atlantic candidate, the black grouper (*Mycteroperca bonaci*, 152 cm).

18.10.16.16

In the Mediterranean region, two species have been investigated. Dusky groupers (*Epinephelus marginatus*, 150 cm, 60 kg) were induced to ovulate, and early larvae were reared. Wild juveniles (7–25 g) grew faster on commercial pellets (7–25 g in 69 days) than on octopus. White grouper (*E. aeneus*, 120, 25 kg) maturation and growth in captivity were studied.

18.10.17 Rachycentridae: cobia

18.10.17.1 Cobia

Cobia (*Rachycentron canadum*, 2 m, 68 kg) have been raised mainly in China, Taiwan, Mayotte, Japan, Vietnam and Thailand, with a developing industry in the Caribbean region and USA (Benetti *et al.*, 2008). Wild female cobia can reach 0.4 kg in 1 years, 2.4 kg in 2 years, 6.0 kg in 3 years, and 31 kg in 7 years, but males only 0.3, 1.5, 3.7 and 15.0 kg. Market size is 6–10 kg. Cobia have voluntarily spawned (all months) in ponds at 23–27 °C, and in tanks during March–November at 26–28 °C. They probably will spawn in tanks during all months. Cobia (25 °C) hatch from planktonic eggs of 1.2–1.4 mm diameter in 30 h at 3.0–3.5 mm TL, first feed 3 DAH, reach EYS 3–4 DAH and transform 30 DAH at 29 mm TL. Larvae can be reared on rotifers (until 4–9 DAH) and *Artemia* (by 7 DAH), or rotifers and copepods. Cannibalism can be worst during 45–75 DAH, and require weekly grading. Usually, ponds or tanks are used for nursery culture and cages for grow-



Fig 18.17 Underwater view of cobia in seacage. (Courtesy of Dr Benetti.)

out. The best temperature for larvae is about 28 °C. Grow-out is good at 25–30 °C (tolerance range 18–32 °C). Cobia do not have a gas bladder and have to expend energy to maintain their horizontal position, but they often rest on the tank bottom. Good starter feed contents are 50–60% crude protein, 12–15% fat. A US grower feed might contain 55% crude protein, 15% fat, and a Taiwanese grower feed 45% crude protein, 18% fat. Cobia can grow from hatching to 5–6 kg in 1 year and 8–10 kg in 2 years. Nursery tank harvest density can be up to 28 kg/m^3. They can be grown out (usually in cages, Fig. 18.17) at densities up to 10–15 kg/m^3. Cobia are susceptible to viruses, Gram-negative bacteria, Gram-positive bacteria, internal protozoans, digenean trematodes, cestodes, nematodes, and ectoparasitic dinoflagellates, ciliates, monogeneans, acanthocephalans and copepods.

Global production was 22 554 and 29 859 t in 2006 and 2007, respectively.

18.10.18 Carangidae: jacks

Jack larvae have been fed oyster larvae, rotifers, copepods, *Artemia*, and fish eggs and larvae. Most probably can be reared on rotifers and *Artemia*, but copepods can increase vigour and survival. Grower feeds for *Seriola* species in Asia can contain 45% crude protein, 15–17% fat, and in the Caribbean 50% crude protein, 10–15% fat, both with less than 20% carbohydrate. Crevalle jacks (*Caranx hippos*, 124 cm, 32 kg) have been raised mainly in Mexico. Miscellaneous crevalle jacks (*Caranx* spp.) have been raised mainly in Egypt, Brunei Darusallam and Philippines. Miscellaneous amberjacks (*Seriola* spp.) have been raised mainly in China, Taiwan and Mayotte. Japanese jack (horse) mackerels (*Trachurus japonicus*, 50 cm, 660 g) have been raised mainly in Japan and the Republic of Korea. Miscellaneous jack and horse mackerels (*Trachurus* spp.) have been raised mainly in Japan.

18.10.18.1 Japanese yellowtail (Japanese amberjack, hamachi)

Japanese yellowtails (*Seriola quinqueradiata*, 150 cm, 40 kg) have been raised mainly in Japan, the Republic of Korea, Taiwan and China. Market size is 1–8 kg (farmed). They have voluntarily spawned in tanks at 18–20 °C and can be induced to ovulate, but mostly are raised from wild mojako (late larvae to juveniles) because hatchery production is insufficient. In Japan, juveniles (often about 10 g, 25–40 mm TL) are caught mostly in purse seines during May–June. Those are grown in small cages for about 30–40 days on minced fish and shrimp and/or soft or granulated dry feeds before grow-out.

Global production was 155 070 and 157 906 t in 2006 and 2007, respectively.

Japanese yellowtails (20 °C) hatch from planktonic eggs of 1.1–1.3 mm diameter in 2.8 days at 3.5 mm TL, first feed 3 DAH, reach EYS 4 DAH and transform 50–55 DAH at 45 mm TL. Wild larvae eat mostly planktonic crustaceans, but at 30 mm TL they begin eating fish larvae, too; by 130 mm, fish are the main food. Cannibalism can start at 1 month. Usually, rotifers, *Artemia* nauplii and older *Artemia* seem indispensable and other zooplankton (e.g. copepods) necessary for yellowtail larvae. Oyster and sea urchin eggs have been used at first feeding. Fish larvae have been used as food, but microfeeds can take their place. Hatchery production of 300–500 20-mm fish/m3 (5–10% survival) is possible.

Larvae do well at 20–22 °C. Juveniles can tolerate from 7–9 to 30–31 °C and 16‰ salinity, but higher (33‰) is better. Best temperatures for growth are ca. 24–26 °C overall or ca. 22–28 °C (range 17–30 °C) at 200–2000 g and ca. 20–27 °C (range 15–30 °C) at more than 2000 g. Dissolved oxygen should be at least 6 mg/L. Yellowtails are grown out mainly in cages with volumes up to 2000 m^3, and sometimes in large pens, ponds, or impounded bays. Because of cannibalism, grading is necessary. Typical cage rearing density is 7–10 kg/m^3 (up to 20 kg/m^3). Previously, they were fed trash fish, but now nearly half of the food used is extruded pellets. Preferred fresh foods have been sardinella and mackerels (considered of better quality than sardines and anchovies). Raw food or moist pellets still often are used at temperatures <15 °C, and raw food for fish larger than 3 kg so that they will eat better. Because of their large appetite and high growth rate, they need to be fed often. They can reach 1.5–2 kg within 1 year and 4–7 kg in about 2 years. Japanese yellowtails are susceptible to viruses, Gram-negative bacteria, Gram-positive bacteria, fungi, internal protozoans, nematodes, and ectoparasitic monogeneans and copepods.

18.10.18.2 Greater amberjack (kampachi)

Greater amberjacks (*Seriola dumerili*, 190 cm, 81 kg) have been raised mainly in Japan, Spain, USA and Taiwan. Market size is 3.5–5.5 kg (farmed). They have voluntarily spawned in tanks at 19–25 °C and can be induced to ovulate. They (23–24 °C) hatch from planktonic eggs of 1.0–1.1 mm diameter in 30–35 h at 2.9 mm TL, first feed just before or at 3–4 DAH, reach EYS 3–4 DAH and transform 31–36 DAH at 16–19 mm TL. Larvae have been reared in tanks on rotifers, *Artemia* (by 11 DAH), and fish eggs or larvae, with or without greenwater. They can reach 860 g at 7 months and 3.0 kg at 18 months.

Global production was 66 and 68 t in 2006 and 2007, respectively.

18.10.18.3 Banded trevally

Banded trevallies (*Gnathanodon speciosus*, 120 cm, 15 kg) will spawn voluntarily in tanks, and their culture is established in Japan.

18.10.18.4 Goldstriped amberjack (hiramasa, yellowtail kingfish)

Goldstriped amberjacks (*Seriola lalandi*, 250 cm, 97 kg) have been raised mainly in Japan, Australia, the Republic of Korea, China and New Zealand. Market size (farmed) is up to 4 kg. They have voluntarily spawned (any month) in tanks at 22–25 °C and can be induced to ovulate. Goldstriped amberjacks (18 °C) hatch from planktonic eggs of 1.3–1.5 mm diameter in 4 days at 3.8–5.0 mm TL, first feed 3–4 DAH, reach EYS 4–5 DAH and transform 30 DAH at 15 mm TL. At 24 °C, first feeding is 3 DAH and they transform at 20–23 DAH, 10–12 mm TL. Larvae can be reared on rotifers (until 15 DAH) and *Artemia* (by 10 DAH), but mixed zooplankton, copepods, fish larvae and minced fish also are used. At 77 DAH, the juveniles were as large as 195 mm TL (120 g). They can grow from 5 to 3000 g in 13–14 months.

18.10.18.5 Mazatlan yellowtail (Pacific yellowtail, Almaco jack)

Mazatlan yellowtail (*Seriola mazatlana*) has been raised in Ecuador, Latin America and the USA, and is probably conspecific with the goldstriped amberjack. They have voluntarily spawned (any month) in tanks at 25–27 °C. The planktonic eggs (27 °C) hatch in 25 h. Larvae can be reared on wild zooplankton, rotifers and *Artemia* in greenwater (25–26 °C). Juveniles were stocked at only 42/m^3 in 12-m^3 tanks to help reduce cannibalism. They can reach 0.5–1 kg in 4 months. Two of the main problems are epitheliocystis infections during weaning (28–42 DAH) and cannibalism beginning at the same time. They can reach 2.5 kg at 10–12 months.

18.10.18.6 Snubnose pompano

Snubnose pompanos (*Trachinotus blochii*, 120 cm, 3.4 kg) have been raised mainly in Singapore, Hong Kong and Taiwan; market size is 400–600 kg. They have been induced to ovulate, and larvae were reared. They (28 °C) hatch from planktonic eggs of 0.9 mm diameter in 20 h at 2.0 mm TL and first feed at 2 days. They have been grown out in brackish or saltwater ponds. They can reach 400–600 g at 8–13 months.

Global production was 141 and 212 t in 2006 and 2007, respectively.

18.10.18.7 Florida pompano

Florida pompanos (*Trachinotus carolinus*, 64 cm, 3.8 kg) have been raised mainly in the Bahamas. Spawning and larviculture methods are fairly reliable, but growth and FCR can be improved. They can be induced to ovulate (any month). Larvae can be reared on rotifers and *Artemia* in greenwater. Best grow-out temperature is near 27–29 °C (about 12–34 °C for survival). The ectoparasitic ciliate *Cryptocaryon irritans* can be a problem, but reducing salinity to less than 20‰ can help reduce the danger.

Global production was 22 and 0 t in 2006 and 2007, respectively.

18.10.18.8 Permit

Permits (*Trachinotus falcatus*, 135 cm, 3.6 kg) have been raised in south-east Asia. They have voluntarily spawned in a large aquarium at 24 °C. Larvae were reared on oyster eggs and larvae, protozoans, rotifers, *Artemia*, fish meal and dry feed. They can reach 900 g in 12 months and 1200 g in 18 months.

18.10.18.9 Palometa

Palometas (*Trachinotus goodei*, 50 cm, 560 g) have voluntarily spawned in tanks at 24 °C. They can reach 500 g in 12 months.

18.10.18.10 Striped jack

Striped jack (*Pseudocaranx dentex*, 122 cm, 18 kg) culture is established in Japan. They have voluntarily spawned in tanks at 15–19 °C and are raised mainly in cages from both wild and hatchery juveniles. For grow-out, 24–28 °C is best. They can reach 400–650 g at 1 year, 700–1000 g at 2 years, and 2–2.5 kg at 3 years.Global production was 3300 and 3100 t in 2006 and 2007, respectively.

18.10.18.11 Rainbow runner

Rainbow runner (*Elagatis bipinnulata*, 180 cm, 46 kg) wild juveniles can reach 1.7 kg at 1 year.

18.10.19 Coryphaenidae: dolphinfishes

18.10.19.1 Common dolphinfish

Common dolphinfish (*Coryphaena hippurus*, more than 45 kg) have been raised in pilot projects in Hawaii, Florida and the Bahamas. They have some very good characteristics, but also some handicaps. With good nutrition, female dolphinfish will voluntarily spawn (any month) every other night for long periods at 26 °C. Broodfish (usually two females and one male) should be kept in a large doughnut-shaped tank to minimise collisions with the tank wall. They (26 °C) hatch from planktonic eggs of 1.5 mm diameter in 40 h at 4.2 mm TL, first feed 2–3 DAH, reach EYS 3 DAH and transform at ≥20 DAH at about 16–24 mm TL. Larvae have been reared on *Artemia* alone (nauplii to 10 days old), but survival is better when rotifers (2–8 DAH) and copepods are included. Aggression, biting and cannibalism can be high during 20–45 DAH, but diminish after complete weaning. Older juveniles (≥120 days) and adults can injure or kill themselves by ramming tank walls. Dolphinfish need very clean water and cannot be crowded. DO should be >6 mg/L for larvae and >5 mg/L for juveniles. Juveniles survive at temperatures of 13–29 °C and higher, but growth is best at about 29 °C. Salinity of 15–35‰ and possibly lower is acceptable. Grow-out feeds can contain at least 50% crude protein and near 10% fat. At 45 DAH, juveniles weigh about 5 g and can grow on pellets to as much as 1.7 kg at 180 DAH (density decreasing from 50 to 9/m^3) and 5 kg at 9 months. Larvae are especially susceptible to bacterial infections, and older dolphinfish are susceptible to Gram-negative bacteria, and ectoparasitic protozoans, copepods and isopods. Adults are easily injured by handling.

18.10.20 Lutjanidae: snappers

Snapper larvae typically are small, slender and fragile, and do not have much time between first feeding and EYS. Some have small mouths and extended larval periods like groupers. Small foods such as small or sieved rotifers and/or copepod nauplii, possibly supplemented with oyster eggs and larvae, can be necessary. Snappers are susceptible to Gram-negative bacteria, Gram-positive bacteria, internal protozoans, and ectoparasitic protozoans, monogeneans and copepods. Spotted rose snappers (*Lutjanus guttatus*, 80 cm, 1.3 kg) have been raised mainly in Costa Rica, Russell's snappers (*Lutjanus russelli*, 50 cm) in Hong Kong, two-spot red snappers (*Lutjanus bohar*, 90 cm, 12.5 kg) in Taiwan, Papuan black snappers (*Lutjanus goldiei*, 100 cm, 19.2 kg) in Singapore, miscellaneous snappers (*Lutjanus* spp.) in Brunei Darussalam and the Philippines, and miscellaneous snappers (Lutjanidae) in Taiwan.

18.10.20.1 Asian (mangrove) red snapper

Asian red snappers (*Lutjanus argentimaculatus*, 150 cm, 8.7 kg) (Fig. 18.18) are farmed mainly in Malaysia, Hong Kong, Singapore, Thailand, Philippines, Taiwan and nearby countries. Global production was 4683 and 4620 t in 2006 and 2007, respectively.

Market size is 500 g. They have voluntarily spawned in tanks and can be induced to ovulate. They (28 °C) hatch

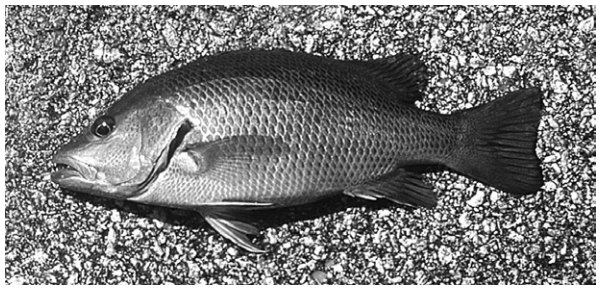


Fig. 18.18 Asian (mangrove) red snapper (*Lutjanus argentimaculatus*). Reproduced with permission from *World Aquaculture Magazine*.

from planktonic eggs of 0.75–0.8 mm diameter in 18 h at 1.8 mm TL, first feed 2–3 DAH, reach EYS 3–4 DAH and transform ≥35 DAH at not less than 19 mm TL. Maximum food size at first feeding is 60–70 µm wide and 100 µm long. Larvae can be reared on rotifers (until 20–28 DAH) and *Artemia* (by 17–21 DAH) in greenwater (25–29 °C). Growth in cages with raw fish was reported to be from 34 to 963 g at 10 months and 2303 g at 22 months. Growth was better for 50 fish/m^2 than with 110 or 152 fish/m^2. Fish stocked at 100/$m^2$and fed once per day can grow from 20 to 500 g in 9–10 months.

18.10.20.2 Golden (John's) snapper

Golden snappers (*Lutjanus johnii*, 97 cm, 10.5 kg) have been raised mainly in Singapore. They have been induced to ovulate and were stripped or allowed to tank spawn. They (28 °C) hatch from planktonic eggs of 0.8 mm diameter in 17 h at 1.6 mm TL, first feed and reach EYS 3 DAH and transform 35 DAH at 20 mm TL. Cannibalism can be a problem after 25 DAH. Larvae have been reared on mixed zooplankton (28–29 °C). They can grow from 90 to 600–800 g in 6–8 months when fed trash fish in cages.

Global production was 20 and 66 t in 2006 and 2007, respectively.

18.10.20.3 Atlantic (Caribbean) red snapper

Atlantic red snappers (*Lutjanus campechanus*, 23 kg) have voluntarily spawned in tanks at 23–26 °C and can be induced to ovulate. They (24 °C) hatch from planktonic eggs of 0.8 mm diameter in 25 h at 2.2–2.3 mm TL, first feed and reach EYS 3 DAH and transform at 22 mm TL. Larvae have been reared on copepods only. They can grow to 500 g in 1.5 years (24–28 °C).

18.10.20.4 Mutton snapper

Mutton snappers (*Lutjanus analis*, 94 cm, 16 kg) have voluntarily spawned in tanks at 24 °C and can be induced to ovulate. They (28 °C) hatch from planktonic eggs of 0.75–0.8 mm diameter in 17 h at 2.5 mm TL, first feed 1–2 DAH, reach EYS 2.0 DAH and transform 30–38 DAH at 29 mm TL. Larvae were reared on wild zooplankton, rotifers, *Artemia* and dry feed in greenwater. They can grow to 500 g at about 1 year, 800 g at 2 years, and 1600 g at 3 years.

18.10.20.5 Yellowtail snapper

Yellowtail snappers (*Ocyurus chrysurus*, 86 cm, 4.1 kg) have voluntarily spawned and can be induced to ovulate. They (27 °C) hatch from planktonic eggs of 0.95–1.0 mm diameter in 23 h at 2.2–2.4 mm TL, first feed 2–3 DAH, reach EYS 3 DAH and transform 35–45 DAH at 20 mm TL. Larvae can be reared on mixed zooplankton, rotifers and *Artemia* (27–28 °C). They can grow to 450 g within 2 years.

18.10.21 Gerreidae: mojarras

Mojarras (Gerreidae) have been raised mainly in Mexico.

18.10.21.1 Striped patao

Striped pataos (*Eugerres brasilianus*, 50 cm) can be induced to ovulate. They (28 °C) hatch from planktonic eggs of 0.64 mm diameter in 19 h at 1.4 mm TL, first feed 2–3 DAH, reach EYS 3 DAH and transform 48 DAH at 20 mm TL. Larvae can be reared on rotifers, copepods and *Artemia* in greenwater.

18.10.22 Haemulidae: grunts

18.10.22.1 Threeline grunt

Threeline grunt (*Parapristipoma trilineatum*, 45 cm, 1.05 kg) culture is established in Japan.

18.10.22.2 Silver grunt

Silver grunt (*Pomadasys hasta*, 60 cm) farming in brackish and saltwater ponds is established in Taiwan.

18.10.22.3 Yellowspotted grunt

Yellowspotted grunt (*Plectorhinchus cinctus*, 60 cm, about 4.6 kg) farming in brackish and saltwater ponds and cages is being developed in Taiwan. They have been induced to ovulate. They (22 °C) hatch from planktonic eggs of 0.8 mm diameter in 34 h at 2.2 mm TL, first feed 2–3 DAH, reach EYS 3 DAH and transform 24 DAH at 9 mm TL. Larvae can be reared on rotifers, *Artemia* and compound

feed in greenwater tanks, or ponds stocked with algae, rotifers and copepods. Pond-reared fish can reach 1.2–1.6 kg at 2 years.

18.10.23 Sparidae: sea breams, porgies

Sparids tend to spawn readily in captivity, and larvae of most can be reared on rotifers and *Artemia*. Although some are small at hatching, they become effective feeders. Juveniles and adults of most cultured species are omnivorous, and weaning of late larvae is relatively easy. Some species can become territorial or cannibalistic as juveniles.

Blackspot sea bream (*Pagellus bogaraveo*, about 77 cm, 40 kg) have been raised in Spain, common pandoras (*Pagellus erythrinus*, about 66 cm, 3.2 kg) in Greece, white sea bream (*Diplodus sargus sargus*, 45 cm) in Greece, sharpsnout sea bream (*Diplodus puntazzo*, 60 cm, 1.7 kg) in Italy and Cyprus, and miscellaneous sea breams (Sparidae) in China, Taiwan, the Republic of Korea and Italy.

18.10.23.1 Gilthead sea bream

Gilthead sea bream (*Sparus auratus*, 70 cm, 17.2 kg) have been raised mainly in Greece, Turkey, Spain, Italy, Egypt, Israel, France, Portugal, Cyprus, Croatia, Tunisia, Malta, Dominican Republic, Morocco, Albania, United Arab Emirates, Kuwait, Oman, Bosnia/Herzegovina and Slovenia. Global production was 107 437 and 125 355 t in 2006 and 2007, respectively.

The usual market size is 350–400 g. It is a protandrous hermaphrodite. They can be conditioned to spawn (any month) in tanks at 16–20 °C when daylength is quickly shortened to 9 h and then maintained. Ovulation can be induced with implanted GnRHa pellets or microspheres. Then, after 2–3 days, they can spawn every day for up to 4 months, and consistently high-quality eggs can be obtained every day of the year. They (18 °C) hatch from planktonic eggs of 0.9–1.1 mm diameter within 2 days at 2.4–2.7 mm TL, first feed 4–5 DAH, reach EYS 5 DAH and transform 40 DAH at 12 mm TL. Larvae can be reared on rotifers (until 14–20 DAH) and *Artemia* (by 10–15 DAH) in greenwater. Weaning can be accomplished just before transformation, but microfeeds can be given by 14 DAH. The range 18–22 °C is good for larvae, rising to as much as 30 °C for juveniles. Early grower feeds might contain 50–60% crude protein, 22% (15–27%) fat, 10–15% carbohydrate, and later grower feeds, 45–50% crude protein, 12–24% fat, 15–20% carbohydrate. Hatchery survival to fully developed juveniles can be 40% or more. Nursery production density at 2–3 g can be as high as 20 kg/m^3. Grow-out is in coastal ponds or lagoons, pens, tanks (harvest 15–45 kg/m^3), but mainly in cages (harvest 10–15 kg/m^3). They can reach about 450 g in 14–15 months. Gilthead sea bream are susceptible to viruses, Gram-negative bacteria, Gram-positive bacteria, internal protozoans, and ectoparasitic dinoflagellates, ciliates, monogeneans and copepods.

18.10.23.2 Red sea bream (madai)

Red sea bream (*Pagrus auratus major*, 110 cm, 97 kg) have been raised in Japan, the Republic of Korea, Taiwan, China and nearby countries. Global production was 75 754 and 74 378 t in 2006 and 2007, respectively.

Market size usually is 1 kg. They have voluntarily spawned at 15–22 °C and can be induced to ovulate. Red sea bream (18 °C) hatch from planktonic eggs 0.85–1.2 mm diameter in 2 days at 2.1 mm TL, first feed 3 DAH, reach EYS 4 DAH and transform 30 DAH at 10–14 mm TL. Larvae are reared in tanks up to 200 m^3 or larger. Hatchery production of one to five thousand 20–mm juveniles/m^3 (30–80% survival) is possible. In the first stage, they usually are reared (12–72 hatchlings/L) on rotifers (until 14–20 DAH) and *Artemia* (by 10–18 DAH) in greenwater, to transformation. Microfeeds are given as early as 8 DAH. Wild copepods are good but not necessary. Cannibalism can begin as early as 20 DAH (5–7 mm) and density might need to be reduced. In the second stage, juveniles (10–50 mm TL) are weaned in cages or tanks with a mixture of minced fish and clams and/or krill and then are raised on minced fish or compound feeds to 60–80 mm or larger. Larvae do well at 15–20 °C, and juveniles at 20–28 °C. Starter and grower feeds might contain 50–55% crude protein, 15% fat, with as much as 20% of the fat as *n*–3 HUFA. Red sea bream usually are grown out in cages (50–300/m^3), pens, or impoundments. They can reach 600–700 g within 18 months, 1 kg within 2 years, and 1.2–1.5 kg after 30 months (≥14 °C). A good harvest density for cages is 7 kg/m^3. Red sea bream are susceptible to viruses, Gram-negative bacteria, and ectoparasitic dinoflagellates, ciliates, monogeneans and nematodes.

18.10.23.3 New Zealand snapper

New Zealand snapper (*Pagrus auratus major*, 20 kg) is conspecific with red sea bream and has similar characteristics, but lives near New Zealand and Australia. They have been induced to ovulate. They (22 °C) hatch from planktonic eggs of 0.95 mm diameter in 28 h at 3.1 mm TL, first feed and reach EYS 3 DAH and transform 25–35

DAH at 9–14 mm TL. Larvae can be reared on rotifers and *Artemia*.

18.10.23.4 Black (blackhead) sea bream

Black sea bream (*Acanthopagrus schlegeli schlegeli*, 55 cm, 3.2 kg) have been raised mainly in the Republic of Korea, Taiwan and Japan. Global production was 3221 and 3396 t in 2006 and 2007, respectively.

Market size is 200–1000 g. They have voluntarily spawned in tanks at 17–21 °C and can be induced to ovulate. They (21 °C) hatch from planktonic eggs of 0.85–0.9 mm diameter at 1.8 mm TL, first feed 2–3 DAH, reach EYS 2–3 DAH and transform 40 DAH at 13–18 mm TL. Larvae can be reared on rotifers (until 24–30 DAH), *Artemia* (by 20 DAH) and microfeed (17–24 °C), with survival as high as 75%. They can reach at least 190 g at 1 year. They often are grown out in brackishwater ponds.

18.10.23.5 Silver (goldlined) sea bream

Silver sea bream (*Rhabdosargus sarba*, 80 cm, 12 kg) have been raised mainly in Taiwan and Hong Kong. They have voluntarily spawned in tanks at 13–26 °C and can be induced to ovulate. At 21 °C they hatch from planktonic eggs of 0.95–1.05 mm in 1.4 d at 2.3–2.4 mm TL, first feed 3–4 DAH, reach EYS 3–4 DAH and transform 42–45 DAH at 17–19 mm TL. Larvae can be reared on rotifers (until 24–30 DAH), *Artemia* (by 20 DAH) and microfeed in greenwater (19–22 °C is good). They often are grown out in brackish or saltwater ponds.

Global production was 52 and 8 t in 2006 and 2007, respectively.

18.10.23.6 Yellowfin sea bream

Yellowfin sea bream (*Acanthopagrus latus*, 50 cm, 1.5 kg) have been raised mainly in Oman, Japan and Taiwan. They have voluntarily spawned in tanks at 17–21 °C and can be induced to ovulate. They (22 °C) hatch from planktonic eggs at 2.0 mm TL, first feed 3–4 DAH, reach EYS 2–3 DAH and transform 38 DAH at 12 mm TL. Larvae have been reared on oyster larvae, rotifers, *Artemia*, copepods and microfeed in clearwater (20–24 °C). They often are grown out in fresh or brackish water ponds.

18.10.23.7 Sobaity sea bream

Sobaity sea bream (*Sparidentex hasta*, 50 cm) have been raised mainly in the United Arab Emirates. They have voluntarily spawned in tanks at 20–22 °C. Reported densities were 200–300/m^3 at 1–60 g and 60/m^3 for grow-out.

Global production was 191 t in 2006 and 2007.

18.10.23.8 Red porgy

Red porgies (*Pagrus pagrus*, 91 cm, 47 kg) have been raised mainly in Cyprus. They have voluntarily spawned in tanks at 12–18 °C (planktonic egg diameter 0.8–1.1 mm).

18.10.23.9 Common dentex

Common dentex (*Dentex dentex*, 100 cm, 14.3 kg) have been raised mainly in Bosnia and Herzegovina. They can be induced to ovulate. They (17 °C) hatch from eggs 0.96 mm diameter in 3.3 d at 2.2 mm TL, first feed 6–8 DAH, and reach EYS 7–8 DAH. Larvae can be reared on rotifers (until 20 DAH), *Artemia* (by 14 DAH) and microfeed (22–23 °C).

Global production was 14 and 10 t in 2006 and 2007, respectively.

18.10.23.10 Sheepshead

Sheepshead (*Archosargus probatocephalus*, 91 cm, 9.6 kg) is an excellent food fish ranging from Latin America to the north-eastern USA but is not a targeted species and has limited commercial availability. Ovulation has been induced with HCG, followed by stripping or voluntary fertilisation, and they probably will spawn voluntarily. They (23 °C) hatch from planktonic eggs of 0.8–0.85 mm diameter in 28 h at 1.6 mm TL, first feed 3 DAH, reach EYS 4 DAH and transform on or after 39 DAH at 14 mm TL. Larvae can be reared on rotifers and *Artemia*, but copepods increase survival. Juveniles and adults can be raised in hard freshwater. Sheepshead can reach 500 g in 9–11 months (23–30 °C).

18.10.24 Lethrinidae: emperors, sweetlips

18.10.24.1 Trumpet emperor

Trumpet emperors (*Lethrinus miniatus*, 90 cm, 9.6 kg) have been raised mainly in Taiwan. Global production was 0 and 182 t in 2006 and 2007, respectively.

18.10.24.2 Spangled emperor

Spangled emperors (*Lethrinus nebulosus*, 87 cm, 8.4 kg) have been raised mainly in Japan and Taiwan. They have voluntarily spawned in tanks at 21–23 °C (egg diameter 0.82 mm) and can be induced to ovulate. They often are grown out in brackish or saltwater ponds.

18.10.25 Sciaenidae: drums

Many sciaenids voluntarily spawn in captivity. Hormone treatment has been used mainly to induce ovulation in wild fish and to synchronise spawning of captive ones. Drum

larvae typically are robust, and easy to rear on rotifers and *Artemia*.

18.10.25.1 Red drum

Red drum (*Sciaenops ocellatus*, 155 cm, 45 kg) have been raised mainly in China, USA, Mauritius, Israel, Mayotte and Martinique. Global production was 44 712 and 52 177 t in 2006 and 2007, respectively.

The most common market size is 1.4–1.8 kg. They have voluntarily spawned in tanks at 25–26 °C (with constant daylength of 12–13 h) and can be induced to ovulate. They have been conditioned to spawn every 3–4 months at a rate of 10–20 times a month, spawn every month, spawn for about 3 days every week for 14 months (on a cycle of 25 °C for 4 days and 21 °C for 3 days) and spawn 360 times in 71 months. Usually, two or three males and two or three females (8–18 kg) are stocked in a 6-m^3 or larger tank. Red drum (25 °C) hatch from planktonic eggs of 0.9–1.0 mm diameter in 24 h at 1.7 mm TL, first feed and reach EYS 3 DAH and transform 35 DAH at 25 mm TL (Fig. 18.19). Larvae can be reared on rotifers (until 7–12 DAH) and *Artemia* (by 10 DAH) or rotifers and microfeed (started by end of first week). Survival from 5 DAH to transformation has been as high as 90% in ponds, but is usually 30–70%. Juvenile temperature range is 8–33 °C, but they can survive 4 °C for 1–2 days and 0 °C for a few hours if the change is not too sudden. Feeding and growth decrease at <20 °C and are negligible at <10 °C. Optimal temperature for growth of all stages is near 28 °C (25–30 °C). Juveniles and adults can be raised in freshwater if hardness is 250 mg/L or higher (Fig. 18.20). Grower feeds might contain 40–45% crude protein, 10% fat, and possibly up to 35% carbohydrate (less is better). They have been grown out in ponds, cages and tanks. They can reach 3 kg at 18–20 months and 5 kg within 30 months (Fig. 18.21), and an increase in harvest size from the usual 1.0–2.0 kg range up to 4.0–5.0 kg is feasible. Harvest densities of 30 kg/m^3 at 350 g and 28 kg/m^3 at 800 g in cages and 50 kg/m^3 in recirculating tanks are possible. Red drum are susceptible to viruses, Gram-negative bacteria, Gram-positive bacteria, fungi, internal protozoans, and ectoparasitic dinoflagellates, ciliates, cestodes, branchiurans, copepods and isopods. Too much handling or crowding can promote disease.



Fig. 18.19 Thirty-day-old red drum as they are becoming juveniles. (Reproduced from Tucker and Kennedy (2004) with permission.)



Fig. 18.20 Four-month-old red drum (13 cm, 21 g). (Reproduced from Tucker (1998) with kind permission from Springer Science+Business Media B.V.)



Fig. 18.21 Adult red drum. They were raised by the author to 4.7 kg (average) at 30 months and 6.1 kg at 36 months.

18.10.25.2 Large yellow croaker

Large yellow croakers (*Larimichthys crocea*, 80 cm) have been raised mainly in China (Hong and Zhang, 2003). Starter and grower feeds may contain 45–50% crude protein and 12% fat.

Global production was 61 067 and 61 844 t in 2006 and 2007, respectively.

18.10.25.3 Small yellow croaker

Small yellow croakers (*Larimichthys polyactis*, 45 cm) have voluntarily spawned in cages at 17–20 °C and can be induced to ovulate. They (19 °C) hatch from planktonic eggs of 1.15 mm diameter in 1.8 d at 3.2 mm TL, first feed and reach EYS 4 DAH. Larvae can be reared on rotifers and *Artemia*. Caged yearlings grew from 48 to 290 g in 21 months.

18.10.25.4 Cuneate drum

Cuneate drums (*Nibea miichthioides*) have been raised mainly in China (Hong and Zhang, 2003). They usually are grown out in cages and fed raw fish. Grower feeds might contain 45% crude protein and 15–17% fat.

18.10.25.5 Japanese croaker

Japanese croaker (*Nibea japonica*) culture is established in Japan; farming in brackish and saltwater ponds is established in Taiwan.

18.10.25.6 Meagre

Meagres (*Argyrosomus regius*, 230 cm, 103 kg) have been raised mainly in Spain, France and Italy. Global production was 966 and 750 t in 2006 and 2007, respectively. Market size is 600–5000 g. They spawn at 17–22 °C. Egg diameter is 0.99 mm; at normal temperatures, first feeding and EYS both occur around 4 DAH. The nursery stage usually is conducted in ponds or cages and grow-out in sea-cages. Grow-out is best at 17–21 °C (range 14–23 °C). A grow-out feed can contain 45% crude protein, 22% fat. In tanks they can grow from egg to 1 kg within 29 months. Meagres are susceptible to Gram-negative bacteria, and ectoparasitic dinoflagellates and monogeneans.

18.10.25.7 Spotted seatrout

Spotted seatrout (*Cynoscion nebulosus*, 100 cm, 79 kg) have been raised for stock enhancement in the southeastern USA. They have voluntarily spawned (any month) in tanks at 26–32 °C and can be induced to ovulate. They (28 °C) hatch from planktonic eggs of 0.7–0.8 mm diameter in 17 h at 1.5 mm TL, first feed 2 DAH, reach EYS 2–3 DAH and transform 37 DAH at 38 mm TL. Larvae can be reared on rotifers (until 10–12 DAH) and *Artemia* (by 7 DAH). They can be very cannibalistic. For juveniles, 27–30 °C seems best. Juveniles and adults have been raised in hard freshwater, but handling tolerance is better if salinity is at least 5–10‰. A starter feed can contain 50–55% crude protein, 13% fat, 15% carbohydrate, and a grower feed 40–45% crude protein, 11% (9–13%) fat, 20% carbohydrate. They can reach 500 g at 10–11 months.

18.10.25.8 White seabass

White seabass (*Atractoscion nobilis*, 166 cm, 41 kg) have been raised for stock enhancement in California. They have voluntarily spawned in tanks at 15–17 °C and can be induced to ovulate. Larvae (16 °C) hatch from planktonic eggs of 1.25–1.3 mm diameter at 2.7 mm TL, first feed 4.8 DAH (4 mm TL), reach EYS 5–6 DAH and transform 42–53 DAH at 22–35 mm TL. Larvae can be reared on rotifers and *Artemia*, and can eat small *Artemia* nauplii at first feeding. Cannibalism can start causing high losses by 30 DAH. A good temperature for juveniles is 19–23 °C. Juveniles grew from 4.5 to 76 g during 100–190 DAH.

18.10.26 Monodactylidae: moonyfishes

18.10.26.1 Eastern pomfret

Eastern pomfrets (*Schuettea scalaripinnis*, 20 cm) have been raised mainly in Indonesia.

Production from Indonesia was 14 990 and 15 546 t in 2006 and 2007, respectively.

18.10.27 Oplegnathidae: knifejaws

18.10.27.1 Striped knifejaw

Striped knifejaw (*Oplegnathus fasciatus*, 80 cm, 6.4 kg) culture is established in Japan. They have voluntarily spawned in tanks and can be induced to ovulate. Striped knifejaws (22 °C) hatch from planktonic eggs of 0.85 mm diameter in 1.5 days at 1.7 mm TL, first feed 3–4 DAH, reach EYS 4 DAH and transform 35 DAH at 15 mm TL. Larvae can be reared on rotifers and *Artemia* in greenwater (21–27 °C). An early grower feed can contain 45–55% crude protein and 10% fat.

18.10.28 Mugilidae: mullets

Mullets have been farmed in more than 50 countries. A few species have been induced to ovulate and can be routinely reared from eggs, but most have been raised from wild juveniles (about 16–20 mm). Larvae are robust and

can be reared on rotifers and *Artemia*; growth is best in greenwater. Mullet are easy to wean by the juvenile stage, after which they continue feeding in the water column but also scour the walls of the rearing unit for food. Larvae can be reared in salt or brackish water, and juveniles of many species grow well in freshwater, but mullet mostly have been grown out in brackishwater ponds. In extensive pond culture, without supplemental feeding, annual productions of 230–300 kg/ha have been obtained. They can be polycultured with shrimp and non-piscivorous fish (e.g. tilapia, carp, milkfish and pearlspot). Because they jump frequently, net covers are needed for tanks. Mullet are hardy and adaptable, and grow well in confinement, but adults are very sensitive to handling and can quickly develop external ulcers and bacterial septicaemia. Mullet are susceptible to viruses, Gram-negative bacteria, Gram-positive bacteria, fungi, internal protozoans, digenean trematodes, and ectoparasitic dinoflagellates, ciliates, monogeneans, branchiurans and copepods. Miscellaneous mugilids have been raised mainly in Indonesia. Global production of miscellaneous mullets was 13 445 and 7 213 t in 2006 and 2007, respectively.

18.10.28.1 Striped (flathead grey) mullet

Striped mullet (*Mugil cephalus*, 120 cm, 8 kg) have been raised mainly in Egypt, the Republic of Korea, Taiwan, Israel, Hong Kong, Greece, Tunisia, Singapore, Italy, Japan, Philippines, Hawaii, Russia, Saudi Arabia, Mexico and Peru. Global production was 244 091 and 264 383 t in 2006 and 2007, respectively.

Market size is 750–2000 g. They have not been reported to voluntarily spawn in captivity, but hormone-induced tank-spawning (any month) is routine. A 6-h day and 21–22 °C were found to be good for maturation. At slightly lower (17–20 °C) or higher (24 °C) temperatures, females can maintain vitellogenic oocytes for as long as 5 months. High-quality eggs can be produced with the least effort and damage to broodfish by injecting a reared or wild female (with oocyte diameters of 600 µm or more) with carp pituitary, then GnRHa, putting it in a tank with one or two reared or acclimatised males and allowing voluntary fertilisation to occur.

Striped mullet (24 °C) hatch from planktonic eggs of 0.88–1.0 mm diameter in 34 h at 2.4–2.8 mm TL, first feed 3 DAH, reach EYS 5 DAH and transform 28 DAH at 35–45 mm TL. Larvae can be reared on rotifers (until 20–30 DAH) and *Artemia* (by 9–15 DAH), but will grow better with enriched *Artemia* and can be fed cultured copepods or wild zooplankton. By 40–45 DAH, juveniles will eat detritus and almost anything organic, and grow well on complete or supplementary feeds with low to medium protein (e.g. 30% crude protein) and low to medium fat (e.g. 8%). They do not tolerate handling well until 40–50 DAH. Nursery and grow-out are good at 20–28 °C. They often are grown in nursery ponds to 10 g. During nursery and grow-out, ponds are fertilised and sometimes rice or wheat bran is given to the fish. Striped mullet can reach 450 g in 10 months, 800 g in 12 months, and 1.5 kg in 15 months. Grow-out usually is in ponds or pens (some in tanks), and harvest density can be up to 5000–6000 kg/ha in ponds.

18.10.28.2 So-iuy mullet

So-iuy mullet (*Mugil soiuy*, 80 cm, 5 kg) have been raised in China and the Ukraine. Larvae have been reared in concrete tanks (e.g. on oyster and mussel larvae, rotifers and brine shrimp), and in fertilised ponds in China. Soy or peanut paste and sometimes rice bran is given to juveniles. Global production was 395 and 876 t in 2006 and 2007, respectively.

18.10.28.3 White mullet

White mullet (*Mugil curema*, 90 cm) (26–27 °C) hatch from planktonic eggs of 0.8–0.85 mm diameter in 40 h at 2.0 mm TL, first feed 1.5 DAH, reach EYS 3.5 DAH and transform 30 DAH at 30–40 mm TL. They have been reared on wild copepods in greenwater (26–27 °C).

18.10.28.4 Liza

Lizas (*Mugil liza*, 80 cm, 9 kg) (24 °C) hatch from planktonic eggs of 0.85–0.9 mm diameter in 35 h at 2.3 mm TL, first feed 3–4 DAH, reach EYS 8 DAH and transform 40 DAH at 22 mm TL. Larvae can be reared on rotifers and *Artemia* in greenwater (23–24 °C). They can reach 800 g at 10–11 months.

18.10.28.5 Largescale mullet

Largescale mullet (*Liza macrolepis*, 65 cm) culture in fresh- and brackishwater ponds is established in Taiwan.

18.10.28.6 Redlip (so-iny) mullet

Redlip mullet (*Chelon haematocheilus*) can be induced to ovulate. They (18 °C) hatch from planktonic eggs of 0.95–1.0 mm diameter in 3.6 d at 3.0 mm TL, first feed 6 DAH, reach EYS 7–9 DAH and transform 40–76 DAH at 22–46 mm TL. Larvae have been reared in tanks on oyster larvae, rotifers, *Artemia*, copepods and minced fish (19–25 °C). Larvae have also been reared in ponds.

18.10.29 Polynemidae: threadfins

18.10.29.1 Fourfinger threadfin

Fourfinger threadfins (*Eleutheronema tetradactylum*, 200 cm, 145 kg) have been raised mainly in Taiwan.

Global production was 322 and 914 t in 2006 and 2007, respectively.

18.10.29.2 Sixfinger (Pacific) threadfin

Sixfinger threadfins (*Polydactylus sexfilis*, 61 cm, 3.2 kg) have been raised in Hawaii, and market size is 450 g. They have voluntarily spawned in tanks and ponds and can be induced to ovulate. They have planktonic eggs and are 2.2 mm TL at hatching. Larvae (26 °C) first feed 3 DAH and transform ≥26 DAH. Larvae can be reared on rotifers (until 14 DAH) and *Artemia* (by 7–10 DAH) in greenwater (26–27 °C). Cannibalism can be bad during 26–40 DAH. They can grow to 500 g at 8 months with harvest density as high as 25/m^3. Grow-out is mostly in cages.

Global production was 2 and 0 t in 2006 and 2007, respectively.

18.10.30 Anarhichadidae: wolffishes

18.10.30.1 Spotted wolffish

Spotted wolffish (*Anarhichas minor*, 180 cm, 28 kg) have been raised mainly in Norway and Iceland, and market size is 3–5 kg. Their normal temperature range is 6–10 °C. They have been grown out in cages, but tanks probably are better.

18.10.30.2 Atlantic (common) wolffish

Atlantic wolffish (*Anarhichas lupus*, 150 cm, 24 kg) have been raised mainly in Iceland. They have voluntarily spawned in tanks at 4–11 °C. Larvae have been reared on *Artemia* and compound feed. Good temperatures are 11–14 °C for larvae and 10–11 °C for juveniles. An early grower feed can contain 50% crude protein, <20% carbohydrate.

18.10.30.3 White sea wolffish

White sea wolffish (*Anarhichas lupus marisalbi*) (synonym of *A. lupus*) have voluntarily spawned in tanks at 12–14 °C. They (6 °C) hatch from sinking adhesive eggs of 5.1 mm diameter in 116–163 days at 22 mm TL and first feed 0–6 DAH.

18.10.31 Gobiidae: gobies

Gobies spawn voluntarily in tanks (sinking adhesive eggs), and larvae typically are easy to rear on rotifers and *Artemia* (sometimes copepods). Miscellaneous gobies (Gobiidae) have been raised mainly in the Ukraine and Taiwan. Bluespotted mudhopper (*Boleophthalmus hexanema*, 20 cm) and flathead goby (*Glossogobius giurus*, 55 cm) pond farming is established in Taiwan.

Global production of gobies for human consumption was 25 and 24 t in 2006 and 2007, respectively.

18.10.32 Siganidae: rabbitfishes, spinefoots

Rabbitfishes are raised mainly from wild juveniles in cages in the Indo-Pacific and eastern Mediterranean regions. Larvae have small mouths at first feeding but can be reared on rotifers and *Artemia*, if enough small rotifers are available during the first few feeding days. Juveniles and adults are mainly herbivorous, and feed and grow well on a wide variety of algae. Cages, pens and ponds are used for grow-out. Rabbitfish tolerate handling and crowding, but grow slowly. They are susceptible to Gram-negative bacteria, Gram-positive bacteria, internal protozoans, nematodes, and ectoparasitic monogeneans, acanthocephalans and copepods. Miscellaneous spinefoots (*Siganus* spp.) have been raised mainly in the Philippines and Saudi Arabia.

18.10.32.1 Yellowblotch spinefoot

Yellowblotch spinefoots (*Siganus guttatus*, 42 cm) have been raised in south-east Asia. Typical market size is 200–300 g. They have voluntarily spawned (any month) in tanks at 25–30 °C and can be induced to ovulate. They (27 °C) hatch from sinking adhesive eggs of 0.55–0.6 mm diameter at 1.8 mm TL, first feed 2–3.5 DAH at 3 mm TL (mouth width 187 µm), reach EYS by 5 DAH and transform 35–45 DAH at 22 mm TL. Larvae can be reared on rotifers (until 25 DAH) and *Artemia* (by 15 DAH) in greenwater (26–30 °C). Juveniles can eat live filamentous algae and dry feeds (e.g. 40% crude protein, 10% fat, 40% carbohydrate). From 55 mm, they can reach 200–300 g in 5–8 months.

Global production of miscellaneous rabbit fishes was 221 and 241 t in 2006 and 2007, respectively.

18.10.32.2 Pearly (whitespotted) spinefoot

Pearly spinefoots (*Siganus canaliculatus*, 30 cm) have been raised in Japan and Bahrain. They have voluntarily spawned in tanks at 30–31 °C and can be induced to ovulate. They (27 °C) hatch from sinking adhesive eggs of 0.5–0.55 mm diameter in 30 h at 1.7 mm TL, first feed 1.6 DAH, reach EYS 2 DAH and transform 21 DAH at 20–24 mm TL. Larvae can be reared on rotifers, copepods and *Artemia* in greenwater (28–32 °C). Trout feed was eaten as early as 20 DAH and the macroalga *Enteromorpha* as early

as 23 DAH. Biting can be a problem during 15–20 days or longer. They can grow from 10 to 114 g in 4 months.

Global production was 1 and 0 t in 2006 and 2007, respectively.

18.10.32.3 Sandy spinefoot

Sandy spinefoot (*Siganus fuscescens*, 40 cm) farming in brackish and saltwater ponds is established in Taiwan. They (24 °C) hatch from sinking adhesive eggs of 0.6–0.65 mm diameter in 30 h at 2.3 mm TL, first feed and reach EYS 2 DAH, and transform 17 DAH at 10 mm TL.

18.10.32.4 Marbled (black) spinefoot

Marbled spinefoots (*Siganus rivulatus*, 27 cm) have been raised mainly in Cyprus. They have spawned voluntarily in tanks and can be induced to ovulate. They (27 °C) hatch from sinking adhesive eggs of 0.55–0.60 mm diameter in 1.2 days at 2.0 mm TL, first feed 1.8 DAH, reach EYS 2.8 DAH and transform 32 DAH at 29 mm TL.

18.10.33 Hexagrammidae: greenlings

18.10.33.1 Okhotsk Atka mackerel

Okhotsk Atka mackerels (*Pleurogrammus azonus*, 62 cm, 1.6 kg) have been raised mainly in the Republic of Korea.

18.10.34 Scombridae: mackerels

Several scombrids have been spawned in captivity, but mainly by stripping ripe wild fish. Larvae have been reared from those eggs and from collected wild eggs, but usually not for long. Although scombrid larvae are fairly robust at hatching, survival in captivity is variable. Prey quality must be high, and water movement and cannibalism seem to be factors needing control.

Larvae have large mouths at first feeding and will eat rotifers, *Artemia* and copepods. Oyster larvae have been considered preferable as a first food and rotifers satisfactory. Copepods are considered indispensible as intermediate food. Weaning is easier with moist than with dry pellets.

Oceanic-quality water seems necessary for raising tunas. Most farmed ones are relatively large, captured wild fish that are grown out in large open-ocean cages. Grow-out food mostly has been raw, including sardinellas, pilchards, sardines, anchovies, mackerels, horse mackerels, herrings, sprat and squid. Resulting problems have been rancidity and disease transmission. Some needed improvements are routine controlled reproduction, better larval nutrition, consistently better survival during 0–10 DAH (e.g. stop surface sticking), reduction of cannibalism in late larvae and early juveniles (2–4 weeks), less colliding with tank and cage walls (1–2 months), and good acceptable pellets. They are very sensitive to handling (especially when >1 kg), bacterial infections and parasites. Tunas are susceptible to viruses, Gram-negative bacteria, internal protozoans, digenean trematodes, cestodes, nematodes, and ectoparasitic protozoans, ciliates, monogeneans and copepods. Miscellaneous tunas (*Thunnus* spp.) have been raised mainly in Spain. Miscellaneous mackerels (Scombridae) have been raised mainly in the Republic of Korea and Spain.

18.10.34.1 Yellowfin tuna

Yellowfin tuna (*Thunnus albacares*, 250 cm, 200 kg) have been raised in Mexico and Oman. They have spawned voluntarily in tanks (all months). They (26 °C) hatch from planktonic eggs of 0.9–1.0 mm diameter in 1.3 days at 2.7 mm TL, first feed and reach EYS 3 DAH and transform 58 DAH at 51 mm TL or more. Larvae were reared to the juvenile stage on rotifers, copepods and fish larvae. Wild yellowfin tuna can grow to 64 cm fork length at 1 years, 105 cm at 2 years, 135 cm at 3 years, 156 cm at 4 years, 169 cm at 5 years, and 177 cm at 6 years.

Global production was 1190 and 1210 t in 2006 and 2007, respectively.

18.10.34.2 Pacific northern bluefin tuna

Pacific northern bluefin tuna (*Thunnus orientalis*, 330 cm, maybe 1000 kg) have been raised mainly in Japan and Mexico. They have spawned voluntarily in a large cage and in tanks at 22–29 °C (24–28 °C might be best). Larvae have been reared on rotifers, *Artemia* and fish larvae. They can reach 60 mm at 6 weeks, 2–3.5 kg in 1 year, 7–15 kg in 2 years, 15–45 kg in 3 years, 30–70 kg in 4 years, and 40–90 kg in 5 years. Although they can survive 12 °C, exposure to <15 °C can cause a reduction in growth later; 25–28 °C is good for grow-out. Sawada *et al.* (2005) reported closing the life cycle of this species.

Global production was 3552 and 3600 t in 2006 and 2007, respectively.

18.10.34.3 Atlantic northern bluefin tuna

Atlantic northern bluefin tuna (*Thunnus thynnus*, 300 cm, 680 kg) have been raised mainly in Croatia, Spain, Italy, Tunisia, Cyprus and Libya. They can grow from 6 to 28 kg in 1.4 years or 12–45 kg in 1.5 years.

Global production was 3423 and 3454 t in 2006 and 2007, respectively.

At 24 °C both species of northern bluefin tuna hatch from planktonic eggs of 1.0 mm diameter in 32 h at 2.8 mm

TL, first feed 2–3 DAH, reach EYS 5 DAH and transform 30 DAH or later at 13–17 mm TL.

18.10.34.4 Southern bluefin tuna

Southern bluefin tuna (*Thunnus maccoyii*, 2 m, 200 kg) have been raised mainly in Australia. Wild juveniles have been grown out in cages at several farms. They usually are caught in purse seines at 5–10 kg and towed in transport cages to the rearing cages of up to 140 000 m^3. Grow-out density can be 2–5 kg/m^3. They have spawned in cages after injection of GnRHa by speargun.

Global production was 3611 and 2139 t in 2006 and 2007, respectively.

18.10.34.5 Black skipjack

Black skipjack (*Euthynnus lineatus*, 90 cm, 9.1 kg) have voluntarily spawned in tanks at 26–29 °C. Wild juveniles reared in tanks on wild zooplankton, molly larvae and chopped fish grew from 50 mg to 320 g in 90 days (24–29 °C).

18.10.34.6 Kawakawa

Kawakawas (*Euthynnus affinis*, 105 cm, 14 kg) have voluntarily spawned in tanks at 23 °C. They (24 °C) hatch at 2.8 mm TL, first feed 2–3 DAH and reach EYS 4.0 DAH.

18.10.35 Paralichthyidae: largetooth flounders

Largetooth flounders are susceptible to vibriosis, fungi, mycobacteria, and ectoparasitic dinoflagellates, ciliates and branchiurans. Miscellaneous largetooth flounders (was Bothidae, now Paralichthyidae) have been raised mainly in China (Hong and Zhang, 2003). Miscellaneous flatfishes (Pleuronectiformes) have been raised mainly in Chile.

18.10.35.1 Hirame

Hirame (*Paralichthys olivaceus*, 103 cm, 9.1 kg) have been raised mainly in the Republic of Korea, Japan and China. The usual market size range is 400–1500 g.

Global production was 48 465 and 45 772 t in 2006 and 2007, respectively.

They have voluntarily spawned (most months) in tanks at 12–18 °C and can be induced to ovulate. Hirame (18 °C) hatch from planktonic eggs 0.9–0.95 mm diameter in 2.0 days at 2.0 mm TL, first feed 2–3 DAH, reach EYS 3–4 DAH, and metamorphose and transform during 24–50 DAH, beginning at 10–14 mm TL. Larvae can be reared on rotifers (until 25 DAH) and *Artemia* (by 12 DAH) in greenwater. Microfeeds can be given as early as 8 DAH. By 30 mm, cannibalism can be a problem. Hatchery production of 1500–5000/m^3 at 20 mm (25–80% survival) is possible. Larval rearing is good at 16–18 °C and grow-out at 20–24 °C. Starter feeds for hirame can contain 50–60% crude protein, 10–14% fat, 10–15% carbohydrate, and grower feeds 45–60% crude protein, 8–9% fat, 10–15% carbohydrate. Raw fish, dry pellets, or moist pellets are used. Hirame can be fed four to six times a day until they weigh 100 g, three to four times until 300 g, and once or twice thereafter. Grow-out usually is in tanks or cages, but cage bottoms need to be smooth and the waves small. They can grow from 2 g to 1 kg in 15 months. Stocking density in tanks can be up to 200/m^2 at 10 g and 38/m^2 at 800 g. Hirame are susceptible to viruses, Gram-negative bacteria, Gram-positive bacteria, and ectoparasitic flagellates, ciliates and monogeneans.

18.10.35.2 Summer flounder

Summer flounders (*Paralichthys dentatus*, 94 cm) are being brought into commercial-scale production in the north-eastern USA. Both voluntary spawning in tanks and hormone-induced ovulation of captive broodstock are possible year-round. They (17 °C) hatch from planktonic eggs of 1.02 mm diameter in 3.1 days at 2.6 mm TL, first feed 3–4 DAH, reach EYS 4 DAH, then metamorphose, settle and transform during 35–65 DAH at 25–35 mm TL or more. Larvae can be reared on rotifers (until 20–23 DAH) and *Artemia* (by 14–16 DAH) in greenwater (16–20 °C); 18 °C is good for juveniles. Cannibalism among juveniles can be bad. They can reach 1 kg in 2 years.

18.10.35.3 Southern flounder

Southern flounders (*Paralichthys lethostigma*, 83 cm) have voluntarily spawned in tanks at 15–24 °C and can be induced to ovulate. They (17 °C) hatch from planktonic eggs of 0.9 mm diameter at 2.2 mm TL, first feed by 5–6 DAH and transform 48–52 DAH at >15 mm TL. At 24–26 °C, they hatch 26 h after fertilisation. Larvae can be reared in tanks on rotifers (until 17–20 DAH) and *Artemia* (by 14–17 DAH) or in ponds with mixed zooplankton. Larval rearing is good at 24–26 °C and grow-out at 22–26 °C. Southern flounders can reach 500 g at 16–18 months and 1 kg at about 2 years.

18.10.35.4 California halibut

California halibut (*Paralichthys californicus*) have voluntarily spawned in tanks at 15–21 °C (range 11–21 °C) and can be induced to ovulate. Larvae (18–20 °C) hatch from planktonic eggs of 0.7–0.8 mm diameter in 2 days at 2.0 mm TL, first feed 3–4 DAH, reach EYS 5 DAH, settle around 19 DAH, metamorphose during 27–42 DAH and

transform 45–50 DAH. A temperature range of 24–28 °C is good for juveniles.

18.10.35.5 Ecuadorian flounder

Ecuadorian flounders (*Paralichthys woolmani*, 80 cm) voluntarily spawn (most months) in tanks at 20–21 °C. Larvae have been reared on wild zooplankton, rotifers and *Artemia* in greenwater (25–26 °C). Wild juveniles in tanks grow to 1 kg in 1 year.

18.10.35.6 Brazilian flounder

Brazilian flounders (*Paralichthys orbignyanus*, 50 cm) voluntarily spawn in tanks (19–23 °C) and can be induced to ovulate. They (23 °C) first feed 4 DAH. They can be reared on rotifers and *Artemia* (by 14 DAH). Grow-out is good at 26 °C, and they can reach 500 g in 1 year.

18.10.36 Scophthalmidae: turbots

18.10.36.1 Turbot

Turbot (*Psetta maxima*, 110 cm, 25 kg) have been raised mainly in Spain, France, Portugal, Netherlands, Germany, the UK, Iceland, Denmark, Italy, Norway and China. Market size is 700–5000 g.

Global production was 7803 and 8205 t in 2006 and 2007, respectively.

Turbot ripen in captivity without hormones, but voluntary spawning is uncommon and strip-spawning is standard. The natural spawning season is for 2–3 months in the summer, with each female spawning six to eight times. With increase in daylength (from 6–8 to 16–18 h), and temperature (from less than 10 to 13–14 °C, range 7–16 °C), and good nutrition, turbot have voluntarily spawned (any month) in tanks. Turbot usually are strip-spawned when ovulation is detected, especially with off-season broodfish, because the artificial conditions in tanks can reduce fertilisation rate.

Turbot (19 °C) hatch from planktonic eggs of 0.9–1.2 mm diameter in 3 days at 2.7–3.1 mm TL (0.1–0.2 mg wet weight), first feed 2–3 DAH, reach EYS 7 DAH, metamorphose 30 DAH, begin benthic feeding 40 DAH (≥18 mm TL) and transform 40–50 DAH at 18–25 mm TL. They are relatively fragile until about 40 DAH, when they can be moved to larger tanks. Rotifers (or rotifer-sized food) are needed for about the first 10 days of feeding. Turbot have been reared on rotifers and *Artemia*, with 40% survival from hatching to metamorphosis. Enrichment of *Artemia* and possibly rotifers is recommended. Larvae also have been reared on copepods alone. Greenwater can help. A typical feeding schedule is rotifers, 2–13 DAH; *Artemia* nauplii, 8–26 DAH; enriched metanauplii 18–30 DAH; weaning to microfeeds, about 25–30 DAH. Intensive systems with small to medium tanks, stocking 20–40 larvae/L, and enriched rotifers and *Artemia* (sometimes copepods) for food have been popular in southern European hatcheries. Extensive systems with large tanks, bags or impoundments, stocking <1 larva/L, and wild zooplankton for food have been popular in northern Europe. The highest losses usually occur during 9–16 DAH, mainly because of nutritional inadequacy of *Artemia* and/or bacterial infections. Malpigmentation can be reduced by ensuring enough balanced *n*–3 HUFA with a good DHA : EPA ratio (e.g. by using natural zooplankton or enriching *Artemia* very well).

Good temperatures are 14 °C for eggs, 18–20 °C for larvae, 18–22 °C for up to 50 g, decreasing to 16–17 °C for more than 100 g (juvenile range 11–23 °C). An early grower feed might contain 55% crude protein, 15–17% fat. Grow-out is mostly in concrete tanks or lined ponds and sometimes flat-bottomed cages. They have been raised to at least 200 g in stacked tanks 20 cm deep. Turbot can reach 1 kg at 13–14 months and 3 kg at 2.5–3 years. Possible tank stocking densities are 1000/m^2 at 2 g, 100/m^2 at 125 g, and 120 kg/m^2 (or 55 kg/m^3) for larger fish. Yield at 1–2 kg can be 60 kg/m^2, or 200 kg/m^3 and higher. Turbot are susceptible to viruses, Gram-negative bacteria, Gram-positive bacteria, fungi, internal protozoans, and ectoparasitic flagellates, ciliates and leeches.

18.10.37 Pleuronectidae: righteye flounders

Miscellaneous righteye flounders (Pleuronectidae) have been raised mainly in China. European flounders (*Platichthys flesus*, 60 cm, 14 kg) have been raised mainly in the Ukraine.

18.10.37.1 Atlantic halibut

Atlantic halibut (*Hippoglossus hippoglossus*, 470 cm, 333 kg) have been raised mainly in Norway, the UK and Iceland. Market size is 2–5 kg or larger.

Global production was 1528 and 544 t in 2006 and 2007, respectively.

They have voluntarily spawned (most months) in tanks at 4–7 °C, but typically captive broodfish are strip-spawned to allow immediate egg care. They (5–6 °C) hatch from planktonic eggs of 3.0–3.4 mm diameter in 15–16 days at 6.5 mm TL, first feed during 30–50 DAH (15 mm TL), reach EYS 45–48 days and transform by 90–100 DAH at 30–50 mm TL. Development is slow, sometimes causing problems. Turbulence or light can inhibit hatching. Because yolksac larvae are sensitive and have to be incubated for a long time before first feeding, incubator tanks

need to provide gentle upwelling to keep the larvae off the bottom, but without battering them. Increasing egg buoyancy by slightly increasing salinity to 35.5‰ also can help. Yolksac larvae are susceptible to mechanical injury and bacterial infection, which can cause permanent damage and dysfunction, especially to the mouth. Too much light also can hurt yolksac larvae. They are transferred from incubators to rearing tanks before first feeding. Larvae have been reared in tanks and plastic bags. They can be reared on *Artemia* alone, but other cultured or wild zooplankton have often been given to ensure good survival, growth and pigmentation. Greenwater also improves survival and growth. Cannibalism is possible among juveniles. Mortality peaks can occur, especially during 15–35 DAH and several days after first feeding. Mortality at hatching can result from defects, bacterial infection, or low oxygen. Larval mortality during 10–14 DAH is likely a result of defects, bacterial infection or water flow. Mortality 25–35 DAH can result from energy depletion, behavioural problems, light or water quality. Mortality 45–60 DAH can result from starvation. Survival from egg to juvenile might reach 35%.

Good temperatures are 5–6 °C for eggs, 5–7 °C for yolksac larvae, 11 °C for first feeding larvae, 13–14 °C for juveniles of 10–60 g, decreasing to 10 °C (7–11 °C) at 3–5 kg. They are best at more than 2 °C but can tolerate −1 °C. Starter feeds can contain 55–65% crude protein, 18% fat, and grower feeds 40–55% crude protein, 14–16% fat. Some grower feeds have as much as 48–67% crude protein and 16–27% fat. Atlantic halibut can grow from egg to 40 g in 7 months, 1 kg in 1 year, and 3–5 kg in 3 years. Females grow faster than males. Wild females fed capelin, herring, squid and shrimp in tanks grew from 2 to 11 kg in 3 years, and males from 2 to 6 kg. In cages, they can grow from 1–1.5 to 5–7 kg in 5 years. For the best grow-out yield, stocking density should be one or two layers of fish on the bottom. Tank density can be up to 50 kg/m^2 at 2 kg and up to 100 kg/m^2 at 10 kg. Atlantic halibut are susceptible to viruses, Gram-negative bacteria, internal protozoans, digenean trematodes, cestodes, nematodes, and ectoparasitic monogeneans, acanthocephalans and copepods.

18.10.37.2 European plaice

European plaice (*Pleuronectes platessa*, 100 cm, 7 kg) have voluntarily spawned in outdoor and indoor tanks. They (9 °C) hatch from planktonic eggs of 2.0 mm diameter in 18 days at 5.8 mm TL, first feed 5 DAH, reach EYS 8 DAH, metamorphose 35 DAH and transform 40–75 DAH at 10–14 mm TL. Rotifers are optional during 5–7 DAH, then *Artemia* nauplii are given (by 5 DAH). For larvae, 12 °C is good, and for juveniles, 18–20 °C. Grower feed can contain 50% crude protein.

18.10.37.3 Marbled flounder

Marbled flounder (*Pseudopleuronectes yokohamae*, 45 cm, 1.9 kg) culture is established in Japan. They can be induced to ovulate (sinking adhesive eggs, 0.70 mm diameter). Larvae have been reared on rotifers, copepods, *Artemia* and minced sandlance (a small fish) in greenwater (12–14 °C).

18.10.37.4 Barfin flounder

Barfin flounder (*Verasper moseri*, 70 cm, 4 kg) culture is established in Japan. They have voluntarily spawned in tanks at 6 °C.

18.10.38 Soleidae: soles

Miscellaneous soles (Soleidae) have been raised mainly in Spain.

18.10.38.1 Common (Dover) sole

Common sole (*Solea solea*, 80 cm, 3 kg) has been raised mainly in Portugal and Spain. Minimum market size is 125 g. They have voluntarily spawned (most months) in tanks at 8–20 °C and can be induced to ovulate. They (19 °C) hatch from eggs of 1.3–1.4 mm diameter in 13 days at 3.6 mm TL, first feed 2 DAH at 4 mm, reach EYS 3 DAH, have exhaustion of the oil globule 10 DAH, metamorphose and begin benthic feeding by 23–28 DAH and transform (have scales) 42–56 DAH at 9–10 mm TL. Larvae have been reared on a simple diet of *Artemia* during 2–15 DAH and semi-moist crumbles beginning 10 DAH. Copepods can improve growth and pigmentation after metamorphosis. Weaning is possible at 30–40 DAH with conventional dry feed and about 10 DAH with microfeeds (but possibly with reduced growth). Normal temperatures for eggs are in the range 10–19 °C, depending on the region. Juveniles grow best at 20–25 °C. Larvae are easy to raise and survive well, but juveniles grow slowly. Larvae can be stocked at high density (70/L), and early juveniles can be crowded (up to 4000/m^2) more than plaice and turbot. A grower feed can contain 55% crude protein, ≥10% fat. Common soles can reach 20 g at 6 months and 250 g at about 2 years. Grow-out is mostly in tanks. Common soles are susceptible to Gram-negative bacteria, and ectoparasitic monogeneans, copepods and leeches. Larval and juvenile culture of this species was described by Palazzi *et al.* (2006).

Global production was 9 and 19 t in 2006 and 2007, respectively.

18.10.38.2 Senegal sole

Senegal soles (*Solea senegalensis*, 65 cm) have been raised mainly in Spain. They have voluntarily spawned in tanks at 16–22 °C. They (18 °C) hatch from planktonic eggs of 0.95–1.0 mm diameter at 2.7 mm TL, first feed 2 DAH at 2.8 mm TL, settle 19 DAH and transform 30 DAH or later. Larvae can be reared on rotifers (until 9–12 DAH) and *Artemia* (by 4 DAH) in greenwater (18–20 °C). Rotifers might not be necessary. Weaning is not easy, and training the fish every hour might be necessary. They can reach a harvest density in tanks up to 20–30 kg/m^3.

Global production was 32 and 36 t in 2006 and 2007, respectively.

18.10.39 Balistidae: leatherjackets

18.10.39.1 Threadsail filefish

Threadsail filefish (*Stephanolepis cirrhifer*, 30 cm) have been raised mainly in the Republic of Korea. They (20 °C) hatch from sinking adhesive eggs of 0.66 diameter in 2.4 days. Small prey like trochophores is necessary at first feeding.

18.10.40 Tetraodontidae: puffers

Miscellaneous puffers (Tetraodontidae) have been raised mainly in China and Japan. Global production was 23 350 and 20 560 t in 2006 and 2007, respectively.

18.10.40.1 Tiger (ocellate) puffer

Tiger (ocellate) puffers (*Takifugu rubripes*, 80 cm) have been raised mainly in Japan, China and the Republic of Korea; market size is 1–1.5 kg. They have voluntarily spawned in tanks at 17–20 °C and can be induced to ovulate. The eggs have been stripped from ripe females. They (17 °C) hatch from sinking adhesive eggs of 1.0 mm diameter within 8.5 days at 2.7 mm TL, reach EYS 6 DAH and transform 30 DAH at 9–15 mm TL. Larvae can be reared on rotifers (until 21–25 DAH) and *Artemia* (by 10–14 DAH) in greenwater. Biting and cannibalism can occur among late larvae and early juveniles. For juveniles, 25–26 °C is suitable. A grower feed can contain 50% crude protein. Grow-out has been mostly in cages, but there has been a shift towards tanks to reduce losses from parasites. They can reach 1 kg at 20 months. Tiger puffers are susceptible to viruses, Gram-negative bacteria, internal protozoans, and ectoparasitic dinoflagellates, ciliates, monogeneans and copepods.

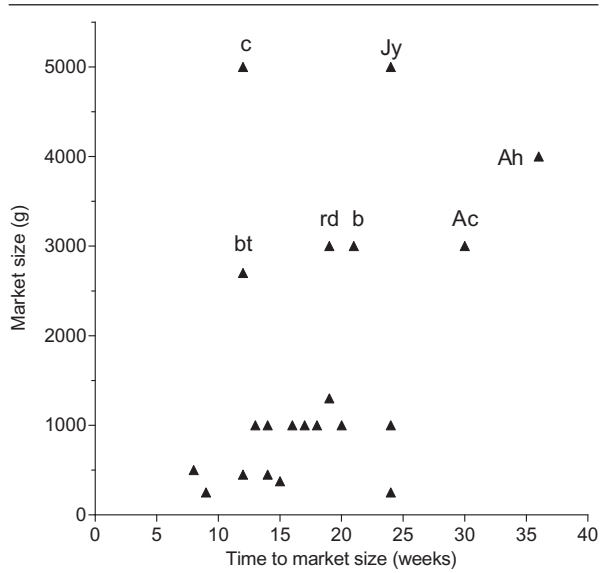


Fig. 18.22 Approximate values for market size versus time to reach market size. This figure shows the great range of growth rates and market sizes. They tend to fall into three groups: (1) very fast growers, cobia (c) and Japanese yellowtail (Jy); (2) fast growers, Pacific northern bluefin tuna (bt), red drum (rd), barramundi (b), Atlantic cod (Ac) and Atlantic halibut (Ah); (3) slow to medium growers. (Figure by John S. Lucas.)

18.10.40.2 Obscure puffer

Obscure puffers (*Takifugu obscurus*, 45 cm) have been raised in Japan. They have been induced to ovulate. They are anadromous, but eggs can survive in low salinities.

In conclusion, Fig. 18.22, based on Table 18.1 and the details above, presents market size versus time to reach market size.

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19
Preventing Diseases in Fish by Vaccination

Andrew Barnes

19.1 DEFINITION

A vaccine, strictly defined, is a cowpox virus or lymph containing it, based on the successful attempts by Edward Jenner to prevent infection with smallpox by administration of the closely related cowpox virus. Indeed the word vaccine is derived from the Latin *vacca*, a cow. The term 'vaccine' is now used more generally to define any preparation used to confer immunity to a disease by inoculation and the principle relies on the recipient having an adaptive immune system which initiates a response to the specific components of the vaccine that results in memory of those components. The immune system of the vaccinated individual is then able to respond more quickly and with greater magnitude on subsequent encounters with the same patterns or structures.

19.2 HISTORY OF FISH VACCINES

Fish vaccination has a surprisingly long history, with Duff (1942) reporting successful specific protective immunity in trout against furunculosis in the USA caused by '*Bacterium salmonicida*' (*Aeromonas salmonicida*) (Ellis, 1988). Further advances were made with early vaccines against bacterial diseases in fish in the 1960s and 1970s when bacterins were used to control yersiniosis in trout cultivated in the USA for sport fisheries (Busch, 1978; Ross and Klontz, 1965; Stevenson, 1997). Indeed the first commercial fish vaccine was introduced by Colorado-based Wildlife Vaccines Inc., granted a United States Department of Agriculture license in 1976 for vaccines against *Yersinia ruckeri* and *Vibrio anguillarum*. Mass vaccination programmes against bacterial diseases followed the rapid expansion of the intensive Atlantic salmon industry in Norway and Scotland. Vaccines against vibriosis in the late 1980s and early 1990s in Norway and Scotland were followed in the mid-1990s with efficacious vaccines against furunculosis caused by *A. salmonicida*. Successful control of these bacterial diseases through vaccination revolutionised the Atlantic salmon sector, and brought forth a wealth of research and development in the field of fish immunology, pathology and vaccination.

Perhaps the most significant indication of the success of the vaccination programme is the decline in chemical use in the Atlantic salmon industry. The Norwegian Office of Statistics keep excellent records of antibiotic use that illustrate the remarkable effect of the advent of bacterial vaccines on chemical use in Norwegian salmonid aquaculture (Statistics Norway, August 2005). From the height of the furunculosis era in 1987, to the latest statistics released for 2003, antibiotic consumption by the industry dropped by 98%. Currently average antibiotic use is less than 0.001 g/kg of salmon produced, down from 0.9 g/kg in 1987. Most of this reduction in use occurred between 1987 and 1994 and directly reflects the introduction of oil-adjuvanted furunculosis vaccines and the improved husbandry techniques implemented to ensure the success of the vaccination programmes (Grave *et al.*, 1999; Sommerset *et al.*, 2005). This represents not only a phenomenal reduction in environmental impact and a greatly reduced potential public health risk resulting from maintenance of antibiotic resistance determinants in the aquatic environment (Gordon *et al.*, 2007), but also a more economically sustainable industry. Many bacterial diseases of fish are now successfully prevented through vaccination.

19.3 FISH IMMUNOLOGY IN A NUTSHELL

Immunology is fascinating but vastly complex and there are excellent textbooks that cover this system in detail

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(Janeway, 2001; Roitt *et al.*, 2001). For this chapter we will focus on the aspects of the immune system we can exploit through vaccination and what makes the fish immune system different from mammals, particularly as it pertains to successful vaccination.

Most vertebrates have both an innate and adaptive immune system that act together in a rapid non-specific defence (innate system) followed by longer-term specific immune 'memory' (adaptive system). At its most basic, the immune system functions around three activities:

1. Pattern recognition: recognising there is a problem.
2. Signalling: ensuring the correct systems are informed and the information becomes systemic as well as localised.
3. Effector: doing something about the problem (i.e. killing the invading organism) and cleaning up afterwards.

During all parts of this process, systems are activated that will begin the process of forming memory so that a similar invader can be recognised quickly and eradicated on subsequent encounters. It is this memory formation that we exploit by vaccination.

We start with a few definitions. There are several cells and molecules within the immune system that we need to be familiar with to understand the fundamentals of vaccination; these are summarised in Fig. 19.1. First, what are the molecules that are needed to recognise and respond to a potentially infinite range of biological patterns and structures that can occur on pathogen surfaces?

- The most important ones are **immunoglobulins** (which include **antibodies**), the **T-cell receptors**, and the **major histocompatibility complex** (MHC). The mechanisms by which these highly varied molecules arise are intriguing but highly complex and will not be covered here.

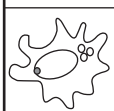
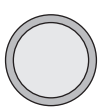
Key molecules		
	Immunoglobulin	Protein family involved with recognition and binding. Antibodies comprise a major group of immunoglobulins found on the surface of and secreted by B lymphocytes. Antigens are recognised by the multivalent F_{ab} end, while the conserved F_c region can interact with specific receptors on effectors eg. complement and macrophages.
	Major histocompatability complex	MHC is critical for antigen presentation to T cells. Two classes:MHCI is expressed by almost all cell types and presents antigens of intracellular origin. MHCII is expressed only by specialist antigen presenting cells that can engulf and and process exogenous invaders
	T-cell receptors	T cell receptors can only recognise antigens when they are bound to MHC molecules on the surface of antigen presenting cell (APCs)
Key cells		
	B lymphocytes	B lymphocytes play dual roles in both antigen presentation by MHCII and as immune effectors sectrting specific antibody. They have immunoglobulin on their surface (Ig^+) that binds antigens which the cell then processes and presents to T cells via MHCII.
	Macrophages	The key effector cells of the immune system, ultimately most invading organisms are destroyed by the macrophages. They are also one of the limited cell repertoire that express MHCII, and may be the major antigen presenting cells in fish.
	T lymphocytes	Naïve T cells cannot recognise antigens unless presented via MHC. When primed by APCs they mature into onf of several different types, $CD4^+$ helper T cells are mediators of immune response, initiating and coordinating the adaptive response. $CD8^+$ cytotoxic or killer T cells are activated inf response to MHCI presentation of intracellular antigens and will kill the infected cell.

Fig. 19.1 Key molecules and cells of the adaptive immune system.

However, through their complex genetic structure and somatic recombination (swapping around and selecting from different chunks of genetic material) these systems can generate structures that can potentially recognise any pattern in nature.

- An **antigen** is any molecule or part of a molecule that interacts with an **antibody** or MHC. An antigen can be made up of one or many **epitopes** – patterns that are recognised specifically by complementary **paratopes** on the MHC or antibody molecule.
- The term '**immunogen**' refers to any molecule that elicits an adaptive immune response by itself, and has been used interchangeably with antigen throughout the literature. However, an immunogen is always an antigen, but an antigen might not necessarily be an immunogen as it is possible for antigens not to induce adaptive immunity.

An adaptive immune response is provoked when an immunogen (such as parts of the surface of an invading organism) interacts with a specific pattern recognition receptor and is presented **T lymphocytes** by an antigen presenting cell. T lymphocytes are major mediators of adaptive immunity in fish. The way in which the particular immunogen is presented depends upon its origin.

- Those immunogens that originate *exogenously*, by ingestion, inhalation or injection, are presented by the MHC class II (MHCII) molecule on the surface of specific **antigen-presenting cells** (APCs). In mammals the major APCs are the **dendritic cells** though definitive markers for this cell type have not yet been found in fish. It is possible that **macrophages**, the dominant phagocytic cells of the immune system, are the major APCs in fish as MHCII is expressed in activated macrophages. The **B lymphocytes** also present antigens by MHCII in fish.
- Immunogens that arise *intracellularly*, for example viral proteins that are synthesised by the host cell, are presented by that cell by the MHC class I (MHCI) molecule.

Essentially there are two types of adaptive immunity that work together:

1. The humoral immune response, which is generally activated in response to antigen presentation by MHCII and is mediated by B lymphocytes and characterised by generation of antibodies. B lymphocytes have specific immunoglobulin molecules (antibodies) on their surface.
2. The cellular response, often activated in response to antigen presentation by MHCI, is mediated by T lymphocytes including helper T cells ($CD4^+$ because they have the glycoprotein marker CD4 on the cell surface) and cytotoxic T cells ($CD8^+$ because they have the CD8 glycoprotein on their surface) and characterised by maturation of naïve T cells to cytotoxic or killer $CD8^+$ T cells that kill the infected host cells. This type of response might be critical for immunity to viruses and intracellular bacteria.

Immune memory can form in both the humoral system and cellular system by formation of long-lived memory cells, and T lymphocytes are always involved in the process. In the humoral system the key cells are the B lymphocytes and $CD4^+$ helper T cells. Antigen presenting cells present antigenic epitopes on their surface by the MHCII molecule. These epitopes are recognised by specific paratopes of the immunoglobulins (or antibody) on the surface of the B cells. Signalling molecules (cytokines) secreted by the T cells induce the B lymphocytes to proliferate and differentiate. Proliferation of the lymphocytes is clonal thus each new lymphocyte will also carry the immunoglobulin paratope on its surface specific for the antigen that triggered the initial response. Most of the new B lymphocytes will be relatively short-lived effector B lymphocytes or plasma cells. These cells secrete antibody that is specific for the antigen that bound the initial paratope on the surface and this is how we derive the specific antibody response that we see after vaccination. Memory is induced because some of the new B lymphocytes are long-lived memory cells. These can respond rapidly upon subsequent challenge by the same antigen. The specificity of the antibody response and subsequent memory is due to the *clonal* differentiation and proliferation of the B cells, that is, all the new memory B cells recognise the original antigen. A simplified diagram of this process is given in Fig. 19.2(a–c).

Immune memory in the 'cell-mediated' response occurs when the receptor (T-cell receptor) on a naïve T cell binds an antigen presented by an MHC molecule. The T cell becomes activated and proliferates into many new clonal T cells. As with the B cells, some of these clones will be effector cells that perform the normal T-cell functions (killing infected cells in the case of $CD8^+$ cytotoxic T cells, or signalling to B and T lymphocytes in the case of $CD4^+$ helper T cells). Other clones will be long-lived memory T cells that can be rapidly reactivated on subsequent encounters with the same antigen. Once again it is the fact the differentiation is clonal, all of the daughter cells have the

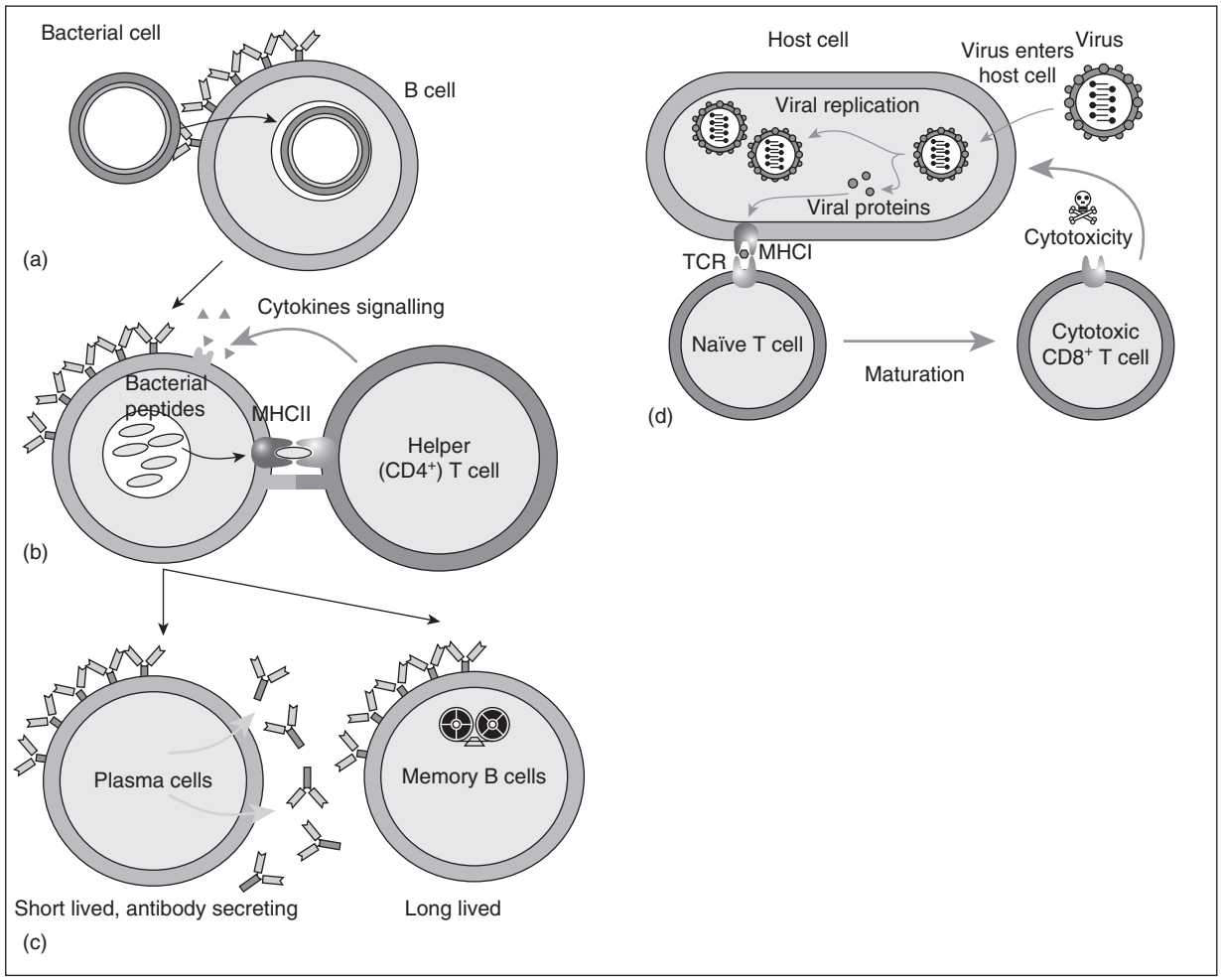


Fig. 19.2 Simplified schematic of immune responses mediated by MHCII (for external antigens) and MHCI (for those that arise inside the host cell). Antigen presentation by MHCII can only occur in specialist antigen-presenting cells such as B lymphocytes (a) and macrophages in fish. Presentation by MHCII to the T-cell receptors on helper T cells (b) results in activation of B-cell differentiation and proliferation, thereby increasing the number of B cells that recognise the original antigen. Effector B cells or plasma cells secrete antibody, which is how we detect increasing specific antibody response to a specific antigen after vaccination (c). As effector B cells are short lived, the specific antibody response declines over time, but memory B cells are long lived and can respond rapidly, producing more antibody on subsequent encounters with the same antigen. Antigen presentation by MHCI can occur in almost any cell but can only present antigens that originate from within the cell. Cells infected by viruses or intracellular bacteria will present processed antigens from the infecting agent by MHCI (d). Presentation to T cells by MHCI will lead to maturation of $CD8^+$ cytotoxic T cells that the kill the infected cell, preventing further replication of the virus.

Table 19.1 Kinetics of immune response to vaccination in fish. The kinetics of immunity in fish are very important when considering how and when to use vaccines. The process is temperature dependent so water temperature must be taken into account in any calculations. Kinetics are measured in degree days (°d), which is the water temperature in degrees Celsius multiplied by the time in days. Key factors to know are how soon the fish will be protected after vaccination (the response time), and for how long (the duration of immunity). This depends on what type of vaccine, how it is given and whether a booster vaccine is used. The table shows some indicative examples of response and duration of immunity. These are general indications only and must be determined experimentally for each vaccine.

	Immersion (aqueous)	Injection (aqueous)	Injection (oil emulsion)
Response time: time from delivery to full protection	Primary ca. 500 °days Booster ca. 150 °days	Primary ca. 500 °days Booster ca. 150 °days	Primary ca. 1000 °days Booster not necessary
Duration of immunity: the length of time protection will last	Primary ca. 1000 °days Booster ca. 2000–3000 °days	Primary ca. 1500–3000 °days Booster ca. 3000+°days	>8000 °days

same T-cell receptor recognising the same epitope as the original activated T cell, which leads to the specificity of the cellular immune memory. A representation of how humoral and cellular systems can be activated by a virus is shown in Fig. 19.2d.

In both cases the initial expansion of the specific *effector* B- and T-cell populations results in antibodies and cytotoxic cells that clear the initial infection, the primary immune response. After these effector cells die and the initial response falls away, the memory cells remain in an inactivated state until reactivated by subsequent infection by the same organism (i.e. with the same antigens or epitopes) that initiated the primary response. The secondary response generated by these memory cells on re-infection is generally more rapid and of higher magnitude than the primary response.

19.4 VACCINATING FISH

During vaccination we are simply introducing an immunogen and rely on the formation of either or both humoral and cellular immune memory. The kinetics of the immune response are very important as these determine how long it takes after vaccination until the fish are protected. It also determines how long the fish will remain protected for (duration of immunity (DOI)). Because most farmed fish are ectothermic (tuna are one of several exceptions), kinetics of physiological processes are in part determined by water temperature. Thus when we measure immune kinetics in fish, we must take this into account and we do so by measuring immune kinetics in degree days (°d). This is simply the time in days multiplied by the water temperature in degrees Celcius. So if the primary response to vaccination takes 600 °d to develop peak primary serum antibody levels, that means it would take 20 days if the fish are in water at 30 °C, but 60 days in a cold water species at 10 °C. The type of immune response we activate and the kinetics of this response, therefore the success of the vaccination, will depend on several factors in addition to temperature that are explored in this section. Some key elements of immune kinetics are summarised in Table 19.1.

19.5 TYPES OF VACCINE

19.5.1 Killed or inactivated vaccines

In their simplest form, vaccines can be a killed version of an infectious agent. The agent is grown under culture in appropriate nutrient medium for bacteria, or in tissue culture using an appropriate cell line in the case of viruses, and then killed or inactivated by one of number of processes. The use of formalin is very common and quite effective for many killed bacterial vaccines and formalin-killed 'bacterins' still comprise the majority of effective bacterial vaccines used commercially in aquaculture today. Killed vaccines constitute exogenous antigens and are thus processed and presented by MHCII, eliciting a strong humoral immune response characterised by increase in circulating specific antibody and subsequent immune memory by B-lymphocyte memory cell induction. They do not tend to elicit a strong cellular immune response, which explains why most killed vaccines against viruses and *intracellular* bacteria have low efficacy in practice.

19.5.2 Live attenuated vaccines

Live attenuated vaccines are simply live agents whose virulence (ability to cause disease) has been attenuated or reduced. This can be done by serial passage *in vitro*, with or without chemicals agents added to the culture medium. For example, one live attenuated bacterial vaccine is prepared by multiple (>16) serial passages on culture medium that contains the antibiotic rifampicin. In other candidate vaccines, key genes essential for virulence can be 'knocked out' by site-directed mutagenesis, which means using a nucleic-acid vector specifically to insert extra DNA (sometimes an antibiotic resistance gene) into the middle of the target gene so that it can no longer be expressed by the pathogen. These attenuated strains no longer cause disease, or only very mild forms of it and are rapidly cleared by the immune system. As they infect in the same way as the original organism and replicate in the host, they tend to activate the same immune pathways. So, as a live virus or intracellular bacterium replicates inside the host cell, components will be presented on that cell by MHCI and thereby initiate cellular immunity. This can get around the problem of only activating humoral immune memory encountered when using killed vaccines. However, there is some concern over potential reversion to virulence in the field, the possibility of over attenuation (so that attenuated strains are cleared too quickly without eliciting immune memory) and, in the case of targeted mutagenesis, the release of live genetically modified organisms into the environment. Although there is a long precedent for using live attenuated vaccines, particularly against viruses in human medicine, the aforementioned concerns have meant that the live-attenuated approach has met with very limited market or regulatory acceptability in aquaculture.

19.5.3 Recombinant or subunit vaccines

Both of the above technologies rely on the delivery of whole cells or viral particles to the animal to gain immune protection. This can be problematical if the organism is very difficult to grow *in vitro*. For example, as viruses need to replicate inside host cells, viral vaccines must be produced in cell culture, and for some fish viruses there are no permissive cell lines in which they can be grown successfully, or they might produce yields so low that they are not economically viable for vaccine production. Alternatively, some whole-cell-based preparations might contain very toxic elements that are not inactivated by the treatment used to kill the vaccine, or they might contain highly antigenic components that do not elicit protective immunity, but swamp any response to potential protective components that may make up a very small proportion of the total components of the vaccine. The advance of molecular technologies has led to the development of subunit or recombinant vaccines, in which only the protective molecules, or the fragments of them that contain the protective epitopes are used in the vaccine. This clearly requires extensive knowledge of the process of infection by the particular pathogen, which components are critical to the process and accessible to the immune system. Once a candidate protein or part thereof has been identified, the simplest means of producing the protein in substantial quantities is by cloning the gene into an expression plasmid and expressing the protein in *Escherichia coli* or in yeast. Generally proteins derived from bacterial pathogens would be expressed in *E. coli*, whereas those from viruses can be expressed in yeasts to exploit the differences in eukaryotic post-translational modification: viral proteins are synthesised by the hosts cell machinery during infection and fish are eukaryotes. The expression process is schematically illustrated in Fig. 19.3. Essentially, expressing the protein in bacteria or yeasts and growing up a large volume of them is the simplest part of the process, although not without its own difficulties. Purifying the recombinant protein from the final culture, and potential further 'downstream processing' such as refolding or conjugation to a hapten to make it more visible to the immune system, can add substantial extra difficulty and therefore cost to the process. This can make the cost of recombinant subunit vaccines prohibitive for fish, which are relatively low value animals. A further disadvantage of recombinant vaccines is that, like killed vaccines, they are processed as exogenous antigens (presented by MHCII) and therefore tend to only activate humoral immune memory, generally making them less effective against viruses and intracellular bacteria. There is, however, one commercial vaccine based on a recombinant subunit of the infectious pancreatic necrosis virus in Atlantic salmon. A recombinant VP2 protein from the virus surface is included as a component of a multivalent vaccine on sale for use in Norway, although the level of protection afforded by the infectious pancreatic necrosis virus component of this vaccine is not clear.

19.5.4 DNA vaccines

The most recent advance in vaccine technology has really been pioneered commercially in fish and is the DNA vaccine, sometimes called a nucleic acid or plasmid vaccine. Like the recombinant vaccines, these vaccines require in-depth knowledge of the structure, virulence and invasive processes of the target pathogen. Once a potential candidate protein (such as a viral coat protein) has been identified, the gene for that protein is cloned into a DNA vaccine plasmid vector (Fig. 19.4). In essence this is very

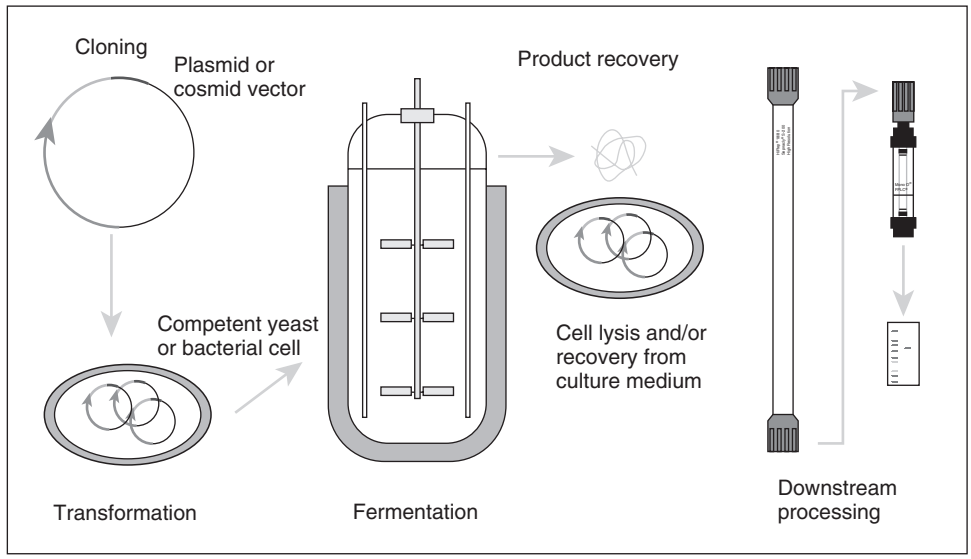


Fig. 19.3 Recombinant vaccine production process. The gene encoding the antigenic peptide or protein is cloned into an expression vector – a plasmid with a very efficient promoter that drives expression of the gene in the host bacterium or yeast during fermentation. The expressed protein is then recovered from the culture. This can be tricky as the protein might be secreted into the medium or compartmentalised in the host cell in the cytoplasm, periplasm (space between the cell and outer membranes) or as inclusion granules which can be very difficult to solubilise. All this must be determined experimentally before scale-up, and the amount of downstream processing required to obtain the antigen in an effective form will determine the cost, and therefore market viability, of the vaccine.

similar to the construction of the recombinant protein vectors. However, the critical difference between recombinant and DNA vaccines is that the promoter ('engine' that drives expression of the desired gene) in the DNA vaccine is a promoter that is active in host (fish or mammalian) cells, rather than in bacterial or yeast cells. In the only commercial DNA vaccine, this promoter is derived from human cytomegalovirus and is incredibly efficient at driving gene expression in eukaryotic cells. In the case of DNA vaccines it is the actual plasmid vector that constitutes the vaccine, rather than the expressed protein. Thus the plasmid vector is injected into the fish muscle and, in this process, a few muscle cells along the injection track become transfected with the plasmid. The cytomegalovirus promoter then drives expression of the target protein *inside the host cell*, making it an *endogenous* antigen. Thus it is presented by MHCI and initiates the cellular immune system that is critical for good protection against many viruses and intracellular bacteria. A simplified theoretical response to DNA vaccination is presented in Fig. 19.5. The first commercial DNA vaccine in any animal, Apex IHN, was licensed by Novartis Animal Health in 2005 for the prevention of infectious haematopoetic necrosis virus (IHNV) in salmon in Pacific Canada. The vaccine comprised a plasmid expressing a major surface glycoprotein from the rhabdovirus IHNV and is extremely effective.

So why are DNA vaccines so effective in fish? Experiments with DNA vaccines against a similar rhabdoviral disease in trout, viral haemorrhagic septicaemia virus, have indicated that antibodies against the virus can still be detected in DNA-vaccinated fish more than 2 years after injection, suggesting possible persistence of the vector in some fish cells. This seems hard to explain, as MHCI presentation of antigens on the surface of transfected muscle cells should result in their rapid eradication by specific $CD8^+$-cytotoxic T cells and macrophages. This appears to be what happens in mice and might explain the lower efficacy of DNA vaccines in mammalian systems.

Are DNA vaccinated fish genetically modified? Certainly not! Although foreign DNA is actively expressed by a few fish cells, the genetic material is never incorporated into

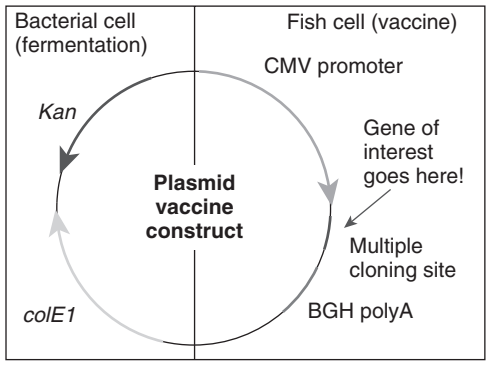


Fig. 19.4 A typical DNA vaccine vector showing the key elements of the plasmid construct. The elements to the left of diagram are active in the bacterial cell during fermentation and are associated with plasmid replication and maintenance. *colE1* is almost all of a very small bacterial plasmid characterised in *E. coli* the 1970s. It contains all of the machinery necessary to replicate the plasmid and mobilise it into daughter *E. coli* cells when the bacteria divide. *kan* is a kanamycin (antibiotic) resistance gene that enables selection during fermentation. The transformed bacteria are grown in medium containing kanamycin during the fermentation so that any daughter cells that do not have the plasmid are killed and will not swamp the culture with empty *E. coli*. The elements to the right of the diagram are those expressed in the fish muscle cells when the plasmid is injected into them. The cytomegalovirus promoter drives expression of any genes downstream of it very efficiently in vertebrate cells. The multiple cloning site immediately downstream of the promoter contains many restriction enzyme cut sites so that vector can be cut with different restriction enzymes to allow directional cloning of the gene for the antigen of interest. It is important that the gene is cloned into the vector in the correct orientation and in frame so that it will be correctly expressed and translated in the fish. In eukaryotes, messenger RNAs are polyadenylated (have a tail of adenines) but they are not in bacteria. The bovine growth hormone polyadenylation signal (BGH polyA) after the multiple cloning site ensures that the gene being expressed has a polyA tail and will be correctly translated in the fish.

the fish genetic material or germline and is therefore never passed on to progeny: the vaccinated fish is genetically identical to the unvaccinated fish and its chromosomes remain unaltered.

Producing the plasmid in large quantity and of sufficient purity for vaccinating hundreds of thousands of fish required some substantial scale-up for commercial use at a price that was cost-effective for farmers. Essentially the DNA vaccine vector is produced in *E. coli* in a standard fermentation. The vector contains a bacterial origin of replication and either an antibiotic resistance gene or complementary gene for an auxotrophic mutant to allow positive selection in culture (Fig. 19.4). The plasmid vector construct is introduced into competent *E. coli* by standard transformation. Thereafter the *E. coli* is grown under appropriate selective conditions to maintain the plasmid in an industrial fermenter and the plasmid is replicated at every cell division with the *Ecolab*. At the end of the fermentation, the bacterial cells are lysed and the released plasmids are purified from the lysate on industrial-scale gel chromatography columns.

19.6 ROUTES OF DELIVERY

Getting vaccines into fish presents several problems, not least because of the numbers in which they are farmed and the low monetary value of the individual. This makes fish vaccines very similar to poultry vaccines in terms of the economics of use. Thus it would seem sensible to use the easiest and least expensive mode of delivery. Unfortunately there is a trade off between low cost, ease of use and actual efficacy in the fish. In other words, the simplest and cheapest form of delivery might not necessarily trigger the most appropriate immune response, or any response at all. In practice, vaccines can be delivered to fish by injection, immersion or bathing, oral (on feed) and occasionally by spraying in an oil emulsion onto the surface of the water.

19.6.1 Injectable vaccines

It is not uncommon, when talking to people outside the field of fish vaccinology, to hear the comment ‘Surely you don’t inject every single fish!’. In fact, the vast majority of immunised fish are vaccinated by injection into the peritoneal cavity (intraperitoneal (IP) or intracoelomic injection). This might seem impossibly labour intensive when the sheer numbers of individuals are considered, yet it has been adopted into the farm management cycle as routine. Use of repeat injectors that control the dose, and vaccination tables that allow unvaccinated fish to remain anaesthetised, while vaccinated fish gently flow to recovery tanks mean that a few skilled individuals can vaccinate

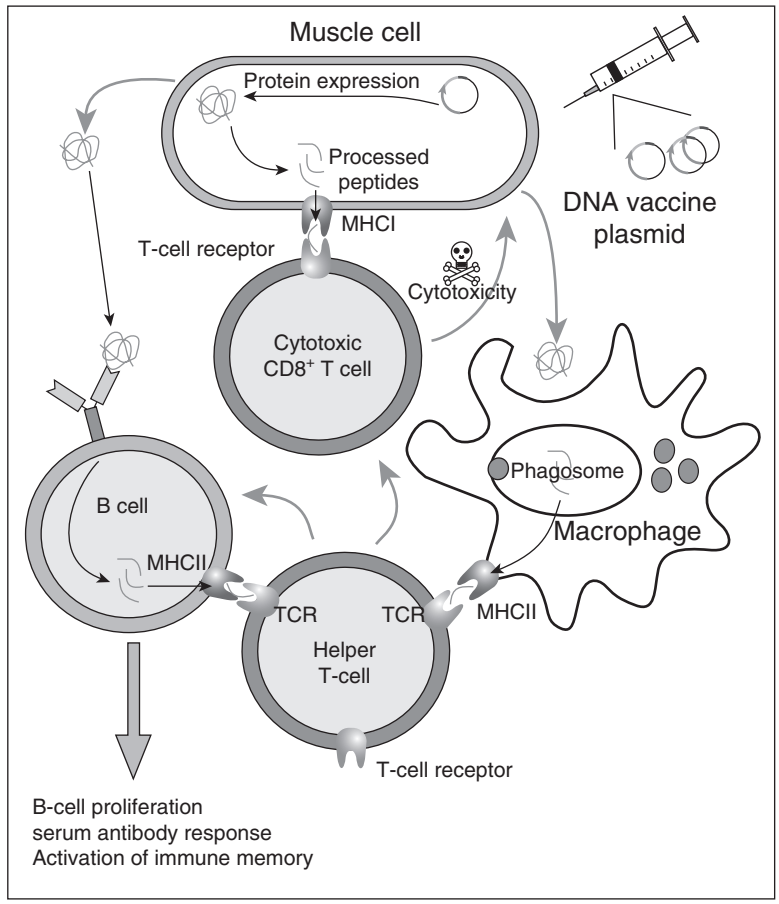


Fig. 19.5 How DNA vaccination might hypothetically initiate both cellular and humoral immune responses in fish. Immune pathways involved in successful response to DNA vaccination in fish are not yet clear. However, research suggests that a signal sequence is important for full protection against rhabdovirus infections in salmonids, implying the secretory pathway might be important, perhaps enabling presentation of the target antigen by APCs by MHCII, in addition to MHCI presentation by the transfected muscle cell.

many tens of thousands of fish per day. A typical small-scale vaccination set up on a barramundi farm in Queensland is shown in Fig.19.6. Occasionally, vaccines can be delivered by intramuscular (IM) injection. This is mandatory for DNA vaccines as host cells need to be transfected by the plasmid, thus IP injection of DNA vaccines appears to be ineffective. Apex IHN is generally given as a separate shot IM at the same time as the fish receive their usual multivalent IP injectable vaccine against a range of other diseases.

The reason farmers have adopted the apparently laborious practice of IP injection is simply because it is very effective. Good vaccines, delivered intraperitoneally to healthy fish with minimum stress will generally protect throughout the lifetime of the fish, and any modern vaccines will protect against five or more different diseases from a single shot. This makes them very cost-effective over the farm cycle.

19.6.2 Immersion, or bath vaccines

It is very difficult to vaccinate small fish by injection, thus juvenile fish <10 g can be vaccinated by immersion in vaccine diluted in clean water from the hatchery. Generally immersions are very short, with 60 s being the usual



(a)



(b)

Fig. 19.6 Vaccination of fish by injection. (a) A typical small-scale commercial vaccination set up at a barramundi farm in Queensland, Australia. The vaccination table has recirculated water containing anaesthetic in the central reservoir, whereas the channels on each side of the central reservoir have flowing, clean, farm water passing through them. Anaesthetised fish are gently lifted from the central reservoir, injected, then released into the channel where the clean water flow transfers them back to their tanks or a recovery tank. Vaccine is delivered by hand with repeat injection guns (b) that can be adjusted to give a precise dose of vaccine. Care is taken to ensure that needles are of appropriate size for the fish being vaccinated and are changed before they become blunt.

pratice and no improvement is seen with longer immersion; indeed, gill irritation can occur with longer immersions. Fish can be lightly anaesthetised and netted into a container with the diluted vaccine. After 60 s the fish are removed, drained and returned to tanks for recovery. Only a defined biomass of fish can be vaccinated per litre of vaccine because the antigen might become depleted as batches of fish are vaccinated in the same vaccine. This biomass has to be determined empirically by the manufacturer and included on the label indications for registration.

Although immersion of fish might seem much more appealing than IP injection, it does have several disadvantages. First, immersion tends only to stimulate the mucosal immune system, with specific antibody-secreting cells (B cells) detected only in the skin epithelium and gills after immersion vaccination. This contrast between the immune responses to injection and immersion is illustrated in Fig. 19.7. Without activation of systemic immunity, duration of immunity might be very short and frequent re-vaccination is likely to be required. This becomes a problem as the fish

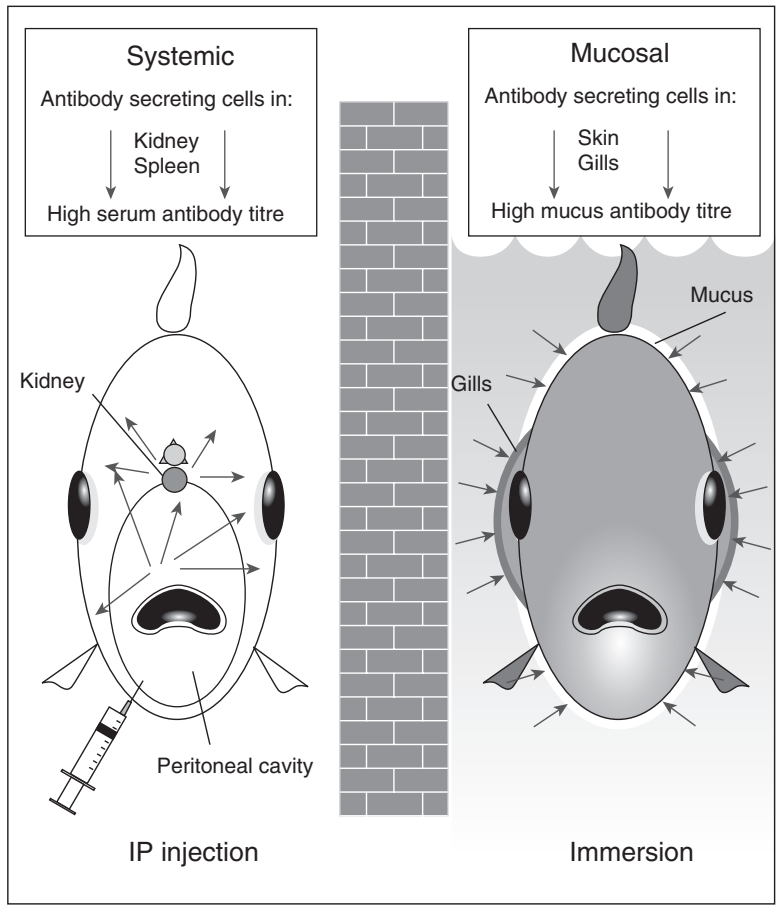


Fig. 19.7 Partition of mucosal and systemic immune responses in fish. The apparent separation of these two systems means that immersion vaccines offer short duration of immunity and are unsuitable for some infections. Injectable vaccines tend to stimulate strong systemic immunity. The 'brick wall' indicates that it is hard to activate systemic immunity by immersion or mucosal immunity by injection: the partition between the systems seems to be quite rigid.

grow because they require a larger volume of water and therefore a much greater volume of vaccine, which is expensive. Furthermore, the mucosal immune system does not seem to protect at all against some pathogens such as *Lactococcus garvieae* and *Streptococcus iniae*. This is indicated very clearly in the experimental data shown in Table 19.2. Rainbow trout vaccinated against *L. garvieae* by injection were highly protected in subsequent challenges (96–100% relative percentage survival). In contrast, when the same vaccine was given by immersion, no protection was achieved (Table 19.2). As a consequence, immersion tends to be used as a primary vaccination to protect fish that are too small to inject through the hatchery, with injection subsequently used as a boost before transfer to grow-out.

19.6.3 Oral vaccination

Considered by many as the 'Holy Grail' of fish vaccination, the ability to deliver vaccines with feed would seem to be the ideal solution. The farmer must feed the fish every day, and sophisticated automated systems exist and are used commercially to deliver precise quantities of feed to appetite, so why not simply include the vaccines in a batch of feed? The answer is that it does not work. Despite years of research throughout the 1990s and 2000s, there is little compelling evidence to suggest that protective immunity

Table 19.2 Difference in protection of rainbow trout from infection by *Lactococcus garvieae* when vaccinated by injection or immersion. Relative percentage survival (RPS) is the percentage of vaccinated fish that survive compared with challenged, unvaccinated control fish. A negative value indicates that more vaccinated fish died than controls! Note how immersion vaccination (Imm) fails to protect the fish against this systemic pathogen, whereas injection (IP) with same vaccine gives very high protection. This is indicative of the different partitions of the immune system (mucosal and systemic) that are activated by the different routes of vaccination. Data from unpublished research by the author, 2001.

Vaccine batch	Adjuvant	Route of delivery	RPS (%)
001/021	–	IP	100.00
001/036	–	Imm	3.05
001/048	M1	Imm	–6.35
001/048	M2	Imm	–23.83
001/048	P1	Imm	–20.63
001/048	–	Imm	3.07
001/048	–	IP	96.40

can be elicited by the oral route in a practical manner in fish. In part this comes down to a degree of immune 'tolerance' to antigens that are ingested, to prevent food from generating a high systemic immune response and potential allergy. It is also partly a result of the hostile environment of the gastrointestinal tract; with the presence of digestive enzymes and extremes of pH, even the hardiest of antigens is likely to be digested long before it encounters any antigen presenting cells. Moreover, the gut-associated lymphoid tissue and Peyer's patches associated with immune processing in the mammalian gut have not been clearly identified in teleost fish. This does not mean that immune protection by the oral route is impossible in fish, simply that it is neither easy nor a certainty. Most research in the field of oral vaccines has centred on protection of the antigen against digestive processes of the foregut and midgut in the hope that if the antigen reaches the more absorptive hindgut intact it will be taken up and processed. Of the many potential 'carriers' that have been used to try to confer immunity on feed, a few are worthy of note. First, antigens have been successfully transported across the hindgut wall in trout and salmon by encapsulation in polylactide co-glycolide microspheres (PLGA), and PLGA particles have been found in the head kidney (the major haematopoietic tissue) of the fish after oral administration. However, there is no evidence of any protective immunity developing by this means. A commercial oil-based system for protecting antigens through the gut has also been marketed. This essentially constitutes oil and a surfactant used to make an emulsion containing the antigen, which is then top-dressed onto feed and given to the fish. Once again, evidence of protective efficacy is scant and the manufacturer generally recommends these formulations as boosters rather than a primary vaccine. More recently, a commercial system that makes the gut more amenable to vaccination has been developed. In this proprietary system, a feed additive is given before vaccination that neutralises gut pH, inactivates gut proteases and increases gut wall permeability to allow antigen uptake. This appears to be an innovative approach but again data on consistently reliable efficacy are not available. For some fish species, such as catfish, injection is quite difficult. With the rapid growth of pangasid catfish aquaculture in Asia (Fig. 1.13) and demand for upwards of 100 million doses of vaccine within this industry in Vietnam alone, research into oral vaccination might enjoy resurgence. However, as hatchery technology and practices continue to improve in Vietnam, the opportunity for reliable injection vaccination using tried and tested technology also increases.

19.7 ADJUVANTS

Adjuvant roughly translates as 'helper'. Adjuvants are substances added to vaccines to improve the immune response.

19.7.1 Adjuvants for killed injectable vaccines

Initially, alum-based adjuvants were favoured and substantially improved immunity to simple, killed bacterial vaccines. However, the advent of oil-adjuvants revolutionised salmon aquaculture. Oil-adjuvanted vaccines are stable emulsions of various types prepared using the aqueous vaccine, a mineral or non-mineral oil, and usually a small amount of surfactant to help the emulsion form and remain stable. The level of protection and duration of immunity afforded with oil-adjuvanted killed bacterial vaccines against a range of bacterial diseases in salmon is unprecedented, but the way in which oil adjuvants work is unclear. They may improve antigen presentation or stimulate better cellular immunity, but one of the major modes of action appears to be a depot effect – the oil emulsion

remains in the peritoneum and prolongs release of the antigen. Indeed tiny amounts of oil emulsion can occasionally be found within the peritoneum of salmon during harvest. This is considered acceptable in salmon as they are generally sold as fillets. However, in fish that are sold round for the plate, market acceptance of oil-adjuvanted vaccines has been slower.

Side effects of oil-adjuvanted vaccines have received a lot attention. In some cases, a localised immune reaction might occur around the oil in peritoneum, resulting in melanotic adhesions that make the fillet unmarketable. Many comparative studies have been performed to try to determine why adhesions occur in some fish and not others, and with some vaccines, but not others. Several hypotheses have been put forward, with explanations ranging from toxicity of the bacterin components (the adjuvants alone do not seem cause adhesions), to poor practice during injection (such as use of blunt or oversized needles) and resulting infection of the injection pore. Regardless of the cause, customer dissatisfaction has prompted development of micro-dose vaccines (given in very small volumes) by some companies, and these seem to reduce the problem.

19.7.2 Adjuvants for other types of vaccine

Currently there are no adjuvants routinely used for immersion vaccines, although adjuvants that stimulate systemic as well as mucosal immunity after immersion vaccination would be extremely useful against some diseases. With the advent of DNA vaccines, inclusion of host cytokines within the vector so that they are expressed along with the target antigen is an intriguing concept. Cytokines are signalling molecules secreted by cells of the immune system to activate immune processes such as T-cell maturation and macrophage activation. Detailed knowledge of cytokine mechanisms of action and discovery of the genes in the target species is an area of research that is progressing rapidly in fish at present and has recently been reviewed (Secombes, 2008).

19.8 VACCINATION IN PRACTICE

The type of vaccine, presence or absence of an adjuvant, route by which it is delivered can be recommended by vaccine manufacturers after extensive experimental and field use of the product. Some indicators of how all of these factors interact and effect time to protection and duration of immunity are presented in Table 19.3.

The development of reliable vaccines, inclusion of adjuvants and a raft of supporting research into virulence of fish pathogens and immunity in teleost species have made a major contribution to the economic success and improved environmental impact of fish farming. However, the technology would not work without carefully developed farming practices that have maximised the performance of the products. It is imperative to vaccinate healthy fish. In sick or stressed fish, at best, immunity will not develop and at worst, the vaccination process will kill the fish. The need for healthy fish for vaccination coincided with significant improvements in hatchery management and biosecurity. Hatcheries are spotlessly clean, broodstock may be routinely tested for notifiable diseases, eggs are disinfected with ozone or licensed biocides before being brought into

Table 19.3 Effect of vaccine type and route of administration on immune response and duration of immunity (DOI) in fish.

	Adjuvant	Systemic immunity	Cell-Mediated	Mucosal immunity	DOI
Killed					
IP injection	Oil	Yes	No	No	Long
IM injection	No	Yes	No	No	Modest
Immersion	No	No	No	Yes	Short
Recombinant					
IP injection	Oil	Yes	No	No	Long
IM Injection	No	Yes	No	No	Modest
Live attenuated					
IP injection	No	Yes	Yes	No	Long
Immersion	No	Yes	Yes	Yes	Modest
DNA					
IM injection	In-line cytokines	Yes	Yes	No	Long

the hatchery, water is treated before flowing through the hatchery and personnel follow strict biosecurity procedures for entry and introduction of equipment. In this way, disease is excluded from the hatchery until juveniles (now specific pathogen free) are large enough to vaccinate. Stress of the animals during vaccination is eliminated by careful fish handling and injection under anaesthesia. The advent of highly selected domesticated lines of fish has also had a significant impact on practical use of vaccines. Domesticated lines of salmonids are much harder to stress than their wild counterparts, evidenced by lower cortisol levels. Coupled with the lower individual variability among domesticated salmon, more uniform response to vaccination with fewer non-responders is generally achieved. Water temperature and size of fish at vaccination are also taken into account to maximise the efficacy of the vaccine. Generally an optimum size range of fish and optimum water temperature range for safe vaccination will be recommended by the vaccine manufacturers' technical teams or by experienced veterinarians after careful experimental laboratory and field validation. Provided hatchery procedures have been followed, the label indications for the vaccine adhered to and the fish are handled carefully during and after the procedure, the vaccine will provide protection against the disease agents it contains.

19.9 RESEARCH AND DEVELOPMENT TRACK FOR COMMERCIAL FISH VACCINES

Projects to develop and register new vaccine products have to be carefully managed to keep them progressing and make sure that they do not cost more money than the final product market is worth. The research and development stages for a typical commercial vaccine project are shown in Fig. 19.8.

19.9.1 Research phase

The early phase of research or discovery phase is essentially screening and experimentation to find key antigens, strains with particular properties or culture conditions with promise for a defined problem within a key market. The most promising candidates will then be taken forward for further research and evaluation to deliver proof of concept, preliminary safety and efficacy information and to allow some optimisation of growth and delivery conditions. At this stage, provisional production outlines may be drawn up to enable potential cost-of goods to be estimated for internal rate of return calculations on the potential product. This is required even at this very early stage, as the key decision on whether to proceed into development needs to be made at this point in the project. Research might seem expensive, but once into the development phase, costs really start to escalate. Moreover, researchers tend to become very attached to projects and an objective decision to let a project lapse if necessary is critical. Therefore the market potential of the product needs to be established, the likelihood of high efficacy/ease of use needs to be clear, and feasibility of production scale-up and registration needs to be considered.

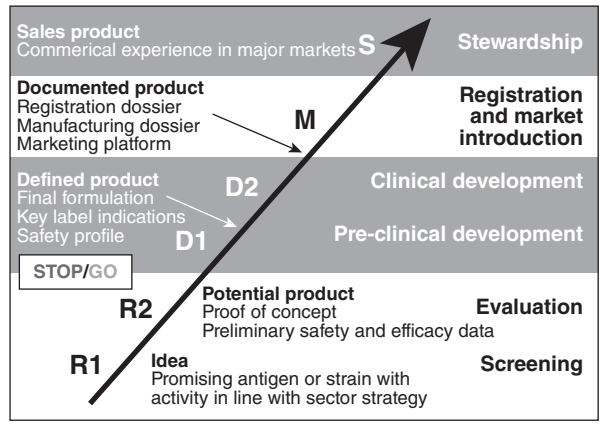


Fig. 19.8 Flow diagram showing a typical research and development track for a new commercial vaccine for aquaculture.

19.9.2 Development phase

Pre-clinical development will involve laying down documented master seed stocks of any organisms in the product. The formulation of the end-product must be finalised (e.g. concentration, adjuvants, etc.). The production outlines will be finalised and scaled-up production batches made under good manufacturing practice. Quality control and quality assurance methods and parameters must be set. The label indications will need to be determined and any label claims proven experimentally under controlled laboratory conditions using good manufacturing practice production batches. This will include duration of immunity, time to onset of immunity and shelf life (it is important to store documented batches of vaccine early in this phase because to determine shelf life one has to prove there is no loss of efficacy on storage – it can take 18 months to claim a 12 month shelf-life for a product). The safety profile must be determined and specified. Essentially, at the end of the pre-clinical development phase, there must be a fully defined product.

Clinical development is the generation of all the required documentation for registration in target countries, developing a manufacturing dossier and creating a marketing

platform in anticipation of product launch. The registration dossier will include safety and efficacy data determined under laboratory conditions (usually under some form of quality assurance scheme such as good laboratory practice or those of the International Organization for Standardization (ISO)). It will also include clinical efficacy and safety in the field after granting of necessary animal test certificates, often conducted under the reporting conditions of good clinical practice. A marketing platform will be established using field and laboratory efficacy data to support the product. The culmination of this phase is the full registration and product launch.

19.9.3 Stewardship

The project management plan does not stop with the product launch. Most vaccine companies have skilled technical teams comprising scientists and veterinarians who will steward the product through its lifespan in the field. Only extensive experience of the product in key markets will enable any problems to be identified and remedied. This is an ongoing process that requires a good working relationship and flow of information between the vaccine company and farmers, and with regulatory agencies should modifications be required.

19.10 CONCLUSIONS

A former colleague once explained to me that vaccinating fish was rather like buying an insurance policy: the farmer was buying reassurance in case disease arose during the production cycle. To my mind, fish vaccines are very much better that. An insurance policy is designed to minimise the risk that it actually has to pay out. In contrast a vaccine is designed (and rigorously tested) to *guarantee* that it will perform every single time: it always pays out. The quality of the science behind commercial fish vaccines therefore has to be excellent, as its validity will be scrutinised by farmers, health managers and veterinarians time and again through the performance of the vaccines in the field. Peer review for academic scientific publication does not even come close in terms of repetition and rigour.

History shows that the advent of reliable oil-adjuvanted injectable vaccines was one of the key enabling technologies in salmonid aquaculture. Their development allowed expansion where previously there was constraint by disease, and reduced chemical use for disease control to negligible levels by exploiting the natural adaptability of the fish immune system. Vaccines for salmon now represent some of the most advanced technologies and some of the most cost-effective vaccines in existence. The perseverance and risk-taking in research and development by (at the time) very small biotechnology companies to get the technology to work, the open mindedness of regulatory agencies and most of all the willingness of salmon farmers to change practices to adopt the new technology were essential to this success story. Vaccine use and development has spread into most regions where aquaculture is practised and to most farmed finfish species over the past decade. There are some significant challenges ahead, particularly in warm-water species where strain variability is proving challenging in some diseases, and in the development of vaccines against parasites such as the salmon louse, which will require innovative solutions. But these challenges will be met and the resulting technologies will be adopted by farmers because, as history has demonstrated, the science will be excellent and the price will be right.

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20
Soft-shelled Turtles

Qingjun Shao

20.1 INTRODUCTION

The Chinese soft-shelled turtle, *Trionyx sinensis*, is a highly valued species for farming in the Asia region (Figs 20.1 and 20.2). Traditionally, soft-shelled turtle is considered a medicinal cuisine due to its high nutritional value. In China, soft-shelled turtles were cultured in common carp ponds in order to control fish stocking density. This is according to the first record in aquaculture history by aquaculture founder Mr. Fan Li in 'Fish Farming Experiences', more than 2400 years ago (Ling, 1977). Intensive culture of soft-shelled turtles in China can be traced to the late 1970s, and the development may be divided into three phases.

The first phase of modern development was from the mid-1970s to the early 1990s. In this phase, the culture of soft-shelled turtles depended mainly on wild-caught turtles. Soft-shelled turtles were farmed at low density in polyculture ponds and production was very limited. However, this phase led to the development from polyculture in ponds to low-density monoculture. Soft-shelled turtles, fed mainly with trash fish, were of relatively good quality. Farming areas were mainly distributed in the middle and lower Yangtze River Basin, including the provinces of Hubei, Hunan, Jiangxi, Zhejiang and Jiangsu. Products were mainly sold to local regions, but they were also sold to Guangdong and Hong Kong because these regions provided a higher product price due to the higher income of their people during that time.

The second phase was from the early to mid-1990s. It was a period of rapid development (Fig. 20.3). With the improvement in people's living standards and income, the demand for soft-shelled turtles increased rapidly in Chinese coastal regions and bigger cities. At the same time, aquaculture technology developed into intensive monoculture indoors in an environment with water temperature controlled around 28–32 °C. Stocking density significantly improved, with about 40–80/m^2 of turtles from hatchlings up to body weight 50 g and about 15–30 turtles/m^2 for body weights greater than 150 g each. Trash fish were replaced by high-quality eel feeds, which significantly improved the turtles' growth rates. The culture period from hatchlings to marketable size (ca. 350–500 g) was reduced to 12 months or so. There were, however, frequent outbreaks of diseases due to the high stocking density and poor water quality in indoor concreted tanks. Many kinds of antibiotics and other pharmaceuticals were used to prevent and treat diseases and, as a result, pharmaceutical residues in the soft tissues of soft-shelled turtles were in excess of safety levels.

According to the statistics of the Ministry of Agriculture Fisheries, the annual production in the China mainland in 1996 was about seven times more than that in 1993. The price of the soft-shelled turtle increased to 580 Chinese yuan (CNY)/kg (about US$85). Hatchlings were 30–35 CNY each and the price of broodstock reached more than 1000 CNY/kg in Zhejiang Province, which was the one of the major regions for soft-shelled turtles. Driven by high profits, various regions entered the turtle culture industry, promoting the rapid development of soft-shelled turtle farming.

The third phase has been from the mid-1990s to the present. It is a continuing phase of rapid development (Fig. 20.3). Owing, however, to uneven quality and taste of meat, overproduction and steadying of market demand, prices declined (Fig. 20.4). Farming methods changed from the second phase of high-density indoor or outdoor

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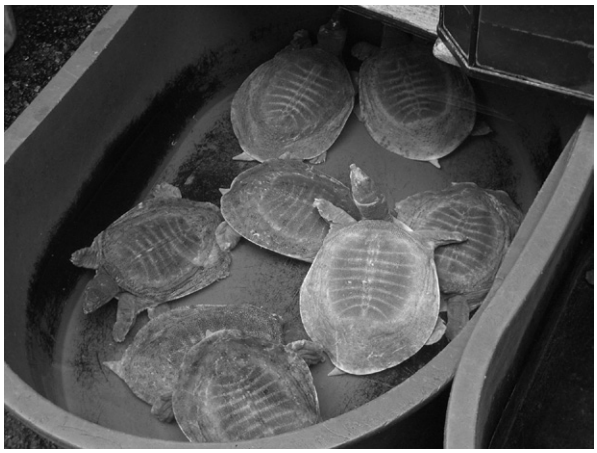


Fig. 20.1 The Chinese soft-shelled turtle, *Trionyx sinensis*. (Photograph by Vladimir Menkov.)



Fig. 20.2 Chinese soft-shelled turtles, *Trionyx sinensis*, including some bagged for sale.

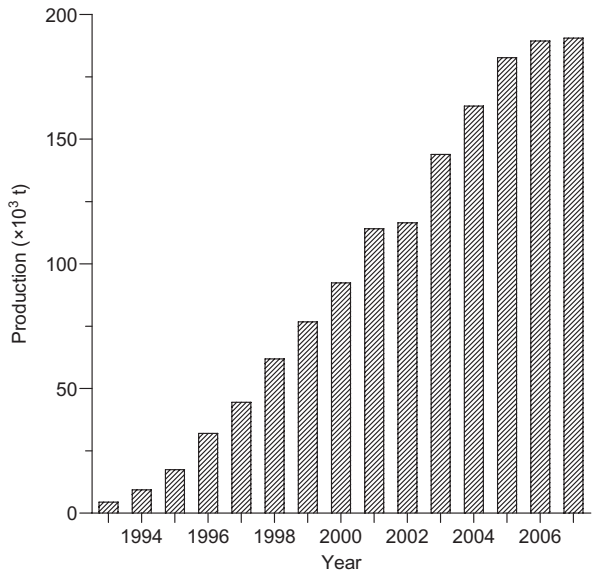


Fig. 20.3 Annual production of soft-shelled turtles in China from 1993 to 2007.

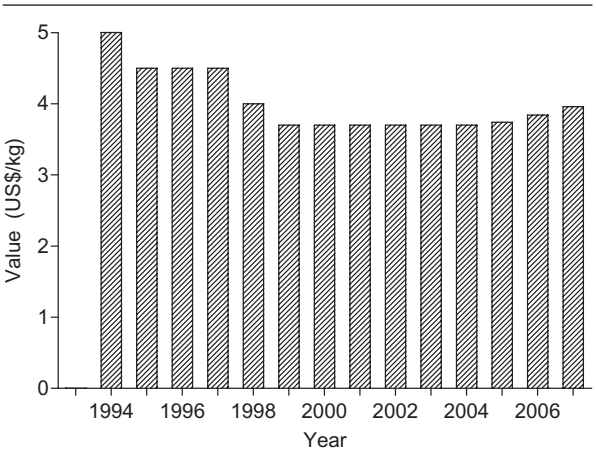


Fig. 20.4 Value per kilogram of soft-shelled turtles in China over the period 1994–2007.

monoculture or both to lower stocking densities together with an increasing level of fish and turtle polyculture. With further improvement of farming techniques, disease prevention, strains and hybrid selection, production and demand for soft-shelled turtles in mainland China were almost balanced. Farming techniques tended to mature and it was almost the most profitable aquaculture practice in China. The result was that soft-shelled turtle culture in indoor dark tanks and outdoor earthen ponds was continuously developed, and more and more companies and businesspeople invested in the turtle farming.

At present, approximately 100 000 people are engaged in the soft-shelled turtle farming industry in China. Now the production techniques are excellent and compare well with those for other cultured species in mainland China.

20.2 BIOLOGICAL CHARACTERISTICS

The Chinese soft-shelled turtle, *Trionyx sinensis*, is known by the colloquial names Zhong-hua-bie, Jia-yu (shelled fish, with 'shell' bone) and Wang-ba in Chinese. In mainland China, there are only two genera and six species of soft-shelled turtles. The two genera are *Trionyx (Pelodiscus)* and *Palea (Trionyx)*. The widespread distribution of *Trionyx sinensis* in China even includes Qinghai, Xinjiang and Tibet in north-western parts of China. Furthermore, farming this species has spread to all provinces and autonomous regions in China. Beyond China, this species is also distributed in Japan, the Korean Peninsula and some south-east Asian countries. *Palea steindachneri* is listed in Appendix III of the CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora) Convention and as an Endangered Species in the IUCN (International Union for Conservation of Nature) Red List (2007). Farming has considerably impacted the wild populations, but farming of *Palea steindachneri* continues.

20.2.1 General

Soft-shelled turtles (family Trionychidae) do not have the usual hard, fused plates that make up the carapace (shell) of most turtles. The central part of their carapace has solid bone beneath it like other turtles, but its surface is leathery and especially flexible along the sides, which are known as 'skirts'.

Chinese soft-shelled turtles are amphibious; they usually live in salinities of not more than 3–5‰. They prefer secluded regions of rivers, lakes, reservoirs, ponds, pools, mountain streams, shore sand and bushes. They are belligerent, aggressive and predatory, but sensitive to the movement of objects and other kinds of animals in their vicinity. Their lungs are used for respiration both on the land and in the water, as they rise to breath. They also use a pharyngeal organ with villi-like gills to get oxygen from the ambient water, especially when immersed during their hibernation.

As reptiles, their body temperature varies and is similar to their immediate environment. Thus, they are sensitive to variations in water temperature. Their metabolic rate declines quickly when the water temperature drops below 20 °C. They stop feeding at temperatures below 15 °C and hibernate under pond mud or in wet soil above the pond water level when the temperature is below 10 °C. When the water temperature is above 35 °C in summer, the turtles retreat under the shade of trees. The suitable temperature range for the turtle's growth is about 20–35 °C and the water temperature for optimum growth is 28–32 °C, especially in indoor culture.

During hibernation, there is little respiration and a very low metabolic rate. The lungs' respiratory function is replaced by the pharyngeal villi for obtaining oxygen in water or in wet sands. After hibernating for 5–6 months over winter, the body weight is reduced by 10–15%, and some physically vulnerable turtles may die during a long period of hibernation, especially when hibernating in anoxic conditions under mud with a high content of organic matter. Soft-shelled turtles usually emerge from the water and bask for 2–3 h/day on sunny days in summer to kill surface pathogens, for example fungi and bacteria. This helps to promote shell growth, and body calcium and phosphorus metabolism. It also increases their body temperature and metabolic rate if the water temperature is not high enough for good growth. So, it is very necessary to keep 5–10% land beside an earthen pond for basking or to build a similar amount of accessible basking roof above water level.

20.2.2 Diet characteristics

Soft-shelled turtles are voracious feeders and omnivorous, but high-protein animal food is favoured. In the wild, juvenile soft-shelled turtles search for large zooplankters, aquatic insects and other invertebrates, such as aquatic worms (*Limnodrilus hoffeisteri*) and invertebrate larvae, as their food. As juveniles grow, they begin to prey on shrimp, tadpoles and small fish. When animal food is short, they will feed on aquatic and terrestrial plants, and scavenge on animal carcasses. In culture conditions, they are fed on high-quality artificial feeds, internal organs of poultry, small fish, shrimp, snails and clams, depending on the culture method. Plant feeds such as various seeds, melons, vegetables, and leaves of aquatic and terrestrial plants may be used as supplementary feeds when necessary.

Although soft-shelled turtles can survive for a long period without any feed, their growth is inhibited and body weight reduced under such conditions. Excessive culture density or food shortage will cause cannibalism. This especially occurs when various sizes of soft-shelled turtles are cultured together: the small individuals may be eaten by the large ones. Also, the small turtles often cannot get enough feed for normal growth under those competitive conditions.

20.2.3 Growth

The growth of soft-shelled turtles is relatively slow in the wild. The hibernation period lasts about half a year in the Yangtze River Basin regions, and the growth period is not more than 6 months each year because the water tempera-

ture is not favourable, being less than 25 °C. In the wild, it usually takes 4–5 years for turtles to reach marketable size in the Yangtze River Basin regions. However, under artificial farming conditions where water temperature is kept around 30 °C, with good water quality and artificial feeds, the turtles grow rapidly and may reach a commercial size of about 400 g in 8–12 months.

Like many other cultured animals, the growth rates of soft-shelled turtle within the same batch of juveniles under the same conditions vary from individual to individual. This can lead to cannibalism and they need to be sorted and cultured in size classes during artificial culture.

20.2.4 Reproduction

Reproduction follows a typical reptilian pattern. The male fertilises the female through copulation. Eggs are fertilised in the female's reproductive system, developing internally for about 10–20 days and then laid to hatch later. Soft-shelled turtles reach sexual maturity in the wild as follows:

- at about 6 years of age in the north of the Yellow River (about 37 °N);
- at about 4 years in the middle and lower Yangtze River region (about 32 °N);
- at 3 years in the south of China;
- at just 2 years of age in Hainan (about 19 °N) and Taiwan (about 23 °N).

These differences result from the temperature regime during the turtles' growth and development. A further effect of temperature regimes between northern and southern China is variation in the oviposition period:

- from June to August in the most northern regions of China;
- from May to August in central and eastern China;
- from April to September in southern regions of China;
- from March to October in the Hainan and Taiwan islands.

In the middle and lower Yangtze River regions, sexually mature turtles mate when the water temperature increases to about 20 °C in April or May.

With daylength and temperature increasing, females gradually begin laying eggs as spring turns into summer. The female turtles lay eggs 2 or 3 weeks after mating. Eggs are usually laid at night when the surroundings are quiet. Laying sites are usually hidden places and eggs are buried in moist soft soil above the water level. To prevent direct sunlight, reduce evaporation, avoid groundwater flooding and maintain enough air in the nest, turtles make their individual burrows in shady places. They put sand back over the burrow with their hind limbs and smooth the surface flat with their plastron (undersurface of the carapace) to erase traces of the burrow and prevent pests from invading it. The hatchlings emerge from the burrow and move immediately down into the nearby water.

About 20 days later, the turtles mate again after the first egg laying. Adults can mate several times during breeding seasons each year or there can be multiple egg batches after one mating. Sperm can survive in the female's fallopian tubes for 5 or more months. Although soft-shelled turtles lay eggs several times a year, the number of eggs per batch, the timing, number of egg batches and egg weight are directly related to the female's body size, age, nutritional status, geographic location and genetics, etc. Approximate numbers of eggs per batch range from several to more than 20, and three to five batches of eggs may be produced in a year by a female turtle.

20.3 COMMERCIAL CULTURE

Chinese soft-shelled turtles have always been a popular cultured species, even in the early history of China more than 2400 years ago. Traditionally, soft-shelled turtles supplied in markets were from local ponds, reservoir, rivers and lakes. The regional popularity of the turtle stimulated interest in pond culture in the early 1950s and 1960s. In the early 1980s, soft-shelled turtles in ponds were popular in the Yangtze Basin and Pearl Basin areas including the provinces of Zhejiang, Hunan, Hubei, Jiangxi, Jiangsu, Anhui, Guangdong and Guangxi. Large-scale indoor culture of soft-shelled turtles on an intensive scale started in the 1990s, as described in section 20.1. Expansion was mainly stimulated by high profits with significantly reduced culture time, lower price of soft-shelled turtle seedlings and development of feed and culture technology.

Local markets remained the outlet for all productions because the incomes of the people in these two basins were higher than other regions in mainland China. The total production yield in mainland China is listed in Table 20.1.

In recent years, the province of greatest soft-shelled turtle production in China has been Zhejiang, which is located south of Shanghai and near Lake Taihu. The Great Canal of Beijing, extending to Hangzhou and the Qiangtang River, crosses the province. Based on estimates from the Zhejiang Bureau of Ocean and Fisheries, the total amount of soft-shelled turtle production in Zhejiang Province in 2008 was between 110 000 and 120 000 t (about 52% of the total soft-shelled turtle production in mainland China). In terms of marketing, there were about 41 000 t from

Table 20.1 Total production of Chinese soft-shelled turtles in China from 1993 to 2008. Data from Statistics of China Fisheries, 1993 to 2008, Bureau of Fisheries, China Agriculture Ministry.

Year	Yields (t)
1993	4427
1994	9360
1995	17445
1996	32004
1997	44460
1998	61881
1999	76731
2000	92343
2001	114077
2002	116436
2003	143816
2004	163257
2005	182610
2006	189332
2007	190469
2008	204139

Jiaxin City, 27500t from Hangzhou City, 22000t from Huzhou City and 5200t from Jinhua City. The total value was about 5 billion CNY. The total culture pond area is about 7300ha (7.3×10^7 m^2), and the total area of indoor tank culture with temperature control is about 7.5×10^6 m^2.

20.4 CULTURE METHODS AND FACILITIES

The culture methods for soft-shelled turtles consist mainly of outdoor earthen ponds, indoor concrete tanks, and a combination of both through the phases of development. The quality of turtles cultured indoors in concrete tanks is lower than those from earthen pond culture, and pharmaceuticals are used more frequently to prevent diseases in indoor culture. However, the period of rearing to marketable size is shorter and the costs per kilogram of turtle produced are lower for indoor culture than outdoor pond culture. Thus, indoor culture is attractive as a more economic industry. Currently, indoor cultured turtles account for more than half of the total production.

20.4.1 Indoor culture

The construction of indoor concrete tanks must comply with relevant building requirements for soft-shelled turtles. They must have good insulation properties, and be equipped with appropriate heating, aeration, drainage and other facilities. The size of tanks is standardised at 15–45 m^2 bottom area with 60–120cm height. The upper tank walls curve inwards 8–10cm to prevent the turtles from escaping. Net bags are placed in lines in the water, at 20–40cm spacing, 10–15cm above the bottom of the tanks and 5–10cm above the water surface in order to add hiding places and so reduce the level of biting in high stocking densities of turtles. Net bags used in indoor tanks are shown in Fig. 20.5. Culture in the dark also assists in reducing the level of biting.



Fig. 20.5 Net bags that are used as cover in indoor tanks.

More and more farms are using temperature-controlled indoor tanks to rear the soft-shelled hatchlings up to 50–150g each, at which size they are then transferred to earthen ponds for subsequent grow-out. In this way the hatchlings are cultivated in controlled intensive conditions to improve growth and survival rates. This is called a two-stage method for commercial culture of soft-shelled turtles.

20.4.2 Pond culture

Pond culture of soft-shelled turtles includes monoculture, and polyculture with fish, shrimp and aquatic plants. The quality of the soft-shelled turtles cultured in this way is quite similar to that of wild turtles and is much better than those cultured indoor in dark tanks. Furthermore, this culture method also improves the usage of water, with less water exchange and less energy required to supply water to the culture system. The farmer's income will increase when prices for pond-reared turtles are higher.

Before stocking in earthen ponds, there is work needed such as the following:

- clearing surplus silt;
- checking the water inlet and outlet;
- building escape-proof walls;
- disinfecting the pond with quicklime.

Water must be kept 1.0–2.0 m in depth, with 10–25 cm bottom sludge, and the slope of the pond walls must be 45° or less. It is necessary to keep the dry land or platform about 5–10% of pond water area for the turtles to bask during the daytime. The height of escape-proof walls must be 40–50 cm.

The stocking density depends on turtle size or body weight, pond depth and how much yield the producer hopes to gain. Suggested stocking densities of turtles are shown in Table 20.2.

Turtles are stocked into grow-out ponds when water temperature reaches about 25 °C, from March to May in China, and the optimal body weight of turtles is 150–250 g. Before stocking, turtles are disinfected with 3% salt water or 20 mg/L potassium permanganate for 5–10 min. All kinds of ponds are built with an escape-prevention structure or enclosure in order to confine the turtles (Fig. 20.6).

To have good pond water quality, the soft-shelled turtles are polycultured with other aquatic animals such as filter-feeding fish and bottom feeders, and aquatic plants such as water hyacinth and water lettuce. In general, to avoid fish taking the turtles' feed, feeding sites are established near the pond water level. Feeding sites are shown in Fig. 20.6. To control small feral fish, some carnivorous species of fish, such as mandarin fish, may be stocked at the same time. The stocking densities of the fish are based on what natural food sources are available in the ponds; the suggested numbers are listed in Table 20.3.

Table 20.2 Recommended stocking densities of Chinese soft-shelled turtles in earthen ponds.

Body weight (g)	Stocking density in earthen ponds (number/m^2)	Suitable earthen pond sizes (m^2)
4–50	6–12	50–100
51–150	3–6	500–1 500
>150	1–2	500–5 000
Broodstock	0.5–1	1 000–15 000

In the process of culture, it is necessary to pay considerable attention to management of water quality and disease prevention. There is no need to set up aerating machines in ponds because the various species in polyculture are



Fig. 20.6 The edge of a pond with walls to prevent turtles from escaping and feeding sites for placing food along the slope above the water level, out of reach of fish.

Table 20.3 Recommended stocking densities of various fish species polycultured in earthen ponds with Chinese soft-shelled turtles.

Fish species	Body weight (g)	Stocking density (number/ha)
Silver carp	100–200	500–200
Bighead	100–200	150–450
Crucian carp	20–100	300–750
Common carp	20–100	300–600
Grass carp	150–300	100–500
Mandarin fish	5–10	100–300

feeding in their niches at a reasonable nutritional level, and water quality can be maintained. However, the water quality of ponds requires pH 7–8, total alkalinity 0.5–3.5 mmol/L, transparency 25–35 cm, the water colour being yellow-green or tea green. Quiet circumstances are required when the soft-shelled turtles are drying their backs on sunny days. Particular attention to the water outlet and inlet is required to prevent turtles escaping. Quicklime or biological agents are used to regulate the water quality every 15–20 days if the water is too fertile. Diseases are common in ponds and there may be regular use of quicklime or a mixture of copper sulphate and ferrous sulphate, at a ratio of 5 : 2 and concentration of 0.7 g/m^3. Medicated feeds may be used, such as those used for fish, if the turtles are not in good health.

20.5 CULTURING THE DEVELOPMENTAL STAGES

20.5.1 Breeding

Broodstock turtles are cultivated in specialised outdoor ponds. The pond is disinfected with the application of quicklime or bleaching powder (chlorine) before stocking. The optimum stocking density is 0.5–1.0 turtles/m^2. Broodstock are stocked before the autumn season so that they can have enough time to adapt to the new living environment and get enough nutrients for reproduction in the next summer. They are given specific artificial feed or natural feed, such as fresh trash fish, prawn, snails, earthworms and vegetables in the ponds. Daily management requires strict feeding times and locations, stable feed quality and suitable quantity, and a quiet environment. Meanwhile, the water depth and transparency of broodstock ponds are maintained at 150–180 and 35–40 cm, respectively. Specific attention must be paid to the management of water quality and to disease prevention or control.

All male and female soft-shelled turtles selected as broodstock must be healthy, without any injury and more than 3–4 years old. They must be physically robust, and more than 1.0 and 2.0 kg body weight for males and females, respectively. The appropriate female to male ratio is 3–5 : 1 according to different lines and sexual maturity. The fertilisation rate of eggs and hatching rate are normally higher than 85% if the culture period and fertilisation of eggs are normal. A higher ratio of females to males will reduce the egg fertilisation rate, whereas a lower ratio of females increases fighting among the males, which effects the subsequent survival and recovery of females after laying.

About 3–5% of the broodstock pond area is established at the north or west side of the pond for turtle laying at night during the breeding season, which goes from May to late August. The laying site consists of about 30–40 cm of fine moist sand under a shed with a cover to protect the eggs from sunlight and rain. The laying site environment must be stable for water content, sand temperature, air movement, etc. To prevent water flooding the laying site, it is set about 40–60 cm higher than the pond water level. It is connected by a slope so that turtles can crawl from the pond to the laying site (Fig. 20.7) and return to the pond after laying their eggs. Some specific structures are needed near the laying site to prevent rodents, snakes and other wildlife from eating the turtle eggs.

Twelve to fourteen hours after the female has laid, all the eggs are collected and fertilised eggs selected for hatching. The animal pole of a fertilised egg must be uppermost; otherwise the egg will not hatch.

20.5.2 Hatchery practices

There is natural and artificial incubation. Natural hatching outdoors in pond culture is only 10–40% successful because of unfavourable weather and harmful animals, while hatching success under artificial conditions is normally more than 90%. During artificial incubation, the interior temperature is kept between 28–34 °C, the relative humidity at 75–85% and the moisture content of the sand bed at 8–12%. Ventilation facilities are needed to supply sufficient oxygen to the eggs. Close attention is paid to orient the pale or white animal pole of fertilised eggs uppermost. Two kinds of sand may be chosen for incubation: river sand and soil sand. Clean river sand with grain size 0.5–1.0 mm is best for egg incubation.

The incubation procedure is to first cover the bottom of the hatching box with 5–6 cm of sand, and then the fertilised eggs are put into the sand with animal pole uppermost (Fig. 20.8). Finally, the eggs are covered with 3–5 cm



Fig. 20.7 A laying site for soft-shelled turtles. It consists of a raised area of soil along the edge of the pond. There is a covering roof and ramps for the turtles to crawl up to the laying site.



Fig. 20.8 Orderly rows of fertilised turtle eggs on a base of sand in a hatchery box.

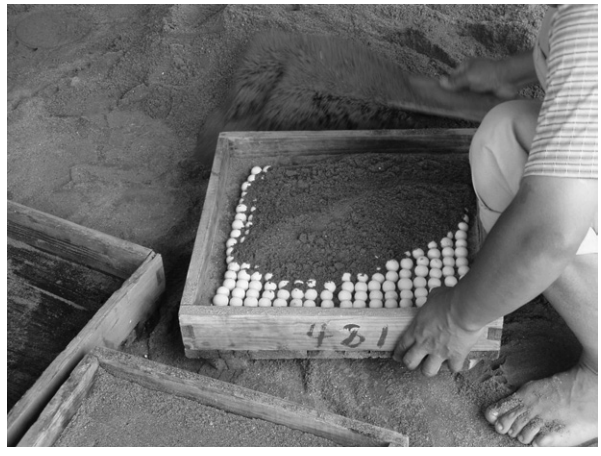


Fig. 20.9 Covering the eggs with more sand.

depth of sand (Fig. 20.9). Several layers of eggs may be kept in a wood container.

The egg boxes are stacked and stored in a hatchery room (Figs 20.10 and 20.11) Incubation time depends on accumulated temperature, i.e. degree–hours. For example, if the average temperature from July 24 to July 30 is 28 °C, the accumulated degree–hours is $28 \times 144 = 4032$ degree–hours. Incubation takes about 36 000 degree–hours. The best temperature for incubation is 30–32 °C, and it takes about 45 days to complete the incubation period (i.e. reach the approximate degree–hours). If sand temperature is a little higher than 32 °C during the incubation period, the proportion of male turtle hatchlings will be more than females.

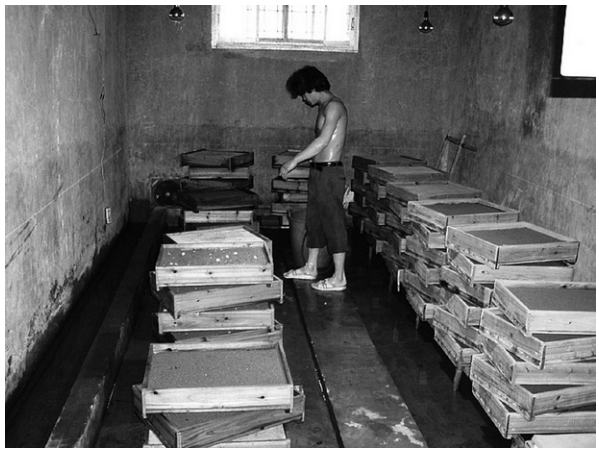


Fig. 20.10 Stacking the egg boxes.

20.5.3 Nursery pond management

The newly emerged hatchling turtles absorb and utilise their remaining yolk, so they do not need any feed during the first 2 or 3 days. After that, they are fed on artificial feed. However, traditional feeds such as zooplanktonic copepods, aquatic worms (*Limnodrilus hoffeiste*), liver, fish, shrimp, snails, etc. may be used. It takes 3 months for the body weight of hatchling turtles to increase from 4 to 50 g in the nursery pond. It is the most critical phase because of easy attacks by predators such as carnivorous fish, birds, frogs and snakes, and by fighting each other. A little negligence of management will result in diseases and high mortality rates.

The turtles are fed twice a day, initially at 2% of body weight and gradually increasing to about 3–5% when using artificial feeds. In practice, the amounts of feed are based on many factors, such as feed palatability, nutrient content, water quality, weather condition, turtle health condition and so on. In optimum culture conditions, water temperature is about 30 °C, transparency is 25–35 cm, while water colour is light green or yellow green. As they grow, there is size variation within batches. Turtles must be sorted and separated into similar-sized groups to prevent them from fighting and harming smaller ones. Because of the high-density and ready morbidity, it is very important to prevent disease outbreaks, especially from hatchling to juvenile stages.

20.5.4 Grow-out

Soft-shelled turtles are generally reared indoors up to 150 g body weight. When the outdoor temperature reaches 25 °C in the middle of May to early June, turtles are transferred from indoors to outdoors. To reduce the stress of

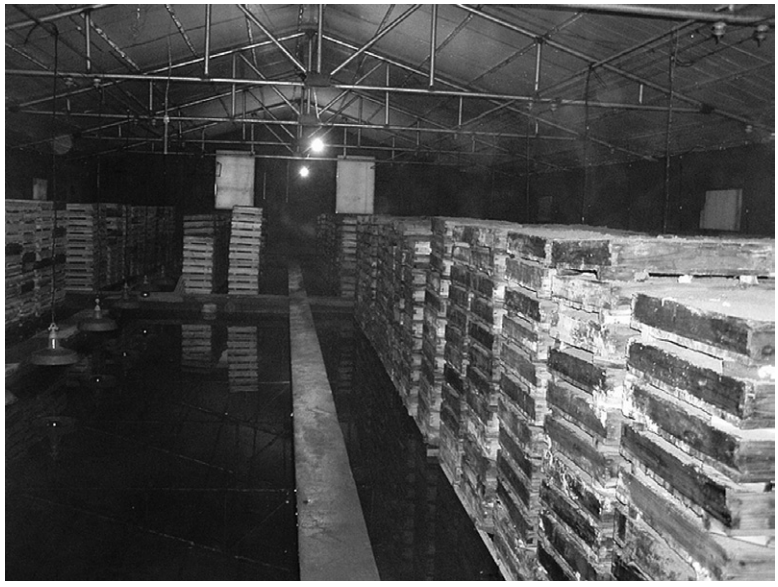


Fig. 20.11 Tall stacks of egg boxes in a hatchery room.

water temperature change, the temperature difference between indoor and outdoor is kept to less than 3 °C. By the end of October, turtles reach marketable size (above 400 g). The optimum stocking density in earthen ponds is two to four turtles/m^2. Turtles are fed twice a day, according to 2–3% of body weight, at 07:00 and 17:00. In the process, it is desirable to keep quiet and not disturb the turtles from feeding and basking in the sun during the daytime. Basking areas are set near the feed platform or a feed platform may also be used for basking to reduce the amount of activity. As in cultivating broodstock turtles, pharmaceuticals are used for disinfection in water and medicated feeds are administered according to the government regulations when turtles are infected.

20.6 WATER QUALITY

20.6.1 Management

Water management in earthen ponds is very important for soft-shelled turtles culture, as it is for fish culture in earthen ponds. It is necessary to keep water transparency to about 25–35 cm to avoid turtles biting each other, and to avoid fungal and parasitic diseases. If the water contains too much plankton, however, there is need to stock with more filter-feeding fish such as silver carp and bighead. Alternatively, 5–25% of the pond surface area may be stocked with water hyacinth or water cabbage or other water plants to absorb nutrients from the water pond (Wang *et al.*, 2007). Meanwhile, quicklime at 20–30 g/m^3 may be used every 2 weeks or some of the pond water exchanged with new water. After harvesting, the pond bottom mud is removed and the pond bottom left to dry in the sun for 20 or 30 days so that bottom organic matter can be broken down and be disinfected. Fertilisers such as pig manure or poultry manure are used when filling a pond to promote plankton growth or these are used if the water has poor plankton content during the turtle farming period. Water transparency of the turtle pond decreases as the plankton grows. It is undesirable to have trees near earthen ponds, especially on the east and south sides, in order to maximise the sunlight on the ponds and thus promote plankton and warm water.

20.6.2 Water parameters

Dissolved oxygen (DO), nitrogenous compounds, pH, hardness and other parameters of waters strongly influence the soft-shelled turtle's feeding and growth. Generally, DO is maintained at not less than 5–6 mg/L. Although soft-shelled turtles mainly use their lungs to breathe, they have requirements of DO in the water for using pharyngeal villa respiration, especially during hibernation. High DO can also effectively prevent the anaerobic decomposition of organic matter in the water, reducing the levels of NH_4^+/NH_3, NO_2^-, H_2S, CH_4 and other toxic compounds. Intensive farming, with high-density stocking and artificial feeding, results in considerable organic waste and low DO. So the process of renewal of DO is blocked and toxic substances, such as those above, which cannot be converted into non-toxic substances, result in 'low-dissolved oxygen syndrome', thereby affecting the healthy growth of soft-shelled turtle. Therefore, it is essential to maintain the DO of the culture water in the progress of soft-shelled turtle breeding.

Soft-shelled turtles prefer neutral or slightly alkaline water, pH from 7.5 to 8.0, because alkaline water can inhibit the growth of bacteria. The culture water is treated with lime or bleaching powder (chlorine) every 15 days at a dose of about 2–3 g/m^3 to regulate the pH and kill pathogenic microorganisms and parasites.

Ammonia nitrogen (NH_3-N) is the main toxic substance in the water that causes turtles to be poisoned if excessively accumulated. Therefore, the content of ammonia nitrogen in water is tested regularly to keep it less than 0.02 mg/L or keep water transparency at 25–35 cm. Other water parameters such as hardness and NO_2^- must also be regulated by water-control methods.

20.7 NUTRITION, FEEDING AND FEED FORMULATION

Feed costs are the largest cost in the production of Chinese soft-shelled turtles, accounting for 35–45% of the total cost. Efficient and economic feeds, together with effective feeding practices, are essential. Nutrition is the most advanced field of soft-shelled turtle culture and their general nutrient requirements have been established in recent years (Table 20.4). As for other animals in aquaculture, proteins, lipids, carbohydrates, vitamins, minerals, calcium and phosphorus are necessary to meet

Table 20.4 Nutrient requirements of Chinese soft-shelled turtles.

Nutrient	Requirement
Protein	43–50% (Qian and Zhu, 2002; Sun *et al.*, 1997)
Lipid	7–8% (Qian and Zhu, 2001, 2002)
Carbohydrate	18–25% (Sun *et al.*, 1997)
Ascorbic acid	186–192 mg/kg (Shao *et al.*, 2004)

the requirements for good growth and health of soft-shelled turtles.

20.7.1 Nutritional requirements

Protein is the most expensive component in balanced fish feeds and often the most important factor affecting growth rate of cultured species, and thus there are many studies about the optimal protein requirements of soft-shelled turtles. Most researchers consider that the optimal protein levels of this species are at or exceed 43% (see, for example, He *et al.*, 2002). Basically, the protein requirements of Chinese soft-shelled turtles reflect their requirements of the essential and non-essential amino acids. These amino-acid requirements have been estimated through studying the amino-acid content of muscle and 'skirt' (leathery edging of the carapace) tissue (He *et al.*, 2002).

There is no systematic report on requirements for lipids such as eicosapentaenoic acid and docosahexaenoic acid for soft-shelled turtles. However, additions of soybean or corn oil and fish oil in the feed are very helpful for supplying essential fatty acids and energy requirements.

There is a dietary requirement for ascorbic acid (vitamin C) to obtain maximum growth of soft-shelled turtles when cultured indoors in dark conditions, although soft-shelled turtle can synthesise a small amount (Shao, 2007; Shao *et al.*, 2004). Ascorbic acid has been provided in feeds in the form of phosphorylated L-ascorbic acid at 184–192 mg/kg of turtle.

20.7.2 Feed formulation and manufacture

The first stage of the feed industry for soft-shelled turtles, the extensive feed stage, was from the 1970s to the early 1990s. Its main features were directly feeding many ingredients with high food conversion ratios and high levels of waste. The second stage was from the early 1990s to the middle 1990s, the formulated feed stage. It was characterised by using compound feeds, such as commercial eel feed, and gradually optimising ingredients for soft-shelled turtles. The third stage of the industry is a relatively low-protein, high-quality stage, which is characterised by feed with reduced crude protein content and feed costs correspondingly lowered.

Feeds used in intensive pond culture of soft-shelled turtles are formulated to provide almost all required nutrients in the proper proportions. Typical commercial feed formulations are shown in Table 20.5. The feed protein is mainly provided by expensive imported white fishmeal. The crude protein content of white fishmeal is about 65%, while that of brown fishmeal (from oily fish) is about 55%. If a proportion of white fishmeal is replaced by a plant source of protein or microbe protein, the feed costs can be decreased to the advantage of the industry. The commonly

Table 20.5 Typical commercial feed formulation for Chinese soft-shelled turtles.

	Ingredient (%)		
Ingredients	Hatchling ≤50 g body weight	Turtle 50–150 g body weight	Turtle >150 g body weight
White fishmeal	60	52	48
Brown fishmeal	–	3	6
α-Starch	22	22	20
Wheat flour	–	–	2
Soybean meal	–	2	3
Fermented bean meal	2	3	5
Animal liver meal	5	3	2
Corn protein meal	–	2	3
Wheat gluten	3	3	1
Yeast meal	1	3	3
$Ca(H_2PO_4)_2$	1.5	1.5	1.5
Mineral premix	2	2	2
Vitamin premix	2	2	2
Zeolite powder	1.5	1.5	1.5
Total	100	100	100

used ingredients in commercial compound feeds are mainly white fishmeal, brown fishmeal, soybean meal, fermented bean meal, wheat gluten, corn protein meal, yeast, animal liver meal, α-starch, wheat flour, corn or soybean oil, mono-calcium phosphate ($Ca(H_2PO_4)_2$), vitamins and minerals.

All the solid ingredients need to be ground into fine particles smaller than 200 µm, and then mixed well. Then every 100 kg of compound powdered feed needs the addition of about 2–4 kg of plant oil or some fish oil to supply sufficient fatty-acid requirements and energy. About 20% water is added and a machine is used to made semi-moist feed pellets. The feed must be sufficiently viscous so that it is not easily spread by the turtle crawling around during its long period of feeding, which is about 30 min to 2 h according to its body size. Fine particles are required so that the feed can be digested and absorbed easily by the turtles.

20.7.3 Feeding

During the nursery stage, the turtles are fed three to five times per day. This begins in the early morning (06:00) and ends at night (22:00), especially in indoor culture. At body weight more than 150 g it is sufficient to feed the turtles twice a day, i.e. in the morning (07:00) and in the afternoon (17:00), as is practised in feeding fish. Feeding must be under water during the nursery stage to encourage the juvenile turtles to feed and grow well. At body weight more than 100 g, the feed is placed on a slope above water level where the turtles emerge to feed. This reduces feed waste and, in ponds, avoids the possibility of wild fish taking the expensive turtle feeds.

The food intake per day of the turtles during the suitable culture season is about 2–3% of body weight with artificial particle feeds or 5–10% of body weight with fresh feeds. However, the feeding rate needs to be adjusted according to weather condition, water temperature and water quality, turtle health and other factors. In farming practice, this means that the feed needs to be enough for a feeding period of about 2 h for nursery turtles and at least an hour for turtles with body weight of more than 150 g. Otherwise, there is insufficient feed and more is required. Alternatively, it is necessary to avoid overfeeding with feed loss and wastage, and potential loss of water quality.

Cleaning and using quicklime are very necessary to disinfect the feeding site after the turtles have finished feeding in order to reduce or prevent disease breakouts.

20.8 INFECTIOUS DISEASES

Soft-shelled turtles grow slowly and are seldom ill in their natural environment. However, with the development of intensive culture and modified environmental conditions in culture, there has been a significant increase in diseases of soft-shelled turtles. According to incomplete statistics, the overall disease incidence in farmed soft-shelled turtles is 20–40%. The average mortality rate may be 20–50% in some poorly managed farms. There are some common diseases, such as parotitis, white plastron (ventral portion of carapace) disease, haemorrhage, perforation disease, enlarged and red neck disease, skin ulcer disease, fungus disease (dermatomycosis), etc. During culture, one disease often results in many symptoms, and one symptom results from several diseases, so that it may be very difficult to diagnose and treat turtle diseases (Ye, 1996).

Controlling water quality is the most important way to avoid disease outbreaks.

20.8.1 Viruses

20.8.1.1 Parotitis ('mumps')

Symptoms: parotitis is evident in sick turtles that are generally drowsy and with a floppy neck so that the turtle's head cannot draw back into its shell. Their parotid (salivary) glands, mouth, oesophagus and intestines are abnormal. The liver is grey to brown colour and fragile. Turtles in the same pond will sometimes present two kinds of disease symptoms:

1. Parotid glands congested with blood and reddish; eroded, with secretions; mouth, oesophagus and intestines inflamed; plastron, limbs and tail are swollen with blood.
2. Parotid glands whitish and eroded, with secretions; oesophagus, stomach and intestine congested with necrotic tissue; plastron white without any blood colour.

There have been several studies on the prevention and control of parotitis, including Chen (1994).

20.8.2 Bacterial diseases

20.8.2.1 White plastron disease (haemorrhagic intestinal necrosis)

Pathogens: *Aeromonas hydrophila*, *Edwardsiella tarda*, *Proteus vulgaris*.

Symptoms: the bodies of sick turtles are pale, showing a state of extreme anaemia and oedematous. The liver is grey and brown with little or no blood; the gallbladder is swollen; the kidneys and spleen are dark. The body musculature is pale and bloodless. Juvenile turtles (100–200 g) are susceptible, particularly from May to July when the water temperature is 25–30 °C (Chen, 1994).

20.8.2.2 Putrid skin disease

Pathogens: *Aeromonas hydrophila*, *Aeromonas sobria*, *Pseudomonas* species and other bacteria.

Symptoms: surface erosion and ulceration are the main symptoms of this disease. Lesions can also occur on limbs, neck, carapace, skirts and tail. Initially, part of the skin is inflamed. Then there is necrosis followed by ulceration of the affected area, which gradually increases until muscles and bone are exposed. In severe cases, the neck bones are exposed, limbs rot away and claws fall off. This disease frequently occurs in high-density indoor culture (Wang and Fan, 1994).

20.8.2.3 Furunculosis disease

Pathogen: *Aeromonas hydrophila punctata*

Symptoms: in the early stage, small white bulges appear on the surface of the turtle's skin. They gradually become more prominent, expanding and forming boils. In severe cases these become festering holes in the carapace concurrent with the skin disease. This disease infects both juvenile and adult turtles, and is most common from May to July, when the temperature is at 20–30 °C (Li and Dong, 1994).

20.8.2.4 Perforation disease

Pathogens: *Aeromonas hydrophila*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Alcaligenes* species

Symptoms: in the early stage, white sores of 2–10 mm diameter appear on the soft-shelled turtle's dorsal shell, plastron and skirt. The sores develop scabs and bleed. The scabs soon fall off, with the original sore site left as a small hole with inflamed edge. There is bleeding from the interior of the hole in severe cases. This disease can infect all stages of the soft-shelled turtle, but occurs especially in juvenile turtles and especially during indoor culture (Li and Dong, 1994).

20.8.3 Fungal diseases

20.8.3.1 White speckle disease

Pathogen: *Mucor* species

Symptoms: white spots appear on the carapace of soft-shelled turtles in the early stages and are not easily detected. Then the white spots gradually increase and extend to other parts of the body, forming a white-spotted necrotic epidermis, which peels. This disease is common from April to June and mainly affects juvenile turtles in the nursery phase (Chen, 1994).

20.8.4 Protozoan parasites

There are several types of endoparasitic protozoa, such as *Trypanosoma* sp., Myxosporidia and Coccidia; whereas *Vorticella* is an ectoparasite.

20.8.4.1 Vorticella disease

Pathogen: *Vorticella* species

Symptoms: this stalked ciliate infests the surface of turtles at very high densities. The limbs, carapace, plastron, skirts and neck of the sick turtles have white lesions. When the water is deep green, the vacuoles of the ciliate are densely green and the whole surfaces of the soft-shelled turtles are green. The disease does not lead to the death of soft-shelled turtles, but their feeding and growth rate decrease (Wang and Fan, 1994).

20.8.5 Metazoan parasites

20.8.5.1 Leeches

Leeches are common ectoparasites of soft-shelled turtles. They infest the turtles' body surface, skirts and limbs, causing irritation, loss of appetite and body weight. This leads to malnutrition and, if serious, can cause death (Wang and Fan, 1994).

20.9 HARVESTING AND PROCESSING

After soft-shelled turtles reach the desired market size, they are harvested, transported to the processing plant and processed. These practices are relatively standardised across the industry.

20.9.1 Harvesting

Soft-shelled turtles may be harvested year around, according to consumer demand. However, they tend not to be harvested during the rapid growth period from July to September. There are many methods for harvesting. Hand capture or rake capture are effective when small numbers are requested. When large numbers of turtles are needed, purse seine capture or drying-pond capture are used.

- Hand capture. Collectors wearing protective pants, going downstream and using their feet in the sediment. When treading on a turtle, they capture the turtle using their hands.
- Rake capture. Rakes made of wood cause less damage to the soft-shelled turtles than metal ones. The rake handle length is about 1.5 m, the space between teeth is 10–20 cm. Rake teeth are inserted deep into mud. The soft-shelled turtles are detected by contact with the rake's teeth and then captured by hand. This method is used to capture adult turtles, broodstock turtles and turtles after hibernation.
- Purse seine capture. The meshes of the purse seines are slightly larger than the meshes of fish nets. The seine net is set, and taken in smoothly and rapidly because turtles will go into the sediment if there is some sound.

- Drained-pond capture. If large numbers or a whole pond of turtles are to be captured, the pond is drained and dried, and the turtles collected from around the pool during the day and by using lights at night.

20.9.2 Processing

There are hundreds of processing and cooking methods in Chinese cuisine.

The soft-shelled turtle is killed by cutting the neck or opening the ventral shell. The turtle is cleaned by removing the viscera, and it is then ready for processing and cooking. The main processing and uses of soft-shelled turtle include the following:

- cooking to produce a great variety of dishes;
- health-food medicines;
- health-food medicines through processing with traditional Chinese medicines;
- mixing with other traditional Chinese medicines as a kind of medicine for treating some specific human diseases.

20.9.2.1 Cooked as dishes

There are many ways of cooking soft-shelled turtles, including boiling, frying, steaming, soaking (e.g. in rice wine) or making into soup, for a great variety of dishes. These may involve the whole or parts of the turtle cooked with other edible animal tissues, vegetables or edible plant seeds. Cooked soft-shelled turtles may be vacuum packed for sale.

20.9.2.2 Healthy food medicines

The soft-shelled turtle is cooked with traditional Chinese medicines, plant seeds or tissues as medicinal food for sick or weak persons. It is a food to support body health and food for health recovery.

20.9.2.3 Soaking in alcohol with traditional Chinese medicines

Slaughtered and cleaned soft-shelled turtles or their organs are soaked in alcohol (e.g. rice wine) with traditional Chinese medicines for daily drinking to support the human body's health.

20.9.2.4 An ingredient of medicines

Whole or parts of the soft-shelled turtle are used as an ingredient of traditional Chinese medicines by powdering and specified processing. In addition, the turtle's shell is traditionally used as a medicine for children and old people as a calcium supplement.



Fig. 20.12 A local market with various sizes of soft-shelled turtles for sale. The largest turtles are enclosed in baskets.

20.9.3 Marketing

Usually, soft-shelled turtles are harvested and sold alive after they weigh 400 g. The main marketing methods are the following:

- The turtles are sold alive by the farmer at the pond side or farm gate to middle-men or restaurants. Then the middle-men wholesale the turtles to the local market dealers, who finally sell to the consumers.
- The farmer takes the turtles to the wholesale market of vegetable, meat and aquatic products, and there sells to the consumers (Fig. 20.12).

A soft-shelled turtle production company sells them through the company's specialised shops in cities, with a marked brand and quality guarantee or specified years of culture, for a high price. Small quantities of soft-shelled turtle are processed and packed, then sold in local markets or supermarkets, or exported to Japan, South Korea and other south-east Asian countries. The turtles with brand names are sold at three to five times and even ten times the price of turtles without brand names, even in China. These brand-name turtles are of high quality with a culture period of 3–5 years in earthen ponds or polycultured with fishes or aquatic plants. The turtle's price at a city supermarket or at a brand-name shop may be 300–400 CNY/kg or even higher if it is polycultured with fish for 4–5 years.

Despite these high prices, the farm-gate price of indoor cultured soft-shelled turtles was only about 30–32 CNY/

kg in Hangzhou in July 2009, because consumers considered that the quality of these turtles was much poorer than those cultured in earthen ponds. The price of soft-shelled turtles depends on many factors that affect quality: period of culture, culture environment (e.g. pond or dark indoors), variety, body weight, sex (males are more valuable), condition of health and status of brand name.

In retail markets, dealers may help customers by slaughtering and cleaning the soft-shelled turtle after weighing if the customer requests this.

20.9.4 Consumption

Most Chinese, Japanese, Korean and south-east Asian people like to consume soft-shelled turtles. During traditional holidays or festivals, banquets, family gatherings, friends getting together, parties for birthdays, business and weddings, and other important activities, soft-shelled turtle is chosen as one of the dishes. This is because soft-shelled turtles are considered to have high nutritional value and good animal protein for human health, and they are tasty. During the meal every participant will usually have one or two pieces of turtle meat by using chopsticks and they will have some turtle soup as well. An example of a dish of cooked Chinese soft-shelled turtle is shown in Fig. 20.13.

20.10 THE FUTURE OF SOFT-SHELLED TURTLE FARMING

Based on Chinese traditional food culture, many people wish to consume soft-shelled turtle if the quality is good and safe. There will be increasing consumption with increasing economic development in most parts of China. More and more soft-shelled turtles will be produced through fish–turtle polyculture or ecological culture methods in earthen ponds and in greenhouses with sunlight, because there are fewer disease breakouts with these methods compared with indoor dark culture. There are needs to improve survival rate during the nursery phase, reduce the period of the nursery phase and improve turtle meat quality. This is likely to be solved by two-stage farming methods with an indoor temperature-controlled nursery phase and then culture in outdoor ponds or in sunlit greenhouses. There is substantial genetic variation in populations of *Trionyx sinensis* (Bu *et al.*, 2011), and some new strains and hybrid strains will be developed for production on a larger scale. Chemical treatments that are more effective and leave less residue must be developed for disease control during culture. Finally, it is most important to train the farmers in basic culture knowledge such as how to control water quality, choose appropriate methods for disease prevention and control, set up turtle farmers' associations, and improve the management of farming risks and other aspects of soft-shelled turtle production.



Fig. 20.13 A Chinese dish of soft-shelled turtle soup.

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21
Marine Shrimp

Darryl Jory and Tomás Cabrera

21.1 INTRODUCTION

21.1.1 History of marine shrimp[1] farming

In the past three decades, farming of various marine shrimp species has developed tremendously. Shrimp farms now contribute about one-half of the world's shrimp demand, and as has been the case for the past three decades or so, continue to rapidly replace traditional fisheries as market demand suppliers. In addition to generating several billions of dollars in trade, shrimp farming also:

- provides employment for millions of people in developing nations;
- brings into production vast areas of previously unused coastal land unsuitable for other types of development;
- produces a valuable commodity that is increasingly marketed locally in many countries;
- generates needed hard currency through significant exports.

The industry has also generated some environmental issues that have been acknowledged and properly addressed in most cases.

Marine shrimp have been grown in south-east Asia for centuries by farmers who raised them as incidental crops in tidal fish ponds. Modern shrimp farming began in the 1930s, when Motosaku Fujinaga, a graduate of Tokyo University, succeeded in spawning the Kuruma shrimp (*Marsupenaeus japonicus*). He cultured larvae through to market size in the laboratory and successfully mass produced them commercially. Dr Fujinaga generously shared his findings and published papers on his work during the next 40 years, and was honoured by Emperor Hirohito with the title 'Father of Inland Japonicus Farming'. In the early 1970s, researchers and entrepreneurs in various countries in Asia and Latin America became involved in promoting development of the industry, which grew steadily. Marine shrimp farming has come a long way in the past 25 years, and enormous progress has been made in developing technologies and methods to culture shrimp. The industry began a tremendous expansion in the early 1980s. Major references on global shrimp farming include Browdy and Jory (2001, 2009) and Rosenberry (2001).

21.1.2 Current status and production

Marine shrimp farming is practised worldwide in over 60 countries, but production is concentrated in about 15 nations in Asia and Latin America. Since 1992, about a dozen countries have contributed approximately 95% of farmed shrimp production. The top world producer of farmed shrimp has changed several times in the past, between Ecuador, Taiwan, Indonesia, China and Thailand. The shrimp farming industry in China has grown extremely rapidly in recent years, and the country is the world's leading producer of farmed shrimp.

Farmed production is still increasing in many countries by responsibly intensifying culture methods, without having to develop large areas. Most of the best locations for farms have already been developed, but the industry can still expand in several countries, including various Latin American and African nations. Shrimp culture is a

[1]The term 'marine shrimp' is used here to distinguish species of the family Penaeidae. 'Shrimp' is the most widely used term for these species, but in some countries they are known as 'prawns'. Most 'marine shrimp' species are euryhaline and are cultured in brackish water. There are a few cases where they are cultured in freshwater.

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very minor activity in a few European countries (Spain, Italy), and Japan and the USA are relatively small producers owing to cool weather, high production costs, limited areas with suitable conditions, and the difficulty of competing cost-efficiently with industry leaders. However, many developed countries are significantly involved in the shrimp culture industry:

- as large consumers of shrimp;
- as producers of supplies and materials used by the industry;
- in providing substantial technical expertise on production and processing techniques.

Between 1975 and 1985, global production of farmed shrimp increased by 300%, and between 1985 and 1995 by 250%. Since 1995, however, industry growth has been slower due to viral and bacterial diseases. Costs have increased and market prices have decreased significantly in the past decade, with uncertainties and fluctuation in the costs of energy and grains, and as the industry moves to comply with new international standards on product quality and the environment. Industry growth in the past decade or so has been around 7–8% and is still slowing down.

Jory (2010) reported on shrimp aquaculture production data by major global producing regions, and in Asia and in Latin America. The data presented cover the years 2003–2011, with the past three years being estimates (Table 21.1a–c). The data are based on several sources, including FAO, as well as information from various national associations, and an extensive survey targeting key industry players along the entire shrimp production and marketing chain.

Total farmed shrimp production for 2009 is estimated at around 3.428 million t (Table 21.1a). By regions, the main producers are south-east Asia with 1 347 698 t, China 1 252 741 t, India and Bangladesh 289 750 t, the Americas

Table 21.1a Marine shrimp (Penaeidae) aquaculture production by major producing regions, 2003–2011.

Region/country	2003	2004	2005	2006	2007	2008	2009	2010	2011
South-east Asia	857 229	993 565	1 150 714	1 333 669	1 353 554	1 382 693	1 347 698	1 362 416	1 444 896
China	789 373	935 944	1 064 949	1 080 479	1 265 636	1 268 074	1 252 741	1 365 069	1 512 877
India/ Bangladesh	169 743	191 064	206 222	209 047	171 265	291 890	289 750	331 250	360 000
Americas	296 461	326 266	379 363	446 471	449 099	470 717	495 033	501 917	523 550
Africa/Middle East	25 257	25 500	26 771	27 790	26 641	28 410	28 250	31 500	38 500
Others	7 703	7 585	9 968	11 751	9 501	14 000	15 000	16 000	16 000

Sources: 2003–2007: FAO Fishstat Plus (2009); 2008–2011: Global Outlook for Aquaculture Leadership estimate. Note that much of this aquaculture is in brackish water.

Table 21.1b Marine shrimp (Penaeidae) aquaculture production in Asia, 2003–2011.

Country	2003	2004	2005	2006	2007	2008	2009	2010	2011
China	789 373	935 944	1 064 949	1 242 385	1 265 636	1 268 074	1 252 741	1 365 069	1 512 877
Thailand	330 725	360 292	401 251	500 800	501 200	473 622	455 000	420 000	420 000
Vietnam	231 717	275 569	327 200	349 000	376 700	391 000	418 148	429 616	475 946
Indonesia	191 148	238 567	279 539	339 803	330 155	315 050	250 000	275 000	302 000
India	113 240	133 020	143 170	144 347	107 665	126 042	106 000	125 000	135 000
Bangladesh	56 503	58 044	63 052	64 700	63 600	165 848	183 750	206 250	225 000

Sources: 2003–2007: FAO Fishstat Plus (2009); 2008–2011: Global Outlook for Aquaculture Leadership estimate. Note that much of this aquaculture is in brackish water.

Table 21.1c Marine shrimp (Penaeidae) aquaculture production in Latin America, 2003–2011.

Country	2003	2004	2005	2006	2007	2008	2009	2010	2011
Ecuador	77400	89600	118500	149200	150000	150000	156500	156000	160000
Mexico	45857	62361	90008	112495	113540	122500	126500	127500	130000
Brazil	90190	75904	63134	65000	65000	65000	65000	70333	76000
Colombia	16503	18040	18040	18050	18050	16750	17500	18500	19500
Honduras	16527	18036	20873	26956	26333	18000	20000	20000	22000
Venezuela	14259	16500	16500	16500	16500	22000	20000	15000	15000

Sources: 2003–2007: FAO Fishstat Plus (2009); 2008–2011: Global Outlook for Aquaculture Leadership estimate. Note that much of this aquaculture is in brackish water.

495033 t, Africa and the Middle East 28250 t, and others 15000 t. Total global production is estimated to increase to 3.608 million t in 2010, and 3.895 t in 2011. Table 21.1b shows shrimp aquaculture production estimates for Asia during 2003 – 2011. The leading producers for 2009, in addition to China's already mentioned 1252741 t, include Thailand with 455000 t, Vietnam 418000 t, Indonesia 325000 t, India 106000 t and Bangladesh 183750 t. With the exception of Thailand, several of these countries will probably increase their production in the next two years. Table 21.1c shows shrimp aquaculture production estimates for Latin America during 2003–2011. All but Venezuela (decreased) and Brazil (remained the same) increased their production from 2008 to 2009. The leading producers in that region in 2009 include Ecuador with 156500 t, Mexico 126500 t, Brazil 65000 t, Colombia 17500 t, and Honduras and Venezuela with 20000 t each. Ecuador, Mexico, Brazil, Honduras and Colombia are forecast to increase production over the next two years, while in other countries like Venezuela it is expected to decrease.

Newman (2010) discussed recent paradigm shifts in global shrimp farming, which has experienced a major shift in the dominant species produced. For many years the black tiger shrimp (*Penaeus monodon*) dominated global production, but around the year 2000 the industry began to significantly shift production to the Pacific white shrimp (*Litopenaeus vannamei*), as this species was widely introduced into various Asian countries. Currently, about 65% or more of farmed shrimp production in the world is white shrimp. This percentage is increasing. Improvements in selective breeding, nutrition, health management, disease diagnosis and prevention, and more efficient and intensive production systems have supported this industry expansion. Currently, the industry in south-east Asia typically consists of the following:

- small, 1-ha or smaller ponds;
- mechanical aeration of around 1 horsepower/400–600 kg of shrimp;
- markedly reduced water exchange rates that also promote biosecurity (disease control);
- stocking densities of 150 or more animals per square metre;
- plastic lined ponds, sometimes with supplementary aeration by the use of blowers pumping air through diffusers on the pond bottoms;
- relatively high protein manufactured feeds.

Asian farmers have demonstrated that specific pathogen free *L. vannamei*, genetically improved in some cases, can be efficiently grown at very high densities with relatively low environmental impacts. Although there are farms in the Americas that have been able to convert successfully from semi-intensive to more intensives production modes, these are relatively rare and there does not appear to be any real move towards the widespread adoption of this technology in the region. Production models in the Americas remain largely based on larger ponds (5–10 ha) stocked at 10–30 animals per square metre using laboratory-reared postlarvae (PL) often selected for growth and survival against prevailing pathogens, with some mechanical aeration, reduced water exchange rates, and continued use of manufactured feeds.

21.2 CULTURED SPECIES

21.2.1 Current taxonomy

A new taxonomy for marine shrimp was prepared by Pérez Farfante and Kensley (1997). It recognises seven families and 56 genera of Penaeoidea and Sergestoidea shrimps. The main change for shrimp farmers in the Western hemisphere is that *Penaeus vannamei* and *P. stylirostris* are now grouped in the new genus *Litopenaeus*. For shrimp farmers

in the Eastern hemisphere, *P. monodon* remains the same, whereas *P. japonicus* is now *Marsupenaeus japonicus*, and *P. indicus*, *P. penicillatus* and *P. chinensis* have been reclassified into a new genus, *Fenneropenaeus.* This new classification has its critics and is not totally accepted by the global shrimp farming community, but is used here.

There are about 2500 species of shrimp worldwide, but only 12 or so species are farmed to some degree. All belong to the family Penaeidae, characterised by a rostrum with ventral and dorsal teeth, and last thoracic segment with gills. Out of the commercially farmed species, two account for probably 90–95% of global production:

1. The black tiger shrimp (*Penaeus monodon*) in Asia.
2. The Pacific white shrimp (*Litopenaeus vannamei*), originally in the Americas but widely introduced throughout Asia, Africa and elsewhere.

Black tiger shrimp alone probably constituted 60–70% of the world's production of farmed shrimp until the end of the twentieth century, when western white shrimp was widely introduced to Asia and have largely replaced black tigers in production and world markets. Both species are popular in local and export markets, and although both are relatively easy to produce, *L. vannamei* has some clear advantages regarding larval rearing, tolerance to high density culture and requirements for lower protein feeds. The seedstock for both species can be produced in hatcheries using relatively simple technologies. Both can tolerate a wide range of salinity, from slightly greater than freshwater (1–2‰) to full-strength ocean water (35–40‰). Both species readily eat formulated commercially manufactured feeds. Information on the major cultured species of shrimp, summarised from Rosenberry (2001) and others, is detailed below.

21.2.2 Black tiger shrimp[2] (*Penaeus monodon*)

Penaeus monodon is the largest (363 mm maximum length) and fastest growing (up to 5.5 g per week) of the farmed shrimp (Fig. 21.1). The species is native to the Indian Ocean and the south-western Pacific Ocean, from Japan to Australia, and dominates production everywhere in Asia except for Japan and China. It can tolerate a wide range of salinities and is grown in inland low-salinity ponds in some countries. It is, however, very susceptible to two of the most lethal shrimp viruses, yellowhead virus (YHV) and whitespot syndrome virus (WSSV) (section 21.8.4). Breeding in captivity is difficult to induce and hatchery survival is low (20–30%). Seedstock production is mainly from wild broodstock, which are often in short supply in many areas. The protein requirement of *P. monodon* in formulated feeds is ca. 35%, although research is ongoing to reduce this level. They are widely exported from south-east Asia and marketed in Japan, the USA and Europe.



Fig. 21.1 The black tiger shrimp (*Penaeus monodon*).

21.2.3 Western white shrimp[3] (*Litopenaeus vannamei*)

This species, also known as the Pacific white or whiteleg shrimp, is the primary species farmed worldwide (Fig. 21.2). Its original range is from the Gulf of California southwards to northern Peru, but it is now a cosmopolitan species. Males reach a total length of 187 mm, and females 230 mm. It is well suited for farming, because it:

- breeds well in captivity;
- can be stocked at small sizes;
- grows fast and at uniform rates;
- has comparatively low protein requirements (20–25%) and is considered among the most omnivorous of the penaeid species cultured, together with *F. indicus*;
- adapts well to variable environmental conditions.

L. vannamei is the leading farm-raised species in the world. It breeds in captivity better than *P. monodon*, but not as readily as *Fenneropenaeus chinensis* and *M. japonicus* (see following). In hatcheries, overall survival

[2]Known in FAO statistics as the 'giant tiger prawn'.

[3]Known in FAO statistics as the 'whiteleg shrimp'.



Fig. 21.2 The Western white shrimp or whitteleg shrimp (*Litopenaeus vanamei*). These shrimp were sampled from semi-intensive culture to assess quantity and quality. Note the full digestive tracts and excellent condition.

is retively high at 50–60%. Some hatcheries in Latin America have captive stocks of *L. vannamei* broodstock, some pathogen free, some pathogen resistant, with some in captivity for 30 years. Production is mostly exported to the USA.

21.2.4 Western blue shrimp (*Litopenaeus stylirostris*)

Another formerly important species in the Western hemisphere is the western or Pacific blue shrimp, *Litopenaeus stylirostris*. It occurs from the Gulf of California south to Peru. Males reach 215 mm in total length, females 263 mm. They have a faster growth rate than *L. vannamei*, but their growth is not uniform, and this results in harvests with very broad size distributions. *L. stylirostris* tolerates lower water temperatures than *L. vannamei,* but requires higher oxygen levels, turbidity, salinities, deeper ponds and formulated feeds with higher protein levels. It was often grown in combination with *L. vannamei* (at around 20% and 80% stocking, respectively) and produced excellent results in many farms in several Latin American countries. It was a commonly farmed species until the late 1980s, when the infectious hypodermal and haematopoietic necrosis (IHHN) virus appeared, to which *L. stylirostris* is highly susceptible. Captive stocks kept at several locations around the world, developed resistance to the IHHN virus through selective breeding. During 1992–97, when *L. vannamei* stocks were being devastated by the Taura syndrome virus (TSV) everywhere in the Western hemisphere, some shrimp producers determined that some of the



Fig. 21.3 The Japanese kuruma shrimp (*Marsupenaeus japonicus*).

captive stocks of *L. stylirostris* were resistant to both IHHN and TSV. Between 1997 and 2000, *L. stylirostris* made a comeback on farms throughout the Western hemisphere, especially in Mexico. In the USA market, *L. stylirostris* and *L. vannamei* are often mixed together and sold as western white shrimp. If *L. stylirostris* is still farmed commercially, it is only at a very small scale, although there are still stocks of selected IHHN virus-resistant lines at some hatcheries.

21.2.5 Chinese white shrimp (*Fenneropenaeus chinensis*)

Fenneropenaeus chinensis occurs off the coast of China and the western coast of the Korean peninsula. It tolerates muddy bottoms and very low salinities, and can withstand much lower water temperatures (down to 16 °C) than *L. vannamei* and *P. monodon*. It readily matures in ponds, unlike most other commercially farmed species. However, it requires high protein levels (40–60%) in formulated feeds, reaches a relatively small size (183 mm maximum length) and has a lower meat yield (56%) than *P. monodon* (61%) and *L. vannamei* (63%). Between 1988 and 1993, this species was marketed extensively around the world from China. Although *L. vannamei* is by far the predominant species now farmed in China, *F. chinensis* is still farmed and is the subject of significant research.

21.2.6 Japanese kuruma shrimp (*Marsupenaeus japonicus*)

Marsupenaeus japonicus is native to the Indian Ocean and the south-western Pacific Ocean from Japan to Australia. It is commercially cultured in Japan, China and on a small scale in some southern European countries (Fig. 21.3).

Attempts to farm Kuruma shrimp elsewhere, including Latin America, met with limited success and were discontinued. They are relatively easy to ship live without water and packed in chilled sawdust and other materials (section 11.8 and Fig. 11.2), and they will mature and spawn in ponds. They need clean, sandy bottoms and formulated feeds with very high (55–60%) protein levels. They can tolerate low water temperatures (down to 10 °C) better than any other farmed species. Marketed live, they command extremely high prices in Japan.

21.2.7 Indian white shrimp (*Fenneropenaeus indicus*)

This species is native to the Indian Ocean from southern Africa to northern Australia and to all of south-east Asia. It is one of the major species in the commercial fisheries of these regions. *F. indicus* is primarily grown on extensive farms throughout south-east Asia, and it is widely cultured in India, the Middle East – especially in Saudi Arabia – and eastern Africa (section 5.7 and Fig. 5.10). It tolerates low water quality better than *P. monodon*, grows well at high densities and salinities, reaches sexual maturity and spawns in ponds, and is widely available in the wild.

21.3 GROW-OUT SYSTEMS

Shrimp grow-out operations span a wide continuum in terms of intensity, complexity and technology. Globally, four grow-out production systems are generally recognised, which share some characteristics, but differ in other aspects (Table 21.2).

Moving from the lowest towards the highest stocking systems, there is:

- progressive reduction in the area of ponds used, eventually moving towards more intensive systems like tanks and raceways;
- progressive increase in capital, production costs and stocking densities, and risk;

Table 21.2 Various shrimp farming systems, based on stocking density, production inputs and operational parameters.

	Intensity			
Parameter	Extensive (low density)	Semi-intensive (medium density)	Intensive (high density)	Intensive (very high density)
Stocking density (PL/m^2)	1–5	5–25	26–120	120–500
Pond/tank area (ha)	Ponds (5–100)	Ponds (1–25)	Ponds/tanks (0.1–5.0)	Tanks (0.1–1.0)
Seedstock source	Wild	Some wild/mostly laboratory	Laboratory	Laboratory
Water exchange (% daily)	Tidal (ca. 5%)	Pumping (5–10%)	Pumping (up to 25%)	Pumping (25%+)
Management requirements	Minimal	Moderate	High	Very high
Feed	Natural productivity	Natural productivity and formulated feeds	Natural productivity/ mostly formulated feeds	Natural productivity/ mostly formulated feeds
Fertilisation	No	Generally yes	Sometimes	Sometimes
Mechanical aeration	No, minimal water exchange	Water exchange and some mechanical aeration	Mechanical aeration	Mechanical aeration
Aeration levels (horsepower/ha)	0–2	2–5	6–20	20–60
Production cycle (days)	100–210	100–210	80–140	80–120
Annual production (kg/ha)	50–500	500–5000	5000–20 000	20 000–40 000

PL, postlarvae.

Modified from Jory (1993) and Fast and Menasveta (2000).



Fig. 21.4 Water pumping station for a shrimp farm. Farms in Latin America tend to operate with greater volumes of water exchange than their counterparts elsewhere.

- a trend for increased use of intermediate nursery or multiple phases;
- an overall intensification in production practices, management and technology.

The last involves a more thorough preparation of ponds or tanks, total use of laboratory-produced seed-stock, use of better formulated and more nutrient-dense feeds, improved pond management practices and others. Up to the mid- to late 1990s, it also involved higher rates of water exchange, but for the past decade or so there has been a strong global trend towards reducing exchange rates and the volume of water required to produce shrimp (i.e. cubic metres of water per kilogram of shrimp) (Fig. 21.4). This has even developed to the point of reaching zero exchange (except for replacement of water lost to evaporation and other processes) and recirculation to improve biosecurity and operational cost-efficiency. There are also trends towards increased use of technology, aeration, technical and professional labour, and a tendency to move away from the coastline to higher ground, and even towards ocean cages. Most farms produce at least two crops per year, although some farms can reach or surpass three or more annual crops, particularly those using nurseries or multi-phase production systems.

Early on, when the industry was young, farms were mostly extensive, and there are still many such farms in Thailand, Indonesia, India, Vietnam, Bangladesh, Central and South America, among other countries. Since around 1980, however, there has been a trend towards refitting farms to increase intensity of production. The more intensive farms are more typical in countries such as Taiwan, Japan and the USA (Jory *et al.*, 2001; Rosenberry, 2001).



Fig. 21.5 A paddy in which shrimp are cultured extensively after rice harvest (Mekong, Vietnam). (Photograph by Dr Michele Burford.)

21.3.1 Extensive systems (low stocking densities)

Shrimp farms with low stocking densities are typically located in tropical water impoundments ranging from ca. 2 ha to >100 ha and located adjacent to estuaries, bays, and coastal lagoons and rivers. They frequently involve polyculture with herbivorous fishes such as mullet, milkfish, tilapia and others. At some of these farms, liming materials are applied to ponds if soils are acidic or have accumulated considerable amounts of organic matter that is not removed, and some operations may sometimes use animal manures or other organic materials to stimulate production of natural food for their shrimp (section 9.9). Ponds are filled by tides and any water exchange (typically <5% per day) is also by tidal action. Ponds are typically stocked with wild shrimp PL, when naturally available, by opening pond gates to incoming tides. Fig. 21.5 shows a pond in the Mekong region, Vietnam, where shrimp are cultured extensively in ditches and paddies after the rice harvest. After the harvest, saline water from the Mekong River penetrates up into ditches and paddies containing rice stubble. The water brings shrimp PL for culture.

Alternatively, or additionally in various regions, juvenile shrimp may be netted from shallow coastal waters and stocked into ponds (Fig. 21.6).

In all cases, these shrimp feed on natural phyto- and zooplankton, small plants and animals living in or on the pond substrate (particularly amphipods and polychaetes),



Fig. 21.6 Sieving wild-caught juvenile shrimp to sell for stocking extensive shrimp farms (Chalna, Bangladesh). (Photograph by Professor Clem Tisdell.)

and particulate organic matter suspended in the water or lying on the bottom. This natural production may be promoted with applications of organic or chemical fertilisers. It is extensive aquaculture, as defined in Chapter 2 (section 2.3.3), for example the cultured shrimp are essentially part of a natural ecosystem that provides their nutritive and other requirements. Construction and operating costs are typically low, and production rarely surpasses 400–500 kg/ha in production cycles that last 100–160 days. Almost no new extensive shrimp farms are being built anymore, because it is now illegal – and inefficient – in several countries to build new shrimp farms in tidal and mangrove areas (Boyd, 1998; Rosenberry, 2001).

21.3.2 Semi-intensive systems (medium stocking densities)

Shrimp farms that operate at medium stocking densities (Table 21.2) are built above the high-tide line and include a pumping station and water distribution canals and reservoirs, and use of formulated feeds. In general, farm layout is relatively more symmetrical than extensive farms and ponds are harvested by draining through a net or by using a harvest pump. Pond preparation may be elaborate, with dry-out once or twice a year, tilling and liming with various liming materials, and fertilisation with N, P, Si and other compounds to promote natural production. Producers may also apply various extracellular enzyme preparations and bacterial inocula to improve water quality, but the benefits of these treatments remain to be conclusively established. Organic fertilisers (mostly manure and agricultural by-products) are sometimes used. Although the water exchange rates typically used are 5–15% of pond volume per day, in recent years many farmers have adopted exchange practices of 2–5%. These lower exchange rates reduce pumping costs, and minimise fertiliser needs and the possibility of pathogen introduction. Formulated and pelleted feeds with 15–35% crude protein are usually applied one to three times per day, typically by manual broadcasting over pond surfaces from boats and levees. Feed quantity applied is calculated and adjusted based on the shrimp biomass estimated from results of cast net sampling and feeding charts typically provided by feed manufacturers, and from historical data collected at each farm. Natural productivity in the ponds is important for juvenile shrimp growth during the early weeks at all intensities of shrimp culture.

Subsequently, there is variation in the degree of dependence on formulated feeds. Dependence on formulated feeds is not as great at this moderate density as it is at higher culture densities; hence, in the continuum of culture practices, this is semi-intensive culture (section 2.3.4).

21.3.3 Intensive systems (high stocking densities)

Shrimp farms using intensive culture (section 2.3.2) and high stocking densities (Table 21.2) typically have ponds of 0.1–2 ha, although various designs of raceways and above-ground tanks are also used, sometimes in greenhouses or other enclosures. Preparation before stocking is more meticulous, and management is often more elaborate, with feed applied six to eight times a day. Mechanical aeration is needed to support the large shrimp biomass and heavy feeding rates needed. Aerators and/or air-diffuser systems are placed in ponds and operated throughout the cycle, usually with increasing number of units and longer hours of operation as the cycle progresses. Generally, 4–12 horsepower/ha is used, with the amount increasing as the biomass of shrimp increases (Table 21.2). In Asia, several chemicals, including calcium peroxide, burnt lime, zeolite, chlorine, iodine, formalin and bactericides, are applied to ponds to improve water quality and prevent water quality deterioration and disease. Various probiotics, such as bacterial inocula and enzyme preparations, are added as in semi-intensive systems.

21.3.4 Intensive systems (very high stocking densities)

At the upper end of the continuum, systems with very high stocking densities (Table 21.2) include the highest level of environmental control, to the point of some being located indoors in greenhouses and other structures. Annual



Fig. 21.7 The Labelle facility owned by OceanBoy Farms, Florida, shows recirculation and reduced water exchange technologies on a commercial scale. The treatment raceway (long and narrow on the right) is where suspended solids are removed and water is treated before re-use in lined and highly aerated ponds. (Photograph by OceanBoy Farms.)

production can reach 20–100 t/ha and higher, but there are currently only a few of these farms, mostly in various Asian countries. Examples of these advanced farms and technology include the pioneer Belize Aquaculture Ltd. (BAL) in Belize and OceanBoy Farms in Florida, USA (Fig. 21.7). Pond management at these farms is based on zero water exchange, heavy aeration (up to 50 or more horsepower/ha) and the promotion of a bacteria-dominated and stable ecological system. This compares with the highly unstable and traditional phytoplankton-dominated system. The feeding regime used promotes the growth of heterotrophic bacteria, and essentially makes the pond into a large outdoor bioreactor, akin to a sewage oxidation pond. In these high-density, zero-water-exchange systems, the pond ecology shifts (at weeks 9–10 after stocking) during the production cycle from an autotrophic phytoplankton-based community to a heterotrophic bacteria-based community. This shift improves water quality through fast digestion (oxidation) of organic waste without production of toxic metabolites. At BAL, feeding rates have exceeded 350 kg/ha/day without resulting in deterioration of water quality once this heterotrophic bacterial community has been developed and established in ponds. The shift also recycles wastes into nutritious bacterial flocs, the basis for 'natural production' in this system (McIntosh, 2000).

21.4 PREPARATION OF PONDS

General details of ponds, site requirements, layout, design and water turnover are given in sections 2.2.1–2.2.4.

Adequate pond preparation provides young shrimp with an environment that is relatively free of predators and competitors, with an ample supply of adequate natural food organisms, and environmental conditions that minimise stress and promote growth and survival. Pond preparation involves several sequential procedures including the following:

- pond draining and drying;
- pH mapping;
- soil tilling;
- disinfection and liming;
- fertilisation;
- weir gate preparation and maintenance.

Some excellent reviews of these procedures are available, including Clifford (1992) and Cook and Clifford (1998).

21.4.1 Pond draining and sludge disposal

Effective removal of sludge that has built up on the pond bottom over a previous crop cycle is a very important step. If not removed, it will become an anaerobic, reduced sediment (Fig. 3.7). This will generate toxic metabolites such as methane, hydrogen sulphide, ammonia, nitrite, ferrous iron, etc., affecting pond water quality and production during the following crop cycle. This can be particularly relevant with some species of shrimp, which burrow periodically over the day/night cycle and after moulting. After a production pond is harvested, all water inlet/outlet gates are opened to generate a strong water flow through the pond, to resuspend and flush out as much accumulated sludge as possible. It is important to do this before the sludge dries out and solidifies. One technique that works in ponds with heavy sludge accumulation is to use heavy chains and rakes dragged along the bottom. Several people may walk along the internal canals to assist in resuspending sludge material as the pond is flushed. Most farms are located in areas with clearly defined dry and wet seasons, and it is not possible to dry pond bottoms effectively during the wet season. In addition, many ponds will not drain adequately because of defective design, construction or both. Low areas with standing water can be pumped dry or drainage ditches can be dug to facilitate drainage. These methods are only appropriate for production areas with sedimentation ponds and discharge canals that meander

through coastal land, allowing sediment and organic matter to settle out before entering the coastal waters.

21.4.2 Pond drying and pH mapping

Earthen ponds must be allowed to dry out for 2–4 weeks to:

- promote decomposition of organic matter by bacteria;
- eliminate pathogens and eggs, larvae and adults of predator and competitors;
- dry out undesirable filamentous algae.

The rate of organic matter decomposition is greatest at a soil pH of 7.5–8.5 and, for ponds built on acidic soils, farmers add various lime products to improve soil pH and promote decomposition of organic matter (Fig. 21.8).

Optimal microbial action for decomposition of organic matter occurs at about 20% soil humidity. Ponds infested with burrowing callianassid shrimp (snapping shrimp) must not be allowed to dry out, as this will cause these animals to burrow even deeper. Pond dry-out and disinfection may be the most effective methods for controlling epidemics of various shrimp pathogens, as well as various predators and competitors. Ultraviolet radiation (from sunlight) and temperatures above 55 °C will destroy several pathogens. If diseases have been detected within the last production cycle, a longer dry-out (2–3 months) may be helpful. The pond surface must be cracked to a minimum of 5 cm, to oxidise the soil and eliminate anaerobic conditions (Fig. 21.8) (Clifford, 1992). In acidic soils with pH ca. 7, pond bottom pH must be mapped promptly by sampling soil pH at various stations within the pond while soil humidity is about 30%, to calculate lime requirements. Once the pond surface is hard enough to walk on, soil pH is measured using standardised procedures. This technique involves collecting representative pond bottom soil from different sites throughout the pond. Appropriate addition of lime is then calculated (section 21.4.4).



Fig. 21.8 Drying of pond bottoms until they crack and subsequent application of liming materials are important steps in pond preparation.

Ploughing or turning the bottom soil of ponds (top 10–20 cm) is another optional step, which will depend on soil condition (section 5.7.3 and Fig. 5.13). Tilling the bottom soil of ponds can significantly promote oxidation of the lower layers of anaerobic sediments. Alternating cycles of tilling and flushing is a common action to reduce levels of iron compounds in acid-sulphate soils. It is also common to incorporate a nitrogen source such as urea into the soil before tilling, to increase the rate of organic matter decomposition. Some farmers will add 50% of the calculated lime requirements before tilling.

21.4.3 Disinfection

Disinfection is important to eliminate eggs, larvae, juveniles and adults of species of fish, crustaceans, insects, and other predatory and competitor species. Inadequate disinfection of ponds can considerably affect the yield of a pond. For example, the gobiid mudfish known as ‘chame’ (*Dormitator* species) can be a serious problem in Latin America. These fish can complete their life cycle in ponds, and will bury in the bottom mud and survive extreme conditions, including near desiccation, until the pond is filled again. They compete for feed and space, and can significantly affect the carrying capacity of a shrimp pond. Mudfish harvests of several tonnes have occurred where ponds were not adequately disinfected during preparation.

Several commercial products have been used to disinfect ponds before stocking shrimp. The use of pesticides (particularly chlorinated hydrocarbons and organophosphates) is not recommended because of their slow biodegradation, potential to accumulate in sediments and bioaccumulate in the food web. Chlorination has been the disinfection method of choice for many farmers, and its use has become prominent in the fight against WSSV, although lately it has been significantly discontinued in Latin America.

About 7 days into the dry-out period, all cement structures (inlets and outlets) must be cleaned, and mud, barnacles, oysters and algae removed. It is important to remove bivalves because they can reproduce in ponds, their filter feeding activity can quickly deplete plankton

and they can be intermediate hosts of some shrimp parasites.

Applying calcium oxide or calcium hydroxide at 5000 kg/ha will raise the pH to greater than 10 and will destroy pathogens. Only muddy areas must be treated like this, not the entire pond bottom, as this process will destroy desirable bacteria needed to promote the development of productive benthos (section 21.4.6). Piscicides such as rotenone and teaseed cake are routinely used to eliminate finfish, including mudfish.

21.4.4 Liming

Liming pond bottoms is a critical step in preparing earthen ponds. When calculating liming requirements the type of lime product to use must be considered, as this will influence the amounts required (Table 21.3). One lime application is generally added 2 days after tilling with lime spreaders, applying lime more heavily to wet low areas than to higher dry areas. One week is allowed for the lime to react with the soil before applying fertiliser. The calculated neutralising values of various compounds used in pond liming range from 59% (sodium bicarbonate) to 208% (calcium magnesium oxide) (Table 21.3).

When liming requirements cannot be calculated, the following liming rates, using calcium carbonate, $CaCO_3$, can be used (Boyd, 1995):

- for pH 6.5–7.5 (500 kg/ha);
- for pH 6.0–6.5 (1000 kg/ha);
- for pH 5.5–6.0 (2000 kg/ha);
- for pH 5.5–5.0 (3000 kg/ha);
- for pH < 5.0 (4000 kg/ha).

Table 21.3 Neutralising values of various compounds used in pond liming. Reproduced from Boyd (1995) with permission of the World Aquaculture Society.

Compound	Chemical name	Neutralising value (%)
Agricultural limestone	Calcium carbonate	100
Agricultural limestone	Dolomite	109
Burnt lime	Calcium oxide	179
Burnt lime	Calcium magnesium oxide	208
Hydrated lime	Calcium hydroxide	135
Hydrated lime	Calcium magnesium hydroxide	151
Soda ash	Sodium carbonate	94
Baking soda	Sodium bicarbonate	59
Other compounds	Calcium silicate	86
Other compounds	Calcium phosphate	65

21.4.5 Weir gate preparation and entrance screening

One of the most important aspects of pond preparation is the maintenance and preparation of weir gates (monks) (Fig. 2.3). Configuration and placement of weir gate restriction boards and screens to prevent escapes and entry of predators, while allowing water to enter and exit continuously, is vital for cost-effective production. Entrance and effluent structures are scraped clean of barnacles, bivalves and filamentous algae. Screens with mesh of varying sizes are used to keep unwanted organisms out. Water filtration has become an important tool in recent years to exclude WSSV carriers from ponds. Clifford (1999) indicated that most WSSV carriers in the water can be selectively removed by properly screening intake water. Although it may not be feasible to eliminate all WSSV carriers from the ponds by screening, the more their numbers are reduced, the lesser the probability of WSSV transmission. Various sizes of mesh screens are used depending on their positions. Screens are placed:

- after the discharge of the pumps (2-cm mesh);
- in the lateral branches of the main supply canal (1- to 2-mm mesh);
- in the inlet structure(s) of each pond (500-µm mesh as a secondary screen, followed by 300 µm or smaller as a primary screen);
- in drainage canals (2- to 3-cm mesh).

Very fine (200–250 µm) screens are installed in the inlet structure to fill ponds initially. When pond filling is complete, these filters are replaced with 300- to 500-µm screens. Bag net filters are used in the inlet structures to augment effective filtering surface area, and reduce screen clogging. Bag net filters of 2–5 m in length are the best, but there is no limit to the length or size of the bag net filter and, in general, the longer the better (Fig. 21.9). Multiple bag configurations (where a filter bag is placed inside another) are a cost-effective means of decreasing the mesh aperture size without installing a finer screen. Seines or gill nets (2- to 3-cm mesh) are installed in the drainage canals to prevent infected shrimp from escaping (Clifford, 1999). Ponds are inspected for proper completion of necessary preparation steps before filling with water.



Fig. 21.9 A battery of bag nets used to filter the inflowing water at a shrimp farm in north-west Mexico.

21.4.6 Natural productivity

Proper management of natural productivity in shrimp ponds is critical to promote and sustain plankton blooms, and microbial and benthic community productivity. A vigorous phytoplankton bloom will support a healthy benthic community and will contribute significantly to stabilising and maintaining adequate water quality in shrimp ponds. This happens through several mechanisms (Cook and Clifford, 1998):

1. By increasing oxygen production through photosynthesis during daylight hours.
2. By decreasing levels of various metabolites and toxic substances such as ammonia, nitrate, nitrite, hydrogen sulphide, various heavy metals and other undesirable compounds.
3. By regulating pond water and bottom pH (extremely important in ponds with acid-sulphate soils).
4. By providing shade to prevent the establishment of filamentous bottom algae.
5. By increasing turbidity with a consequent reduction in predation by diving birds, among other mechanisms.

Natural productivity is also important because it supports the generation of detritus, the particulate organic material produced from the dead bodies, non-living fragments and excretions of living organisms. In nature, organic detritus is an important food source for many estuarine organisms, and in shrimp ponds it can have an important role. Shrimp feed on detritus, and derive nourishment by stripping the micro-organisms from the detritus as it passes through their gut. In addition, their faecal pellets may be re-colonised and the process repeated until all the organic material has been utilised. By maximising the recycling capability of organic detritus within the culture environment, nutritionists and pond managers have an opportunity to reduce feed and production costs, improve food conversion ratio and reduce environmental impacts.

As a source of carbon for growth, natural productivity is much more important in shrimp ponds with moderate stocking densities than in high densities. In the latter, formulated feeds provide most of the feed consumed by shrimp from the start of the production cycle. In comparison, natural productivity in moderately stocked ponds can sustain shrimp for about 30 days (about 20–30% of the duration of a typical grow-out cycle), depending on the stocking density (10–20 shrimps per square metre). This is until a critical shrimp biomass (100–250 kg/ha) is reached and additional subsidies, in the form of added formulated feeds, are needed to sustain shrimp growth and production. There is extensive evidence that juvenile shrimp feed on plant material and, although generally via intermediate prey species, algae are probably a major source of carbon for these shrimp.

21.4.7 Initial fertilisation

Fertilisation of shrimp ponds is an effective means of stimulating natural food production that can help reduce feed costs (sections 3.4.2 and 9.9). Through fertilisation, managers can promote those pond ecosystem components that are beneficial to shrimp production and discourage those that are detrimental. Several organic and inorganic nutrient sources can be used to fertilise shrimp ponds. They vary in their effectiveness because of differing nutrient density, solubility in water, potential toxicity, C : N ratios and other factors. Appropriate fertilisation rates vary depending on fertiliser type and the ambient nutrient concentration in the water. Various dynamic processes in ponds can also affect fertilisers, producing dissolved nutrient concentrations after fertilisation that may substantially differ from calculated concentrations.

Fertilisation involves the application of fertilisers over the entire pond bottom before filling with water. The objective of inorganic fertilisation during preparation and during the grow-out cycle is to promote and maintain population densities of diatoms and green algae of at least 80 000–100 000 cells per millilitre. These algae groups are reportedly the most nutritionally desirable for shrimp (Clifford, 1994). Desirable phyto- and zooplankton densities in semi-intensive shrimp ponds are shown in Table 21.4.

Table 21.4 Desirable phyto- and zooplankton densities in shrimp ponds with medium culture densities.

	Minimum number of organisms per millilitre	Maximum number of organisms per millilitre
Phytoplankton		
Diatoms: bacillariophytes and chrysophytes	20 000	–
Chlorophytes (green algae)	50 000	–
Cyanophytes (blue-green algae)	10 000	40 000
Dinophytes (dinoflagellates)	–	500
Total phytoplankton cells	80 000	300 000
Zooplankton (copepods, rotifers)	2	50
Protozoans	10	159

Adapted from Clifford (1994) with permission.

There is no universally optimal N : P ratio, and each farm must determine by trial and error the most suitable inorganic fertilisation regime for its prevailing conditions and needs. In areas that have markedly different dry and wet seasons, there are different optimal fertilisation ratios for each season, as well as different optimal stocking densities and other significant management differences.

When selecting inorganic fertilisers, the type (nutrient class, composition and solubility), the N : P ratio, the daily dosage rates and the frequency of application must be considered. The main nutrients needed in shrimp ponds to promote phytoplankton blooms are N, P and Si. Si compounds stimulate production of the very desirable diatoms. Commonly used inorganic fertilisers are urea and sodium nitrate as nitrogen sources, and monoammonium phosphate, diammonium phosphate and triple superphosphate as phosphorus sources. Urea is the most commonly used source of nitrogen because it is widely available, inexpensive and effective. Nitrate-based fertilisers are very effective, but more expensive and less available. The most common silicate fertiliser used is sodium metasilicate, which is expensive and not widely available. An N : P ratio of 15–20 : 1 promotes the development of diatom blooms; however, the N/P ratios used in many shrimp farming areas worldwide can vary from 1 : 1 up to 45 : 1.

Organic fertilisers promote production through direct consumption by the shrimp. Shrimp graze on the bacterial detritus that forms on the fertiliser particles and on other natural food organisms whose populations are promoted by fertilisation. Chicken manure was the main organic fertiliser used in shrimp ponds for many years, with various other manures (duck, cattle and swine) also used to some extent. In recent years, however, farmers have switched to finely ground vegetable meals (wheat, soybean, rice, sorghum and others). These agricultural by-products serve as substrate for bacteria, zooplankton and meiofauna, and they can be consumed directly by shrimp (Cook and Clifford, 1998).

Initial fertilisation enhances the natural productivity of a newly filled pond or tank, reducing the need for costly high-protein start-up feed, and supplying richer more digestible protein, lipids, phospholipids, fatty acids, cholesterol and trace nutrient sources through planktonic and benthic organisms. The juvenile shrimp in a well-fertilised pond that is stocked at 15 PL per square metre can usually reach an average weight of 1–2 g in 30–35 days with minimal supplemental feeding.

21.5 REPRODUCTION AND MATURATION

21.5.1 Hatchery production and the life cycle

The typical life cycle of a marine shrimp in nature begins with adult animals migrating up to several kilometres offshore, maturing and spawning. The eggs initially sink, but after a few hours they hatch and the nauplii (the first larval stage) float to the surface. Marine shrimp usually spawn in areas where favourable currents will eventually bring the developing larval stages inshore into nursery areas such as estuaries, large bays and coastal lagoons. These areas provide abundant natural food and adequate conditions for survival and growth. Developing shrimp remain in nursery areas for several months, and then begin maturing and moving offshore to spawn and complete their life cycle.

Hatcheries in the Western hemisphere are typically large (Fig. 21.10), often belong to a vertically integrated operation that includes a grow-out farm and a processing plant,

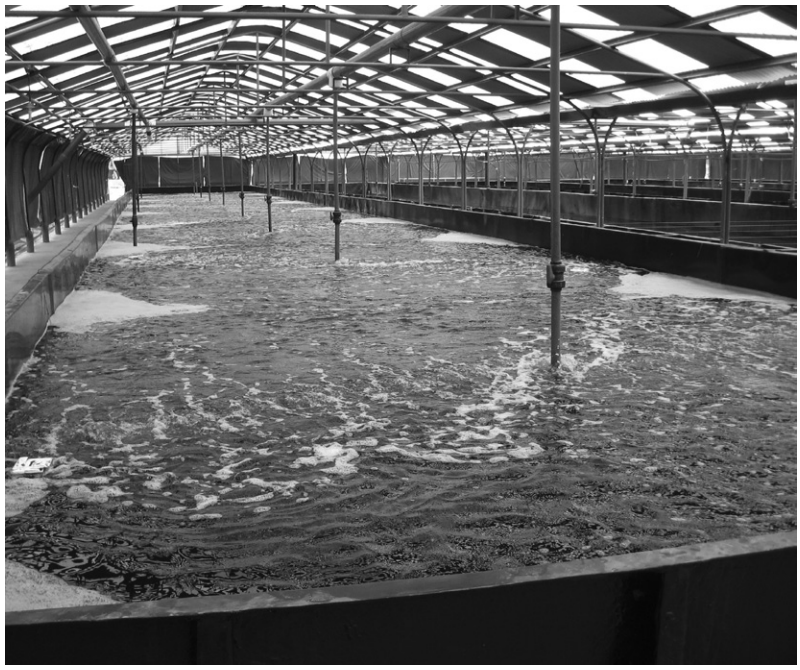


Fig. 21.10 Large tanks for rearing shrimp larvae in a commercial hatchery in Latin America.

and they frequently produce excess nauplii that are sold to smaller hatcheries, or PL that are sold to other farms. In the Eastern hemisphere, backyard and medium-scale hatcheries generally produce most of the seedstock used by farmers (Rosenberry, 2001). In recent years, however, large hatcheries have also started operating in Asia, sometimes as part of integrated operations. Hatcheries produce seedstock ready for stocking into nursery or grow-out ponds and tanks. Shrimp are very fecund animals, producing 100 000–1 000 000 eggs per spawning in the wild, and 50 000–400 000 eggs per spawning in captivity (depending on species, size, water temperature, wild or captive origin, and the number of previous spawns). In the hatchery, gravid female shrimp (wild or matured in captivity) spawn at night, and eggs hatch the following morning.

Larval development from egg to PL is complex and involves three stages: nauplius, zoea and mysis. The first has five or six substages, and the next two generally have three substages each, with each substage lasting some to many hours. The larval development process takes ca. 15 days. As larvae develop and consume the yolk sac, their diet switches to phytoplankton and then to zooplankton. After the mysis stage, they consume a variety of organisms, including the brine shrimp (*Artemia* species). All these live organisms are produced in the hatchery, but major advances have been made recently to develop alternative inert diets (section 9.6). After they become PL, the animals look like small adult shrimp and are able to feed on zooplankton, detritus and commercial feeds. After several days as PL, they are ready to be stocked into nursery or grow-out systems. With the increasing importance of genetic improvement programmes to address biosecurity concerns for various shrimp diseases, hatcheries will play an increasingly important role in the support and expansion of the industry. For detailed information on hatchery procedures refer to Treece and Fox (1993).

It is commonly believed that PL from females matured in the wild are superior and are generally preferred over PL from eyestalk-ablated females (section 21.5.4), which are considered of inferior quality. This ‘lower’ quality of PL from ablated shrimp could be due to less than optimal treatment of broodstock at a time (after ablation) when there are increased energy requirements (proper nutrition) and disease susceptibility. Nauplii production from captive broodstock maturation varies considerably, but has several advantages, including known origin, species and age, increased biosecurity (disease-free status), and relatively predictable availability. These are all very important for production planning and forecasting, and even more relevant in genetic improvement programmes. The use of

wild seedstock can also introduce pathogens, competitors and predators into grow-out systems. The dependence on wild broodstock used to be more serious in Asia than in the western hemisphere. This was because there was less interest in captive reproduction of cultured broodstock in Asia when *P. monodon* was the dominant species. Most of the *P. monodon* broodstock used by Asian hatcheries were from wild stocks. *P. monodon* has been reared in captivity many times since the 1970s, but a combination of cost, technical constraints and a lack of demand for domesticated stocks were some of the reasons for a lack of application on a commercially significant level. However, in recent years there has been a marked trend to develop domestication programmes for *P. monodon*, with both official and private sector backing, and significant progress has been made in several Asian countries to domesticate and selectively breed this species.

Now with the introduction and widespread culture of *L. vannamei* in Asia there has necessarily been development of hatcheries for this species for which there are no wild populations in the region.

21.5.2 Broodstock maturation

The ability to produce seedstock on demand, consistently and in sufficient numbers to support the industry is extremely important. Maturation of shrimp broodstock is undertaken routinely in many countries, and at least 26 penaeid shrimp species have been matured and spawned in captivity to produce viable eggs. The life cycle has only been closed consistently and dependably for a few shrimp species; however, most shrimp hatcheries around the world still depend significantly on wild broodstock. Some shrimp species appear more suited to captive maturation than others: *P. monodon* is comparatively difficult to mature in captivity, whereas *L. vannamei* and *L. stylirostris* are relatively easier. There are three approaches to maturation:

- procure wild, sperm-bearing females that can spawn right away; this usually produces high quality eggs and larvae;
- obtain wild adults, and mature and spawn them in captivity;
- mature and spawn adult shrimp that have been grown in captivity.

The first two approaches are widely practised in most shrimp farming countries. The third strategy, producing succeeding generations in captivity, is the only one that will lead to successful stock domestication and selection for commercially important traits such as fast growth and disease resistance.

Large hatcheries typically have separate infrastructure to carry out broodstock maturation. The maturation section of a hatchery is normally isolated from other sections, to reduce noise levels and stress caused by human activity (section 2.7). Maturation tanks are typically round, about 3–5 m in diameter and 60–100 cm in height, and gently sloped towards a central drain to facilitate removal and siphoning of uneaten food and other undesirable debris. They are generally arranged in batteries or rows in a dedicated room. Tanks are generally black on the inside, and vinyl or PVC liners are commonly used to reduce construction costs, reduce injuries to animals caused by jumping into tank walls, and to facilitate cleaning and disinfection. A sand or pebble substrate is sometimes provided for burrowing species.

Environmental conditions in maturation facilities duplicate or even intensify conditions known to stimulate reproduction (section 6.3). Parameters such as water temperature, salinity, photoperiod, light intensity, lunar phase, maturation room and tank characteristics, and diet may be manipulated and adjusted. Each species has optimum ranges at which maturation will be facilitated. The value of these parameters and their rate of variation over time are critical to stimulate the reproductive process. The water supply system, closed or open, must continuously provide maturation tanks with clear, unpolluted water with oceanic characteristics and a daily exchange capacity of 200–300%. Optimum water temperature for maturation is typically around 28–29 °C. An oceanic salinity of 30‰ is considered optimum, although maturation can occur between 28‰ and 36‰, and pH must be maintained between 8.0 and 8.2.

21.5.3 Open versus closed thelycum species

Penaeid shrimp belong to two groups based on the structure of the female thelycum. This is a receptacle structure on the ventral thorax of females where the spermatophore is deposited by the male at mating. The open-thelycum species are indigenous to the Western hemisphere and include *L. vannamei*, *L. stylirostris* and *L. occidentalis.* The closed-thelycum or 'brown' shrimp species include *P. monodon*, *M. japonicus*, *F. merguiensis*, *F. indicus* and several others:

- Open-thelycum species: moult → mature → mate → spawn.
- Closed-thelycum species: moult → mate → mature → spawn.

Consequently, the management of maturation procedures is different for open- and closed-thelycum species. For example, natural photoperiod is normally used in maturation facilities for most closed-thelycum species, whereas for open-thelycum species a reversed photoperiod regime is typically used (with artificial lights) so that animals will spawn during daylight or normal working hours.

21.5.4 Maturation procedures

The maturation process is relatively simple and includes selecting or sourcing good prospective broodstock (screened for absence of viruses) and holding them under stable, optimal environmental conditions, minimal stress and adequate nutrition using both natural and artificial diets. Exclusion and control of opportunistic shrimp pathogens, such as various bacteria, fungi and protozoea, is critical. It is accomplished by maintaining the best water quality possible and by periodic prophylactic treatments. Depending on animal size, maturation tanks are stocked at three to eight animals per square metre and at ratios from 1 : 1 to 1 : 4 males to females for closed-thelycum species, or 1 : 1 to 3 : 1 males to females for open-thelycum species. Eyestalk ablation is a relatively simple procedure and is normally done only on female shrimp that are hard shelled (section 6.2.2), not on animals that are about to moult or that have recently moulted. Prospective broodstock are normally acclimated for several weeks in maturation tanks before undergoing ablation. It is possible to mature, mate and spawn several shrimp species without resorting to eyestalk ablation, by subjecting animals to a controlled, manipulated photoperiod and water temperature regime (Treece and Fox, 1993).

Adequate nutrition is another factor critical for shrimp maturation, promoting sexual maturation and mating, and improving fertility and offspring quality. Maturation diets typically include combinations of commercial dry and semi-moist pelleted feed supplements, together with natural organisms such as various molluscs (clams and other bivalves, squid), crustaceans, fish and bloodworms. Bloodworms are marine polychaetes rich in the polyunsaturated long-chain fatty acids that are essential for shrimp to mature, and they are commercially available.

There are five ovarian maturation stages in female shrimp: from stage I (immature) to stage IV (ripe or mature) and stage V (spent after spawning). Assessment of the progression in ovary development can be performed by holding animals against a light source, or by shining a flashlight from above over animals in the tanks to determine ovary size and colouration. In a stage I animal, the ovaries are very thin and look like almost invisible strands. As the ovaries develop through stages II to IV, they become progressively thicker and darker as seen through the animal's exoskeleton.

21.5.5 Mating and spawning

Mating in shrimp is characterised by particular courtship behaviour, and various pheromones are involved in sex attraction. Elaborate behaviour with extended and elaborate chasing, contortions and other activities has been described for different species. Mating in closed-thelycum shrimp normally happens at night. In closed-thelycum species, a hard-shell male mates with a soft-shell female that has just moulted, inserting its spermatophore into the female's thelycum near the female's gonopores. The female carries the sperm packet internally until it spawns or moults, and mating typically occurs again shortly. Mating in open-thelycum shrimp normally occurs late in the day between hard-shell males and hard-shell females. Males attach the spermatophore to the ventral surface of the female's body and close to the gonopores. As females of both types spawn and discharge their eggs through their gonopores, sperm exude from the spermatophore and fertilise the eggs. The species and size of the spawner determines spawn size, with larger species and larger animals producing more eggs per spawn. Ablated females will normally spawn multiple times over a period of several weeks. Nauplii can be collected directly from maturation tanks, but in most commercial operations mated females are usually removed and placed in individual spawning tanks of 100–500 L. This is particularly important in operations implementing selective breeding programmes.

21.6 HATCHERY DESIGN AND LARVAL CULTURE

21.6.1 Hatchery design

Shrimp hatcheries can be set up in a warehouse structure or building shell, and have indoor and outdoor infrastructure and facilities. Hatchery design follows the typical pattern (section 2.7), with various areas for microalgae culture, laboratory, brine shrimp production, seawater treatment and holding, spawning, larval and PL rearing. Support infrastructure includes storage space, offices, and aeration and electrical power generating capability. There are small, medium and large shrimp hatcheries, and all have the same basic infrastructure:

1. Small hatcheries are usually a family operation, with very low set-up and operating costs, using simple techniques and untreated water, low culture densities, and

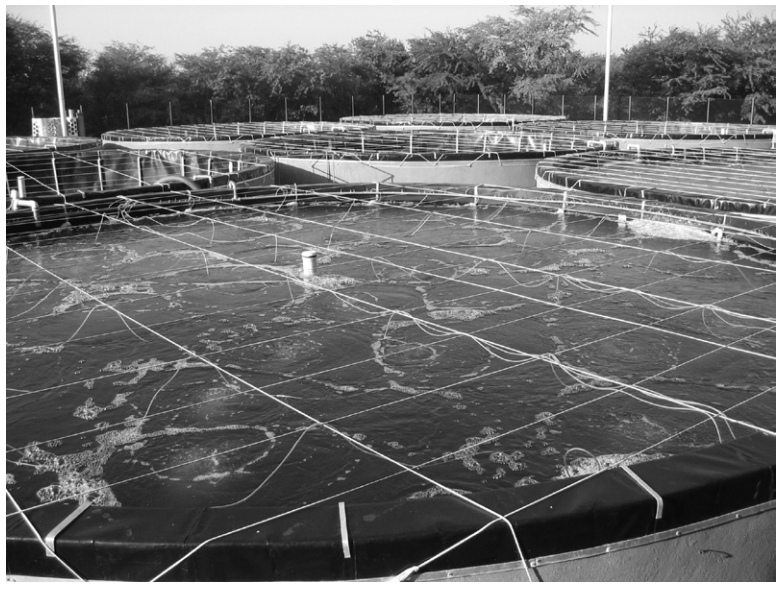


Fig. 21.11 Large outside tanks for rearing shrimp PL, Brasil.

with larval culture tank capacity under 50 000 L. These generally produce either nauplii or PL, and often suffer disease outbreaks and water quality problems. However, they can readily shutdown and restart in a relatively short time. These hatcheries have been very successful in south-east Asia, but are not so common in Latin America.

2. Small- and medium-scale hatcheries are usually based on the Taiwanese design (section 21.6.2), with large culture tanks between 5000 and 50 000 L.
3. Large hatcheries use elaborate techniques to operate controlled culture environments (Fig. 21.11). They target annual production of 100 million PL or more and are typically based on the 'Galveston' system (section 21.6.2). When large hatcheries have water quality and disease problems, they usually require several months to get back on line (Rosenberry, 2001).

21.6.2 Larval culture methods

Worldwide, various methods are used to produce shrimp larvae in hatcheries. These are all modifications of the two basic methods, the Taiwanese and the Galveston methods. The latter is a modification of the former and differs from it in that microalgae are cultured outside the larval tank and are added as required. Over the years, many researchers have helped modify and refine these techniques:

1. The Galveston or 'clear water' method is based on the procedures developed at the Galveston Laboratory (National Marine Fisheries Service) in Texas in the 1970s, and has been successfully adapted to local conditions and implemented throughout the Western hemisphere. It uses high densities of 100 or more larvae per litre, relatively high water exchange rates and elaborate water filtration and conditioning, and produces 6- to 12-day-old PL. Microalgae and brine shrimp nauplii are cultured separately and added to larval culture tanks as required.
2. In the Taiwanese method, lower stocking densities and larger larval culture tanks are used. This method requires large broodstock numbers, and the microalgae blooms are promoted '*in situ*' by directly fertilising larval culture tank water. The Taiwanese method performs well in temperate areas, where there is a short production time, but it can be comparatively harder to manage because of the large water volumes involved. This method is generally not used in tropical areas because of the management difficulties inherent to tropical conditions (Jory, 1993; Treece and Fox, 1993).

21.6.3 Larval nutrition

The larval feeding regime of shrimp hatcheries is typically based on microalgae and brine shrimp (sections 9.3 and

Table 21.5 Feeding schedules of microalgae and brine shrimp nauplii used successfully by several hatcheries for rearing shrimp larvae and PL. In addition, the diet included a dry commercial diet. Z, zoea; M, mysis, PL, postlarvae. Reproduced from Laramore (2000) with permission.

Day	Stage and substage	Estimated survival (%)	Cells per millilitre[a]		Brine shrimp per millilitre[b]
			Chaetoceros gracilis	*Tetraselmis chuii*	
1	Z1	90	40 000	–	–
2	Z1/Z2	85	50 000	–	–
3	Z2	82	60 000	–	–
4	Z2/Z3	80	70 000	–	–
5	Z3	78	80 000	–	–
6	Z3/M1	75	70 000	10 000	0.1
7	M1	70	60 000	15 000	0.2
8	M2	65	50 000	20 000	0.4
9	M3	62	40 000	25 000	0.7
10	M3/PL1	60	20 000	25 000	1
11	PL1–2	58		20 000	2
12	PL2–3	56		10 000	3
13	PL3–4	54			3
14	PL4/5	52			4
15	PL5/6	51			3
16	PL6/7	50			2
17	PL7/8	49			0
18	PL8/9	48			0
19	PL9/10	47			0

[a]Algae count expressed as desired number of cells.

[b]Frozen or killed *Artemia* are used to feed Z3/M1.

Temperature, survival and water quality may affect consumption and, therefore, feeding rates may be modified to prevent overfeeding and tank fouling.

9.4.3) or on live feeds combined with formulated/prepared diets (Table 21.5). The latter can be produced at the hatchery, although in recent years several commercial brands have become available. Most are 'dry diets' packaged under vacuum or in nitrogen-filled cans or pouches, and are produced in a range of particle sizes suited to the feeding habits of the larval stages for which they are intended. Although the sizes of particles may vary from one manufacturer to another, in general zoea diets are <50 µm, mysis 50–100 µm, PL1–PL4 100–150 µm and PL4–PL8 150–250 µm. Crude protein levels typically range from 42 to 48%, whereas lipids are 12–16% and fibre is less than 5%. Artificial diets are not a complete replacement for natural food, but when used supplementary to natural food they can increase both survival rate and growth, when compared with the feeding of natural diets (algae and brine shrimp) alone. Determining which diet to use on a cost–benefit basis should be a high priority with the hatchery manager, and diets have been used successfully by several hatcheries feeding several dry formulations (Laramore, 2000). It is likely that in the near future these diets will totally replace live feeds in hatcheries.

Several species of microalgae are commonly produced in shrimp hatcheries, including species of the genera *Tetraselmis*, *Isochrysis*, *Chaetoceros*, *Skeletonema* and others. Table 21.6 shows some of the microalgae species typically cultured and used in shrimp hatcheries. *Isochrysis galbana* and *Chaetoceros gracilis* are among the best, because of their relatively small size and significant content of highly unsaturated fatty acids. It is common to

Table 21.6 Microalgae species typically used in penaeid shrimp hatcheries. Reproduced from Treece and Fox (1993) with permission of the Texas Sea Grant College Program.

Class	Species
Bacillariophyceae	*Skeletonema costatum*
	Thalassiosira pseudomonas, *T. fluviatilis*
	Phaeodactylum tricornutum
	Chaetoceros calcitrans, *C. curvisetus*, *C. neogracile*, *C. simplex*
	Ditylum brightwelli
	Scenedesmus sp.
Haptophyceae	*Isochrysis galbana*, *Isochrysis* species (Tahitian)
	Dicrateria inornata
	Cricosphaera carterae
	Coccolithus huxley
Chrysophyceae	*Monochrysis* species
Prasinophyceae	*Pyramimonas grossii*
	Tetraselmis suecica, *T. chuii*
	Micromonas pusilla
Chlorophyceae	*Dunaliella tertiolecta*
	Chlorella autotrophica
	Chlorococcum species
	Nannochloris atomus
	Chlamydomonas coccoides
	Brachiomonas submarina
Chryptophyceae	*Chroomonas* species
Cyanophyceae	*Spirulina* species

enrich brine shrimp nauplii with highly unsaturated fatty acid before feeding these to larval shrimp (section 9.4.7).

21.6.4 Probiotics, vaccines and immunostimulants

The ability to control pathogenic organisms, particularly bacteria, has been very important to the success of commercial-scale hatcheries. Until recently, the general method to control pathogenic and undesirable bacteria was through various water filtration and disinfection techniques (e.g. ultraviolet treatment, chlorination). Other common procedures include implementation of hygienic procedures (personnel and equipment disinfection) and use of non-infected cultures of microalgae and brine shrimp. Various biocides and chemotherapeutics are also used to indiscriminately eliminate or reduce the numbers of pathogenic bacteria, frequently as a routine procedure and not necessarily when needed. This practice can bring serious consequences, including development of resistance, chemical traces in shrimp tissues, and environment impacts. Probiotics are single or mixed cultures of some harmless or beneficial bacterial strains that are widely used to promote survival and growth of larval shrimp. Probiotics can be an effective hatchery tool by:

- competitively excluding pathogenic bacteria;
- producing substances that inhibit growth in opportunistic pathogen species;
- providing essential nutrients;
- promoting digestion by supplying essential enzymes;
- direct uptake of dissolved organic material mediated by bacteria.

There is much potential for improvement of probiotics, but more research is needed to identify suitable species and how to promote their population growth in larval culture tanks.

The immune system of crustaceans does not produce antibodies and is relatively primitive, with non-specific immune responses, which means that they cannot be 'vaccinated' in the traditional sense. In recent years, several commercial products have claimed the ability to stimulate disease resistance and promote shrimp health by preventing and minimising the impact of outbreaks of pathogenic bacteria both in hatcheries and during grow-out. In hatcheries, these products can be administered to late mysis and PL by immersion or by microencapsulation using brine shrimp. Results reported from field-testing indicate that some of these products can stimulate non-specific immune responses and improve survival and growth of treated animals, thus enhancing production.

21.7 SEEDSTOCK QUALITY AND STOCKING

21.7.1 Seedstock packing, transportation and reception

It is important that the delivery of seedstock (PL) is well coordinated with the farm, and good planning and communication are required to ensure a smooth transition from the protected conditions of a hatchery to the conditions of an outdoor pond, tank or raceway (Figs 21.11). Timing is critical to ensure that the PL are going into a grow-out environment that has been adequately prepared and has the proper conditions to promote acceptable survival and production. Of particular importance, in the case of outdoor ponds, is that there is sufficient natural productivity to provide adequate nutrition to the animals being stocked. There is a window of about 10 days, starting about 10 days

after pond filling begins, when pond conditions should be adequate for stocking. In general, ponds should not be stocked beyond 20 days after filling has begun.

Seedstock of great quality may be severely stressed by inadequate water quality or high packing densities. Inadequate packing and transportation of shrimp seedstock will significantly affect survival and health. Care must be taken to minimise stress and to provide the best handling and conditions possible. When possible, a farm representative should be present during harvesting of larval tanks and packing of PL for transport to the farm. They should also be involved during counting of PL, to calculate numbers as accurately as possible, and to observe any undesirable conditions and practices. The farm representative may also accompany the shipment, to ensure fast movement without unnecessary delays and to maintain adequate transportation parameters (mainly temperature and dissolved oxygen (DO)) during transit (Fig. 21.12).

Methods of packing and transportation of shrimp PL can vary significantly, depending on origin, species, distance to farm, resources available and other factors. PL are typically transported to farms from hatcheries or from collection and consolidation stations (wild PL):

- in plastic bags with water and added oxygen, within polystyrene foam or cardboard boxes and cooled to 18–22 °C (down from the typical 28–29 °C in hatchery larval culture tanks);
- in tankers ranging in water capacity from 2 to 20 t and set up to provide water aeration/oxygenation.

Feed (e.g. frozen brine shrimp) is added to prevent cannibalism. Packing densities vary between 500 PL per litre and 2000 PL per litre, depending on species, age and estimated time in transit. When possible, PL are transported early in the morning or in the evening, to avoid high temperatures. Transport time should be as short as possible, ideally not exceeding 6–8 h. Reduced water temperature (18–22 °C) and the addition of various compounds to shipping water (including ammonia suppressants, buffers and activated carbon) increase PL survival over extended shipping schedules.

Upon arrival, parameters such as water temperature, salinity, dissolved oxygen, pH and alkalinity must be determined to serve as the baseline for acclimation adjustments to match pond water characteristics. Upon arrival, PL may be transferred to counting or acclimation tanks, which can be located next to the pond(s) to be stocked. Care must be taken to maintain water parameters and avoid any sudden drastic changes.



Fig. 21.12 Shrimp PL held up for inspection.

21.7.2 Counting and quality control

There are various methods used to count shrimp PL, and controversy about the accuracy of each. According to Cook and Clifford (1998), volumetric subsampling is the most common technique. It involves concentrating all or most of the PL in a known volume of water, and drawing a fixed number of subsamples (around five) using a beaker or similar container of known volume. The subsamples are counted and their average is extrapolated to the larger volume.

Stocking only the best-quality shrimp PL is critical to the success of a shrimp farm. Several well-established criteria are used to assess PL quality, including the following:

- their origin and hatchery reputation;
- visual evaluation;
- stress tests;
- various tests to detect the presence of pathogens.

Strict use of PL quality assessment criteria in the evaluation and selection of PL for stocking, and a careful acclimation procedure using the best-quality seedstock available, will have a significant effect on the production and profitability of a shrimp farm. Detailed information on PL quality assessment procedures is given in Clifford (1992).

The strength or 'hardiness' of PL from different hatcheries or batches can vary significantly and the acclimation schedule must be tailored to the PL 'fitness'. Stronger animals can be acclimated at a faster rate than weaker PL. Various stress tests are used to challenge PL and determine a suitable acclimation schedule. These tests typically involve subjecting a PL sample of 100–200 individuals to a thermal, osmotic or chemical shock for 1–4 h and counting the survivors. For example, Clifford (1992) proposed a standardised 'stress test' method whereby a sample of PL is placed in a container and the salinity and temperature are simultaneously brought down to 20‰ and 10 °C, respectively, for 4 h (a test lasting under 4 h does not adequately account for lingering PL mortalities). Survival of 80–100% of the test animals indicates high-quality PL, but 60–79% survival is considered acceptable. A stress test should always be combined, when possible, with laboratory tests to ensure the absence of pathogens.

21.7.3 Acclimation and stocking

Most shrimp farmers spend substantial resources and effort during pond preparation to enable them to stock their PL into a grow-out environment with the best possible environmental conditions, as free of predators, competitors and stress as possible, and with ample supply of adequate food organisms. Still, the transition from relatively benign conditions in hatcheries to those prevailing in open grow-out systems, such as tanks and ponds, where water conditions continually or unpredictably change (day/night, dry/rainy seasons over the production cycle) can be a traumatic experience for PL unless the transition is gradual and stress is minimised.

Proper preparation of the acclimation station and equipment is a critical step in PL acclimation. The acclimation station, including all tanks and other water reservoirs and equipment (nets, siphons, buckets, tubing, others), is thoroughly cleaned and disinfected by scrubbing with chlorine or other disinfecting agent. Well-functioning and calibrated equipment to monitor water parameters (temperature, salinity, pH and dissolved oxygen) before the PL arrive at the acclimation station is critical.

The typical acclimation process involves holding the PL for a period in tanks and slowly adding water from the pond to be stocked to equalise various parameters (mainly salinity and temperature). General acclimation recommendations that have been used for many years include the following:

- increase/decrease salinity by no more than 3‰/h;
- avoid sudden temperature changes (>3–4 °C);
- maintain DO levels at 6–7 ppm.

Acclimation densities should not exceed 300–500 PL per litre depending on animal size and duration of acclimation.

Salinity is probably the most critical parameter to manipulate during PL acclimation. Table 21.7 suggests salinity acclimation rates for various scenarios, including when handling PL of different ages and condition (strong, weak). These recommended schedules may be used as the basis for developing in-house acclimation procedures. That is rapidly developed tolerance of changed conditions, rather than natural acclimatisation to prevailing environmental conditions.

Acclimated PL may be released into the pond using buckets or other containers at points at least 50 cm in depth, at 50-m intervals and on the upwind side of the pond to maximise PL distribution throughout the pond. Excessive turbulence at release must be avoided to prevent damage to animals.

Table 21.7 Suggested salinity acclimation rates for 'strong' and 'weak' PL. 'Strong' refers to animals older than 10 days (>PL10) or strong larvae. 'Weak' describes animals younger than 8 days (<PL8) or weak animals. Reproduced from Clifford (1992) with permission of the World Aquaculture Society.

Salinity change (‰)	Suggested acclimation rate (‰ per hour)	
	Strong PL	Weak PL
From 35 to 20	5	3
20–15	4	2
15–10	3	2
10–5	2	1
5–2	1	0.5
2–0	0.5	0.2
30–40	4	2
40–50	2	1

Survival cages, buckets or net enclosures are commonly used to estimate PL survival 24–48 h after stocking. This information is very important because it is the basis for additional compensatory stocking if initial survival is not satisfactory. Clifford (1992) provides detailed descriptions of the use of survival cages in Latin America.

21.8 PRODUCTION MANAGEMENT AND HARVEST

21.8.1 Water and sediment quality

Some water quality parameters must be routinely monitored to effectively manage shrimp production systems during grow-out. Controlling various parameters can be difficult, but, if not managed properly, pond carrying capacity can be rapidly exceeded and ponds can crash within a few hours or days. Shrimp mariculture requires high water quality to function efficiently and maintain farm productivity and profitability. Therefore, monitoring the quality and properties of intake water, pond water/soil conditions and organics and other parameters in effluent are essential for good animal husbandry. It is also important for farms to be environmentally aware and maintain water management programmes to minimise any potential 'downstream' impacts (section 4.2.1). Table 21.8 shows the general range and monitoring frequency recommended by Clifford (1997) for various water quality parameters that are important in shrimp production systems. These standard parameters are described in section 3.2. They are measured in the field with probes and meters or by taking water samples that are analysed in a laboratory on site. Measurements and samples are taken at several places within a pond, including the water intake, the central region, drain, and surface and bottom layers. This will provide a more representative assessment of the parameter. Boyd (1998) provides detailed information on pond water parameters and their monitoring.

DO is one of the most critical physical parameters of a pond culture. Low DO is one of the most common causes of mortality and poor growth in high-density shrimp ponds. Lethal levels seem to vary from about 0.5 to 1.2 ppm, depending on the species and hardiness of a particular population. DO levels can be relatively unstable as a result of wide fluctuations in photosynthetic oxygen production and the bacterial population affecting BOD. DO levels in the water column fluctuates over a day/night cycle, from a low at dawn to a high in mid-afternoon, particularly owing to phytoplankton photosynthesis during the day and then respiration at night. Hence it is appropriate to sample DO at dawn and mid-afternoon. DO readings in the water column immediately adjacent to the bottom are essential, because shrimp spend most of their time feeding and resting in or on the sediment (Fig. 21.13).

Salinity and temperature are extremely important parameters that affect several biotic and abiotic processes

Table 21.8 General range and recommended monitoring frequency for various water quality parameters in shrimp ponds.

Parameter	Minimum value	Maximum value	Monitoring frequency
Water temperature (°C)	24	30	2× per day
Salinity (‰) (very species specific)	15	45	1× per day
DO (ppm)	3	12	2× per day
pH	8.1	9.0	2× per day
Secchi disc (cm)	30	50	1× per day
Alkalinity (mequiv.)	100	200	1× per week
Total ammonia-N (ppm)	0.1	1.0	2× per week
Non-ionised ammonia-N (ppm)	–	0.2	2× per week
NO_3 (ppm)	0.6	1.2	2× per week
NO_2 (ppm)	–	0.5	1× per week
Total N (ppm)	0.6	2.5	2× per week
Phosphate (ppm)	0.2	0.5	2× per week
Silicate (ppm)	1.0	4.0	1× per week
H_2S (ppm)	–	0.1	1× per week

Adapted from Clifford (1997).

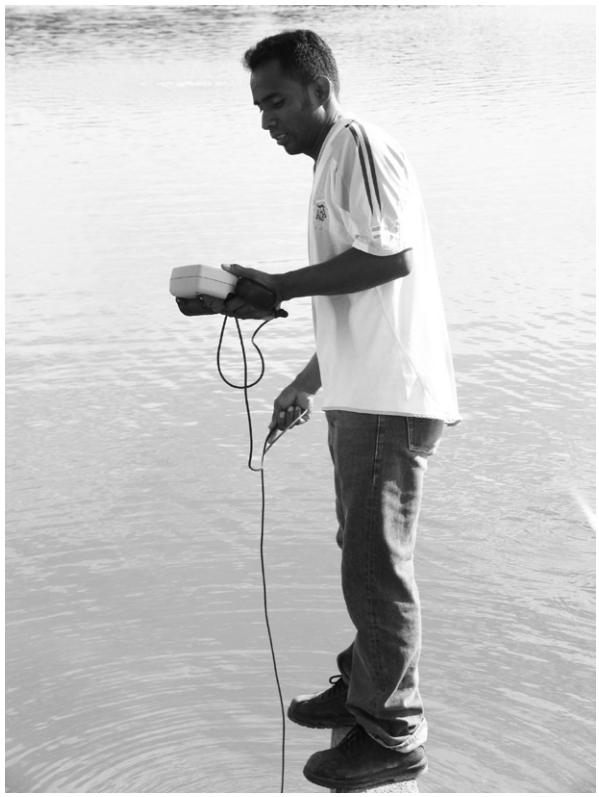


Fig. 21.13 Standing on a plank above the pond with oxygen meter in hand and DO probe down in the water column.

in the production system environment, but there is usually relatively little that can be done to modify them. Water temperature, however, may be controlled in indoor systems of high stocking density. Farms with free access to both good-quality seawater and freshwater can mix these to desired salinities.

pH does not usually reach levels that affect the shrimp. Values seldom exceed the range of 6–9 in sediments or the water column (except in acid-sulphate soils). High pH (>9) is a lesser risk than low pH (<6). Low pH may affect the mineral deposition of the shrimp's exoskeleton after moulting, resulting in 'soft' shrimp, and can destabilise some phytoplankton species that prefer alkaline conditions. pH in the water column fluctuates with phytoplankton photosynthesis and respiration in proportion to dissolved CO_2, which is the reciprocal of DO. pH is lowest at dawn when dissolved CO_2 is highest and highest in mid-afternoon when dissolved CO_2 is lowest. Sediment pH varies less on a diel basis than water column pH, but sediment pH tends to decrease progressively towards the end of the production cycle, due to accumulation of organic acids and nitrification of ammonia.

Nitrogenous wastes resulting from protein digestion can accumulate and even reach dangerous concentrations, particularly at high stocking densities. Nitrite and un-ionised ammonia can accumulate to toxic levels periodically, particularly during massive die-offs of phytoplankton. Nitrate is usually not toxic at levels typically found in ponds. Total ammonia and nitrate are managed through water exchange.

Water transparency is an index of plankton biomass present and is measured with a Secchi disc, typically once per day at mid-morning (Fig. 3.2). Acceptable values are between 30 and 50 cm. Readings of less than 30 cm indicate high phytoplankton biomass or suspended sediment or both in the water column. Pond water colour usually reflects the predominant phytoplankton species: golden brown means diatoms; green means green flagellates; blue-green is blue-green algae; and red is dinoflagellates.

Pond sediments tend to deteriorate with successive cycles and within production cycles owing to accumulation of organic matter and sludge deposition. Sediment quality can be determined from hydrogen sulphide level and redox potential (E_h) (section 3.2.14), and by visual examination of sediment cores. Smelling a scoop of surface mud from various areas of the pond bottom is a quick check for hydrogen sulphide sediment. Redox potential of sediment is another quantitative indicator of sediment conditions. It is a measure of the proportion of oxidised to reduced substances, and is an indicator of the relative activity of aerobic and anaerobic bacteria in the sediment profile (Fig. 3.7).

21.8.2 Water management

Water exchange is an effective tool for pond management, namely to flush out wastes and, in some cases, to improve DO levels. At many large farms, water exchange often follows a fixed schedule rather than being a flexible alternative used when pond conditions require it. High exchange rates can be wasteful and have a negative effect on fertilisation and natural productivity by flushing out nutrients. As much as 35–40% of pond volume may be exchanged daily in some ponds stocked at high and very high densities. More typical exchange rates for medium and high stocking densities are, however, about 5–25%. With the continuing incidence of various shrimp viruses and declining shrimp prices, the global trend to reduce water exchange rates to maximise biosecurity through exclusion of pathogens continues.



Fig. 21.14 Liming shrimp ponds on a regular basis to improve water quality is a common management technique.

Fertilisation of ponds with inorganic and organic nutrients is an effective means of stimulating the production of natural foods and reducing feed use. In the first few weeks, it may be necessary to fertilise a pond every day, then every other day, and eventually biweekly. Routine fertilisation should be enough to maintain adequate water transparency (30–50 cm). Proper maintenance fertilisation can provide a continual natural food supplement and improve water quality. Once feed applications reach about 25–30 kg/ha/day, no additional nitrogen or phosphorus fertilisation is usually needed, because uneaten feed and shrimp faeces supply enough. Liming compounds are added to ponds in some farms (approximately 20–100 kg/ha/week) during the cycle to increase alkalinity, particularly during the rainy season or in areas where low-salinity waters are prevalent. Liming compounds are also added under the presumption that water quality will be improved and pathogens in the water reduced (Fig. 21.14).

21.8.3 Water aeration and circulation

Pond aeration is the primary life support system of many aquaculture ponds, including shrimp ponds. Management of DO aims at maintaining DO levels over 4.0 ppm or higher, if possible, throughout the pond, using the least equipment and with minimum cost. Efficient use of aeration and circulation equipment should take advantage of the oxygen supplied by photosynthesis during the day and by diffusion across the pond surface at night. Aerators are mechanical devices that act as the heart and lungs of an aquaculture pond, providing distinctive circulation and aeration effects, increasing the rate at which oxygen enters water. There are two main techniques for aerating pond water: one is to splash water into the air and the other is for air bubbles to be released into the water, so there are 'splasher' and 'bubbler' aerators (section 3.4.3).

Shrimp ponds require mechanical aeration when production biomass exceeds 2 t/ha and additional aeration at a rate of 2 kW for each additional tonne. The moderate circulation effect provided by one or two aerators is desired from the time of stocking. Surface circulation must be ca. 4 cm/s during feed applications. This speed will not disturb fresh pellets, but will keep finer particles and organics in suspension. Excessive water circulation can be managed by arranging aerators in uniform configurations or by other means. In a typical configuration, aerators generally create a central area of sediment deposition, and sometimes the sludge deposited may be removed by using suction hoses. There are central drains in some ponds with high stocking densities. Proper positioning of aerators and circulators is very important, because the operating efficiency of aerators is highly dependent on achieving adequate water circulation (Fig. 21.15). They must be positioned to generate a whole-basin flow pattern and produce an efficient water movement. This is achieved by aligning the current from each unit so that it supplements rather than conflicts with the overall flow pattern in the pond (Figs 5.7 and 21.15). Aeration equipment must be placed at the points of lowest DO within a flow pattern that encompasses the entire pond.



Fig. 21.15 Semi-intensive shrimp ponds with paddlewheel aeration. Note the feed station poles where feed trays are used, and the feed technician in a kayak servicing the trays.

One of the needs for circulation and aeration is thermal stratification. Thermal stratification can occur in ponds when surface waters warm faster than deeper waters during the day (Fig. 3.1). This may lead to DO depletion in the bottom water, because most of the DO in pond water originates from photosynthesis in the upper stratum of water or by diffusion from the air through the water surface. One kind of mechanical device often accomplishes aeration and circulation. Paddlewheel and propeller–aspirator pump aerators are especially efficient in circulating pond water. There are also circulators that supply little aeration but can prevent stratification. These are generally large, slowly revolving (50–150 rpm) propellers installed in ponds to quietly move large volumes of water.

21.8.4 Population sampling and health assessment

Periodic sampling of shrimp populations is an important management tool during the production cycle. Cast netting is about the only effective sampling tool currently available and it is widely used. The process of sampling has the objective of generating information about a large group of individuals, a shrimp population in a pond, by looking at a small number of individuals. Most shrimp farms routinely perform sampling programmes to:

- monitor population size;
- monitor individual and average size/weight of animals;
- evaluate the animals' physical condition, appearance and product quality;
- assess the animals' overall health, and to test for the possible presence of known pathogens or diseases.

In the past few years, and as their use becomes widespread, it has been realised that properly managed feed trays (section 21.9.8) or lift nets can provide adequate population estimates by combining daily feed consumption rates with percentage body weight curves.

One of the most important considerations is to eliminate or minimise sampling bias. In general, the biases are relatively consistent and, with experience, correction factors can be developed to compensate and produce good workable approximations of shrimp size and populations. Estimates of shrimp population size and survival rates can be remarkably accurate if properly done or very inaccurate owing to variable shrimp activity, for example moulting, lunar and tidal cycles of behaviour, size differences in habitat preference and catchability. In general, to improve the validity of a shrimp population sampling programme, sample collection must be performed after lowering the

pond water level by experienced personnel using a large and heavy cast net, and the number of sampling stations and frequency of sampling must be as large as possible. It is important for a farm to establish adequate in-house sampling methods that adequately reflect its needs and capabilities (Clifford, 1997).

Health assessment and management on shrimp farms has become an important issue in the past 12 years or so, because of the increased importance of various diseases, particularly of viral origin. There are now around 25 distinct viruses (or groups of viruses) known to infect marine shrimp. Viral diseases have had a severe impact on the shrimp farming industry worldwide, causing very important production and economic losses.

- Viruses belonging to the WSSV, MBV (monodon baculovirus), BMN (baculoviral mid-gut gland necrosis), HPV (hepatopancreatic parvovirus), IHHNV, the yellowhead virus (YHV) group, and TSV (Taura syndrome virus) have been important pathogens of cultured shrimp in Asia and the Indo-Pacific regions.
- TSV, IHHNV and BP (baculovirus penaei), and more recently the IMNV (infectious myonecrosis virus) have been the most important in the Americas.

Once thought to be limited to Asia, WSSV and YHV have been found in cultured and wild shrimp in the Americas and both viruses have been shown to be present in commodity shrimp imported from Asia and sold directly on the USA market. WSSV was first reported in Central America in 1999, and from there it expanded to most of the shrimp-farming countries in the region, where it still is causing significant economic losses.

Several strategies have been tried to control viral diseases in shrimp farming, ranging from improved husbandry practices to stocking 'specific pathogen-free' or 'specific pathogen-resistant' species or stocks. Further information on shrimp diseases is provided by Lightner (1996) and in section 10.5.2.1.

A cost-effective health management and biosecurity programme requires reliable diagnostic tools that shrimp farmers can use to make adequate and timely decisions on management procedures to control or exclude pathogens. Virulent pathogens can produce catastrophic mortalities very rapidly, and shrimp farmers need this fast diagnostic capacity in order to respond effectively. Practical diagnostic methods that are accurate, sensitive, rapid and economical to conduct are already available, including polymerase chain reaction, dot–blot gene probes and various methods for rapid fixation and staining (Fegan and Clifford, 2001).

Early signs of many health problems can be promptly observed by examining shrimp during regular feed tray monitoring or from weekly sampling. Some of these signs include the following:

- loss of appetite (empty guts);
- changes in colour (blueish or reddish);
- persistent soft shells or shell fouling;
- red discolouration (particularly in appendages and uropods);
- lethargic or disoriented, behaviour;
- fouled or discoloured or black gills;
- blackened lesions on shell;
- opaque or white tail muscle;
- various morphological deformities, such as cramped tail.

21.8.5 Harvest and transport to processing plant

Shrimp are highly perishable and delicate, and no amount of manipulation can restore product quality once it is lost. Therefore, proper preparation, harvesting and preservation are critical for the product to be the best quality and command the best price. This preparation can be quite elaborate, because a pond ready to be harvested may have 10–20 t or more of shrimp, which must be properly collected, handled and packed (Fig. 21.16). The most important objectives at harvest are to:

- minimise the quantity of shrimp left on the pond bottom;
- immediately chill shrimp to near freezing to prevent deterioration due to bacterial activity;
- pack the shrimp in a manner that avoids physical damage.



Fig. 21.16 Harvesting procedures are critical for the best-quality product that commands the best price.

The most important considerations of when to harvest a pond are shrimp size, price and maximising the economic return of the production cycle. Sometimes ponds need to be emergency-harvested because of a disease outbreak. Many shrimp farmers use the moon phase to programme their harvests, targeting periods of full and new moon. About 3–5 days before the harvest date, the texture (representative of the stage in the moulting cycle) of shrimp to be harvested is monitored by daily collection of a sample (100–300 animals per pond) to determine the percentage that are moulting. The particular requirements of the processing plant and the intended market determine what percentages of hard, soft and semi-hard (post-moulting) shrimp, are acceptable to proceed with a planned harvest. For example, for shrimp to be marketed whole, typically less than 5–8% should be soft, and less than 15–20% should be semi-hard. Some farmers also stop feeding a few days before the harvest, but this is a decision that really depends on each pond, its biomass and its overall condition (Clifford, 1997).

Most shrimp are harvested by draining the production ponds, tanks and raceways. In preparing ponds for harvest, water levels are typically lowered to approximately 70% of their operational levels, beginning 24–48 h before the harvest. In larger ponds it is often necessary to reduce water levels to 50% or so before beginning the harvest, so that the harvest does not last excessively long, which would stress the shrimp and reduce their quality. Most pond harvests are undertaken during the night to avoid high water temperatures. A net is placed covering the outflowing water and any shrimp remaining are picked up by hand or dip nets. The quality of these residual animals is generally reduced because of mud embedded in their gills and joints between tail segments, and delayed chilling. Harvested shrimp are immediately separated into size classes and layered with ice or immersed in an ice slurry for transport to the processing plant. Within minutes after death, shrimp begin to deteriorate at normal pond temperatures (25–30 °C) that promote bacterial decomposition. Initial spoilage, however, is due to digestive enzymes from the hepatopancreas. These break down proteins and reduce product weight, and quickly break down tissue at the junction of the head and tail, making the head appear loose and sagging, which is unacceptable to the European market for head-on shrimp. Bacterial and enzymic activity can be stopped by immediately reducing temperature to near freezing. It is very important that shrimp are killed by thermal shock when immersed in chilled water (4 °C is maintained from the time the animals are harvested to the time they reach the plant). Often additional processing takes place at the pond bank. This includes dipping the shrimp in chlorinated water (50–60 ppm) or a solution of sodium bisulphate to reduce blackening of the head for head-on shrimp product, removing crabs, fish and plant material, and selecting the largest animals to be used as potential broodstock (if needed). Shrimp are handled carefully during packaging to minimise damage to the product and thus reducing its acceptability in markets. Mechanical harvesting systems are being developed to harvest shrimp ponds and assure product quality.



Fig. 21.17 Shrimp processing plant.

Further details of post-harvest technology for shrimp, including safety and health, live transport, post-mortem processing and chilled storage life, are provided in Chapter 11. A processing plant that operates on a QA/QC (quality assurance/quality control) basis is shown in Fig. 21.17.

Shrimp are marketed in a variety of forms: heads-on shrimp (including live), shell-on tails, peeled tails (included canned) (Fig. 21.18), breaded tails and other value-added products. These are transported to various markets in a variety of forms and packaging.

21.9 NUTRITION, FORMULATED DIETS AND FEED MANAGEMENT

21.9.1 Nutritional requirements and formulated diets

The nutritional requirements of marine shrimp, their feed formulation and manufacture have been extensively discussed and documented, but much still remains to be learned. With industry expansion there has been an intensification of production and an increased dependence on the use of manufactured dry feed, which often represents the highest production cost. Protein is typically the most expensive macronutrient in shrimp feeds, and dietary protein levels from 18 to 60% have been recommended for various species and sizes of marine shrimp species, possibly because of their wide range of natural feeding habits. PL shrimp require a higher dietary protein level than older shrimp. Formulated shrimp feeds are complex products and the main components typically are wheat flour (20–35%), soybean meal (15–45%) and fish meal (10–25%). These few ingredients contribute most or all the protein, amino acids and energy. In recent years there has been a strong effort to reduce the inclusion of fish meal and fish oil and replace these with new, alternative ingredients, including various meals and oils from agriculture products as well as rendered ingredients from the processing of other terrestiral meat industries. The remaining ingredients used in typical formulae include various lipids and micro-ingredients that provide essential fatty acids, vitamins, minerals, attractants, binders, preservatives, pigments and health additives (Table 21.9).

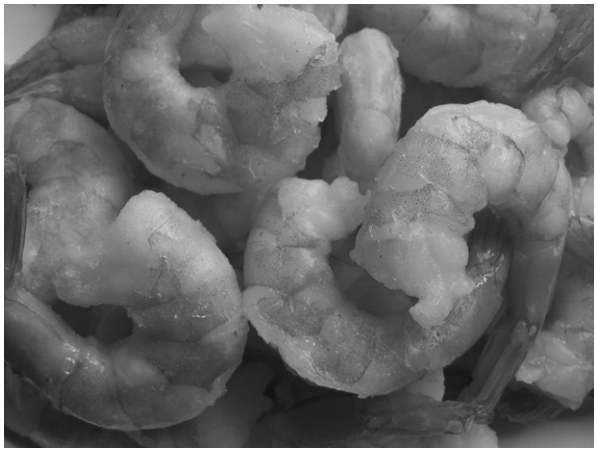


Fig. 21.18 Peeled and cooked shrimp is a common value-added market form.

Table 21.9 Recommended nutritional composition of shrimp feeds. Only critical nutrients for feed formulations are listed on as-fed basis. Reproduced from Akiyama (1992) with permission of the World Aquaculture Society.

Nutrient	Shrimp 0–3 g	Shrimp 3–15 g	Shrimp 15–40 g
Protein (minimum)	40.0	38.0	36.0
Lipid (minimum)	6.2	5.8	5.5
Lipid (maximum)	7.2	6.8	6.5
Fibre (maximum)	3.0	4.0	4.0
Ash (maximum)	15.0	15.0	15.0
Calcium (maximum)	2.3	2.3	2.3
Phosphorus available (minimum)	0.8	0.8	0.8
Potassium (minimum)	0.9	0.9	0.9
Lysine (minimum)	2.12	2.01	1.91
Arginine (minimum)	2.32	2.20	2.09
Threonine (minimum)	1.44	1.37	1.30
Methionine (minimum)	0.96	0.91	0.86
Methionine/cysteine (minimum)	1.44	1.37	1.30
Phospholipid (minimum)	1.0	1.0	1.0
Cholesterol (minimum)	0.35	0.3	0.25
20:5 *n*–3 (minimum)	0.4	0.4	0.4
22:6 *n*–3 (minimum)	0.4	0.4	0.4

Chamberlain and Hunter (2001) reviewed additives used in formulated shrimp feeds. In total, approximately 110 additives are commonly used as ingredients in shrimp feeds today.

1. Attractants include animal by-products (crustacean tissue meals, squid by-products, fish and meat extracts of low molecular mass), and purified compounds (free amino acids, artificial flavours, betaine and nucleotides).
2. Enzymes supplements improve digestibility of phytate, fibre, indigestible sugars and other components.
3. Various additives, including coccidiostats, antibiotics and hormones, have been used as growth promoters in shrimp.
4. Other additives, including immunostimulants, probiotics and vaccines used to stimulate the immune system of shrimp and improve resistance to disease. This has become especially important to reduce outbreaks of shrimp viral diseases. The immunostimulants used include β-glucans, bacterial extracts, blood plasma, seaweed by-products and yeast.
5. Wheat flour is the principal binder used in shrimp feeds but, by itself, may not provide proper water stability, especially if the manufacturing process does not include fine grinding, extended conditioning and post-pellet conditioning. Therefore, additional binders are often needed to assure water stability.
6. Additives are used to improve shrimp pigmentation, which is significantly determined by dietary carotenoids. Some markets have a premium demand for shrimp with strong natural pigmentation, and ovarian pigmentation is important for maximum reproductive performance.
7. Finally, because shrimp feeds are typically stored for several weeks in humid tropical environments, antioxidants (butylated hydroxytoluene, butylated hydroxyanisole and ethoxyquin) and preservatives are generally added to prevent oxidation of fats and mould infestation (Chamberlain and Hunter, 2001 and section 9.10.5).

21.9.2 Feed management

Shrimp feed management is a critical aspect for cost-efficient, environmentally responsible shrimp production. Appropriate practices will produce maximum shrimp growth and survival concurrent with the lowest feed conversion, while reducing feed inputs and minimising impact of effluents. Efficient feed management is the summation of several sequential steps, including feed selection, storage and handling, application methods, and feeding regimes. Determining when to feed requires knowledge of diel activity patterns, feeding frequency and time (subject to change with geographical location, season, species, size, age, stocking density, unusual environmental conditions and other stimuli). Calculating feed rations involves estimating survival, population size and biomass, and size distribution. Monitoring and continuously adjusting the amount of feed applied, according to changes in consumption caused by various biotic (e.g. amount, quality and availability of natural food items) and abiotic (water quality and other environmental parameters) factors, is important for effective feed management. Evaluating and adjusting feed input involves regular population sampling and proper monitoring of various water quality parameters. Shrimp are bottom feeders, and it is difficult to estimate their feed consumption, unless feed trays (section 21.9.8) or lift nets are used. Inadequate feed management may promote the onset of various diseases and water quality-related problems, and may adversely affect production. Ineffective practices often include the following:

- applying feed during times convenient for employees (during daylight hours), but not necessarily at the best times for shrimp;
- inadequate handling and storage practices during bulk feed storage and after feed distribution to pond side;
- overfeeding.

The best shrimp feed in the world will yield poor results if it is not handled, stored and used properly.

21.9.3 Factors that affect feed consumption

Several factors can affect shrimp feeding behaviour, and it is important to understand these to make proper and timely management adjustments (Clifford, 1992, 1997; Jory *et al.*, 2001). The major factors affecting feeding behaviour are as follows:

1. Species, age and size. There are marked differences between species. Some species are much more active and aggressive while foraging for food, and this has to be incorporated into a feeding strategy. Feeding rate is a physiological function dependent on the growth stage of the animal: it decreases as the animal grows and approaches maturity. Growth immediately after pond stocking can be 12–15% per day, decreasing to 1–2% per day towards the end of the production cycle.
2. Availability of natural food. When natural food is very available, the demand for formulated feeds is reduced. This is typical when the biomass of stocked shrimp is

low during the first few weeks after stocking and until the natural carrying capacity of the pond is reached.

3. Water quality. The most important parameters are temperature, DO, pH and salinity, but other parameters also influence the shrimp (Table 21.8). For each parameter, animals have a range of tolerance and a narrower optimum range that promotes optimum feeding, growth and overall well-being (section 3.3).
4. Moulting. Shrimp moult periodically (days–weeks) throughout their lives, and this is a stressful period during which their appetite diminishes markedly. It can take 2–5 days for normal feeding to resume after moulting. Thus, it is important to recognise when there is a significant reduction in feed consumption (use of feed trays is a good method) indicating high incidence of moulting in a pond.
5. Quality of commercial feeds. Shrimp eat to fulfil their nutritional needs, and if the feed they consume does not have enough energy or appropriate nutritional profile, their feeding activity will increase. Feed attractability and palatability are also important factors.

21.9.4 Feed handling and storage

Feed management at a shrimp farm begins upon arrival of a feed shipment. Poor storage and handling of feeds will result in product deterioration, reduced feed attractability and palatability, possible nutritional deficiencies and disease outbreaks, and reduced growth rates and overall production (sections 9.10.3 and 9.10.4). Upon reception of a feed batch, a few randomly selected bags are examined for physical integrity. In addition, twice a year feed samples are collected from newly received shipments (or when using a new feed) and analysed for proximate composition, mycotoxins and selected pesticides if pertinent (Jory *et al.*, 2001).

Feed is ideally used within the first 2–4 weeks after manufacture and must not be stored for more than 2–3 months. At farms that feed several times over 24 h, the total feed ration is often distributed from the farm warehouse to the ponds once, usually early in the morning. The feed bags must then be protected from sunlight and rain by storing them off the ground in simple pond-side sheds.

21.9.5 Application and distribution

Formulated feeds can be applied in a variety of ways to shrimp production systems (section 9.11.2). Feeds may be distributed manually in large ponds from boats or mechanically using blowers mounted on vehicles and boats (Fig. 21.19). Even crop-dusting aeroplanes have been used to distribute feed to very large ponds. In small ponds, tanks and raceways, automatic feeders with timing mechanisms



Fig. 21.19 A feed blower used to feed shrimp.



Fig. 21.20 Manually broadcasting feed from a boat.

can be used (section 9.11.2). Feed is applied exclusively using feeding trays at some farms. Broadcasting feed from small boats is used by some farms (Fig. 21.20).

It is important to distribute the feed evenly early in the grow-out cycle, but, as the cycle progresses, shrimp react to changing pond microhabitats. They avoid areas where anaerobic sediments accumulate and noxious compounds, such as H_2S, are produced, including internal drainage canals and areas close to the drainage structures. In addition, many shrimp will move to the deeper areas of ponds during the day to avoid light. Therefore, it may not be appropriate to provide feed to very shallow areas during daylight hours, because it is unlikely shrimp will consume it. It is also important to recognise that shrimp distribution in ponds is generally not uniform along the linear dimensions of the pond.

21.9.6 Frequency and timetables

All formulated feeds have an ideal feeding rate range that optimises growth and feed efficiency. This range varies with species, age and weight, stocking density, water quality, availability of natural foods, stress and other factors. At many farms, feeding is based on tables that do not properly consider natural feeding habits of shrimp or their physiological state. Increasing the frequency of feeding generally produces immediate benefits, including reduced nutrient and feed loss, and increased growth and feed utilisation efficiency. In Asia, it is common practice to feed up to six to seven times over a 24-h period regardless of the species farmed. Because *L. vannamei* appears to be less nocturnal than *P. monodon*, most shrimp farms in Latin America feed one to three times per day, usually between the morning and late afternoon. Because of the higher degree of activity shown by *P. monodon* at night, farms in Asia that still culture this species may provide most of the daily ration during night hours.

How many times and when to feed is an important decision that each shrimp farm must determine, based on experience, production system used, season, environmental conditions, species, stocking densities and available resources. Feeding during the night typically becomes more important as the production cycle progresses and the availability of natural feed diminishes. In general, a minimum of two feed applications per day are needed at the beginning of the production cycle, increasing as the cycle progresses to at least three or four applications per day.

21.9.7 Feed rations

Feed rations can be calculated using a set schedule that accounts for animal weight and estimated biomass/survival in the pond. Feeding based on tables is still widely practised (section 9.11.1).

There are several problems with relying on feeding tables:

1. There are problems in accurately estimating survival, particularly when dealing with small animals in large (approximately 5-ha) ponds.
2. Various factors (section 21.9.3) affect feeding rates.

Feed consumption changes can usually be detected by proper monitoring of feed trays (section 21.9.8). Feeding rates are adjusted periodically (usually weekly), based on sampling estimates for individual average body weight, population size distribution and pond shrimp biomass. As shrimp grow, the feed amounts used decrease as a percentage of the total shrimp biomass, but the absolute amount of feed increases together with increasing shrimp biomass (Jory *et al.*, 2001).

21.9.8 Use of feed trays

Feed trays are also called feed monitoring trays, observation or lift nets, feed inspection trays or umbrella nets. They are simple devices usually consisting of a frame and fine mesh that is lowered to the floor of the pond (Fig. 21.21). There are several ways feed trays can be used, including the following:

1. As indicators of feed consumption, population and health assessments and other observations.
2. To apply all feed (Peruvian system).



Fig. 21.21 Using a feed tray at set locations.

3. A combination of these two.

In using the feed trays as indicators of feed consumption, about four to eight trays per hectare are used in ponds less than 5 ha, whereas two to five trays per hectare are used in larger ponds (10–20 ha). A small percentage of the ration is placed in the trays and the ration is distributed throughout the pond. Trays are checked after 1–3 h and the data are used to adjust rations. In the Peruvian system, the entire ration is applied in trays, which requires many more trays per pond.

The reliability of feed trays for estimating food consumption has been questioned. It has therefore become common for most farms, when feeding a pond for the first time, initially to follow their own or the feed manufacturer's guidelines and tables, and then begin adjusting daily rations using feed trays (Jory *et al.*, 2001). However, in general, observation of feed consumption from a small number of trays is not an adequate measure of actual feed consumption, especially in large ponds. This is because there are considerable day-to-day variations in feed consumption, as outlined in section 21.9.3.

21.10 EMERGING PRODUCTION TECHNOLOGIES AND ISSUES

21.10.1 Diseases and biosecurity

Viral diseases have had a considerable impact on commercial shrimp farming during the past two decades (section 21.1.2), significantly affecting the operation, management and design of shrimp farms. Another resulting consequence is increased awareness of the need for better husbandry methods to reduce the risks of exposure to pathogens, and of the need for improved management practices to enhance shrimp health. Shrimp farming is a relatively new industry and has lagged behind in the development of practices for standard health management. This in part resulted from the relatively poor level of understanding of shrimp physiology and their production systems. Also, there had been limited involvement by veterinarians and animal health specialists to develop health management practices like those in use in terrestrial animal husbandry. But in recent years tremendous progress has been made in shrimp health management.

The modern poultry industry, with its considerable advances in production and disease control, best exemplifies the advantages of biosecure animal production, and shrimp farming must adopt similar practices to become more competitive. Biosecurity in shrimp aquaculture involves those practices that will reduce the probability of introduction and dissemination of a pathogen. Shrimp producers often give only limited attention to routine biosecurity on their farms. This is because of the misconception that the potential costs of implementing biosecurity measures will outweigh the benefits or because they do not have appropriate knowledge. Effective implementation of biosecurity protocols requires awareness, discipline and a commitment by farm owners to implement them. We have improved our knowledge of shrimp viral diseases significantly during the past 20 years, mainly owing to their negative impact on the industry, but biosecurity is still very new to shrimp farming (Fegan and Clifford, 2001).

A cost-effective, biosecure shrimp-farming protocol involves the following:

- aggressive methods for pathogen exclusion from production systems;
- effective screening of seedstock;
- appropriate environmental management;
- effective health management, integrating genetic selection;
- specific pathogen-free and/or pathogen-resistant stocks;
- limited or zero water exchange;
- stocking strategies;
- feed management and use of immune stimulants to increase host defences;
- strict and proactive health monitoring and farm management strategies.

Also important are farm location (site selection) and design. Currently, only a few shrimp farms have been specifically designed to prevent diseases, although it is more cost-effective to incorporate disease prevention and treatment aspects during the planning stage than to redesign or refit existing farms. Eliminating or reducing water exchange is an important aspect to prevent viral diseases, to exclude viral carriers or free virions from production systems and to minimise stressful variations in water quality, which may trigger disease outbreaks. There are several examples of successful commercial farms in Asia and Latin America that use very low water exchange (section 21.10.5).

21.10.2 Probiotics and microbial management

As described, the use of probiotics is relatively common to limit pathogenic bacteria in the disease-prone intensive systems used to produce shrimp PL in commercial hatcheries. For some time now, the use of bacterial amendments has been recommended for use in aquaculture ponds to obtain several benefits (Boyd, 1995), including the following:

- reducing blue-green algae populations;
- preventing off-flavour;
- reducing nitrogen and phosphorus levels;
- increasing DO;
- promoting decomposition of organic matter.

There is ongoing research on the use of probiotics, which could be important in promoting shrimp yield in intensive systems, in the implementation and optimisation of low- and zero-water exchange production systems, and in managing the quality of pond effluents.

The pond microbial community plays a major role in the natural food availability, mineral recycling rates and DO dynamics in shrimp ponds. Effectively managing the microbial community can help prevent or reduce the risk of a disease outbreak, but, if mismanaged, the microbial community can also promote disease by creating conditions that facilitate growth of pathogenic bacteria. Microbial management is a relatively new but most promising field for the shrimp farming industry.

21.10.3 Nursery systems

Jory and Dugger (2000) reviewed the use and advantages of nursery systems for PL shrimp. These systems generally produce higher overall survival rates per production unit area and more efficient capital utilisation than direct stocking into grow-out systems. They also provide better management of environmental conditions, feeding, and exclusion of pathogens, predators and competitors. It is critical to know the survival, quantity, condition and quality of seedstock before stocking into ponds before investing several months of effort and resources into a production cycle. Nursery strategies involve holding seedstock at very high densities (5000–10 000 PL per square metre) in specially designed facilities for 20–40 days, with precise technical management, feeding and water quality monitoring.

A two-stage grow-out system using a nursery phase as a quarantine area increases biosecurity. Indoor nursery systems increase turnover (number of grow-out production cycles) by reducing culture time to market size in grow-out ponds. Therefore, the grow-out pond is being used more efficiently as a biological system, and with greater capital and operating efficiency. A nursery system also provides improved accuracy to estimate the juvenile population before actual stocking in grow-out ponds. Thus, stocking juveniles allows for a more accurate estimate of the initial population and biomass, and improving feeding rate estimates when formulated feed becomes up to 60% of the production cost. Indoor, intensive nursery systems can further broaden the effective temperature, stocking windows for seasonal hatchery outputs, allowing greater efficiency for the hatchery and farm. Shrimp farms in areas of lower salinities can use the nursery as an acclimation system. Nursery head-start strategies may allow farms without hatcheries to purchase seedstock in advance of the peak demand periods, possibly at lower cost and with improved certainty of seedstock delivery.

Managing nursery systems in tanks and raceways is relatively more difficult than standard grow-out ponds stocked directly, but the many benefits derived from a two-phase grow-out strategy, using first a nursery system (indoor) followed by final grow-out to market size (outdoor pond) can significantly improve production and profitability.

21.10.4 Inland shrimp production

Marine shrimp farms have traditionally been built in tropical coastal areas, very close to the ocean or to an estuary or river. Recently, shrimp farms have been built in other environments such as inland areas and desert coastlines that, in principle, do not appear suitable for this activity. Some of these farms are located in inland deserts with available undergroundwater having specific chemical characteristics. They could provide a new direction for the expansion of the industry, because deserts and other dry lands constitute over 40% of the global land area.

Establishing shrimp farms far from the ocean using low-salinity waters was successfully implemented with *P. monodon* in south-east Asia before the introduction of *L. vannamei*, which are now routinely grown in these areas The effluent can be used to irrigate various crops, thus minimising effluent disposal efforts. These emerging technologies offer opportunities to establish shrimp culture operations on marginal arid land or agricultural sites, reducing demand for shrimp farming on limited, high-cost coastal land. Limited seawater use during a relatively short acclimation phase and complete re-use of effluent water for irrigation of agricultural crops can provide for environmentally friendly integrated systems. *L. vannamei* is being successfully raised in low-salinity groundwater with varying ionic compositions and salt concentrations in several regions of the world.

Chapter 5 gives further examples of inland aquaculture in saline water and its integration with agriculture.

21.10.5 Recirculation and reduced water exchange systems

The shrimp farming industry can benefit significantly from improved water management regimes. In particular, these can help address viral disease problems and issues raised by environmental groups that have targeted marine shrimp farming, pressuring the industry to adopt more sustainable production practices.

Large-scale application of zero-exchange and recirculation technologies on existing farms has already increased producer confidence in the potential for reducing or eliminating routine water exchange through most or all of the growing season. There are several successful examples of the implementation of zero-exchange production systems, including those in Belize, Panama and Florida (Fig. 21.7). In particular, McIntosh and co-workers at Belize Aquaculture Ltd. have shown the tremendous potential these production systems have (section 21.3.4).

Browdy and Jory (2001) recently reviewed various aspects of intensive closed technologies for shrimp production. Nutrient-rich effluents from intensive production systems can contribute to the eutrophication of receiving waters, potentially affecting both natural biota and local culture operations. Water exchange can be reduced or eliminated, and supplementary aeration can have an essential role in the successful operation of intensive closed systems. Paddlewheel aeration must be increased by 10% or more over levels traditionally applied in intensive culture to maintain appropriate DO levels. Better placement of mechanical aerators and use of back-up aeration and alarm systems are also necessary. Formulated feeds are the main nutrient input into shrimp production systems and, as water exchange is reduced or eliminated, feed formulations and feed management fast become critical factors as stocking densities increase. The design and management of production facilities to re-use water, minimise exchange and eliminate discharge will improve the outlook for more profitable and sustainable production technologies.

21.10.6 Effluents

General impacts of effluents from coastal aquaculture farms on marine environments are considered in section 4.2.1. This is an issue that shrimp producers and processors need to address while they are discharging effluents.

Settling basins are especially efficient for treating shrimp farm effluents because the high concentrations of cations in seawater and brackish water tend to neutralise the negative charges on suspended clay particles, which will flocculate and settle. Plankton cannot be removed efficiently by sedimentation, and products such as aluminium sulphate, lime and selected organic colloids, often used in wastewater treatment to promote sedimentation, are not needed in shrimp farm settling basins.

According to Burford *et al.* (2001), the shrimp farming industry will face increasing pressure to develop new practices and technologies that more efficiently convert the nitrogen in shrimp feeds to shrimp biomass, and to minimise or eliminate residual nitrogen waste before effluent discharge into receiving waters. Meeting these goals will require an understanding of the fate and transformations of dietary nitrogen in shrimp production ponds and effluent treatment systems. These authors discussed the development of new technologies to reduce nitrogen waste from

shrimp ponds, including improvements in feed formulations and feed management, genetic selection for improved food conversion ratio, improved in-pond processing of nitrogen, and improved design and management of effluent treatment systems.

21.11 RESPONSIBLE SHRIMP FARMING AND THE CHALLENGE OF SUSTAINABILITY

21.11.1 Domestication and genetic improvement

During its first three decades, commercial shrimp farming depended significantly on wild seedstock and broodstock. Wild seed supply was often unreliable and limited, and PL shortages severely afflicted the industry. Many factors may affect broodstock and wild larvae supply, from global weather phenomena, such as El Niño and annual monsoons, to localised pollution and environmental degradation, and overfishing or over-regulation of fisheries.

Marine shrimp are promising candidates for domestication and genetic improvement, because of their high fecundity, short generation interval and the presence of additive effect of genetic variance for growth rate) (Fig. 21.22). When compared with most livestock industries, however, shrimp farming is still a newcomer to domestication and selective breeding. International discussion and collaboration in both conventional selective breeding techniques and the application of the tools of modern molecular biology are being widely used to promote global progress and efficiency of genetic improvements in the industry.

A global survey of shrimp farming practices conducted by the Global Aquaculture Alliance showed the significant differences in progress in the genetic improvement of farmed shrimp between geographical regions and among the dominant farmed species. In the Western hemisphere, the domestication and selective breeding of the dominant species (*L. vannamei* and *L. stylirostris*) have become a widespread practice with clearly demonstrable benefits. The benefits include improved production efficiency and a more reliable supply of seedstock, the results of selection of genotypes with improved tolerance to disease and faster growth rates. Domesticated lines of *L. vannamei* have been reared in captivity for >40 generations in various countries. In the Eastern hemisphere, progress in the domestication and selective breeding of *P. monodon* has been slow but has gained strength in recent years. Significant advances have been made, however, in the domestication and selective breeding of species that are less commonly farmed there, including native species such as *M. japonicus* and *F. chinensis*, and on the introduced Western species *L. vannamei*.

Achieving domestication of selected shrimp species, together with selection, genetic improvement and, possibly, hybridisation and ploidy manipulation, should be major research objectives. An industry with the global importance that shrimp farming has achieved cannot depend on nature to supply its seedstock reliably.



Fig. 21.22 Genetically based variation in size among sibling *Penaeus monodon* PL. (Photograph by Dr Nigel Preston.)

21.11.2 Nutritional requirements and formulated feeds

The development and use of compound feeds have been major factors in the expansion of the industry and will continue gaining importance. Growing demand for formulated feeds will increase competition for component resources, particularly fish meal. There is an enormous but still unrealised potential to reduce the production cost and improve the nutritional performance of compound feeds for shrimp. Extensive research must continue to improve our knowledge of the nutritional requirements of shrimp and to develop new diets that are species, area and even season specific. These diets could include innovative processing methods, lower-cost and innovative ingredients from various agriculture crops and byproducts from the various meat processing industries, health additives and growth promoters that improve survival, growth, yield, food conversion ratio and disease resistance while reducing environmental concerns. Reducing the cost of feeds is an aspect critical to expanding the industry and improving its competitiveness relative to other protein sources, such as beef, pork and poultry. Low-pollution or 'environmentally friendly' feeds are being developed to reduce the environmental impacts of shrimp feeds. Currently, emphasis is on 'least cost' feed formulations for minimising feed

cost while optimising aquaculture production. In the future, emphasis will also be on 'least polluting' feed formulations for minimising environmental impacts with the greatest compatible aquaculture production.

21.11.3 Disease prevention, diagnosis and control

As described earlier, historically there have been spectacular collapses of shrimp farming industries in several countries, including the top-producing countries, China, Thailand, Indonesia, Taiwan and Ecuador. Standard denominators among the shrimp farming industries of these countries were very fast, unregulated development and an increased incidence of diseases, particularly viral diseases. There still are very few alternatives to deal with viral infections, and the best procedure for disease management is exclusion (section 21.8.4). There will undoubtedly be new pathogens that the industry will have to confront and manage. Accurate and prompt diagnosis of infectious agents continues to be an industry priority.

The application of effective pathogen detection and disease diagnostic methods, particularly those based on molecular biology and recently developed by the industry (section 21.8.4), is essential to understand diseases better and prevent losses caused by them.

21.11.4 Best management practices

The resolution of environmental and social conflicts can be accomplished through regulations, technical assistance, education and voluntary measures. Some local and regional governments are already exercising increased pressure in regulating the shrimp farming industry. For example, there are regulations governing water quality parameters in effluent waters from shrimp aquaculture operations in several areas, and these will become increasingly strict. On the other hand, because shrimp farming is such a young industry, there are still inadequate regulations and guidelines in some countries. The industry needs to continue being proactive and voluntarily adopt measures of self-regulation.

Best management practices (BMPs) are a practical way to approach environmental management for shrimp farming. They are practices considered the most effective methods of reducing environmental impacts, while being compatible with resource management goals. Producers may adopt them voluntarily to show environmental stewardship and reduce the urgency for governmental regulations. BMPs may also be the backbone of environmental management in activities whose effects are diffuse and located over large areas. The shrimp farming industry, by voluntarily preparing and adopting BMPs, is demonstrating environmental responsibility to reduce the need for future regulations, and to provide a basis for the form of future regulations. A system of BMPs, however, is typically needed to prevent a particular type of farming from causing negative impacts. Shrimp farming is conducted over a wide range of coastal environments, with significant differences in resource patterns, and physical, chemical and biological conditions, so a single system of BMPs for use in all situations is not possible. Systems of BMPs must be developed by country or by region, and the national or regional system must be customised for site-specific conditions on each farm. Where voluntary adoption of BMPs is used to improve environmental management, it is convenient to establish it as a Code of Practice. This document usually asserts the commitment of adherents to the code to use environmentally and socially responsible management, states the purpose of the code and provides a list of BMPs to be used by the industry. Codes of Practice for shrimp farming already exist in Australia, Belize, Thailand and other countries, and shrimp farming organisations in several more countries are intent on producing their own Codes of Practice (Boyd, 1998).

Lee (2010) recently discussed the relevance of certification to the aquaculture industry, with special reference to shrimp farming. According to him, when consumers select seafood products from displays or from restaurant menus, they base their choice on a quick assessment of key attributes such as price, quality, convenience and origin. But refining their choices further – based on food safety, ethical, environmental or animal welfare grounds – is more difficult because of a shortage of reliable, independent information. In essence, it is this information gap that opens the way for seafood certification and eco-labelling schemes. Such schemes have the potential to link a consumer's responsible choices with a producer's responsible practices and to deliver market rewards accordingly. But in reality it is not clear if consumer choice alone is enough to drive and sustain all certification schemes. In the absence of consistent market incentives, what drives the current proliferation of aquaculture certification programmes? There are underlying environmental and economic pressures that support certification. Although some non-governmental organisations have adopted prominent roles as catalysts, mounting successful campaigns to draw public attention to negative environmental, health and social consequences of some aquaculture systems, including shrimp farming, these groups lack the technical expertise and resources needed to deliver regular, reliable and independent assessments of aquaculture ventures. Typically, their campaigns are sporadic and, in the media

attention they generate, important information is often lost among unsubstantiated or exaggerated claims.

Concerns about the unintended consequences of aquaculture certification schemes have been raised, particularly for the developing world. These concerns should not be underestimated and they are already influencing the spread of certification in the shrimp industry. For example, most farms that have entered the Best Aquaculture Practices (BAP) programme of the Global Aquaculture Alliance (www.gaalliance.org) are large ventures, often parts of vertically integrated business structures. Small-scale farmers, particularly common in Asia, are constrained because of the associated costs and because of the requirements for improved record keeping and new management procedures. The solution is for small farmers to come together in clusters or co-operatives and seek certification in groups, thereby spreading the costs of certification among many participants and promoting communal responsibility for the environment. The BAP programme is now actively promoting this solution in south-east Asia including, very importantly, for shrimp farmers in Thailand.

Despite important policy caveats, many international bodies such as the Food and Agriculture Organization of the United Nations recognise that BMPs play an important part in the sustainable development of aquaculture, for both large- and small-scale producers. At the same time, third-party certification of compliance with these practices can help improve public confidence in the management of aquaculture facilities provided that the certification systems function independently of the standard-setting organisations. Hopefully the rapidly expanding and evolving aquaculture industry, with the assistance of certification schemes, will continue to learn lessons from other industries and will increasingly follow the path towards environmental and social sustainability.

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22
Other Decapod Crustaceans

Chaoshu Zeng, Yongxu Cheng, John S. Lucas and Paul C. Southgate

22.1 INTRODUCTION

22.1.1 Morphology

The crustacean order Decapoda is characterised by having five pairs of legs, as its name describes. The pairs of legs of a decapod often vary in form and function from anterior to posterior, with the anterior legs typically being more involved in feeding. Often one or more pairs of legs have chelae: pincer-like structures at the appendage tip that are formed by the terminal segment articulating with a projecting 'finger' from the second last segment (Fig. 22.1). These anterior chelipeds are enlarged in many species, with one of the pair being larger than the other. They may also be larger in males than females and of a different shape. Large chelipeds may be used in crushing robust prey such as bivalves, for example feeding by portunid crabs such as the mud crab (Fig. 22.1) (section 22.6), or in aggression and display by males, for example *Macrobrachium rosenbergii* (section 22.4). Where the anterior legs are highly specialised for feeding or display, it is left to the posterior legs to carry the role of locomotion. These walking legs may also be specialised, for example in portunid crabs the last pair of legs are specialised as paddles and the crabs swim laterally when moving rapidly. In other cases the legs are adapted for digging into the substrate, as in marine shrimp.

The regular number of paired legs reflects the conservative body plan of decapods. They have a set number of body segments and associated appendages (Table 22.1). There is, however, great adaptive variation based on this body plan, for example in crabs the abdomen in reduced to almost a flap which is flexed under the cephalothorax.

22.1.2 Habitats

Some decapods are terrestrial, except during reproduction when they move into the sea to release the larvae from their eggs, for example land hermit crabs and other land crabs. Most are aquatic, inhabiting environments from wet soil near stagnant pools, to the intertidal zone, to the abyssal depths of the ocean. They occur in salinities from freshwater to hypersaline conditions and, while having defined salinity ranges, many brackish water and coastal species are distinctly euryhaline.

22.1.3 Life cycles

As described in section 6.4.2, decapod crustaceans grow in a staircase-like fashion with smaller and more frequent 'stairs' at the beginning of growth. The concept of growth is confusing in that the period of no external growth, when the exoskeleton (shell) is hard, the period between moults, is the period when the animal is feeding and laying down tissue. Moulting or ecdysis, when the completely enclosing exoskeleton is shed and the animal abruptly expands in size is not a period of tissue growth: the animal's body and appendages are soft and not functioning normally. Immediately after moulting, the protein tissue content of the body is relatively low and the fluid content is high. Large decapods that are harvested commercially may be known as 'empty' during this period, because of their relatively low meat content. The high fluid content is because the body's expansion is by rapid intake of fluid, often by drinking. Fluid intake not only provides the mechanism for expansion of the body after moulting, it also provides force during moulting to split the old exoskeleton along

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predetermined lines and push the body out of the old exoskeleton through the splits.

This pattern of growth occurs even in the earliest larval instars, but the process of moulting is very rapid and there is hardly any disruption to feeding. As the decapod gets larger, the period of moulting and recovering a strong exoskeleton takes longer and the animal spends longer in a phase where it is vulnerable to predation through poor mobility and defence. Some large decapods seek concealment for the period of moulting.



Fig. 22.1 A mature male specimen of the mudcrab, *Scylla serrata*, showing its first pair of legs adapted as huge chelae, with the larger right-hand one especially enlarged for crushing. The next three pairs of legs are for walking. The fifth pair of legs, paddle-like and adapted for swimming, are obscured except for part of the left one adjacent to the man's hand. The abdomen is much reduced and evident as a small triangular flap. (Photograph © 2011 The State of Queensland, Department of Employment, Economic Development and Innovation (Fisheries Queensland).)

As in most other arthropods, the presence of an all-enclosing exoskeleton has another profound morphological effect: there are no cilia, flagella or similar organelles on the surface of the skin beneath the exoskeleton. This absence extends to the gametes, which have no flagellum and are thus non-motile. The male crustacean must pass sperm to the female in packed masses, known as spermatophores. This is sometimes accomplished while the female is soft, immediately after moulting, and the copulating male may push the spermatophores into the female's reproductive system through her genital apertures to be stored in her seminal receptacles. An alternative used with hard intermoult females is for the copulating male to put a sticky spermatophore mass on the female's exoskeleton near her genital apertures, for example in freshwater crayfish (section 22.5). The alternatives of copulation with soft and hard exoskeleton females are demonstrated in different species of marine shrimp (section 21.5).

Like many crustaceans, the larvae of marine decapods are typically members of the zooplankton. The earliest larval instar of Crustacea is the nauplius, which hatches from the egg. It has only three pairs of appendages, which correspond to antenna 1 and 2, and mandible appendages of the adult decapod. The larva then goes through a series of moults and stages (instars), adding segments and appendages, and modifying its appendages to suit the needs of the particular larval instars, until it reaches the adult number of body segments. This is the pattern of development in marine shrimp (section 21.5). It is

Table 22.1 Generalised morphology of decapods crustaceans.

Body region	Body segments	Appendages	Typical functions
Head, enclosed by carapace	1–2	Antennae	Sensory
	3–5	Mandibles, maxillae	Mastication of food
Thorax, enclosed by carapace	6–8	Maxillipeds	Collection and mastication of food
	9–13	Legs (often some are chelate)	Locomotion, collecting food, display, aggression
Abdomen	14–18	Pleopods	Swimming, brooding eggs (females), spermatophore transfer (males)
	19	Uropods	Making a tail fan with telson for rapid backward locomotion with abdomen flexing
Telson			

atypical, however, of other decapods. Most other decapod crustaceans suppress the nauplius and earlier stages as embryonic stages within the egg, and the larva that emerges has the adult or near adult number of segments, although its form is often entirely different to the juvenile. Virtually the whole of larval development is suppressed within the egg in some species and near-juveniles emerge from it.

Environment is clearly important in the nature of early development in decapod crustaceans in freshwater. Three groups of freshwater decapods are considered in this chapter. Freshwater crayfish remain in this environment throughout their life cycle and have no free living planktonic larval instars (section 22.5.2). The Chinese mitten crab and the giant freshwater prawn have larval instars, but they move downstream to release their larvae into brackish water.

22.2 CULTURED SPECIES

FAO FishStat Plus (2009) lists 36 decapod species that are currently or have been cultured:

- 17 are species of marine shrimp;
- seven are species of freshwater crayfish/crawfish;
- five are crab species including one freshwater species;
- four are species of freshwater prawns;
- three are species of spiny and squat lobsters.

Although the number of species cultured is reflected in the annual production of marine shrimp (penaeids), this is not the case for the next three most important groups (Fig. 22.2). One species of freshwater crab, *Eriocheir sinensis*, the Chinese mitten crab, is second in global production. Two species of freshwater prawn, *Macrobrachium rosenbergii* and *M. nipponense*, are next in importance. The freshwater crayfish/crawfish are next in importance of global production with production primarily from one species, *Procambarus clarkii*, the red swamp crawfish. Apart from the freshwater mitten crab, brackish and marine crab production is from swimmer crabs (Portunidae) and mostly the mud crabs, *Scylla* species, and the swimming crab, *Portunus trituberculatus*. Although farming of mud crabs has a long history and is practised in many countries, swimming crab culture has undergone rapid expansion in China in recent years with annual aquaculture production almost matching that of mud crab production at nearly 100 000 t (China Fisheries Yearbook, 2007). Production of spiny and squat lobsters is in the early stages of development. There is also some preliminary mariculture of the marine lobsters: the American lobster (*Homarus americanus*) and the European lobster (*H. gammarus*).

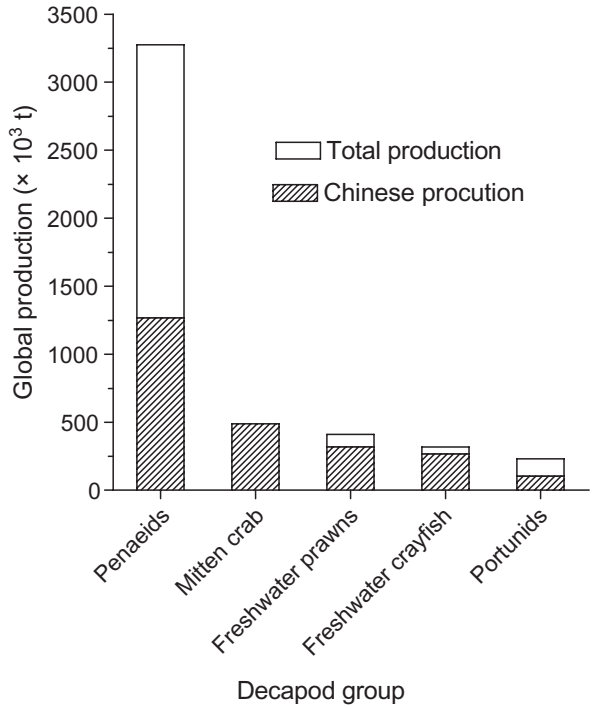


Fig. 22.2 Global production per year of major decapod crustacean groups from FAO Fishstat Plus (2009). Penaeids, marine shrimp; portunids, swimming crabs.

Thus, the production of decapod crustaceans other than marine shrimp is dominated by relatively few species.

China dominates the global production of decapod crustaceans, with ca. 40% of marine shrimp production, all the mitten crab production, and a high proportion of the *Macrobrachium*, freshwater crayfish/crawfish and swimmer crab production (Fig. 22.2). *Macrobrachium rosenbergii* is not native to China but was introduced for aquaculture from south-east Asia. Its production boomed during the 1990s owing to the widespread diseases of marine shrimp in the country during this period. However, production of the native species, *M. nipponense*, has caught up in recent years. Similarly, the red swamp crawfish, native to southern USA and Mexico, was introduced to China and the country has become the dominant producer of freshwater crayfish/crawfish through this species. The USA state of Louisiana is particularly noted for its production and special meals of red swamp crawfish, and was traditionally the largest global producer, but this is no longer the case.

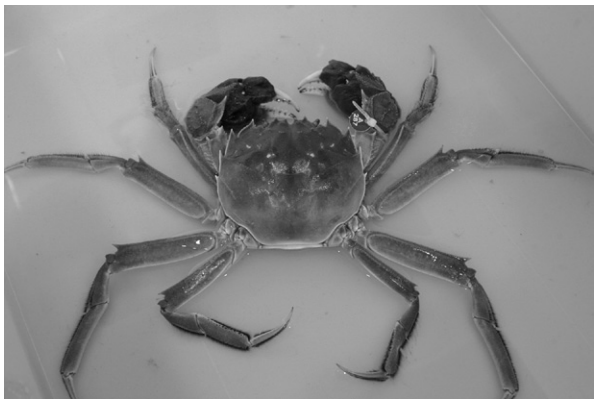


Fig. 22.3 Adult Chinese mitten crab, *Eriocheir sinensis*.

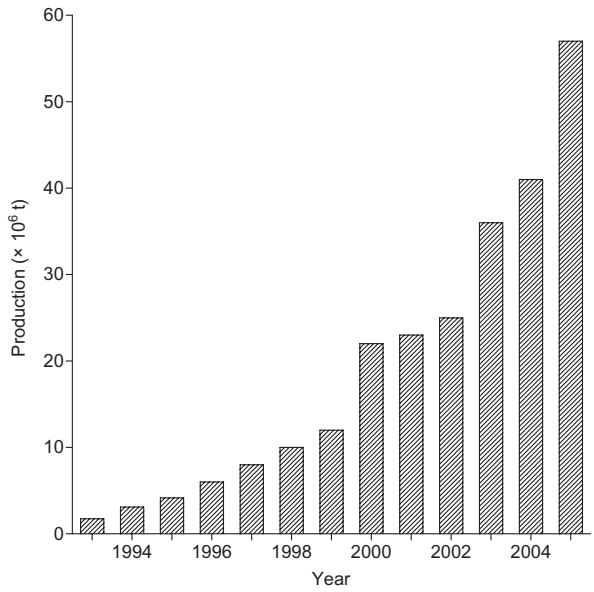


Fig. 22.4 Annual aquaculture production of the Chinese mitten crab in China from 1993 to 2005.

22.3 THE CHINESE MITTEN CRAB

The Chinese mitten crab, *Eriocheir sinensis*, won its name by the distinguishing feature of dense patches of hairs on its chelipeds (Fig. 22.3). This freshwater crab species is native to China and distributed widely along the eastern coasts in streams and rivers that are connected to the sea. As a medium sized crab (50–80 mm carapace width as adults), the Chinese mitten crab has traditionally been regarded as a culinary delicacy in China. However, during the 1960s and 1970s, the *E. sinensis* fisheries collapsed owing to overfishing and habitat destruction. This stimulated increased research efforts to develop aquaculture of this species in the 1970s and 1980s, with particularly emphasis on hatchery techniques. Successful mass production of crab 'seed' in hatcheries was reported in both natural and artificial seawater in the 1980s. Chinese mitten crab 'seed' are in the form of the transitional megalopal instar between the final zoeal instar and the first crab instar (section 22.3.2). This was an important breakthrough that enabled hatcheries to be established inland. However, it took time for the new techniques to be adopted and improved, and it was only after 1990 that the Chinese mitten crab farming industry began to develop rapidly and spread far beyond the natural distribution range of *E. sinensis* (Cheng *et al*., 2008a).

22.3.1 Production status

Chinese mitten crab farming now occurs in all provinces and regions of mainland China. The most important provinces for the farmed production of mitten crabs are Jiangsu and Zhejing provinces in the south and the Liaoni province in the north. Annual farming production has reportedly reached 570 000 t in 2005, which is a more than 40-fold increase on production in 1991 (Fig. 22.4) (China Fisheries Yearbook, 2007). Based on statistics from the China Fisheries Bureau, in 2001, hatchery production of Chinese mitten crab seed (megalopae) exceeded 200 000 kg/year (ca. 140 000 megalopae/kg) for the first time. Subsequent annual production more than doubled to reach 522 893 kg in 2003.

Despite grow-out of the Chinese mitten crabs being practised all over China, the hatcheries are mainly located in the Yangtze River Delta in southern China, where most megalopae are produced and transported to other parts of the country for grow-out. For example, among 522 893 kg of megalopae produced in 2003, 360 586 kg were produced in Jiangsu province located in the Yangtze River Delta. Outside the Yangtze River Delta, the most important region for hatchery production of Chinese mitten crab is along the Liao River in northern China.

22.3.2 Biology

The Chinese mitten crab inhabits fresh water streams and lakes, favouring habitats with clean water and lush aquatic plants. They are omnivorous and nocturnal. Their diet includes aquatic plants and insects, small molluscs, fish and shrimps, as well as earthworms. Aquatic plants often

make up the bulk of their gut contents, probably because of their ready availability. The crab has broad temperature tolerance and can survive between 1 °C and 35 °C; however, in winter when temperature drops below 5 °C, the crabs often hide in holes and feeding ceases. The Chinese mitten crab can tolerate long periods of starvation and can survive without feeding for more than 1 month.

The Chinese mitten crab is catadromous; they spend most of their life in fresh water, but must return to the sea to breed. In the wild, the sexually mature crabs begin migrating downstream in late autumn and females attain ovarian maturity when they reach tidal estuaries. After mating and spawning, the extruded eggs are attached to the female's pleopods. Females may continue seaward, overwintering in deeper waters but returning to shallow water in the spring for the hatching of their eggs.

Larval development of the crab occurs in brackish water. The newly hatched larvae are the planktonic first instar zoea (zoea I). They go through five zoeal instars (zoea I–V) (cf. Fig. 22.15) before metamorphosis into the megalopa stage, which is more crab-like and swims with its abdominal appendages. Megalopae migrate with tides upstream and metamorphose to become the first instar crab, which is benthic. The juvenile crabs continue migrating inland into various freshwater systems where they will grow until they reach sexual maturation with a terminal moult. First instar crabs undergo 15 moults over a period of about 2 years to reach the terminal moult, which indicates sexual maturity and maximum size.

22.3.3 Culture methods

The production cycle for *E. sinensis* normally takes 2 years and involves three distinct culture phases: hatchery, nursery and final grow-out.

22.3.3.1 Hatchery

Obtaining high quality broodstock crabs is the first step to successful hatchery operation. Ideally, female broodstock crabs should be 125–150 g and males should be larger than 150 g. Only healthy active crabs with intact appendages are selected and crabs collected from lakes and streams are generally preferred to those from pond cultures. Broodstock nutrition is crucial during the ovarian maturation process in late September to mid-November. The diets commonly used to feed broodstock are fresh natural food, such as trash fish, *Chaeturichthys stigmatia*, the razor clam, *Sinonovacula constricta,* and sandworm, *Nereis japonicus*. Specific formulated diets supplemented with essential fatty acids have been developed for female broodstock (Wu *et al*., 2007, 2009).

After conditioning, male and female broodstock crabs are put together with a female : male sex ratio of ca. 2–2.5 : 1 and brackish water is introduced to initiate mating. The optimal salinity and temperature range for inducing copulation are 14–20‰ and 8–12 °C, respectively. Hard sandy or muddy substrate is provided to facilitate the attachment of newly extruded eggs to the female abdomen. Soon after brackish water is introduced, the crabs will pair, mate and spawning ensues (females usually spawn within 2 weeks of mating). Egg-carrying crabs can be found the next day after mating, and in about 1 week 70–80% of females will carry eggs. Such a spawning induction process helps generate synchronised spawning and enable hatcheries to arrange the most suitable time for larval culture. The fecundity of Chinese mitten crab is relatively high, with females of 100–200 g producing between 200 000 and 900 000 eggs.

After spawning, female crabs carry their eggs on pleopods beneath their abdomen while the embryos develop. This takes 2–4 months depending on temperature; incubation temperature can be manipulated in the hatchery either to speed up or slow down embryonic development.

Two major larval culture techniques, namely indoor intensive and outdoor semi-intensive/extensive, have been developed for Chinese mitten crabs:

22.3.3.2 Indoor intensive larviculture

Indoor intensive larviculture is mainly performed in indoor concrete ponds or tanks with temperature control. A typical pond size is 5 × 5 × 2 m; temperature is normally controlled at 18 °C for zoea I but increased by 1 °C as larvae moult to each of the next zoeal instars. It is therefore about 24 °C by the time larvae moult to megalopae. Microalgae, live prey such as rotifers, *Artemia* nauplii and copepods (harvested from wild), frozen rotifers and egg yolk, are used to feed the larvae. The initial stocking density is typically 200 000–500 000 larvae/m^3, and megalopa production is usually 150–500 g/m^3, or 20 000–70 000/m^3. Survival is generally between 10 and 15%.

There is limited water exchange from zoea I–III; however, a higher percentage of water is exchanged after larvae reach zoea III and, by the megalopal instar, water is exchanged twice daily. Antibiotics, such as tetramycin or tetracycline, are often used. The optimal salinity range for larval rearing is 20–25‰, although megalopa can be reared in 10–30‰ salinity. Both natural saltwater or specially formulated artificial seawater can be used. The latter is mainly for the inland areas and incurs higher costs; therefore it is less commonly used. To acclimatise megalopae to freshwater, salinity reduction typically begins 3

Table 22.2 Main features of three different larviculture techniques of the Chinese mitten crab, *Eriocheir sinensis*, in China.

		Outdoor culture	
Culture methods	Indoor intensive	Outdoor semi-intensive	Outdoor extensive
Culture ponds	Concrete ponds or tanks	Earthen ponds	Earthen ponds
Pond areas (m^2)	12–30	400–700	10 000–15 000
Stocking density (individuals/m^3 of newly hatched zoea I)	200 000–500 000	20 000–30 000	≤10 000
Diets of zoea I–V larvae	Algae, egg yolk. *Artemia* nauplii, rotifers and copepods	Algae, soybean milk, *Artemia* nauplii	Live rotifers (the main diet) and algae
Diets of zoea V and megalopae	*Artemia* nauplii, frozen adult *Artemia* and copepods	*Artemia* nauplii, frozen adult *Artemia* and copepods	Live rotifers (the main diet), frozen adult *Artemia* and copepods
Antibiotic used?	Yes	No	No
Probiotic use?	Only for zoea I–II	Yes	Sometimes
Megalopal yield (g/m^3)	150–500	15–30	1–7.5
Survival to megalopae (%)	10–15	5–10	2–4
Cost for producing 1 kg of megalopae (Chinese Yuan)	600–1200	500–1000	300–500
Megalopa quality	Variable	Good	Good
Duration of larviculture (days)	22–24	28–30	28–30
Temperature (°C)	18–24 (controlled)	10–23 (not controlled)	10–23 (not controlled)

days after most larvae have become megalopae and salinity is reduced to <5‰ by the time megalopae are 6–7 days old. The whole larval culture cycle, including the time for megalopa acclimatisation, is about 22–24 days. Megalopae of 6–7 days old, weighing 140 000 megalopae/kg, can be sold to farmers for nursery culture in freshwater.

22.3.3.3 Outdoor larviculture

Outdoor larviculture is mainly in outdoor earthen ponds without temperature control. The earthen ponds are normally built near estuarine areas, ideally with smooth hard clay bottoms and capable of taking in both saline water (20–25‰) and freshwater. This outdoor larviculture technique has set a new hatchery trend in China in recent years and become very popular because it requires fewer facilities (e.g. building, heating and aeration systems). It is also substantially cheaper to setup and run, and is easier for farmers to operate. In addition, antibiotics are not used in such systems, the quality of the megalopae produced is generally better and they are therefore favoured by the crab farmers. This method has recently become the dominant hatchery technique, producing the majority of megalopae for farming in China.

There are two kinds of outdoor larviculture technique: semi-intensive and extensive. The former uses small ponds of 400–700 m^2 equipped with aeration, whereas the latter is practised in bigger ponds of 1–1.5 ha without aeration. Production of megalopae per unit volume of water is higher for the former, but the costs are also higher (see Table 22.2). Outdoor larviculture takes place mainly during mid- to late spring when water temperature ranges between 10 and 23 °C.

Typical semi-intensive larviculture ponds are either square or round, with 1.5 m water depth, whereas the ponds for extensive larviculture are often rectangular and normally built in high intertidal zones. As such, a 1.5 m deep channel is dug around the perimeter of each extensive pond (loop channel), which buffers the pond water from abrupt temperature fluctuations that could lead to mass larval mortality. Twenty to 50 days before larval stocking, ponds are disinfected by applying 1500–2000 kg lime (CaO)/ha or 750–1000 kg bleaching powder (Cl > 30%)/

ha. The ponds are then filled with 40–50 cm of filtered seawater about 7 days before stocking and may be fertilised to stimulate algal growth. Normally there is little water exchange during the larval culture period.

For semi-intensive larviculture, initial stocking density is 20 000 to 30 000 newly hatched zoea/m^3. If the ponds are well fertilised with a good algal bloom (often *Isochrysis* sp. and *Platymonas* sp. at concentrations of 2×10^5 to 4×10^5 cells/mL), larvae can moult to zoea II in about 5 days without additional feeding. If algae are not growing well, zoea I and II larvae may be fed supplemental feeds, such as soybean milk and dry power of *Spirulina*. From zoea III onward, larvae are fed mainly with live *Artemia* nauplii as well as frozen adult *Artemia* and copepods harvested from the wild. The yield of megalopae is normally 15–30 g/m^3.

For extensive larviculture, initial stocking density is generally less than 10 000 larvae/m^2. For all larval instars except zoea I (not fed), live rotifers, *Brachionus* sp., are the main feed; zoea II and III larvae are fed about 35–40 kg of rotifers (wet weight)/ha/day to maintain a density of 2 000–3 000 rotifers/L. Rotifer feeding increases to 70–80 kg/ha/day and 120–130 kg/ha/day at zoea IV and V, respectively. At later larval instars, especially for zoea V and megalopae, rotifers may be supplemented with frozen copepods or adult *Artemia*. The rotifers used to feed the larvae are often reared in earthen ponds next to the larviculture ponds, with a 1 : 1 ratio of pond areas. Additional rotifers can also be purchased from farmers who specialise in producing rotifers in earth ponds to supply the mitten crab hatcheries. The rearing of rotifers is largely based on fermented organic fertiliser and in a period of 20–35 days, 900–1 000 kg/ha can be produced. The yield of megalopa is more variable for extensive larviculture, ranging from 1 to 7.5 g/m^2.

The major features of different larviculture methods for the Chinese mitten crab are summarised in Table 22.2.

22.3.3.4 Nursery

The hatchery-produced megalopae that are sold to nursery farms are normally carried in wooden boxes. The wooden boxes are about $60 \times 46 \times 10$ cm in size with an opening on each sides of the box to allow air exchange. The bottoms and the sides of the boxes are lined with mesh net to prevent escape. Before transport, the boxes are soaked in water for 12 h and a layer of clean aquatic plants or wet towel is laid to provide moisture and avoid accumulation of megalopae in one area. Each box can be loaded with about 0.5 kg megalopae and 5–10 boxes can be stacked together. If conditions allow, the temperature during the transportation is controlled between 14 and 18 °C. Using this method, megalopae survival can be near 100% if the transportation time is less than 12 h or approximately 90% if it is about 24 h.

The nursery phase of Chinese mitten crab culture takes 5–8 months and can be divided into two stages. The first lasts about 20–30 days, during which hatchery produced megalopae are cultured in earthen ponds to reach the second to fifth crab stages with a weight between 0.1 and 0.2 g. The size of such juvenile crabs is similar to that of soybeans; therefore, they are named bean-sized crabs. The second stage nursery is to grow bean-sized crabs to bigger 3–10 g juvenile crabs that are ready for stocking into grow-out ponds or enclosures. The second nursery stage takes several months and can either use the same ponds



Fig. 22.5 Coin-sized mitten crabs in a nursery tank. Note that they can be kept at high densities, which is not possible for some other aggressive and carnivorous crabs.



Fig. 22.6 Nursery culture ponds for Chinese mitten crabs.

that were used for the first nursery stage or the bean-sized crabs may be stocked to other ponds or rice fields, which is more common. The sizes of juvenile crabs produced at the end of the second nursery stage are similar to that of coins or buttons, therefore, they are known as coin-sized crabs or button-sized crabs. Most coin-sized crabs are seventh to eighth juvenile stage crabs.

The nursery ponds are generally rectangular and from a few hundred square metres to 0.25 ha in area (Fig. 22.6). If the first and second stages of nursery culture are in separate ponds, the ponds for the first stage nursery are smaller. The second stage nursery can also be conducted in rice fields with appropriate modifications. Firstly, structures to prevent escape and access by predators (e.g. frogs and toads) must be built. This is normally done by setting up a mesh fence of about 1 m high on the edges of either ponds or rice fields. Secondly, an encircling channel needs to be dug around the perimeter inside the fence. The channel is typically 1 m wide and 60–90 cm deep. This deeper channel serves as a refuge for the crabs on hot days. For both the first and second stage nursery, aquatic macrophytes, such as *Vallisneria spiralis*, *Hydrilla verticillata* and *Elodea* sp., are usually planted before stocking, and serve as both food and refugee for the crabs.

The initial stocking density for the first nursery stage is around 100 megalopae/m^2 or 7.5 kg/ha. When megalopae are first stocked, the water depth is normally maintained at 20–30 cm, but this is gradually increased by 10, 15 and 20–25 cm steps when crabs moult to the first, second and third crab stages, respectively. The maximum water level is around 70–80 cm.

During the nursery phase, crabs feed on natural diets, including plankton, plants and small benthic animals that grow naturally in the ponds or rice fields. Supplementary feeds, such as corn, wheat, pumpkin and minced trash fish, as well as formulated feeds are also provided to enhance their growth.

A major problem during the nursery phase culture of Chinese mitten crabs is the precocious puberty of some juvenile crabs. It is common that 15–30% crabs harvested from nursery ponds show this. Such crabs, weighing 15–35 g, have reached the terminal moult (i.e. the maximum size) and will not grow any further. They, therefore, have little or no market value. Research has shown that such a phenomenon is linked to genetics, culture environment (e.g. high temperature) and nutrition (Wu *et al*., 2010); however, techniques are yet to be developed to control its occurrence effectively.

22.2.3.5 Grow-out

Depending on the farm and location, either later in the year before winter sets in or early in the following year, the coin-sized crabs harvested from nurseries are stocked for final grow-out to market size of between 80 and 200 g. The final grow-out of Chinese mitten crabs occurs mainly in three forms: pond, net enclosure and rice field culture.

Pond cultures use earthen ponds for grow-out, the size of the ponds being generally between 0.6 and 2 ha with a water depth of 1.5–1.8 m. Fences similar to those of nursery ponds (Fig. 22.6) are erected to prevent escape and access by predators.

Some time before crab stocking, the ponds are normally disinfected with lime (2000–3,000 kg/ha) and 10 days after disinfection, aquatic plants, such as *Vallisneria spiralis*, *Hydrilla verticillata*, *Ceratophyllum demersum*, *Potamogeton maackianus* and *Myriophyllum spicatum*, may be introduced to the ponds to grow. Meanwhile, 1 month or so before crab stocking, live mud snails, *Bellamya purificata*, a favourite food for mitten crabs, may be stocked in the ponds at the rate of 3500–4500 kg/ha and left to reproduce in the ponds. Finally, fertilisation using fermented organic fertilisers is often applied 10–20 days before crab stocking to increase the natural biomass inside the ponds.

The stocking density of coin-sized crabs is normally around 7500 crabs/ha and they are often polycultured with various freshwater fish and shrimp, which may be stocked at different times. The important consideration for fish species selection is that their feeding habits should complement rather than compete with Chinese mitten crabs. Over the culture period, water quality needs to be closely monitored, dissolved oxygen should be about 5 mg/L, pH 7–8.5 and Secchi disc reading between 50 and 80 cm. The yields per hectare per year are variable, depending on intensity of polyculture, but they are normally close to 1000 kg of market-sized crabs plus twice that weight of various freshwater fish and shrimp.

Net enclosure grow-out now occurs in almost all inland lakes in China. Polythene with mesh sizes of 1–2 cm is a common net material. The nets are fixed in place with bamboo stakes. The net walls are folded inwards and held on the bottom with rocks, causing them to sink into the mud. The upper edge of the net is typically 50–60 cm above the water level. Site selection is important for net enclosure culture. Suitable sites should have good water quality and slow flow, stable water level and suitable depth (1–2 m); and, most importantly, abundant submerged aquatic plants and high benthos biomass.

Stocking density for net enclosures range from 1500 to 9000 crabs/ha. For some enclosures, crabs can totally rely on the natural food. For others with lower natural biomass, the mud snail *B. purificata* may be stocked at 6000–7500 kg/ha before crab stocking and sometimes again after stocking. Other supplemental diets, include chopped trash fishes, and cooked corn and wheat can also been provided at a ration of 7–8% of crab biomass per day.

Survival is normally around 40–50%. This method normally has higher profitability because water quality is better, the crab can utilise abundant natural foods and, owing to lower stocking density, crabs can grow to bigger sizes that have high market values. Generally, the yield from net enclosure culture is about 150–450 kg/ha of market-sized crabs although higher yields of close to 1000 kg/ha have been reported.

The paddy-field culture system integrating rice production and Chinese mitten crab grow-out are popular in northern China (Fig. 22.7). Rice fields need modifications for this including setting up fences, digging perimeter channels and planting aquatic plants; they are similar to those for nursery culture described in section 22.3.3.4. One of the main differences is that the channel needs to be deeper at 1.5 m for grow-out. In addition, a small pond (100–200 m^2 with 1.5 m depth) needs to be dug for acclimation, temporary culture of coin-sized crabs and for temporary storage of market-sized crab during harvesting.

Stocking density ranges from 1 to 1.5 coin-sized crabs/m^2. As the paddy-fields also grow rice simultaneously, the water depth is normally kept low at 5–10 cm. Water is exchanged more regularly owing to lower levels of water in paddy-fields and extra care needs to be taken to avoid large fluctuations in temperature. As the natural benthic biomass in the rice fields is relatively low, the crabs need regular feeding with supplemental feeds, such as corn, wheat, trash fish, trash shrimp or mud snails, once or twice daily. These feeds are normally cooked to avoid water quality problems in the shallow water. With proper management, rice field culture can achieve yields of 300–450 kg/ha, and occasionally 750 kg/ha. Although crabs will feed on rice plants, they also feed on nuisance insects and weeds, and may even improve rice production. As such, the integrated culture system produces rice that is largely 'organic' with higher value and the economic benefits are substantial.

22.3.4 Market and marketing

The Chinese mitten crab is largely consumed domestically in China (Fig. 22.8) although it is also exported to countries or cities that have large ethnic Chinese populations, such as Singapore. Female crabs with well-developed ovaries and large crabs normally fetch the highest prices.

22.4 FRESHWATER PRAWNS

Species of the genus *Macrobrachium* belong to the family Palaemonidae within the infraorder Caridea. They are characterised by their very long, slender, chelate second



Fig. 22.7 Rice (paddy) field culture of Chinese mitten crabs in northern China.



Fig. 22.8 Fresh cultured Chinese mitten crabs for sale at a market in China.

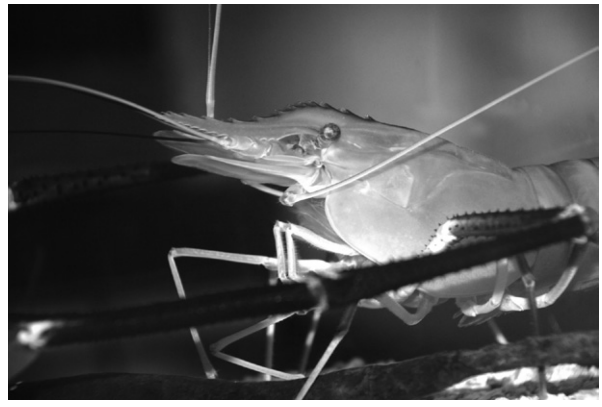


Fig. 22.9 The giant river prawn *Macrobrachium rosenbergii*. (Photograph by Jarek Tuszynski.)

pair of legs (Fig. 22.9). There are more than 150 *Macrobrachium* species, which can be found in inland freshwater bodies and estuaries throughout tropical and sub-tropical zones of the world. The two major species in aquaculture are the giant river prawn or Malaysian prawn, *Macrobrachium rosenbergii*, and the oriental river prawn, *M. nipponense*.

Macrobrachium rosenbergii (Fig. 22.9) is indigenous to south and south-east Asia, northern Oceania, northern Australia and the islands of the western Pacific. It is a traditional food source throughout this range and now supports a significant aquaculture industry over most of its natural distribution. A major reason for the development of *M. rosenbergii* as a successful aquaculture species is its

Table 22.3 World aquaculture production of *Macrobrachium rosenbergii* and *M. nipponense* in 1998 and 2007.

	1998 production (t)	2007 production (t)
M. rosenbergii	79 140	213 274
M. nipponense	–	192 397
Total	79 140	409 801

Table 22.4 Aquaculture production (t) of *Macrobrachium rosenbergii* and *M. nipponense* in 2007 from the major producing countries.

	M. rosenbergii	*M. nipponense*
China	124 520	192 397
Bangladesh	23 240	–
Thailand	27 650	–
India	27 262	–
Taiwan	8 316	–
Indonesia	989	–

rapid growth rate to a large size (up to 200 mm). The potential of *M. rosenbergii* for aquaculture has resulted in its translocation to other regions, including Hawaii, South America and the Caribbean, where it also supports successful aquaculture industries. *M. nipponense* is smaller than *M. rosenbergii*, with a maximum length of around 100 mm. It inhabits fresh and brackish waters throughout much of eastern Asia but is of particular commercial importance, from a fishery and aquaculture perspective, in Japan and China. *M. nipponense* shows relatively broad tolerance to variation in temperature and salinity and its high survival in colder temperate climates has facilitated its development as an aquaculture species, particularly in China. The use of *M. nipponense* in aquaculture has been growing since the 1990s (New, 2005).

22.4.1 Production status

Aquaculture production of *M. rosenbergii* increased more than 2.5 times between 1998 and 2007 to 223 274 t (Table 22.3). Around 60% of this production was from China (Table 22.4). Aquaculture production of *M. nipponense* has increased rapidly during the past decade from 87 139 t in 2000 to 192 397 t in 2007 (Table 22.3). This production comes almost exclusively from China (Table 22.4). Other species of *Macrobrachium* which have shown aquaculture potential or are being investigated for this potential, include *M. malcolmsonii* (in India and China), *M. amazonicum* (in Brazil), *M. vollenhovenii* (in Ghana) and *M. lamarrei* and *M. idella* (in India) (New, 2005).

22.4.2 Biology

There is variability between species of *Macrobrachium* in some aspects of their biology relevant to aquaculture. This is particularly true of the influence of salinity during reproduction which reflects their marine origin and the varying degrees of adaption to freshwater between species (Ogasawara *et al.*, 1979). The following account of the biology and culture of freshwater prawns will therefore focus specifically on *M. rosenbergii* which is the most widely cultured species of freshwater prawn.

22.4.2.1 External anatomy

The rostrum in *Macrobrachium* species is a relatively long, toothed and laterally flattened extension of the carapace. The first and second pairs of legs in *Macrobrachium* are chelate. The first pair is used for picking small epiphytic food organisms from solid substrates whereas the second pair is very long and slender (Fig. 22.9) and are much larger in males than females. The reproductive openings of males and females are located at the bases of the fifth and third pair of thoracic legs, respectively. Backward escape movements result from rapid contraction of the abdomen and tail and large pleopods or swimmerets allow effective swimming.

22.4.2.2 Life cycles

Macrobrachium rosenbergii grows to maturity in freshwater. The courtship display of males includes waving feelers and head bobbing, and copulation between a mature male and a recently moulted, soft-shelled female results in deposition of a spermatophore on the ventral median thoracic region of the female. Eggs are extruded through the genital pores 6–20 h after mating and are fertilised as they pass across the spermatophore into the brood chamber, formed by the downward elongation of the abdominal pleura. During egg laying the female bends the tail forward to the thoracic region to assist this process. Oviposition of a complete batch of eggs usually take around 20 minutes. The eggs are held in bundles by an elastic membrane secreted by the setae to which they are attached. Eggs are slightly elliptical, around 0.6 mm long and bright orange

in colour. A large female may produce up to 100 000 eggs and they can reproduce three or four times a year under natural conditions. Incubation takes around 19 days at 26–28 °C and throughout this period pleopods beat back and forth intermittently to provide water movement. Egg-bearing females move downstream into saline regions of estuaries and the orange colour of the eggs gradually changes to dark slate grey after 16–17 days when the larvae inside the eggs are fully developed. Hatching of the planktonic zoea larvae and their dispersal is assisted by vibrations of the pleopods.

From hatching to metamorphosis into juveniles the actively swimming larvae undergo 11 moults (11 larval stages are recognised) and metamorphose into post-larvae within 3–7 weeks, depending on temperature, water quality and food supply. They feed on zooplankton. Post-larvae become benthic and migrate upstream from saline conditions to complete their life cycle in freshwater.

22.4.3 Culture methods

Farming techniques for *M. rosenbergii* were comprehensively reviewed by New (2002).

22.4.3.1 Hatchery

Because *M. rosenbergii* completes its larval life in brackish water, and post-larvae and adults live in freshwater, hatcheries are usually located near sources of freshwater and seawater (although artificial seawater can be used). Berried females may be obtained from the wild or from captive broodstock and should be held at ca. 5‰ salinity until the eggs hatch. Larvae can be siphoned from hatching tanks counted and placed into separate larval rearing tanks or the hatching tanks may become the larval rearing tanks once the females are removed. In some hatcheries the females are kept in the larval rearing tanks in cages which are simple removed once hatching has occurred. Salinity needs to be around 12‰ for culture of all larval stages of *M. rosenbergii* and the temperature at 28–31 °C. *M. rosenbergii* larvae are cultured in either flow-through or recirculating hatchery systems.

In flow-through hatchery systems, larval culture tanks are stocked at relatively low densities (30–50 larvae/L). Water is exchanged on a regular basis at a rate of around 50% per day. The new water is either filtered ‘clear water’ or ‘green water’ which is enriched with batch cultured micro-algae (e.g. *Chlorella*, *Nannochloropsis*, *Isochrysis* and *Tetraselmis* species) or algae bloomed in outdoor tanks. The algae improve water quality by removing nitrogenous wastes excreted by the prawn larvae. The larvae do not really get any direct nutritional benefit from the algae although it may be eaten by zooplankton, which in turn are consumed by the prawn larvae. The benefits of green water cultured may increase production by 10–20% compared with clear water culture. It is important to ensure that water quality is at its highest at night when the larvae moult.

Recirculation hatchery systems enable larvae to be cultured at higher densities than in flow-through systems. They generally utilise continuous circulation of larval culture water and must incorporate a range of technologies such as particle filtration, biofiltration and ultraviolet irradiation, to maintain adequate water quality (section 2.4.4). Because of this they conserve water and reduce demand for seawater or brine and may be established inland, near grow-out facilities, using seawater transported to the site or artificial seawater.

Newly hatched *Artemia* nauplii (section 9.4.3) are the major live feed used for *Macrobrachium* larvae. Freshly hatched brine shrimp (*Artemia*) nauplii are added to larval culture systems after day 2 and feeding rate is increased as larvae grow. For example, early-stage *M. rosenbergii* larvae may be fed at a rate of five to six nauplii/larva (0.15 nauplii/mL), whereas older larvae may receive 60 nauplii/larva (1.8 nauplii/mL) (Roustaian *et al.*, 2001). Larval cultures are ideally fed up to five or six times a day. Feeds other than *Artemia* are also extensively used in larval culture of *Macrobrachium*. They include fish roes, ‘egg custard’ and artificial diets (New, 2002).

The first post-larvae should be seen in culture tanks at 16–20 days of age and their presence is indicated by a change in behaviour from free swimming to crawling or clinging to the tank surfaces. Newly metamorphosed post-larvae are 7–8 mm long and they are generally acclimated to freshwater before being harvested and transfer to nursery culture systems (New, 2002). Survival during hatchery culture to the post-larval stage is generally around 40–60%.

22.4.3.2 Nursery phase

The nursery phase is concerned with culturing post-larvae to the juvenile stage before stocking into grow-out ponds. Indoor nurseries can utilise a range of tank types with a volume of 10–50 m^2 and a water depth of around 1 m. Tanks can be stocked at densities in the range of 1000–2000 post-larvae/m^2. Artificial substrates such as a nylon screen or netting are often added to provide shelter and increase surface area to support greater survival. Water can be supplied from a flow-through or recirculating system and may be heated and is aerated. Post-larvae are fed at a rate of around 10–20% of their total weight each day.

Foods commonly include pelleted diets as well as fresh foods such as beef liver, egg custard or minced fish (New, 2002). Survival of around 90% can be achieved after 20 days and high survival can be maintained if density is reduced as the post-larvae grow. Post-larvae can be harvested using 3-mm mesh dip nets.

Outdoor nursery facilities utilise earthen ponds stocked with 70–800 post-larvae/m^2. Higher stocking densities are supported by providing additional substrate, supplemental aeration and protection from predators. Post-larvae and juveniles are generally fed with grow-out feeds at a similar rate to those in indoor nursery systems. Survival in outdoor nursery ponds may be around 75% to 0.8–2.0 g size when juveniles can be harvested from ponds using a 5- to 6-mm seine net.

22.4.3.3 Grow-out

Grow-out culture of *M. rosenbergii* may be extensive, semi-intensive or intensive.

22.4.3.3.1 Extensive culture

Extensive culture of *M. rosenbergii* is generally conducted in earthen ponds and impoundments such as reservoirs, irrigation ponds and channels, and rice fields. Post-larvae or juveniles are stocked at a low density (1–4/m^2) and there is little (if any) intervention relating to water quality and stock management and no supplemental feeding. This system is most widely applied in Asia and productivity is generally less than 500 kg/ha/year.

22.4.3.3.2 Semi-intensive culture

This is the widely practised culture system for *M. rosenbergii* and the most common in tropical areas. Semi-intensive culture is usually conducted in purpose-built ponds and involves management interventions relating to water quality maintenance, stocking, feeding, pond fertilisation and predator control. Ponds are usually stocked with hatchery raised post-larvae or juveniles at a density of 4–20/m^3.

In batch culture grow-out systems the whole pond is drain harvested after 6–9 months when prawns have reached a marketable size of 20–60 g (Fig. 22.10). Production ranges from 1000 to 3000 kg/ha/year, depending on the culture period and initial stocking density. However, male *M. rosenbergii* show a phenomenon called heterogeneous individual growth where some individuals grow at a much faster rate than others, become dominant and stunt the growth of others. Batch culture, as a consequence of this, yields a crop of non-uniform sized prawns. Modified batch culture systems have been developed where larger prawns are removed from cultured and placed at reduced densities into different ponds to allow smaller animals to grow to market size (New, 2002).



Fig. 22.10 Batch cultured *Macrobrachium rosenbergii* held in tanks after harvest. Although not yet large prawns, some males are showing an early stage of enlarged arms.

Grow-out ponds for *M. rosenbergii* may be managed using a continuous culture system where there is regular harvesting of market-sized prawns and restocking of post-larvae. This system is widely used in the tropics where prawn growth is continuous and ponds may be emptied only after several years. Selective harvesting usually begins after 4–9 months to remove market-sized prawns of uniform size. This procedure may be repeated monthly and yields around 2000–4000 kg/ha/year. Harvesting costs are increased compared with batch culture and predators and competitors may become established in ponds over longer culture periods. Continuous harvest and the regular removal of larger males support greater productivity by reducing intraspecific aggression and allowing more energy to be captured as growth. However, large dominant prawns may evade capture and influence the growth of underlings.

Semi-intensive grow-out systems that combine some characteristics of both batch culture and continuous culture systems have been developed. In these systems ponds are stocked once and selective harvesting begins when the first prawns reach market size. Smaller prawns are then able to grow and this process supports several harvests. The 9–12 month production cycle is terminated with a final harvest and draining of the pond.

Freshwater prawns are omnivorous and their nutritional requirements are not particularly demanding. Successful

semi-intensive pond culture of *M. rosenbergii* relies on provision of supplemental feeds. These are commonly farm-made feeds formulated from locally available animal or vegetable matter such as rice, vegetable waste and trash fish (section 8.4.1). A protein content of around 30–35% is considered optimal. Commercially made pelleted feeds are also used and some specifically formulated for *M. rosenbergii* are available but are relatively expensive. Feeding rate is usually reduced from around 10–20% of body weight for juveniles to around 2% of body weight at harvest (New, 2002). However, ration should be adjusted in response to observations of feeding activity particularly in ponds where there is regular harvesting of larger individuals.

Grow-out culture of *M. rosenbergii* generally concerns mixed sex populations where males and females are cultured together. However, culture of monosex populations (all male or all female) of *M. rosenbergii* offers potential benefits to farmers. Because males grow to a larger size than females they would produce a greater biomass over a given culture period. This increased production would be assisted because energy that would normally be partitioned into reproductive activities in mixed sex cultures could be used for growth. Growth trials have shown that all-male cultures produced larger prawns, greater biomass and estimated revenue increases of 24.5 and 85.7% compared with mixed populations and all-female populations, respectively. Although monosex populations can be obtained by hand-sexing, this is time-consuming, labour intensive and can be inaccurate. All-male offspring can be produced by mating normal males to 'neo-females' produced by surgical removal of the androgenic glands from juvenile males.

22.4.3.3.3 Intensive culture

Intensive culture of *M. rosenbergii* is generally undertaken in small earthen or concrete ponds up to around 0.2 ha in size. Stocking density is high (>20/m^2) and systems require continuous aeration, high water exchange, strict control of water quality, precise mangement of nutrition and food input and elimination of predators and competitors. Establishment and maintenance costs for intensive systems are high and considerable technical input is required for success. However, production levels of between 5000 and 10 000 kg/ha/year are achieveable. Intensive culture may become more popular for *M. rosenbergii* as the technology required by such systems improves and further developments are made with aspects of the husbandry of this species (e.g. reliable production of monosex populations).

22.5 FRESHWATER CRAYFISH

Freshwater crayfish, also known as freshwater crawfish[1], has been an item of human food for thousands of years. However, of more than 500 recognised species, there are only few from the genera *Astacus*, *Pacifastacus*, *Procambarus* and *Cherax* that are commercially important (Holdich, 2002). Among the most important cultured species, *Astacus astacus* is mainly cultured in Europe, *Pacifastacus leniusculus* has been cultured in Northern America and Europe whereas *Cherax destructor*, *C. albidus*, *C. quadracarinatus* and *C. tenuimanus* are mainly farmed in Australia. However, in terms of global fisheries and aquaculture production, the red swamp crawfish, *Procambarus clarkii*, is by far the most important species. Although the native distribution of *P. clarkii* is north-eastern Mexico and the south-central USA, particularly in Louisiana, the species has been introduced extensively to other parts of the USA and countries in Europe, Asia and Africa. This species is now the most widely distributed freshwater crayfish/crawfish in the world and has become one of the most important freshwater species for both fisheries and aquaculture in the modern world (Huner, 2002).

22.5.1 Production status

The noble crayfish *A. astacus* is widely distributed in Europe and has been an object of trade and commerce in the continent for more than 2000 years. It is recognised as a delicacy and is widely used in French cuisine. However, its abundance in Europe has dramatically reduced owing to the outbreaks of crayfish plague (section 10.5.2) in the twentieth century and by water pollution and habitat changes. Attempts to culture this species during the latter half of the twentieth century in Europe also largely failed for economic and other reasons, including the crayfish plague outbreaks. Current culture activities in Europe are mainly for production of juveniles to restock affected lacks and rivers.

Several native species belonging to the genus *Cherax*, redclaw (*C. quadracarinatus*), marron (*C. tenuimanus*) and yabbies (*C. destructor* and *C. albidus*) are farmed commercially in Australia as well as being introduced overseas for farming. Among them, marron and yabbies originate in south-western Australia and central Australia respectively and are more adaptive to cooler climates whereas redclaw are a tropical species from northern Australia. Although the number of aquaculture farms for

[1] 'Crawfish' is used for red swamp crawfish, *Procambarus clarki*, but since there is no intrinsic difference between this species and the other cultured species that are known collectively as 'freshwater crayfish', the latter will be used.



Fig. 22.11 Female red swamp crawfish, *Procambarus clarkii,* with newly independent juveniles.

these species had increased rapidly during the 1980s and 1990s, with production peaking at several hundred tonnes in the country, the prediction that the industry would further expand and produce thousands of tonnes in following decades has failed to materialise. In fact the number of farms and production have shown a declining trend in recent years. For example, in the state of Queensland, the total production of tropical redclaw has dropped from 100 t in 2006–07 to 67 t in 2007–08. Over the same period, the number of producing farms also decreased. The major problems identified are the generally small scale of farms, best-practice technologies are not widely adopted and the total production volume is too small. The local markets appear satiated while the low volume of production has severely hampered the potential of exploiting lucrative export markets.

The red swamp crawfish, *P. clarkii* (Fig. 22.11), has been commercially cultured in its native USA since the 1950s and is now farmed in most of the states in the southern USA. By 1986, the culture area had reached 50 000 ha, with average annual harvests around 30 000–40 000 t in most years. However, towards the end of the twentieth century, production had declined owing to two major factors: first, large volumes of peeled crayfish meat imported from China were sold for prices below production costs, and, secondly, dry weather patterns had led to a major reduction in both wild and farmed crayfish production in Louisiana (Huner, 2002). Outside the USA, *P. clarkii* has been cultured on a limited basis in Spain, France, Italy and Zambia; however, an explosive growth in aquaculture production of the species in mainland China in recent years has dwarfed the production from these countries. Consequently, the following sections will largely describe its production in China.

P. clarkii was introduced to mainland China from Japan in the 1930s during the Second World War. The establishment of local populations was initially confirmed in Jiangsu and Anhui provinces in south China. Harvested wild *P. clarkii* began to appear in domestic markets in the 1950s and by the 1960s and 1970s, it has become commonly known in the market. Meanwhile, wild populations of *P. clarkii* had become widespread along the middle and lower reach of the Yangtze River and become a dominant species in wetlands of the region. Harvests of wild *P. clarkii* also grew to become one of the most important freshwater fisheries in China, with Jiangsu province producing the largest quantity. It was estimated that, before 1990, the annual harvest was only a few thousand t, but by 1995 it had increased dramatically to 65 500 t and by 1999 it has almost reached the100 000 t mark. The substantial increase in *P. clarkii* fisheries production was largely driven by the establishment and rapid growth of the processing industry, which produced peeled crayfish meat (tail meat) for export to the USA and Europe, during the early 1990s. For example, during the 3 years from 1992 to 1995, the crayfish tail meat exported to the USA increased by eight times; and in 1999 out of the ca. 100 000 t annual production, at least 70 000 t was channelled into export. The large volumes of cheap tail meat imported to the USA from China has seriously impacted the *P. clarkii* culture industry in the USA and led to its downturn (Huner, 2002). Meanwhile, it has stimulated *P. clarkii* fisheries in China, leading to over-exploitation and a sharp decrease in the wild stocks. The decrease in wild crayfish stocks and harvests has generated much interest in aquaculture of the species and significant growth in *P. clarkii* farming developed at the end of the twentieth century.

Although before 2000, *P. clarkii* production in China was mainly for export, since the turn of the century there has been a new phase that is driven by a dramatic increase in domestic market demand. This is largely attributed to the creation of several very popular brand-named culinary delicacies for *P. clarkii*, such as 'spicy small lobster', 'aromatic small lobster' and 'savoury whole small lobster'. There was a very successful marketing strategy of renaming the crayfish as 'small lobster' and it is consumed in restaurants all over the country in recent years. The sharp increase in domestic market demand has seen the price for *P. clarkii* increase dramatically and even the processing industry for export has diminished as it is now more profitable to sell the whole, live crayfish to domestic restaurants than to export their meat. Aquaculture production of

P. clarkii in 2006 was more than 130 000 t; it increased to more than 200 000 t in 2007 and was about 300 000 t in 2008. Hubei province has emerged as the most important region for *P. clarkii* farming with the aquaculture area estimated to exceed 70 000 ha in 2008 (Cheng *et al*., 2008b). Meanwhile, the culture area in Jiangsu province has also nearly doubled from 7 000 ha in 2006 to more than 12 000 ha in 2007.

22.5.2 Biology

Freshwater crayfish occur in a wide range of habitats, including lakes, rivers, swamps, cave pools, temporary ponds and estuaries. There are potentially few freshwater habitats that crayfish could not invade. Some species, such as *P. clarkii* and the yabby, *Cherax destructor*, are well adapted for burrowing.

Freshwater crayfish are opportunistic omnivores, feed on a wide range of food items, including microbially enriched plant detritus, benthic and planktonic invertebrates, plant seeds and succulent green plant material where available (Huner, 2002). Although microbially enriched plant detritus often accounts for the bulk of the stomach contents of crayfish, it is high-energy food sources, i.e. animals and plants, that are critical for crayfish growth (Momot, 1995). The dietary preferences may change at different life stages of the crayfish as animal food occurs more frequently in guts of young crayfish and appears to be more crucial for new hatchlings (Huner, 2002).

Under culture conditions, crayfish readily consume a variety of formulated feeds, including grain-based, low-protein livestock feeds, high-protein fish and crustacean feeds, and raw seeds or by-products of those seeds, such as corn, rice and soybeans (Huner, 2002). However, out of economical consideration, feeding is often only used in intensive culture.

Crayfish are sexually dimorphic. The males and females can be distinguished by the difference of their gonopore location: the gonopores of males are located on the bases of the fourth walking legs whereas they are on the bases of the second walking legs in females. Females do not need to moult in order to mate. During mating, the male deposits a sperm mass on the ventral surface of the female between her walking legs. Several weeks to several months after mating, prominent glair glands develop and oviposition takes place. When eggs are extruded, the female releases a mucus-like substance called glair from the glair gland to attach fertilised eggs to her pleopods for brooding (Fig. 22.12). The incubation period largely depends on temperature: for *P. clarkii*: it takes 18–22 days at 22–24 °C, but more than 4 months when the temperature is lower than 10 °C. Once the young have hatched, they cling to the female's swimmerets and undergo two moults during which they do not feed but rely on yolk reserves to survive. They are unable to survive away from the female until they have completed their second moult (Fig. 22.11), at which time they assume an adult-like appearance and begin to feed independently (Huner, 1994, 2002).



Fig. 22.12 A female Alleghany crayfish (*Orconectes obscurus*) with large eggs attached to the appendages (pleopods) of the animal's abdomen. (Photograph by Justin Montemarano.)

Compared with other commercially important decapod crustaceans, the simplified life cycle of freshwater crayfish is advantageous for aquaculture. The absence of planktonic free-living larval stages makes freshwater crayfish relatively easy to cultivate as populations in culture ponds are normally self-sustaining and mature crayfish can be simply stocked into the ponds to start the population.

Like other crustaceans, growth of crayfish is achieved by periodical moulting (section 6.4.2). One interesting feature of freshwater crayfish in relation to moulting is their ability to reuse calcium from the old exoskeleton. When the moult approaches in freshwater crayfish, the old exoskeleton de-mineralises. Calcium is withdrawn and stored in paired hemispherical stones, known as gastroliths, which are located adjacent to the stomach. After moulting, the gastroliths dissolve and calcium is absorbed into the circulatory system to be re-deposited into the new exoskeleton during the re-calcification process. This mechanism conserves calcium in environments where it is at relatively low levels.

Like other crustaceans, freshwater crayfish suffer from a range of diseases caused by various pathogens, parasites and commensals. Among them, the most devastating of all is the crayfish plague caused by the fungus *Aphanomyces astaci*. Crayfish plague is a fatal disease that can lead to rapid mortality of the infected crayfish and often the loss of whole populations (Evans and Edgerton, 2002). The crayfish plague outbreaks are responsible for elimination of native crayfish populations in many European waters in the twentieth century. However, so far, it has not been reported as a major problem in North America, China or Australia (Evans and Edgerton, 2002). The susceptibility of different crayfish species to the disease varies: for example, the North American species, including the red swamp crawfish *P. clarkii*, are more resistant to the disease (Evans and Edgerton, 2002).

22.5.3 Culture methods

Freshwater crayfish possess several important attributes that make them a very good candidate for aquaculture, which include the following:

- simple life cycle (absence of free-living larval stages) makes them relatively easy to cultivate;
- fed on inexpensive, low protein and grain-based diets;
- physically and physiologically robust with broad environmental tolerances;
- fast growing to large size;
- some species, such as redclaw, are not very aggressive;
- lobster shape helps marketing;
- texture and flavour comparable to marine crustaceans.

The culture of freshwater crayfish outside China and the USA is mainly in earthen ponds. For instance, redclaw (*Cherax quadracarinatus*) farms in tropical Australia are generally about 0.1–0.25 ha with a depth of between 1.3 and 1.8 m. The ponds are elongated for more efficient food distribution and V-shape in the bottom to allow easy drainage. Perimeter fencing is installed around the pond site to prevent escape and control predators. A complete netting enclosure (top and sides) to culture ponds is also essential where bird predators are prevalent. Various hides, such as used car tyres, bundles of synthetic mesh, PVC pipes and crates, are provided to increase water column utilisation and provide refuge for the redclaw (Fig. 22.13). Depending on the farm, feeding may be conducted with commercial grain-based pellets and there may be mechanical aeration.

In China, the culture of *P. clarkii* occurs in various forms. Pond and paddy-field cultures are the two most common methods used, which will be described in more detail below. Other culture systems include the following:

- extensive culture in large natural water bodies, such as shallow or vegetated rivers and lakes, marshes and other wetlands;

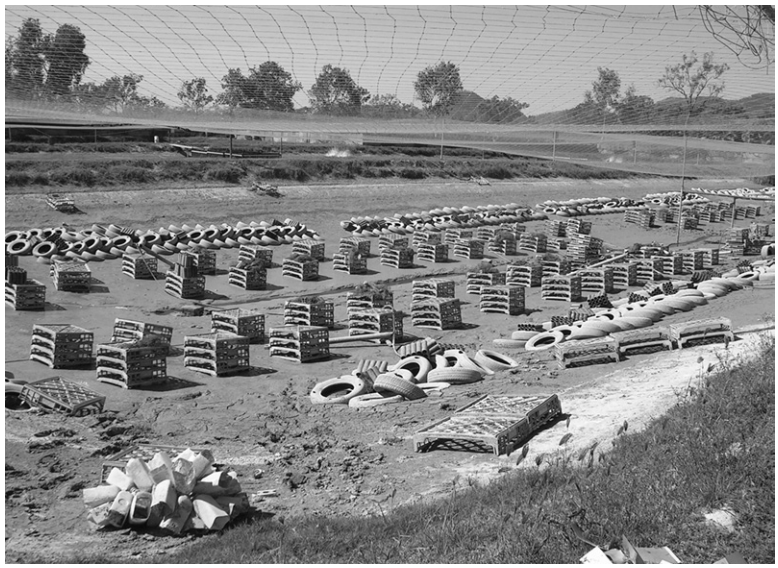


Fig. 22.13 Artificial substrates used to provide hides for pond cultured freshwater crayfish. These are being used for the redclaw crayfish, *Cherax quadracarinatus*, in north Queensland. The netting stretched above the ponds is a barrier to water birds that prey on the crayfish.

- polyculture in ditches set between forests of the poplar tree, *Populus bolleana*;
- polyculture in lotus, *Nelumbo nucifera*, culture fields (the roots and seeds of the lotus are popular foods in China);
- semi-intensive or intensive culture in leveed marshy areas and wetlands, where aquatic plants are planted as food for the crayfish and regular supplemental feeding is undertaken, which in many ways is similar to pond culture.

22.5.3.1 Pond culture

Pond culture is the most common system for *P. clarkii* in China. The silver carp, *Hypophthalmichthys molitrix*, is often stocked for polyculture with crayfish. The stocking rate for silver carp generally ranges from 700 to 1100 fish/ha.

The most commonly used ponds are rectangular earthen ponds of between 0.4 and 1.0 ha. Various structures, such as plastic sheets or aluminium plates about 0.5 m high, are erected around the pond to prevent escape. The length to width ratio of culture ponds is generally about 2 : 1, with pond banks having a slope of at least 1 in 3. Water depth is generally between 1.0 and 1.5 m and the substrate should be no more than 10 cm deep in mud. A channel of about 2 m in width and 0.3 m in depth is dug along the length of the pond. This is used for harvesting crayfish when the pond is drained.

Before stocking with crayfish, ponds are normally drained and disinfected with lime (750–1500 kg/ha), which is followed by fertilisation with organic fertiliser (2250–4500 kg/ha). Several days later, water is introduced through a long sieve bag with a mesh size of ca. 60 µm to prevent introducing predators or their eggs. The pond is first filled to about 50 cm deep and various aquatic plants are then planted. They include *Elodea nuttallii*, *Hydrilla verticillate*, *Alternanthera philoxeroides*, *Pistia stratiotes*, *Ceratophyllum demersam*, *Eichhornia crassipes*, *Zizania caduciflora*, *Vallisneria spiralis*, *Lemna paucicostata*, *Myriophyllum spicatum*, *Potamogeton crispus*, and *Potamogeton* sp. *A. philoxeroides* and *E. crassipes* are normally planted in the shallower areas along the pond banks. A combination of rooted aquatic plants in shallow water with floating plants in deeper water can provide more abundant and diversified food sources for crayfish. Overall, the area for planting aquatic plants is about 30–60% of the total pond area.

The crayfish seed used for pond culture are mainly from two sources. Firstly, juveniles of 2–3 cm length (5 g on average) can be purchased from a hatchery for stocking the ponds. This is normally done in spring, and stocking density is about 20–30 crayfish/m^2. After about 3 months' culture in summer, some crayfish reach market size and can be harvested; the remaining crayfish are cultured further and harvested intermittently.

The other method is stocking sexually mature crayfish of about 30 g in the ponds in later summer. These crayfish are stocked at a density of about 20 000 individuals/ha with a female : male ratio of 1.5–2 : 1. After stocking, the crayfish are fed well to prepare for over-wintering. By spring of next year, the early juveniles produced from breeding adults stocked the year before should be independent of females and the adult crayfish can be harvested. As soon as crayfish juveniles have been found to become independent of females, they are fed with soy milk as well as minced trash fish, pig's blood and soy cakes. With growth of the juveniles, the feeding rate can be reduced from initially 20% of biomass to about 10%. When they grow to about 2 cm, the culture enters grow-out phase. Juvenile crayfish, compared with adults, must be fed more on animal-based diets, such as minced trash fish, freshwater gastropod and bivalve meat, or poultry offal. As they grow larger, the plant component of their diet can be increased to become the major component. However, just before harvest, animal-based diets may be given at an increased ratio to enhance growth and meat quality.

During grow-out, in addition to aquatic plants grown in the ponds, crayfish are fed a variety of other relatively inexpensive diets, such as wheat, corn, peanut cake, wheat bran, trash fish and formulated feed (crude protein content 25–28%). In summer, crayfish are fed twice a day: about 30% in the early morning and 70% in the afternoon. However, in winter, they are only fed once every fortnight and at noon. Feeding is adjusted according to temperature, water quality and observation of the amount being consumed by the crayfish.

Apart from growing aquatic plants and supplemental feedings, live Chinese mystery snail, *Cipangopaludina chinensis*, may also be stocked in crayfish ponds in spring. They are normally stocked at a rate of 300–450 kg/ha and left to reproduce inside the ponds. The early juvenile snails produced have a fragile shell that can be easily cracked by crayfish, thereby providing crucial animal protein. Also, the fine feeding of the snails helps maintain good water quality in the culture ponds.

The yields of pond culture vary depending on intensity of culture, but are generally between 3000 and 6000 kg/ha, although it can be as high as 7500 kg/ha.

22.5.3.2 Paddy-field culture

Paddy-field culture, which integrates rice production and crayfish culture, has been gaining in popularity in China



Fig. 22.14 Paddy-field culture of freshwater crayfish in China integrates crayfish culture with rice production.

over recent years (Fig. 22.14). With vast rice fields available in China suitable for such culture, it has huge potential for further expansion.

In general, paddy-fields of between 0.7 and 1.7 ha are considered most suitable for crayfish culture and daily management. Rice fields need to be modified to be suitable for crayfish culture. The major modifications include a channel dug around the perimeter of the paddy-field about 0.5–1.0 m inside the levee. This channel is typically 3–5 m wide and 0.8–1.0 m deep. For larger paddy-fields, cross channels or double cross channels are also dug in the middle of the paddy-field. In general, the total area of the channels is about 20–25% of the total paddy-field. Inside the channels, aquatic macrophytes are planted before stocking. As in ponds, they serve as food and refuge for the crayfish. However, the area used for planting aquatic plants is limited to about 20–25% of the total surface area of the channels, and aquatic plants should been planted evenly throughout them. In addition, structures must be built to prevent escape; this is normally done by setting up mesh fences on the edges of rice-fields (Fig. 22.14).

Similar to pond culture, there are two ways that crayfish are stocked in rice fields. First, adult crayfish can be stocked in paddy-fields during later summer to autumn to produce juveniles for culture during the following year. In this case, crayfish bigger than 30 g are selected and stocking density ranges from 10 000–20 000 crayfish/ha with a female : male ratio of 3 : 1. Rice is planted the following year without tilling. A second method is to stock paddy-fields with juvenile crayfish in May after rice has been planted. The stocking density is about 75 000–150 000/ha of 2- to 4-cm juveniles.

The water level in the paddy-field needs to be managed. When juvenile crayfish are first stocked it must be kept low, but with the growth of crayfish and rice, the water level is gradually increased to 12–15 cm. Water quality must be monitored closely, and every 3–5 days fresh water must be introduced; however, during mid-summer, this needs to be done more regularly, at 1–2 day intervals. The use of pesticides for rice needs to be avoided whenever possible. If they have to be applied, low toxicity and low residue types are required.

The channels inside paddy-fields are fertilised with organic and inorganic fertilisers to encourage the growth of freshwater rotifers, *Daphnia* and insects, which are important food for early juveniles.

Crayfish are more commonly harvested before the rice is harvested; however, some farmers harvest rice first, and then refill the paddy-field with water to continue crayfish culture for a further 1–2 months to produce larger-sized crayfish. With proper management, the crayfish yield of paddy-field culture can reach 3000 kg/ha with additional rice production of 6000 kg/ha.

22.5.4 Markets and marketing

Freshwater crayfish are sold whole, live or frozen (cooked or uncooked) hard-shelled, and as fresh or frozen abdominal muscle (tail meat) in US and European markets.

Whole, live or frozen soft-shelled crayfish have also been produced and sold in the USA, which fetch a substantially higher price. However, in mainland China, domestic consumption of the red swamp crayfish is mostly live and hard-shelled, and often in restaurants. The popularity as well as the market demand for the crayfish is expected to continue increasing in China, which is likely to continue to drive the fast expansion of the aquaculture industry for the species.

22.6 MUD CRABS

Mud crabs, *Scylla* spp., also known as mangrove crabs, occur widely in estuaries and coasts of tropical, subtropical and warm temperate zones of the Indo-Pacific. They are found from the coasts of eastern Africa to south-east Asia (including south-east China and the southern tip of Japan), to north-eastern Australia and many Pacific island nations, such as Papua New Guinea, Fiji, Samoa and Tonga (Keenan *et al.*, 1998). With large size and a delicate flavour, mud crabs are highly sought-after seafood items that command high market prices wherever they occur. In the recent past there was considerable confusion about their taxonomy, but it is now widely accepted that there are four species of mud crabs: *Scylla serrata*, *S. olivacea*, *S. tranquebarica* and *S. paramamosain*, with different species occurring at the same location in many countries, such as the Philippines and Indonesia (Keenan *et al*., 1998). Among the four mud crab species, the largest and the most broadly distributed is *S. serrata* (Fig. 22.1). However, the most important species for aquaculture is *S. paramamosain*.

During the latter half of the twentieth century, increasing fishing efforts driven by market demands and destruction of mangrove habitats led to the decline of mud crab wild stock in many Asian countries. There are only a few exceptions, such as Australia, where the mud crab fishery is strictly regulated and the wild stock has remained relatively healthy. Increasing market demand and price, coupled with dwindling wild stock, have generated substantial interest in mud crab aquaculture in many countries. Although the mud crabs have been farmed in southern China for more than 100 years and in other south-east Asian countries for a few decades, the rapid expansion of the industry has occurred mainly since the turn of the century. However, further expansion of the industry is highly dependent on whether its current high reliance on wild-caught crab seed can be replaced by hatchery-produced juveniles.

22.6.1 Production status

China is the biggest producer of farmed mud crabs in the world, with annual aquaculture production reportedly reaching 106 000 t in 2003 (China Fisheries Yearbook, 2007). The actual total production could be higher as mud crab farming is traditionally small scale and family run; hence production from some small farms may not be included in the official statistics. Owing to temperature limitations, the production region is mainly in southern China. Other Asian countries, such as Vietnam, the Philippines, India and Indonesia also produce sizeable amounts of farmed mud crabs and the industry is growing rapidly. For example, Vietnam's farmed mud crab production tripled in only 5 years, increasing from 10 000 t in 2004 to an estimated 30 000 t in 2009. It appears that *S. paramamosain* is the most important species in aquaculture, as this is the dominant species in both China and Vietnam, where mud crab farming is most successful.

22.6.2 Biology

Mud crabs inhabit shallow coasts and estuaries, and favour soft muddy or sandy-mud bottoms. They are also commonly associated with mangrove forests, hence their common names of mud crab or mangrove crab. Mud crabs often burrow in the substrate or hide under shelter during the daytime and move out for foraging at night. They are largely omnivorous scavengers with diets including a wide range of animal and plant materials, including molluscs, small crustaceans, polychaetes and detritus (Hill, 1976). Mud crabs have broad tolerance to salinity, with adults venturing far upstream with tides in estuaries to forage. *Scylla serrata* in particular possesses strong osmoregulatory ability and has been reported to survive well in brackish water in salinity as low as 3–5‰. It is also found in seawater with salinities of 34‰ or higher (Keenan *et al.*, 1998).

Under captive conditions, mud crabs can reach sexual maturity within 1 year. The carapace width at sexual maturity is generally between 9 and 14 cm depending on species. Before copulation, male and female crabs often pair up for several days and copulation takes place soon after the female crab undergoes a moult and is still in the soft-shell condition. During copulation, the male crab transfers spermatophores to the seminal receptacles of the female, where they will remain viable for a long time and are used for fertilising eggs during subsequent spawnings of the female until her next moult. After copulation, the newly moulted female crab often remains under the protection of the male for several days until her new shell has become hard. The ovaries of female crabs start to develop after copulation and may take several months to ripen. In nature, females of *S. serrata* with ripe ovaries migrate long distances offshore to spawn (Hill, 1994), which largely explains why egg-carrying females (berried females) are

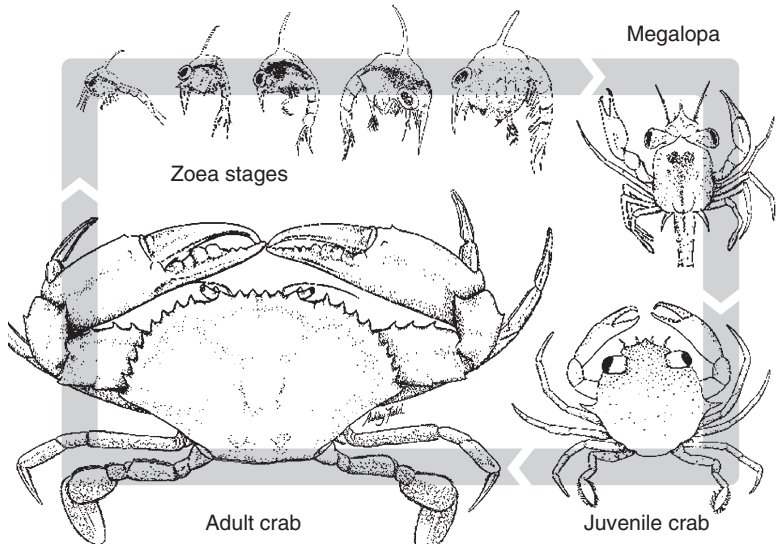


Fig. 22.15 The life cycle of the mudcrab, *Scylla serrata*, showing the successive stages.

rarely found in estuaries and near-shore waters. Mud crabs are highly fecund, producing one million to eight million eggs per spawning, depending on the species and size of the female. After spawning, eggs are attached to the pleopods of the female and cared for by the female until hatching, which can take 10 days to more than a month, depending on water temperature (Zeng, 2007).

The pattern of larval development is like that of the mitten crab (section 22.3.2):

- the eggs hatch out as first zoea, a small planktonic larva (Fig. 22.15);
- the larvae progress through five zoeal instars (zoea I–V) before undergoing metamorphosis to the megalopa stage;
- megalopae can swim in the plankton, but resemble crabs and become increasingly benthic.

Under optimal culture conditions, zoeal stages typically take 3–5 days each, whereas the megalopal stage lasts 7–10 days. It is believed that at the megalopa stage, migration from offshore to coasts and estuaries occurs and upon reaching suitable habitats, megalopae undergo a second metamorphosis and settle as benthic first-stage crabs (Fig. 22.15).

22.6.3 Wild-caught 'seed'

Unlike Chinese mitten crabs, mud crabs have been farmed in many countries, particularly in Asia. However, the industry worldwide still heavily relies on wild-caught small or 'empty' crabs (section 22.1.3) for stocking farms. Traditionally, mud crab farming used wild-caught juveniles to stock their farms. Despite substantial efforts over recent decades to develop hatchery techniques for mud crabs in various countries and commercially viable hatchery operations being recently reported in Vietnam, the industry as the whole still largely relies on wild seed supply for stocking ponds and mangrove pens.

Mud crab juveniles, often between 10 and 100 g, are collected by various means, including traps, various types of net or bare hands, from the field to stock mud crab farms directly. However, because mud crab juveniles are not normally densely distributed, the collection of wild mud crab 'seed' is often very tedious and inefficient. For example, it was reported that in Vietnam, using scoop nets in mangroves, on average only 100–200 juvenile mud crabs could be collected per person per working day.

In recent years, in southern mainland China as well as in Taiwan, collection methods for wild late megalopae have been developed to collect onshore migrating megalopae during their recruitment season. These methods allow the collection of large quantities of wild megalopae, often in millions, in a short period as the recruiting megalopae often form large swarms. However, such large-scale removal of recruiting megalopae will undoubtedly impact natural populations and fisheries and, in the long term, is unsustainable. The collected wild megalopae usually moult to first stage crabs in 2–4 days and are further grown

in nursery ponds for 1 month or more before being sold to grow-out farmers.

The reliance on wild seed supply for mud crab farming is a major bottleneck for the industry. It is clear that even at the current scale of the aquaculture in most Asian countries, quantities of wild caught seed are not sufficient to meet demand (Keenan, 1999). Furthermore, collected juveniles are often not uniform in size and consist of mixed species. Most importantly, there is evidence that wild juveniles of mud crabs are already over-exploited in many places, which will inevitably impact on natural populations and local fisheries.

22.6.4 Hatchery production

The encouraging development is that over the past few years, more than 250 commercial mud crab hatcheries (mainly with *S. paramamosain*) have been established in Vietnam. These hatcheries provide crablets (early juvenile crabs), accounting for one-third of the total demand for mud crab seed in Vietnam. In other Asian countries, such as the Philippines and China, hatchery production of crablets is also increasing, albeit still at a small scale, and this is often done by research institutes and universities subsidised by government funding.

22.6.4.1 Broodstock

Although in nature, mud crab females with advanced ovarian development migrate offshore to spawn, spawning can be readily achieved under captive conditions. The natural breeding season in subtropical and warm temperature regions is limited to late spring and summer. By manipulating water temperature and eyestalk ablation (section 6.2.2), mud crabs can be induced to spawn yearround (Zeng, 2007). Eyestalk ablation may also be used to enhance ovarian maturation and spawning during the breeding season. Although both re-maturation of spent females and closing the life cycle, i.e. broodstock females reared from hatchery spawnings, have been achieved experimentally, commercial hatcheries normally do not try to bring spent crabs back to gonad maturity, but replace them with newly caught crabs with fully developed ovaries as this is more efficient and costs less. Hence, after hatching out larvae, broodstock crabs are discarded and a new batch of mature females are introduced for subsequent spawnings. Because female mud crabs with advanced ovarian development have normally already mated, no males are needed. Healthy, good-sized female broodstock are fed bivalves, squid and other crustaceans (e.g. shrimp) daily until spawning. Sandy or muddy substrate is provided to facilitate the attachment of eggs to the female's pleopods during spawning, and shelters are added to reduce cannibalism.

Newly extruded eggs are brightly yellow to orange and they gradually turn grey and then dark grey when they are about to hatch. After spawning, berried females can either be immediately transferred to separate tanks for hatching or the transfer can occur after eggs turn dark. Berried females do not need to be fed during egg incubation. The hatching time can be accurately predicted with regular monitoring of embryonic development, which has been described in detail (Zeng, 2007). Hatching normally occurs in the early morning, and high fertilisation and hatching rates can often be achieved under captive conditions.

22.6.4.2 Larval culture

Larval culture for mud crabs is usually performed indoors in either concrete ponds or large circular tanks. The size of the culture ponds or tanks varies substantially, depending on hatchery. With slight modifications, marine shrimp hatcheries can be used for mud crab larval culture.

The optimal culture temperature for mud crab larvae is between 28 and 30 °C as high temperature substantially reduces the culture period and improves survival (Hamasaki, 2003; Nurdiani and Zeng, 2007). However, there is controversy over the optimal salinity for early zoea larvae: some research suggests a low salinity of about 25‰ being optimal (see, for example, Nurdiani and Zeng, 2007), whereas others believe that oceanic salinity conditions should be used. The differences may reflect the salinity preferences of different species and broodstock habitat. Despite different views on the optimal salinity for culturing zoeal larvae, it is generally agreed that from megalopae onwards, salinity should be gradually reduced (Nurdiani and Zeng, 2007). Additionally, the background colour of larval culture tanks has been shown to affect larval survival and development, with darker coloured backgrounds producing better results (Rabbani and Zeng, 2005).

The 'green water' method is often used for larval culture, particularly during early larval culture when rotifers are used as the main food for larvae. The basic larval feeding regimen for mud crab larvae are that for early larvae, i.e. zoea I and II, rotifers are used as feed. However, with further larval development and their increased foraging ability, from either late zoea II or zoea III onwards, newly hatched live *Artemia* nauplii are added to feed larvae as rotifers are an inadequate food for the later larvae of mud crabs (Zeng and Li, 1999). In Vietnamese hatcheries, it has also been reported that the umbrella stage of *Artemia* (the first few hours after hatching when the

embryo hangs beneath the cyst shell) (Fig. 9.6) is used for feeding early larvae; therefore, eliminating the need to use rotifers for larval culture.

22.6.4.3 Problems in mud crab hatchery culture

Although successful culture of mud crab larvae on a large scale has been reported in various countries, including Japan, China, Vietnam and the Philippines, the main problem of mud crab hatchery culture is its highly inconsistent and often low larval survival. This can be attributed to the following issues often encountered in mud crab hatcheries:

- significant variation in the quality of newly hatched larvae from different females;
- high mortality rates often occur at the zoea I stage if antibiotics are not used – this is particularly a problem in countries, such as Australia, where the use of antibiotics is strictly controlled;
- mass mortality routinely occurs around the time zoea V metamorphose to megalopae – often linked to 'moulting death syndrome', and then during the megalopa stage;
- high cannibalistic rates at megalopal and early juvenile stages;
- species-specific optimal larval culture condition needs further investigation, for example there is evidence suggesting that larval culture of *S. serrata* is more difficult than *S. paramamosain*;
- larval nutritional requirements and digestive physiology need to be better studied despite some recent effects (see, for example, Holme *et al.*, 2009).

Despite these problems, it is very encouraging to see that in Japan (for stock enhancement) and more recently in Vietnam, more consistent mud crab hatchery production has been achieved, and that especially in the latter case, the operations are commercially viable.

22.6.5 Nursery and grow-out culture

22.6.5.1 Nursery culture

The nursery phase is often required for wild-caught megalopae or hatchery-produced juveniles to grow them to sizes that are acceptable to the grow-out farmers. However, nursery culture of mud crabs is a relatively new development with the recent availability of wild megalopae and hatchery produced early juveniles. Both the nursery systems and sizes used are very diverse in different places and countries. The nursery systems include shallow concrete ponds, earthen ponds, hapa nets and cages, and mangrove pens and enclosures.

The initial stocking density for mud crab nursery culture is generally <100 megalopae (wild caught) or crablets (hatchery produced)/m^2. During nursery culture, substrata and shelter, such as artificial seaweeds, are normally provided to reduce cannibalism. The feeds used in mud crab nurseries are again highly diverse, including minced trash fish and shrimp meat, small bivalves and gastropods, and commercial feeds for marine shrimp. In southern China, small enterprises specialising in the collection and production of small bivalves and gastropods, which are unsuitable for human consumption, but ideal for mud crab juveniles, have emerged to supply feed for mud crab nurseries.

22.6.5.2 Grow-out culture

There are many techniques for growing out mud crabs. These include growing out crablets to market sizes, fattening crabs and soft-shell crab production. The last two practices are more specific, with substantially shorter culture periods, and hence are generally more profitable.

Grow-out of crablets to marketable sizes can be practised either as monoculture or polyculture. For the latter, mud crabs have been cultured with a variety of species, including seaweeds, marine shrimp (e.g. black tiger prawn *Penaeus monodon*), marine fishes (e.g. milkfish *Chanos chanos*) as well as mangrove silviculture (re-forestation of mangroves, including some intended for human use). For example, in Taiwan, polyculture of mud crabs with tiger prawns is practised in ponds with stocking densities of 10 000–16 000 mud crab crablets and 150 000–250 000 tiger prawn postlarvae in a pond of about 0.5 ha. In Vietnam, mud crabs are stocked in mangrove silviculture farms for extensive culture at very low densities of ca. 500 crabs/ha.

Mud crab fattening is either fattening lean or 'empty' crabs for flesh gain or culturing female adult crabs with undeveloped or spent ovaries to produce crabs with ripe ovaries. The former is commonly practised in south-east Asia with newly moulted 'empty' crabs being stocked for a culture period of 1–2 months to fatten them, whereas the latter is mainly conducted in southern China and Taiwan, where crabs with ripe ovaries fetch two to three times higher prices. There are several things in common between the two, including that the crabs stocked are already at market size, and that culture is in ponds, mangrove pens and bamboo cages at relatively high densities but sometimes, also individually, in small baskets. With relatively short culture periods and without moulting, mud crab fattening can normally achieve higher survival rates of 70–85%.

Soft-shell crab production of mud crabs has also been practised for some time in several south-east Asian

countries, particularly Thailand. The principle of such practice is that by regularly monitoring cultured crabs (every 3–4 h), any crabs undergoing moulting are discovered and harvested within a few hours of their moult. Hence, they do not have time to harden their new exoskeleton. As soft-shell crabs can be eaten whole, they fetch a high price and are often exported to lucrative overseas markets. The production of soft-shell crabs is either by holding individual crabs in perforated plastic boxes suspended on the water surface or holding them communally in big cages. In communal holdings, the chelae of the crabs are often removed to prevent cannibalism. The crabs used are often wild-sourced and between 60 and 130 g.

The most common culture systems for mud crab grow-out are pond culture and mangrove culture. Whereas in China pond culture is exclusively used, both pond and mangrove culture are practised in other south-east Asian countries. It is particularly worth mentioning that grow-out culture of mud crabs in mangroves has been dubbed 'mangrove-friendly aquaculture' as opposed to marine shrimp farming, which led to destruction of mangrove forests for pond construction. This practice is mangrove friendly while still generating significant income for the local community.

Earthen ponds are commonly used in pond culture. With simple modifications, existing marine shrimp ponds can be utilised for mud crab grow-out. The size of the ponds are generally between 0.3 and 0.5 ha with a water depth between 0.8 and 1.5 m. Various structures, most often simple net fences, but sometimes also concrete or lined pond banks, are constructed to prevent escape.

Stocking densities between 2.5 and 5 crabs/m^2 are most common for pond culture as higher density is likely to lead high mortality due to cannibalism. Various feeds, such as trash fish, animal offal, cheap molluscs and sometimes formulated feeds for marine shrimp, are used to feed mud crabs. Feeding rate is generally between 3 and 10% of biomass, with a decreasing feeding ratio as crabs grow bigger. The culture period for grow-out from juvenile to market size is generally between 5 and 8 months, and survival of 30–70% is commonly achieved.

There are two types of grow-out in mangrove: extensive culture in mangrove ponds and semi-intensive culture in enclosures. The former is practised with silviculture and the mud crabs are often stocked at very low densities of around 0.05 crabs/m^2. The crabs are normally not fed after stocking as they feed on naturally occurring food in the mangroves. With low culture density and abundance of natural food, both survival and growth rates of mud crabs are often high although there is a low overall yield.

The more commonly used culture system is mangrove enclosures, which are often constructed in intertidal zones using local widely available bamboo (Fig. 22.16). The stocking density is generally between one and five crabs/m^2. Although crabs can utilise natural diets, supplementary



Fig. 22.16 Enclosures for grow-out of mud crabs in a development project in Micronesia. (Photograph by Rohana Subasinghe.)

feeds such as trash fish are also supplied daily at a rate of approximately 5% of cultured biomass. The survival rate for mangrove enclosure culture is generally between 50 and 90% for grow-out from juveniles.

22.6.6 Markets and marketing

Mud crabs are well accepted throughout the Indo-Pacific region, particularly in countries where they occur naturally. They can remain alive out of water for a considerable period (more than 1 week when kept moist) after capture and are traditionally marketed alive. However, they are also sold as a cooked product in Australia and as a frozen product for the newly emerged soft-shell crab market. Although the price may vary substantially from country to country, mud crabs are generally regarded as high-priced seafood items on a local living standard basis. The size and sex of the crabs may affect the price: for example, female crabs with well-developed ovaries may fetch prices several times higher than those for male crabs in markets dominated by ethnic Chinese.

22.7 SPINY LOBSTERS

Spiny, or rock, lobsters (Fig. 22.17) occur from the tropics and subtropics (mainly *Panulirus* species) to temperate waters (mainly *Jasus* species). They inhabit protective habitats in reefs and other hard substrates. They differ from freshwater crayfish and other marine lobsters (and most other large crustaceans) in only having plain legs without chelae.

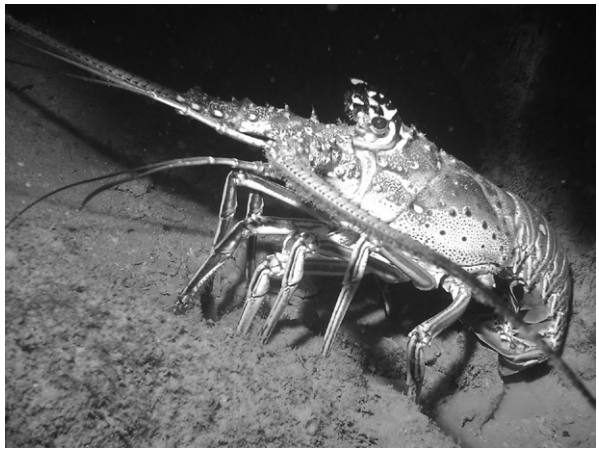


Fig. 22.17 *Panulirus argus* in the field at Morrocoy National Park, Tucacas, Venezuela. (Photograph from Creative Commons.)

There are fisheries based on spiny lobsters in many parts of their distributions and typically the natural stocks are overfished owing to the high market demand for them. Spiny lobsters have the highest or near-highest market value of all decapod crustaceans. A capture fishery of 70 000–80 000 t/year, based on the Western Australian spiny lobster, has a value of ca. Australian $200 million/year. They are very valuable in Asian markets as a live product, and exporters obtain $30+/kg. Live painted spiny lobsters, *Panulirus ornatus*, are popular because of their attractive pigmentation. Aquaculture has a potential advantage over capture fisheries in more readily producing live product and pursuing this lucrative market. Also, importantly, it would overcome the limitations of widespread overfishing. Thus, in countries such as Japan and Australia, there is considerable interest in culturing spiny lobsters and their relatives the squat lobsters (family Scyllaridae), through their life cycle.

22.7.1 Culture methods

Routine culture of spiny lobsters through their life cycle is one of the 'Holy Grails' of aquaculture, i.e. a target that will not be achieved without a great deal of effort and research. Breeding stock can be maintained in broodstock tanks and readily spawned, producing numerous eggs; but the larval stages that follow take 8 months to almost 2 years to complete the planktonic phase of their life cycle. To sustain the larvae over such a long period requires a huge technical commitment. Furthermore, the chances of bacterial infection, contamination, equipment failure and other sources of culture 'crashes' are high over such a protracted period. Having mass mortality in a culture of 7-month-old larvae is catastrophic compared with having mass mortality in a culture of 7-day-old shrimp larvae, which can be discarded and then the culture process begun again quite promptly.

The nature of the larvae, phyllosomas (Fig. 22.18), adds further to the problems of culture. The long period of development involves many larval stages and corresponding numbers of risky moultings as they progressively develop. The phyllosomas are leaf like, transparent, fragile and difficult to feed (Fig. 22.18). Their long thin appendages beat the water as they swim, and they trap slow-moving soft and relatively large particles rather haphazardly. The trapped particles are pushed in towards the mouth region where they are masticated and ingested. *Artemia* nauplii and later stages of *Artemia* are usually used as the food source in culture, but adequate nutrition is a problem as the larvae progressively die during the later stages of development. Research is focusing on the

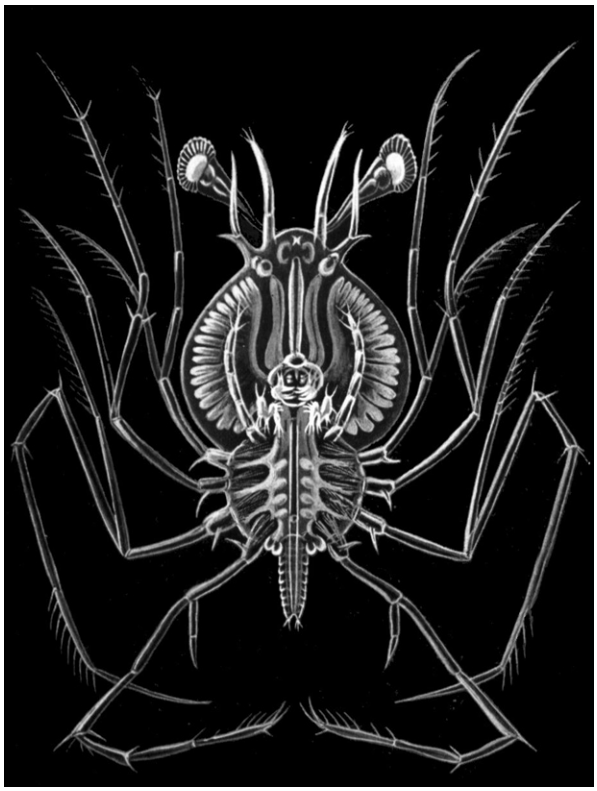


Fig. 22.18 Phyllosoma from *Kunstformen der Natur* (1904), plate 76: Thoracostraca (Ernst Haeckel, 1834–1919).

biochemical composition of the phyllosoma larvae, biochemical supplementation of *Artemia* used as the food source and developing culture techniques that reduce the levels of maintenance and risk.

As with bivalve culture, for instance, it is possible to bypass the hatchery phase and collect the post-larvae in the field. Phyllosoma larvae in the field are transported over vast distances out into the open ocean during their long planktonic period. They tend to occur in current systems that bring them back inshore at the end of the planktonic phase. They are typical of decapod crustaceans with a transitional stage, the puerulus, between the last planktonic phyllosoma stage and the first juvenile instar. The puerulus deliberately settles from the plankton onto a suitable substrate for the juvenile stages. In some areas where the currents bring in large numbers of puerulus larvae, it is possible to capture substantial numbers of them.

In central provinces of Vietnam a spiny lobster culture industry is based on the following:

- puerulus larvae captured in dip nets when they are attracted by lights;
- recently settled juveniles in attraction traps of netting bundles and wood with shelter holes that are suspended in the water column.

There are specialist spiny lobster 'seed' collectors who sell the young juveniles to farmers who grow them to commercial size in seacages. Juvenile and adult spiny lobsters are robust and have been reared in seacages using trash fish. However, as the Vietnam industry has expanded there have been environmental and disease problems arising from this technique and overcrowding. The supply of seed is also a problem, being erratic and declining.

Research on culturing spiny lobster larvae is being funded with millions of dollars from governments and commercial sources. The stakes are high, with the prize of success being an extremely valuable aquaculture industry.

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23
Bivalve Molluscs

John S. Lucas

23.1 INTRODUCTION

Culture of table oysters has a history going back to Roman times (Quayle and Newkirk, 1989). Mussel culture in Europe began more than 700 years ago (Jeffs *et al*., 1999) and probably considerably before this in eastern Asia. It was relatively easy to culture shore-inhabiting species such as table oysters and mussels, and to make the transition from harvesting wild stocks (Fig. 23.1) to farming them. Substrates, such as rocks and stakes, were added to promote larval settlement in areas of natural settlement. Then, where someone or some group had rights to harvest these stocks at consumable size, this constituted a simple form of aquaculture.

Bivalves are excellent aquaculture candidates because they can be reared using relatively simple technology and because they are filter feeders. The simple technology and low labour inputs in the rearing process offset the low value of the product in many cases. Furthermore, since bivalves in the field obtain their food by filter feeding the particulate organic matter (POM) from the water around them, there are no costs for feeds in the grow-out phase of culture. The different modes of life and range of commercial values of various bivalves, however, require different culture methods. The techniques for individually handling pearl oysters, a high value species, are totally inappropriate for lower value species, which must typically be cultured in large numbers for an economically viable operation.

The global aquaculture production of molluscs was 13.1 million t in 2007, valued at US$12.8 billion. This ranks them third in both quantity and value of global aquaculture production (Fig. 1.5). There has been a steady growth in aquaculture production of molluscs in recent decades, i.e. there was mean growth in global mollusc production by weight of 7.5% per year from 1987 to 2007. This was not much less than the 8% per year increase in total global aquaculture (Table 1.1). By far the greatest source of this mollusc production is with bivalves; some culture industries of gastropods are in their infancy (Chapter 24).

Global capture fisheries for molluscs produced 7.6 million t in 2007, i.e. about half the amount from aquaculture. Global capture varied between 6.7 million and 7.7 million t/year over the decade to 2007, reflecting the variability intrinsic to fisheries.

23.2 ASPECTS OF BIOLOGY

23.2.1 Morphology

The following is a brief generalised description of bivalve morphology. Gosling (2003) provides a more comprehensive description for representative species. Fougerouse *et al.* (2008) provide an atlas of soft tissue anatomy for the pearl oyster, *Pinctada margaritifera*.

The name bivalve arises from these animals' most prominent and dominant feature: a shell consisting of two valves that are joined along a hinge and enclose the body. Each valve usually has a prominence near the hinge, the umbo, which is the oldest part of the valve. There are often concentric lines on the shell reflecting previous valve sizes (Fig. 23.2). Each valve consists of three layers: the outer periostracum, the medial prismatic layer and the inner nacreous layer (Fougerous *et al.*, 2008). They are made up of calcareous and proteinaceous material. The most important layer for aquaculture is the inner nacreous layer, which is often lustrous and very hard (the 'pearly' layer of pearl oysters and pearl mussels).

Bivalves have no identifiable head. The body is laterally compressed within the valves and mainly consists of a soft

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Fig. 23.1 A natural population of rock oysters. Croajingolong National Park, Victoria, Australia. (Photograph by Stevage.).

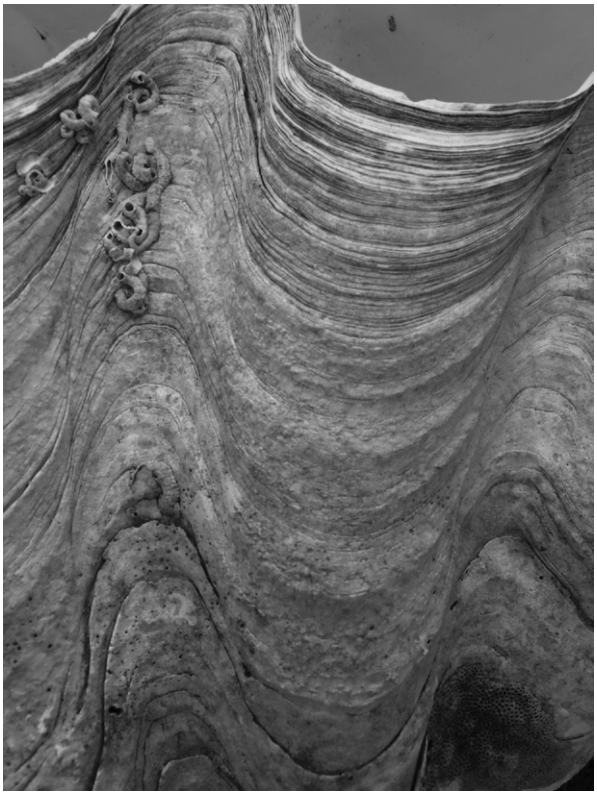


Fig. 23.2 The shell of a giant clam (*Tridacna gigas*) showing numerous growth lines over its ca. 25 years of growth. The growing shell edge is uppermost.

visceral mass: gut, kidney, gonads, blood vessels, etc. There is a ventral foot, which may be insignificant in sedentary species such as mussels and oysters or very well developed in burrowing species, such as cockles and clams. Associated with the foot there may be a gland that secretes attachment threads (byssal threads), whereby sedentary bivalves attach to their substrate.

Extending across between the valves are one or two large muscles, anterior and posterior adductor muscles, which close the valves. Lining the valves and enclosing the visceral mass are two thin lobes, the mantle lobes. These reach to the edges of the valves and secrete the material for shell growth and thickening. The cavity enclosed by the mantle lobes is called the mantle cavity and it contains the gills on each side of the body. The gills consist of fused parallel filaments.

23.2.2 Filter feeding

Bivalves obtain their nutrition by filtering fine (2+μm) POM, including phytoplankton and organic detritus, from suspension in the water column around them. They may also obtain some nutrition by direct uptake of dissolved organic matter from seawater across the large permeable surfaces of their gills and mantle chamber. A water current is created through the mantle cavity by cilia on the gills. The gills' fine structure is basket-like and particles are trapped by cilia lining the microscopic surfaces. These particles are bound in mucus and directed anteriorly to the mouth in food channels. Palps at the mouth sort the parti-

cles, rejecting large particles, inorganic particles and excessive numbers of particles as mucus-bound 'pseudofaeces'. Ingested food particles are digested within the gut and undigested material compacted into faeces and expelled. The volume of water 'pumped' through the gills per unit time may be impressive. Pearl oysters are among the highest with values for large individuals being >1000 L/day (Yukihira *et al*., 1999). The ingestion rate is described by

$$IR = [(FR \times FC) \times PR] - Ps$$

where IR is ingestion rate of food (e.g. mg/h), FR is the flow rate of water through the gills (e.g. L/h), FC is the food concentration in the water (e.g. mg/L) PR is the proportion of food in the water that is trapped by the gills (e.g. 0.9) and Ps is the rate of pseudofaeces production (e.g. mg/h).

There is often an optimum range of particle concentrations that maximises food intake while minimising food wastage, i.e. optimum range of PR values. When feeding bivalves with unicellular algae cultures, e.g. in conditioning bivalve broodstock or rearing larvae and juveniles, it is not sufficient to load the water with as high a density of algae as possible to maximise food intake and growth. This is wasteful and may be detrimental to growth due to clogging of the feeding processes (Fig. 23.3). The optimum cell concentration varies with algal species. This relates to the size, other physical characteristics, acceptability and age of the algal culture (section 9.3.3). The optimum cell concentration usually increases through the life cycle in bivalves. They may range from ca. 10 000 cells/mL for early larval stages, to ca. 50 000 cells/mL for late larval stages, to ca. 100 000 cells/mL for juveniles and 100 000+ cells/mL for adults.

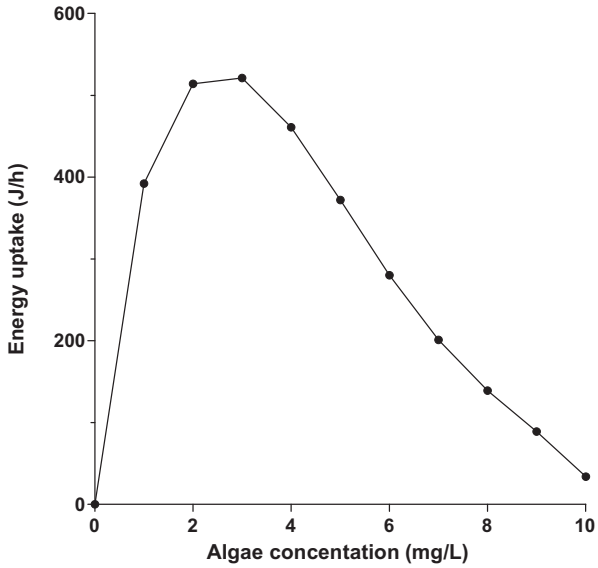


Fig. 23.3 Rate of energy obtained by a bivalve from filter feeding at increasing concentrations of suspended microalgae. As the concentration of microalgae increases the pearl oyster obtains more food, then beyond the optimum concentration the food intake and digestion rate progressively decline as the feeding mechanism is choked. The pearl oyster, *Pinctada maxima*, is feeding on the microalga *Isochrysis* species. (Drawn from data in Yukihira *et al*. (1998).

23.2.3 Growth

Scope for growth is used for physiological assessments of bivalves. It is a measurement of the surplus energy from feeding that is available for growth and reproduction. Scope for growth results from the ingested energy (IR) that is assimilated into the body, less the energy costs of metabolism and excretion (section 6.4.3). Growth in bivalves is such that the size-versus-age relationship follows the pattern described in section 6.4.1).

POM levels strongly influence growth rates and the final size (L_∞) to which bivalves grow. Low POM levels result in sub-optimal food intake, slow growth, stunted size and reduced reproductive output.

23.2.4 Anaerobic metabolism

Anaerobic metabolism is a particular aspect of bivalve physiology. Bivalve morphology gives a level of protection against many adverse conditions: the valves may be held tightly closed for long periods as protection against adverse conditions such as desiccation, salinity variations, pollution and predators seeking entry into the mantle cavity. This behaviour, however, limits oxygen availability and periods of prolonged valve closure are accompanied by anoxic conditions within the mantle cavity, reduced metabolic rate and anaerobic metabolism, which are well tolerated. It is best developed in intertidal and bottom-inhabiting species, such as table oysters, mussels, clams and cockles. A practical consequence of this behaviour and biochemistry for aquaculture is that it is better to hold most bivalves in non-desiccating conditions out of water during transportation and marketing than to keep them crowded together in polluted conditions in water.

23.2.5 Reproduction

Sexuality is not strongly fixed in some bivalves and individuals may change sex during a breeding season, between seasons depending on prevailing conditions, in response

to nutritional status or as an age-related phenomenon. Cupped oysters, pearl oysters, mussels and clams are usually dioecious, with equal numbers of each sex. However, pearl oysters and some oysters and clams change sex according to the pattern described above. Most scallops are simultaneous hermaphrodites, with separate male and female sections to the gonad, and a few species are protandrous hermaphrodites, being males when young then switching to female. Giant clams are another example of protandrous hermaphroditism, with ovary and testis tissue closely interwoven in mature gonads (Lucas, 1994).

Most temperate bivalve species and many tropical species have a distinct breeding season that is primarily related to water temperature. Typically, gametogenesis occurs mainly during the late winter to early summer months, with progressive accumulation of mature gametes in the gonad. Then spawning occurs, with numerous individuals spawning synchronously through being triggered by pheromones released with gametes by nearby individuals (section 6.3.2). Although histological studies may be used in research on bivalve reproductive seasonality, such techniques are usually not available to the industry and simpler methods are used to assess sexual maturity. Condition index (CI) (Quayle and Newkirk, 1989) is a quantitative measure used in commercial aquaculture.

$$\text{CI} = \frac{\text{wet weight of meat (g)}}{\text{volume of shell cavity (mL)}} \times 100$$

or

$$\text{CI} = \frac{\text{dry weight of meat (g)}}{\text{volume of shell cavity (mL)}} \times 1000$$

CI relates the amount of body tissue to the volume of the shell cavity. For the dry weight CI, a value of 70 for a Pacific oyster indicates a 'thin' oyster in poor condition, while a value of 150 indicates a 'fat' oyster (Quayle and Newkirk, 1989). General CI levels can reflect the state of nutrition or health of the bivalve; but seasonal changes reflect the gametogenic cycle, because the mature gonad becomes a major component of the total visceral mass. Even simpler than CI measurements, viewing the size and colour of the gonads through open valves (shell valves may have to be wedged open) may be sufficient to identify their degree of development, and sex, once one is familiar with the morphology.

23.2.6 Life cycles

Most bivalve life cycles consist of planktonic trochophore, veliger and pediveliger larval stages, and then metamorphosis to a juvenile, called a spat (Fig. 6.3). Some variations from this pattern occur in flat oysters and freshwater mussels, where the early larval stages are retained within the mantle cavity of the female and released late in development. These species are exceptional to the normal pattern of eggs being shed into the water to be fertilised by concurrently released sperm.

Bivalve eggs are usually quite small, 40–100 µm diameter, and very numerous. Fecundity ranges from about a million eggs per spawning, for example flat oysters, to tens of millions, for example cupped oysters, to hundreds of millions per spawning in the largest giant clam (*Tridacna gigas*). (In fact, this giant clam species must be among the most fecund animal in existence with tens of billions of eggs released through its decades-long lifespan.) Bivalve sperm are also very numerous. They have a small head, a few µm in diameter, and a long flagellum. The sperm swim vigorously when in good condition.

The mature sperm is haploid (n) but the mature egg is tetraploid ($4n$) until a sperm penetrates its outer membrane. The fertilised egg then undergoes two meiotic divisions. These are evident by the successive ejections from the egg of two small polar bodies, each containing discarded haploid chromosomes from a meiotic division. The resulting haploid egg nucleus is then ready to merge with the sperm nucleus to form a diploid embryo (section 7.7.2).

The embryo commences mitotic divisions involving the whole cytoplasm and passing through 2, 4, 8, etc., cell stages. It develops into a ciliated trochophore larva, usually within 24 hours at normal temperatures for the species. The trochophore rapidly develops into a bivalved veliger, which is also known as a D-stage because of its shape (Fig. 6.3). The veliger swims and feeds on phytoplankton with a ciliated lobe, the velum. Towards the end of its planktonic life, which typically lasts 2–4 weeks, the veliger develops a prominent foot to become a pediveliger. The pediveliger settles on an appropriate substrate and metamorphoses into a spat. Metamorphosis involves absorption of the velum, development of gills and a gill-based feeding mechanism, and attachment to the substrate with byssal threads or other means. During this transformation from velum to gill feeding, they undergo a brief period when they are unable to feed. They must rely on the energy reserves built up in the latter part of larval development.

There follows a period of growth without abrupt changes in morphology (Fig. 6.3). Sexual maturity is usually reached in about 2 years. Thereafter follows a substantial period of sexual maturity. Bivalves are quite long-lived and some are very long-lived, e.g. several temperate clam species have been shown to live for more

Table 23.1 Global aquaculture production of some major bivalve molluscs in 2007.

Species	Quantity (10^6 t)	Value (10^6 US$)
Pacific oyster (*Crassostrea gigas*)	4234	3054
Japanese carpet shell (*Ruditapes philippinarum*)	3044	3044
Yesso scallop (*Patinopecten yessoensis)*	1413	2016
Marine mussels (Mytilidae)	1631	1609
Marine mussels (China)	448	117
Constricted tagelus (razor clam) (*Sinonovacula constricta*)	667	594
Blood cockle (*Anadara granosa*)	424	489
Pearls[a]	n/a	784

Data from FAO Fishstat Plus (2009) and Tisdell and Poirine (2008).
[a]From marine pearl oysters (*Pinctada* species) and freshwater pearl mussels.

than 100 years. Age can be measured from annual growth lines in cross-sections through a valve, like growth rings in a tree stump.

23.3 CULTURED BIVALVES

There are five main 'groups' of cultured bivalves:

1. Table oysters.
2. Marine mussels.
3. Scallops.
4. Clams and cockles.
5. Pearl-producing oysters and freshwater mussels.

The annual production of some important bivalve species is shown in Table 23.1. The most important species in quantity and value, the Pacific oyster is the bivalve most widely cultured and consumed in the world. The Pacific oyster is native to the Pacific coasts of Japan, Korea and China, but has been widely introduced. It has often been intentionally introduced to replace or complement native oysters that have been decimated by disease because the Pacific oyster has greater resistance to the diseases. It also has the advantage of relatively fast growth rates. Introductions, however, are not without problems (section 4.4). For instance, on the eastern coast of Australia the northern spread of Pacific oysters is impacting on the specialist industry based on Sydney rock oysters.

The next most important species in quantity and value is a small clam, the Japanese carpet shell (or Manila clam), *Ruditapes phippinarum*. It has a wide natural distribution in the Indian and Pacific Oceans. Like the Pacific oyster, it has also been introduced to the Pacific coasts of North America and to Europe. China, however, accounts for about 97% of global production. Here the clams are largely sold live in local markets.

The third major bivalve, a scallop, *Pecten yessoensis*, is interesting. Scallops are not regarded as easy material for aquaculture. The yesso scallop is, however, highly valued and produced in large quantities in China (ca. 84% of global production) and Japan. It is notably the only bivalve in Table 23.1 whose in-the-shell value substantially exceeds US$1 000/t. Unlike most bivalves, scallops do not retain mantle water after removal from the ocean. They can be held out of water and sold whole for only a limited period and usually close to the site of production. Most scallop product is sold as meat removed from the shells (shucked) and exported as frozen meat product.

A notable feature of Table 23.1 is the large quantity of marine mussels produced in China for a relatively small return. This is partly because marine mussels are used as feed components, for instance in shrimp culture. By contrast, far fewer Mediterranean mussels are produced for a greater return and New Zealand mussels were developed as a specialist product ('Kiwi clams') and exported to more than 50 countries (Jeffs *et al*., 1999).

The relative importance of the four groups in quantity and value of aquaculture production is shown in Fig. 23.4. The relative importance of clams and cockles, which are not a major component of the seafood industry in many countries, is from China's production of >4 million t/year, much of it for home consumption. China is dominant in bivalve production, being responsible for ca. 77% of production by weight and ca. 64% by value. China also has an industry producing pearls from freshwater mussels and these, together with pearls from marine pearl oysters, constitute a significant component of the value of bivalve aquaculture. World production of freshwater and marine pearls was US$625 million according to Golay's estimates (Anon, 2006).

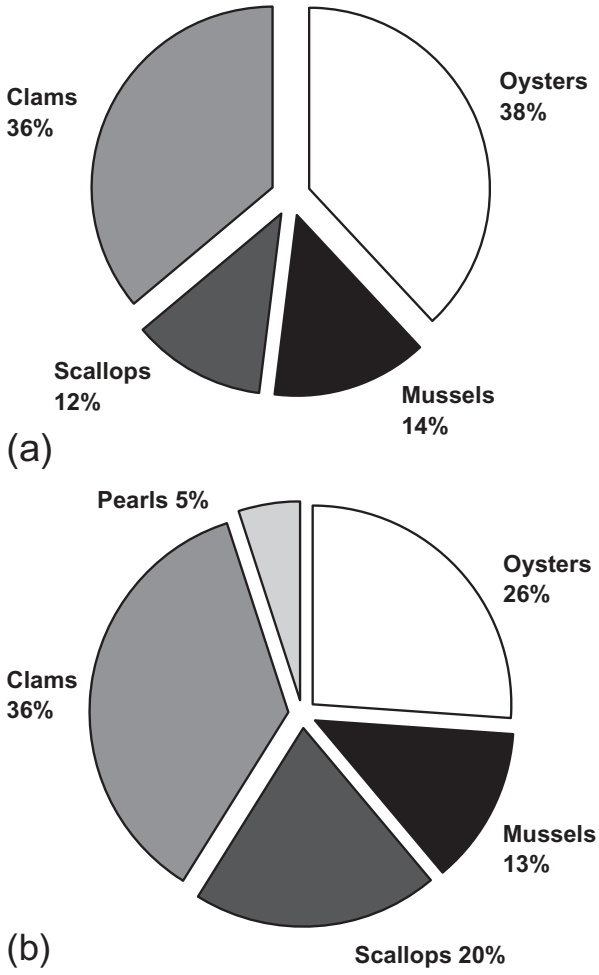


Fig. 23.4 Relative aquaculture production of the four major groups of bivalves in 2007. (a) Quantity; (b) value. (FAO Statistics and Information Service of the Fisheries and Aquaculture Department (2009). Aquaculture FISHSTAT Plus – Universal software for fishery statistical time series [online or CD-ROM]. Available at: www.fao.org/fishery/statistics/software/fishstat/en.)

23.3.1 Table oysters (family Ostreidae)

In table oysters the left valve (called the lower valve) is cemented to a firm substrate. Table oysters are divided into cupped oysters (e.g. *Crassostrea* and *Saccostrea* species) (also known as rock oysters) and flat oysters (*Ostrea* species). As well as shell shape and other morphological differences, they differ in mode of development. Cupped oysters are oviparous, spawning gametes into the water column, whereas flat oysters are larviparous, brooding embryos in their mantle cavity and then releasing larvae.

23.3.2 Mussels (family Mytilidae)

There are two unrelated groups of mussels that are cultured. One group, the family Mytilidae, is marine and cultured for human consumption. The other group, the family Unionidae, is freshwater and cultured for pearl production. Both families have wide geographic distributions. Because these groups share little in common, except that they both happen to be called 'mussels', the unionids will be treated with pearl oysters, for which they at least have a similar reason for culture.

Commercial mytilid species are mainly from two genera of large mussels:

- *Mytilus* spp., which are temperate;
- *Perna* spp., which are mainly tropical.

Mussels have similar valves. They are not motile and attach to a firm substrate by strong byssal threads. Their foot becomes vestigial soon after settlement. As in oysters, the mouth is near the umbo and there is essentially one adductor muscle, the posterior one. Mytilid mussels have gonad tissue located in their mantle tissue as well as in the visceral mass.

23.3.3 Scallops (family Pectinidae)

Species of *Pecten*, *Aequipecten*, *Argopecten* and *Patinopecten* are among those commercially cultured. Most scallops are free-living on soft seabeds. They respond to disturbance with swimming movements caused by rapid 'flapping' of their valves, expelling water jets. This is caused by vigorous contractions of a large single adductor muscle. Scallops tend to be circular in outline with a long prominent hinge, similar to pearl oysters.

23.3.4 Clams and cockles

The terms 'clam' and 'cockle' have no taxonomic significance, and bivalves from a range of families are included in this assemblage. They include the following:

- the blood cockles (*Anadara* species);
- the Japanese carpet shell or Manila clam (*Ruditapes philippinarum*);
- the North American hard clam or quahog (*Mercenaria mercenaria*) (of clam chowder fame).

Their common feature is that they inhabit particulate seabeds, ranging from thick mud to sand. They have a well-developed foot for burrowing through the seabed. They have two adductor muscles. Some variation occurs

in the incurrent and excurrent channels; however, because they are buried in the seabed, their mantle lobes are usually fused to create tubular incurrent and excurrent siphons that connect the mantle cavity to the surface seawater.

Giant clams (Tridacnidae) are an exception to the usual 'clams'. They are a small group of coral-reef inhabiting species that are exceptional in size and in obtaining nutrition by a combination of filter feeding and photosynthesis of symbiotic algae (Lucas, 1994).

23.3.5 Pearl oysters and pearl mussels (families Pteriidae and Unionidae)

As previously indicated, pearl oysters and pearl mussels are unrelated and, apart from common bivalve morphology, they have little in common except for their use in cultured pearl production.

Pearl oysters are tropical and subtropical marine species occurring in a range of habitats from silty seabeds to coral reefs. They have a vestigial foot and at least initially attach to a firm substrate with byssal threads. They are from the genera *Pinctada* and *Pteria* (Southgate and Lucas, 2008). The inner shell layer, the nacreous layer, is very lustrous and this is the basis of pearl production. They have the typical larval development of marine bivalves.

Unionid mussels live in freshwater. They have no byssal threads and burrow in particulate seabeds with a well-developed foot. They share with pearl oysters a nacreous layer that is very lustrous and this again is the basis of pearl production. The life cycle of these mussels involves suppressed larval development, a feature in common with some other freshwater invertebrates (section 6.3.4). Embryonic development occurs in eggs retained in the gills. Subsequently a well-developed and bivalved glochidium larva hatches from the egg and attaches to the gills, fins or surface of a fish host by an adhesive thread and possibly hooks on its valves. The glochidium larva feeds from the fish host as a parasite. The parasite gradually changes into a juvenile bivalve, detaches from the host, falls to the bottom and assumes the adult burrowing habit. Rearing these mussels through the glochidium stage without a fish host as source of nutrients has proved to be very difficult.

23.4 PHASES OF BIVALVE AQUACULTURE

The complete process of bivalve aquaculture involves the series of phases outlined in Chapter 6 (Fig. 6.3). The period for this complete process, hatchery production to marketable size, is at least 18 months for most commercial bivalves. Much bivalve aquaculture, however, does not involve this complete process: the complete process is only used in culturing the higher-value species. For many cultured bivalves the process commences after natural spatfall, and the costly and technically demanding hatchery and nursery culture phases are omitted.

23.4.1 Culture from natural spatfall

As indicated in the Introduction to this chapter, a very simple form of aquaculture for some bivalve species is to provide additional substrates in regions where natural spatfall occurs, i.e. in regions of the environment where larvae are completing development and seeking appropriate substrates on which to metamorphose. These added substrates may be natural surfaces such as rocks and wooden stakes that remain in place.

A further step in technical development is to provide spatfall substrates that can subsequently be moved to other regions or which will allow some thinning or sorting of the bivalves as they grow. These are known as spat collectors or, particularly in relation to oysters, as culch. They take a variety of forms depending on local industry practices and on the species' biology and its value (Figs 23.5 and 23.6). The amount of microalgae and biofouling by larger organisms can have a significant effect on the amount of settlement. The amount of settlement is also strongly related to the density of larvae in the water flow over the spat collectors. It is essential to deploy spat collectors at the appropriate time of late larval development and in areas where the highest concentrations of larvae are known to occur.

Oyster larvae tend to prefer a hard rough surface that is non-greasy and clear of silt and algae. Culch used for them includes lime- or tar-coated wooden sticks, flexible plastic strips, oyster shells, cement tiles, mangrove tree branches and bamboo. There is a feature of the culch material that is important for harvesting and market acceptability of the



Fig. 23.5 Groups of battens in Salamander Bay, New South Wales, to collect Sydney rock oyster spat. (Photograph by John Holliday.)

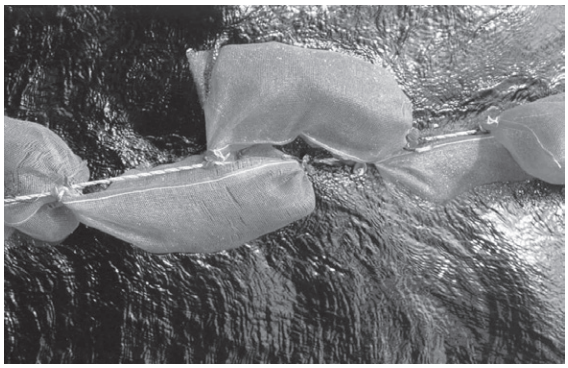


Fig. 23.6 Spat collectors consisting of mesh bags filled with fishing line or similar filamentous material. This kind of collector may be used for scallop and pearl oyster spat, which tend to prefer protected soft substrates for settlement. (Photograph by John Mercer.)

cupped oyster species. The oyster's lower valve assumes the shape of the surface on which it is growing. Thus, cupped oysters growing on a broad flat surface without near neighbours will be broad and flat. Oysters growing on the edge of surfaces will extend around the surface into a bent shape of poor commercial value. Furthermore, oysters attached to culch require individual chipping off the culch, a time-consuming process.

Culchless oysters, i.e. oysters that have been detached from the original settlement substrate or have been allowed to outgrow it early in development, grow into the desirable cupped form. This is the main advantage, but they are also very simple to harvest. Producing culchless oysters in hatcheries is readily achieved (see below). Methods to produce culchless spat from natural settlement include culch of plastic strips, which are flexed to dislodge the juvenile oysters after they have grown to ca. 10 mm shell size.

Marine mussels are an example of bivalves that are grown on fibre substrates. Commonly used spat collectors are frayed ropes, which provide an attractive surface for the larvae, which attach to them in very high densities. The natural densities of spat on the settlement substrates are generally too high, which can jeopardise growth and survival. Furthermore, they may not be in the region for best subsequent growth. Therefore, when the juveniles grow to 20+ mm shell length, they are generally stripped from the settlement substrate, tearing their byssal thread attachments to this substrate. They are re-attached to vertical ropes at controlled densities for further growth. This reattachment is achieved by passing the settlement ropes steadily into the water from the surface. Each rope is surrounded by a sleeve of fine thin mesh and mussels are fed in between the mesh and rope. The mesh and mussels are tied periodically into 'sausages' and pass over a roller into the sea. The mesh holds the mussels in place until they have reattached to the rope by their byssal threads (a matter of hours rather than days) and the mesh subsequently disintegrates.

23.4.2 Culture from hatchery production

Bivalve hatcheries have several common components:

- a source of uncontaminated seawater and pumps;
- treatment of the water intake, e.g. filtration and temperature control;
- broodstock holding and conditioning tanks;
- larval rearing tanks;
- nursery rearing tanks;
- a facility for mass-culturing of microalgae.

The components and their operation are described in detail by Sarkis (2007) in relation to the installation and operation of a modular hatchery.

23.4.2.1 Spawning induction

Often little more than stress is required to induce spawning of ripe bivalve broodstock. The stress may be exposure to elevated temperature for a tolerable period or to air for several hours. Addition of some chemicals to seawater containing the broodstock may induce spawning, for example hydrogen peroxide, potassium chloride and ammonium hydroxide. Ultraviolet irradiation of the seawater, which produces free oxygen radicals, may also stimulate spawning. The precise action of each of these chemical treatments is not clear.

Serotonin, a neurotransmitter, injected directly into the gonad (e.g. 0.2–0.5 mL of 10^{-3} molar solution) is effective in inducing spawning, both sperm and ova release, in a variety of bivalves (section 6.3.2) The action of serotonin is to bypass all normal processes associated with spawning, acting on the gametes and mechanism for expelling gametes.

When one individual sheds eggs or sperm (usually sperm, males tend to be more easily triggered to spawn) (Fig. 23.7), the gametes can be spread into the vicinity of other individuals to stimulate them with the gamete pheromones. This may lead to polyspermy (see below) and fertilisation of many eggs from the gametes of one male. Thus, a male may be sacrificed and its sperm killed by microwaving and then the sperm suspension used as a spawning stimulus.

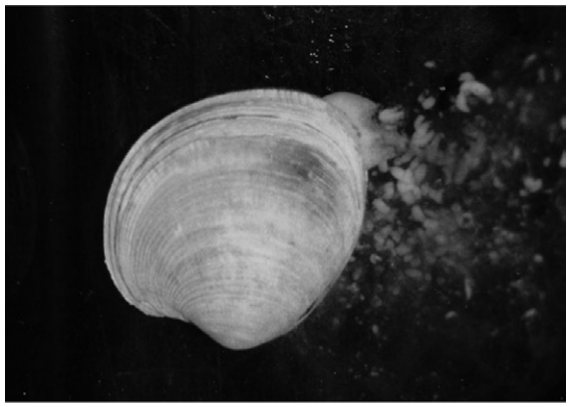


Fig. 23.7 A hard clam (*Mercenaria mercenaria*) releasing gametes in response to elevated water temperature. (Photograph by Paul C. Southgate.)

Spawning induction may be by-passed completely with gonad stripping. When the broodstock are known to contain fully mature gametes, they may be killed, their gonads repeatedly lacerated with a sharp blade, and the gametes washed out into a beaker. This is only appropriate to dioecious bivalves as there would be self-fertilisation (and reduced genetic variability) from hermaphrodite gonads using this technique.

23.4.2.2 Fertilisation

Where broodstock can be sexed, the sexes are often placed in separate containers for spawning induction. Alternatively, individuals are removed from a group as they commence to spawn and the kind of gametes, eggs or sperm, is evident. The objective is to have dense suspensions of eggs in filtered seawater, up to one or two thousand per mL, and very dense suspensions of sperm in separate containers. The eggs may be kept in suspension with gentle aeration and their numbers estimated from microscope counts of small subsamples in a Sedgwick-rafter counting chamber. A few mL of sperm suspension, mixed from several males, are then added to the suspensions of eggs.

There are constraints to the amount of sperm to be added. Too little results in low levels of fertilisation. Too many sperm may result in embryonic abnormalities through polyspermy (section 6.3.3).

23.4.2.3 Triploidy

Triploidy is induced in bivalve eggs by blocking the first or second meiotic division preventing the release of either the first or second polar body, respectively (section 7.7.2). In either case the result is a diploid egg nucleus which becomes a triploid nucleus as it is fertilised by a haploid sperm nucleus. There are various chemical and physical methods for accomplishing this process in bivalves (Gosling, 2003). The most reliable method is by crossing tetraploids and diploids, using tetraploid broodstock. Thus, lines of tetraploid Pacific oysters have been developed for large-scale production of triploids. Triploids are largely infertile (see below), but some produce eggs and these have been used to produce tetraploids by blocking one of the meiotic divisions of the triploid egg nucleus.

The advantages of triploidy have been particularly used for the Pacific oyster. Triploids differ from diploid oysters in having larger cell nuclei and cells to accommodate the additional set of chromosomes; but, more importantly, they show poor gonad development (section 7.7.2) and do not spawn out during the summer months, as do diploids. They maintain good meat condition throughout the year and are available for marketing when the diploids are in poor condition post-spawning. Furthermore, where the market does not want ripe table oysters, as in North America, there is the advantage of lack of gonad development in summer when diploid oysters are ripe. Spawned-out oysters generally have poor market acceptance. With less energy diverted to gametogenesis, more energy is available for other tissue growth and triploids grow more rapidly than diploids, reaching commercial size earlier.

The commercial advantages of triploidy have been studied in four oyster species, but so far triploidy has only been adopted on a large commercial scale in Pacific oysters (Gosling, 2003). This is in particular by the industry on the Pacific coast of North America, but triploids are also available from a large hatchery on the east coast. Obviously, triploidy can only be used on a large commercial scale where a substantial proportion of the industry depends on hatchery-produced spat.

23.4.2.4 Larval rearing

Modern bivalve hatcheries tend to use the following techniques:

1. Seawater is generally filtered down to ca. 1 µm to remove silt, organic debris, zooplankters, which may be predators or competitors, and phytoplankton that the larvae may not be able to feed on. This requires a comprehensive filtration system in the seawater intake.
2. Water temperature is controlled. Temperature fluctuations reduce survival and temperature affects development rate.
3. Larvae are kept at low densities, one to five larvae/mL, in large culture volumes, often 10 000–20 000 L (Fig.



Fig. 23.8 Large tanks (20 000 L) for rearing bivalve larvae. The tank in the left foreground is being drained via a black pipe into sieves to collect bivalve larvae. (Shellfish Culture Pty Ltd., Tasmania, Australia.)

23.8). The culture tanks are typically stocked with embryos at a level that is several times greater than the anticipated final density of larvae. Density is reduced during larval development by the following:

- mortality;
- discarding the slower-growing individuals during the regular sieving programme (see below).

With a final density of about one larva/mL, a 20 000-L tank has an output of 20 million pediveligers. Mass-cultured unicellular algae are added as food for the larvae. Usually a mixture of species is supplied, because mixed species diets give a better balance of nutrients (section 9.3.3). Small unicellular algae, about 5 µm, are used, such as *Isochrysis* species, *Pavlova* species, and single-celled diatoms, *Chaetoceros* species (Table 9.2). Long-chain, spiny diatoms, which are appropriate to the setal feeding structures of crustacean larvae, are quite unsuitable for the ciliary feeding mechanisms of bivalve larvae.

4. Gentle aeration is used to maintain water movement, keeping the larvae in suspension.
5. Culture water is changed regularly, usually every second day, to control the build up of metabolites from larvae and algae, and especially the build up of bacterial populations. The culture water and larvae are discharged through sieves of appropriate mesh size to collect the larvae (Fig. 23.8). The water may be discharged through several sieves of different mesh sizes, with coarsest mesh uppermost, to grade the larvae. Abnormally small larvae pass through the sieves and are lost in the effluent water. The larvae retained on different mesh sizes are transferred to new culture tanks with the objective of having uniform batches of larvae. Sieving the water flowing out of 20 000-L tank may be accomplished in an hour with the water blasting onto the sieves. Surprisingly, the thin shells of the veligers and pediveligers survive this treatment.
7. As in hatcheries for other kinds of aquacultured animal, scrupulous attention is paid to cleanliness to avoid contamination and bacterial blooms in the bivalve larvae tanks. There is generally a separate larval culture or tank room with limited access. The inner surfaces of the culture tanks are scrubbed with hypochlorite solution to sterilise them, or with detergent, between uses. With a culture regime of water changes every second day, each tank is cleaned after it is emptied and then refilled with seawater to be back in use a day later. Sieves, hoses, buckets, etc., are all washed in hypochlorite solution after use.

A highly intensive method for rearing bivalve larvae has been developed. Larvae are reared in relatively small, 100–150 L, tanks in flow-through conditions of 1–2.5 L/min (Fig. 23.9). Thus there is a rapid rate of water turnover. Initial densities are in the order of 1000 eggs/mL. Final densities of larvae may be in the order of 250/mL resulting in an output of larvae from these small tanks that is equivalent to that from 10 000–20 000 L tanks, while requiring far less hatchery space and time-consuming cleaning of tanks. The flow-through system has particular advantages for feeding the larvae in that an automated system may be used to monitor the microalgae density in the outflow water from the chamber. Monitoring levels of microalgae in the outflow is connected to a system that delivers a continuous, but regulated supply of algae to the culture chamber. In this way it is possible to maintain prescribed optimum levels of microalgae in the culture chambers, for example 3000–15 000 cells/mL according to the size and stage of the larvae. In static systems the larvae

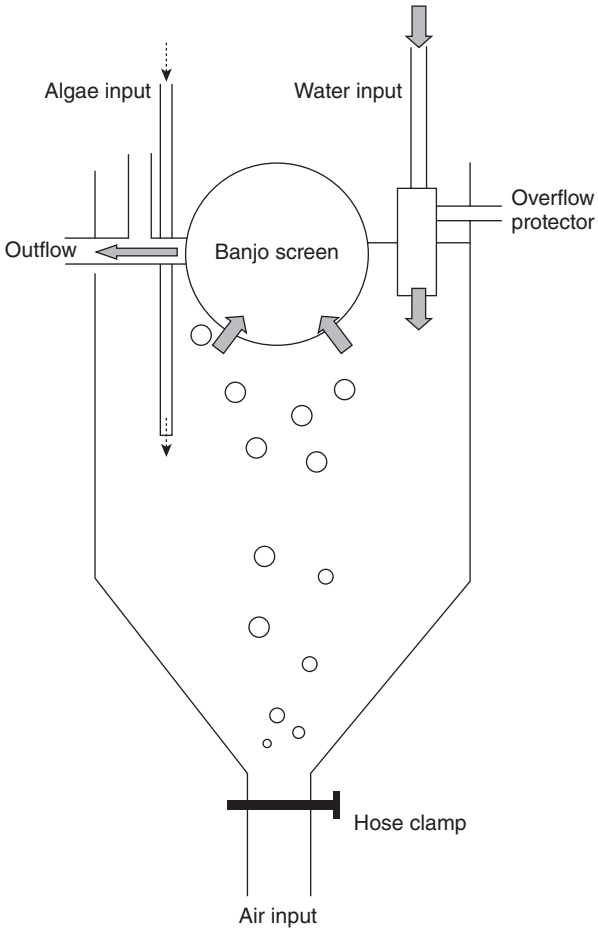


Fig. 23.9 An alternative high-density flow-through culture system for rearing bivalve larvae. The input of microalgae and water exchange are regulated to maintain an optimum level of microalgae. The chamber contents are vigorously stirred with a flow of bubbles. The banjo screen provides a large surface area to prevent larvae from being lost in the outflow, while not accumulating on a small outflow orifice. (Courtesy of Shellfish Culture Pty Ltd., Tasmania, Australia.)

are periodically provided with high densities of algae, which progressively decline as the larvae feed. There is one further energy-saving advantage of the flow-through system. Ambient seawater temperature is often substantially below the optimum temperature for larval rearing. Thus there is a considerable energy budget for water heating. In the large tank systems the energy input is lost when the heated water is discharged from the tank. Water must be heated continuously before it reaches the culture chambers in the flow-through system. There potentially is continuous loss of heat energy through the discharged water. This has been mitigated in some hatcheries by using a counter-current heat exchange system between the intake and discharging water. The two opposing flows of water are brought into close proximity and heat passes progressively from the discharging to the intake water across the temperature gradient.

23.4.2.5 Settlement

Late pediveliger larvae may be sieved from their culture water on an appropriate-sized mesh and transferred to settlement tanks with static water and settlement-inducing substrates. Alternatively, appropriate substrates may be added to the larval tanks. Table oysters, scallops, clams and pearl oysters have their individual requirements for settlement-inducing substrates in the hatchery, as they have in the field. Chemicals may be added to the water in hatcheries to promote settlement and metamorphosis, for example epinephrine (adrenalin) at 100 µM/L and GABA (γ-amino-butyric acid) may be used.

23.4.2.6 Remote settling

In the final stages before settlement, oyster pediveligers are amazingly robust and can be collected into dense balls of millions of larvae, wrapped in damp material and stored in a refrigerator or freighted in small containers. In this way pediveligers of the American cupped oyster (*Crassostrea virginica*) are sent from hatcheries to distant oyster farms in the USA ('remote setting'). The pediveligers are settled at the recipient farms.

23.4.2.7 Culchless spat

A technique used in settling cupped oyster larvae is to provide particles of shell, about 500 µm in diameter, as substrates. The pediveligers settle and metamorphose on these, but within a few days the spat shells have grown larger than the particles on which they settled. The shell particles with and without spat are then passed through an appropriate sieve that separates the spat from the plain particles. Thus, culchless spat are produced.

23.4.3 Nursery culture

Early bivalve spat are 200–500 µm in shell length and very vulnerable to predation and overgrowth by fouling organisms. They need good concentrations of POM to sustain their growth. Thus, best survival and growth may be obtained by maintaining them in onshore facilities with cultured microalgae as food. This is not usually an option with juveniles resulting from natural spatfall.

The spat may be maintained in a flow-through system with high POM content or within a recirculating system with supplementary microalgae. In the latter case, the spat may be held on sieves at high densities with either vigorous upwelling or downwelling flows through the sieves. Upwelling has the advantage of lifting the spat off the mesh and stirring them, whereas downwelling packs them down onto the mesh. Stronger flows, however, can be used with downwelling systems. The mass-cultured microalgae added to the recirculation system does not need to be as 'clean' as those used for larval culture and may be cultured in open tanks (section 9.3.1).

When the spat are of sufficient size they are transferred to offshore sites for ocean-nursery culture. There is pressure to make this transition as early as possible as it is costly to maintain the spat in an onshore facility with feeding and maintenance.

23.4.4 Ocean-nursery and grow-out

Various methods are used for culturing bivalves during their ocean phase. These reflect differences in biology and habitat of the cultured species; they also reflect local factors in the farming process, such as labour and material costs, and market price. A fundamental difference is between culturing bivalves:

- within the seabed;
- on or just above the seabed;
- near the ocean surface.

23.4.4.1 Within particulate seabeds

The bottom-inhabiting cockles, clams, etc., are reared within natural muddy to sandy seabeds with minimal intervention. In some cases the seabed may be prepared for addition of seed bivalves by ponding and fertilisation or by harrowing it to loosen up the seabed and remove predators such as starfish. After seeding, the surface of the culture area may be covered with a mesh to deter predators and the area may be defined with a fence.

23.4.4.2 On or just above the seabed

Some bivalves are cultured on the surfaces of hard substrates, such as cement slabs and horizontal and vertical stakes, which require natural spatfall. Rows of horizontal or vertical stakes (usually wood or bamboo) are used for oyster and mussel culture. These bivalves tolerate intertidal exposure, and stake culture is particularly used for farming in the intertidal zone on sheltered muddy coasts with a good tidal range. There are considerable advantages in that installation, maintenance and harvesting can be performed at low tide.

Bivalves are also cultured on racks above the seabed in mesh boxes, mesh baskets, trays, and horizontal wooden and asbestos-cement batons. Mesh boxes are used for seed bivalves, particularly culchless oysters immediately after transfer from the land nursery. Later, when the oysters are less prone to predation, they can be transferred to open mesh baskets or trays. Horizontal batons are typically used where the batons were previously used to collect spat from natural spatfall (Fig. 23.5).

Scallops are mainly cultured in lantern and pearl nets hanging from longlines, but may be cultured on natural seabeds in mesh enclosures. The enclosures may be boxes or vertical walls. Where only mesh walls are used they must be sufficiently high to exceed the vertical swimming ability of the scallops. The mesh walls must also be of sufficient strength and design to exclude predators. Scallops are intolerant of intertidal conditions and are cultured subtidally on appropriate soft seabeds, typically at ca. 10 m depth.

Bivalves are sometimes cultured attached to ropes that hang down from horizontal racks that are built a short height above the bottom. This is typically in the intertidal zone for mussels and table oysters.

23.4.4.3 Surface or suspended culture

Culture methods at the surface include hanging the bivalves on ropes or in appropriate culture units from rafts, longlines and floats.

1. Rafts are rectangular metal, wooden or bamboo frames with buoyancy provided by large air-filled drums or floats. Ropes with bivalves attached hang down at intervals from cross-members on the raft (Fig. 2.7). In raft culture, the bivalves are typically mussels attached to vertical ropes in vast numbers (Fig. 23.10).
2. Longlines are 50+ m horizontal ropes, supported at the surface by floats at regular intervals (Fig. 4.9). Bivalves are cultured on ropes suspended vertically at regular intervals along the longline. Attached to the vertical ropes are various culture structures depending on the species, such as:
   - scallops in lantern nets (elongate cylindrical nets that are suspended vertically and which have regular horizontal partitions on which the scallops rest);
   - ropes packed with mussels;
   - vertical series of pyramid shaped nets (Fig. 23.11);
   - 'ear-hung' pearl oysters (Fig. 23.12) and scallops.

   Longlines are held in place at their ends by terminal anchors or attachment to points of hard substrate below. Some longlines have their supporting floats held below the surface by anchors, so that they may be invisible at



Fig. 23.10 Mussels (*Mytilus edulis*) cultured on ropes in western Scotland.



Fig. 23.11 Scallops (*Pecten fumatus*) in a pyramid net, Victoria, Australia. (Photograph by John Mercer.)

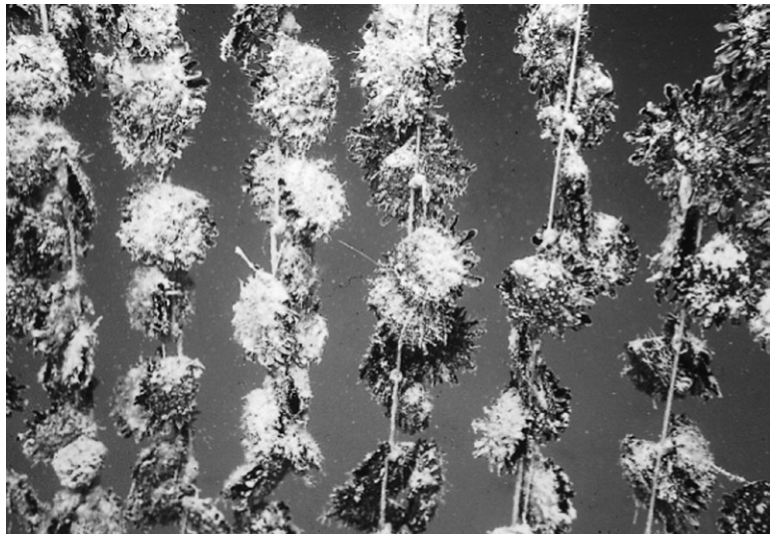


Fig. 23.12 'Ear hung' blacklip pearl oysters on longline, Takapoto Atoll, French Polynesia.

the surface or only indicated by marker buoys. This system has the advantages of being more resistant to storm damage, having less chance of damage from boating, being less conspicuous to poachers and more aesthetically acceptable.

3. Floats are single versions of the multi-float longline system. The buoyancy floats are much larger and directly support the culture structure below. They are employed in similar ways to longlines in that they are often joined in lines.

23.4.5 Breeding programmes

As well as chromosomal manipulations (section 23.4.2.3), there have been selective breeding programmes with bivalves (Gosling, 2003). These have involved the usual procedures of individual selection, family selection and within family selection. Major breeding programmes, especially with table oysters, have been and are being undertaken by large research organisations. The programmes have mainly targeted disease resistance, although growth rate has also been considered. Despite successful

Table 23.2 Predators of the hard clam (*Mercenaria mercenaria*). Reprinted in part from Gibbons and Blogoslawski (1989), with permission from Elsevier Science.

Group of predators	Numbers of species
Decapod crustaceans (crabs, hermit crabs, snapping prawns, etc.)	19
Gastropod molluscs	12
Horseshoe crab	1
Starfish	2
Birds	8+
Rays	8+
Finfish	5

results from research programmes, it seems so far that there are no bivalve industries that are substantially based on selected strains supplied from hatcheries.

23.5 CULTURE PROBLEMS

23.5.1 Predators, parasites and diseases

23.5.1.1 Predators

Bivalves are prone to predation, especially during the early months of development while their shells are fragile. Major predators include gastropod molluscs, starfish, crabs and fish.

As an example of the range of predators, Gibbons and Blogoslawski (1989) listed 34 invertebrate species and 21+ species of vertebrate predators of the hard clam (*Mercenaria mercenaria*) (Table 23.2). It must be noted that the hard clam is a soft seabed inhabitant in the intertidal zone. A different suite of predators will prey on bivalves in other habitats and, of course, other regions.

1. Gastropod predators feed using a toothed rasping radula, in an extensible proboscis. They either drill through one shell of the bivalve to access the animal within or push their proboscis between the prey's valves. The major gastropod predators of bivalves include members of the families Naticidae (moon-snails), Ranellidae (triton shells), Buccinidae (whelks) and Muricidae (murex shells).
2. Crab predators include swimming crabs (family Portunidae), rock crabs (families Cancridae and Grapsidae) and stone crabs (family Xanthidae). Crabs are equipped with two chelate arms ('pincers'); the larger chela being used for crushing and the smaller for manipulating and picking. Other decapod crustaceans, such as hermit crabs, shrimp and snapping prawns, although not equipped with such powerful crushers, will readily consume early juvenile bivalves.
3. Starfish (seastar) predators of cultured bivalves are mainly a problem in temperate regions. They particularly feed on bivalves that inhabit soft-seabeds: flat oysters, clams, cockles and scallops. The starfish attaches to both valves with its tube feet, pulling the valves apart with prolonged tension, and then inserting its extruded stomach between the valves.
4. Octopuses have not been widely reported as predators of cultured bivalves, but they prey on bivalves through prolonged tension in a similar manner to starfish.
5. A variety of rays and fish have been reported to feed on cultured bivalves. The fish include flatfish and whiting (active on soft seabeds), perch, bream, puffer fish, snapper, etc. These fish are bottom feeders that both crush and consume the whole prey or crop the siphons off burrowing bivalves. The latter activity is not necessarily lethal to the prey, but must reduce its growth rate.

Measures against predators include excluding them with appropriate-sized meshes around the bivalves. There is, however, a problem in that meshes can be fouled by algae and epifauna, seriously reducing the flow of water to these filter feeders. Another measure is to culture the bivalves away from the predators' habitats (e.g. above the bottom), and regularly inspect for mortality and identification of its source (e.g. damaged mesh, concealed predatory gastro pods).

The advantage of using racks and other methods for keeping the cultured bivalves off the bottom, where appropriate, is that it is more difficult for predatory gastropods, starfishes, crabs, etc., to gain access to the bivalves. Then, when predators gain access, there is less cover for them to hide. They can be located and removed during regular inspections when localised groups of dead bivalves are observed. The predatory fish that feed on benthic bivalves tend not to swim up off the benthos to structures above their habitat.

Ocean surface culture is a further step in removing cultured bivalves from benthic predators. There is no physical connection with the seabed by which benthic predators can reach the bivalves.

Predators, however, may get around all these measures to exclude them by settling from the plankton as larvae onto the culture equipment. They then grow to become a significant predator on the cultured bivalves.

23.5.1.2 Parasites and diseases

Chapter 10 describes some of the diseases of bivalves. Elston (1990) has detailed the major diseases of table oysters and some other cultured bivalves. Humphrey (2008) describes the diseases of pearl oysters that may result in mass mortalities in some cultured populations, with consequent loss of pearl production and pearl quality, and negative perceptions of investment in the industry.

The parasitic and other disease-causing organisms of hard clams are summarised by Gibbons and Blogoslawski (1989). They include the following:

- herpes-like viruses and *Rickettsia*;
- eubacteria (e.g. *Vibrio* species);
- protozoans (e.g. *Perkinsus marinus*);
- fungi, sponges and polychaete worms (which penetrate the shell and make it more prone to crushing by predators);
- flat, tape, nemertean and nematode worms;
- pyramidellid gastropods (ectoparasites that suck body fluids);
- endoparasitic copepods;
- pea crabs ('food-stealers' among the gills in the mantle cavity).

Some, such as copepods and pea crabs, may have minimal effect on the host: causing some loss of host condition due to loss of nutrients and local irritation. Others, such as some viral and protozoan diseases, can have devastating effects on bivalve aquaculture industries, through mass mortalities, resulting in the industries becoming uneconomic, being closed down or requiring alternative more resistant species. The traditional flat oyster (*Ostrea edulis*) industry in France is an example of the last case (Heral, 1990). The Pacific oyster was imported because of its resistance to two protozoan parasites that were causing havoc with the native flat oyster. Proportions of oyster species in the annual harvests varied, but in 1984, a particularly bad year for the native oyster, the annual harvest was 98% Pacific oysters.

23.5.2 Biofouling

Like boat hulls and jetty piles, aquaculture structures become coated with fouling organisms, which may include communities of bivalves that are not the target species. As described earlier, biofouling is a particular problem for bivalve culture as it restricts water flow to these filter feeders. Furthermore, many of the fouling organisms are themselves filter feeders and compete for available POM and dissolved oxygen with the culture species. They include bacteria and diatoms, macroalgae, sponges, hydroids, hard and soft corals, bryozoans, tube-inhabiting polychaetes, barnacles, mussels (feral species) and tunicates. A surrounding mass of fouling organisms may have a greater filtration rate and oxygen consumption than the cultured bivalves creating a low food and dissolved oxygen environment for them (De Nys and Ison, 2008). Thus, regular removal of biofouling results in more rapid growth in the cultured bivalves.

The problems of biofouling may be acute during the spat and seed stages of bivalve culture. Biofouling of culch surfaces before settlement reduces the sites for pediveligers to settle and metamorphose; and thus spat collection on the culch is reduced. The initial phase of biofouling by bacteria and diatoms may result in a slimy coating that is unattractive to the settling pediveligers of many species. Furthermore, spat and seed bivalves are particularly vulnerable to biofouling overgrowth due to the relative size of macroscopic fouling organisms.

A further aspect of biofouling of suspended culture systems, e.g. longlines and rafts, is that it can add so much weight to the system that it threatens the systems' buoyancy.

Many bivalves are subject to biofouling through their shells. These fouling organisms include boring fungi, sponges and tube worms that may riddle the bivalves' shells. The borers may penetrate through the inner nacreous layer of the shell, necessitating the mantle to secrete new nacreous layers to keep the borers at bay. Borers weaken the shell, making the bivalve more prone to crushing predators, and their burrows detract from the shell's appearance. This is particularly a problem for pearl oyster culture where good quality shells are valuable. In the Australian cultured pearl industry such riddled shells are known as 'chicken shell' (De Nys and Ison, 2008).

One of the simplest ways to control biofouling is to expose the cultured bivalves and their fouled equipment to air and sunlight for a sufficient period to kill most of the fouling organisms without killing the bivalves. These methods rely on the ability of bivalves for sustained closure of their valves, avoiding desiccation. The period required for exposure, however, may be more than a day and this method is time-consuming and costly in requiring regular movements of large numbers of bivalves to hold them out of water. Culturing in the intertidal zone has the advantage that exposure to air occurs regularly without movements of stock and generally biofouling is less of a problem in intertidal culture than in subtidal culture (Fig. 23.13).

Other methods for controlling biofouling are pressure hoses and mechanical scrubbers. As well as these physical



Fig. 23.13 Intertidal oyster farm at Medoc, southern France. (Photograph by Mike Peel.)

methods of biofouling control, chemical and biological methods may be used (Quayle and Newkirk, 1989). Most of these chemical methods, as with exposure to air, rely on the bivalves' capacity to close valves and shut out deleterious environmental factors for some time. The fouled bivalves may be immersed for appropriate periods in freshwater or hypersaline seawater. Toxic solutions such as hypochlorite may also be used. Other antifouling chemicals may be used to treat the culture equipment. These chemicals include tar, some new synthetic organic coatings, and, in the past, toxic paints containing copper, zinc or tin. Tributyl tin, in particular, was a very successful and widely used antifoulant. However, use of these toxic metals as antifoulants is now recognised as being environmentally unacceptable (section 4.4.3). Furthermore, they may cause abnormalities and deaths of cultured bivalves, and the metals can accumulate in bivalve tissues to levels unacceptable for human consumption.

Biological methods are appropriate where there are one or a few major fouling organisms to deal with. Biological methods require knowledge of the breeding season, patterns of larval settlement, and life cycle of the fouling organism. For example, knowing the breeding period of the fouling organism, it may be appropriate to put culch out after this period; or it may be appropriate to use a chemical or exposure treatment while the fouling organisms are recently settled and more vulnerable. Local areas of heavy settlement of fouling organism larvae may be avoided or just avoided during the period of settlement. If the fouling organism larvae tend to settle at particular depths, then these can be avoided in suspended culture.

23.5.3 Biotoxins and gut contents

23.5.3.1 Biotoxins

With appropriate hydrological conditions, some phytoplankton species bloom to cell densities of millions/mL, causing discolouration of inshore waters, a phenomenon often known as red tides. During red tides the harvesting and consumption of bivalves may be suspended owing to potent biotoxins accumulating in the bivalves from their filter feeding on toxic phytoplankters. More insidiously, at other localities there are blooms of toxic phytoplankters without obvious discolouration of the water.

Most sources of these biotoxins are dinoflagellates and about 20 out of the total 1500 dinoflagellate species have been implicated. They include species of *Alexandrium*, *Dinophysis*, *Gymnodinium* and *Porocentrum*. Several species of the diatom *Nitzschia* are also toxic.

Mass mortalities of pearl oyster species have been attributed to biotoxins from phytoplankton blooms (Lucas, 2008):

- seasonally recurrent outbreaks of the dinoflagellate *Heterocapsa cularisquama* caused mass mortalities of all commercial bivalves in the coastal water of Japan;
- blooms of the dinoflagellate *Gymnodinium* sp. have caused heavy mortality of fish and shellfish, including pearl oysters;

- blooms of the cyanobacteria *Trichodesmium* spp. were associated with mass mortalities of pearl oysters in north-western Australia and the Gulf of Manna;
- red tides caused mass mortalities in some Japanese pearl oyster farms.

It appears, however, that biotoxins do not necessarily poison bivalves. Dense phytoplankton blooms may cause mass mortalities by choking the bivalves' filter-feeding and respiratory mechanisms, and by causing very low dissolved oxygen levels in the environment.

Where biotoxins are assimilated, they accumulate in the bivalves' digestive gland and other organs. The biotoxins may then severely affect humans who subsequently consume the infected molluscs. Some of the sources and effects of biotoxins from bivalves are the following.

1. Amnesic shellfish poisoning. This causes gastrointestinal disorders such as nausea, abdominal cramps, diarrhoea. More severe symptoms are neurological, such as headaches, dizziness, disorientation and loss of short-term memory. The symptoms are caused by domoic acid, which is derived from several diatoms (*Nitzschia* species). Poisonings have primarily occurred on the north-eastern coast of North America but the diatoms have a wide distribution.
2. Diarrhoeic shellfish poisoning. This causes gastrointestinal disorders due to okadaic acid, which is derived from species of *Dinophysis*. It has a wide occurrence, but occurs especially in Europe and Japan.
3. Neurotoxic shellfish poisoning. This manifests as neurological symptoms that are milder than paralytic shellfish poisoning. These are caused by a polyether toxin of the same class of compounds as ciguatoxins in fish flesh. The toxin is derived from a dinoflagellate (*Gymnodinium breve*) and primarily occurs in Florida and Texas.
4. Paralytic shellfish poisoning. This causes numbness of lips and tongue, fingers and toes. Extreme symptoms are muscular paralysis and breathing difficulties and, in severe cases, death. Variable complexes of toxins are involved, as are various dinoflagellates, including *Gymnodinium catenatum*.

In recent times, biotoxins in bivalves have been responsible for several thousand cases of severe poisoning and several hundred deaths throughout the world. Apart from these dire effects on seafood consumers the biotoxins have severely affected bivalve culture industries through industry closures or loss of consumer confidence. For example, the Spanish mussel industry was decimated following loss of consumer confidence after mussels infected with paralytic shellfish poisoning were exported throughout Europe in 1976, while the New Zealand shellfish industry, valued at NZ$100 million per year, was closed down following suspected biotoxin poisoning of over 170 people. A problem with biotoxins at one locality can result in loss of consumer confidence that extends to the whole industry.

Biotoxins in bivalves have been a long-standing problem for the bivalve culture industries of Europe, south-east Asia and North America, but the problem seems to have become more common and widespread (Hallegraeff, 1993).

Suggestions for this are as follows:

- increased scientific awareness of the problem;
- increased utilisation of coastal waters for aquaculture;
- increased algal blooms due to eutrophication of coastal waters;
- unusual climatic conditions stimulating algal blooms;
- transport of dinoflagellate cysts in ships' ballast water;
- transport through transfers of bivalve stocks.

Discharge of ships' ballast is blamed for introducing the dinoflagellate *Gymnodinium catenatum* (a source of paralytic shellfish poisoning) to Tasmania, where its first recorded bloom was in 1986 and where it has resulted in closures of Pacific oyster farms in some areas.

The safe level for paralytic shellfish poisoning is 0.8 ppm shellfish tissue (80 µg/100 g). However, although toxins in infected bivalves can be magnitudes above safe levels, after the toxic algal bloom clears from their environment the animals lose their accumulated toxins over periods of weeks or months and become marketable.

Biotoxins are a major problem for worldwide bivalve industries and unfortunately there is no simple solution. The safest, but uneconomical, way to cope with biotoxins in bivalves is to bioassay all suspect product before it is marketed. Practical solutions are to have annual periods of closure in areas of long-standing bivalve culture where the occurrence of toxic algal blooms is regular and seasonal. For example, this applies to areas of the Atlantic coast of Canada and to parts of the Californian and Washington State coastlines (Quayle and Newkirk, 1989). Specific stations in these areas are monitored to adjust the timing of the closures. Regular samples of cultured bivalves from representative sites may be tested when the occurrence of biotoxins is suspected or anticipated. In other areas the occurrence of red tides or the particular conditions that lead to red tides in that locality are carefully monitored.

23.5.3.2 Gut contents

The other possible sources of health problems for bivalve consumers are live micro-organisms in bivalves' guts when the animals are consumed raw. (One does not wish to be accused of trying to turn away 'shellfish' consumers; but it is important that consumers' needs for a safe product are identified.) Bivalve guts contain bacteria and viruses, which they have ingested through their filter feeding. Coastal waters may be subject to some level of sewage input or waste input from animal husbandry. Under these conditions there may be high levels of faecal bacteria and viruses in the water column and hence in the bivalves' guts. The bacteria include *Escherichia coli* and *Salmonella* species, which can cause gastroenteritis and typhoid fever, respectively (Quayle and Newkirk, 1989).

These micro-organisms in the bivalves' guts can be dealt with much more readily than biotoxins accumulated in their tissues. It is a matter of exposing the animals to purified water for several days while they clear their gut contents; a process called depuration. It usually involves a large recirculating seawater system with a means of water treatment, such as ozone, chlorine or ultraviolet radiation, and a system of racks and trays to hold large numbers of bivalves in tanks within the seawater system. The animals are then marketed. In many areas, depuration is used as a routine practice between harvesting and marketing.

23.6 INTRODUCTIONS AND OTHER ENVIRONMENTAL ISSUES

23.6.1 Introductions

The Pacific oyster (*Crassostrea gigas*) has many desirable traits for culture and market-acceptability, and it has been introduced over large distances from its original distribution, apparently without adverse environmental effects (section 23.3). This must, however, not be taken as support for introductions. The prevalence of diseases has major implications for introductions and re-introductions of cultured bivalves and other aquaculture organisms (section 4.4.2). There are temptations to introduce species that are being cultured successfully in other regions, and translocations are relatively simple because many bivalves tolerate extensive periods out of water if handled correctly. Introductions and re-introductions, however, cannot be undertaken without comprehensive quarantine preparations and assessment of potential ecological effects. This is in view of the suite of noxious organisms associated with bivalves, and their potential for devastating effects on bivalves at the recipient locations (Carriker, 1992). Translocations to regions where there are existing populations of a species also have genetic implications. It is possible to destroy regional genetic variability through this process (section 4.4.2). It is impossible to prevent spawning in translocated bivalves in the open aquatic environments where they must be cultured and spawning will lead to vast numbers of 'foreign' gametes entering the environment.

There is a further aspect to deliberate or accidental introductions of bivalves that warrants caution. The introduced bivalve species can proliferate to the extent of becoming a pest in its new environment, where factors controlling it within its normal range are absent. The proliferation of introduced species can be at the expense of related local species. The zebra mussel in the Great Lakes system, North America, is a well-known example. Since it was introduced in the mid-1980s, probably from ballast water of a trans-Atlantic ship or ships, it has become a disastrous fouling organism in this environment with both ecological and economic consequences (Connelly *et al.*, 2007). Furthermore, it has the potential to cause the extinction of some endemic mussel species. It has eliminated most endemic unionid populations within the lower Great Lakes.

23.6.2 Environmental issues

One potential environmental problem with many aquaculture operations, the input of large quantities of nitrogen-rich feeds, is not present in bivalve aquaculture. There may be artificial feeding of bivalve larvae and early juveniles in hatcheries, but this is only with relatively small biomasses of bivalves and they are fed live microalgae. By the time the cultured bivalves reach a substantial size they are totally dependent on natural phytoplankton. There can, however, be deleterious environmental effects from the filter feeding. Large marine mussels may each filter 2–5 L/hr. In very general approximation, a raft of mussels may filter 70 million L/day, ingest 180 t of organic matter/year and produce 100 t of pseudofaeces and faeces/year. The 'rain' of faeces and pseudofaeces from these rafts endangers the benthic environment below. It may transform the seabed below into anaerobic conditions, just as this may occur below cages in fish culture with the 'rain' of uneaten feed and faeces. The solutions are as follows:

- locate the culture structures in regions of high current velocity to promote dispersal of faeces and pseudofaeces in the water column;
- limit biomass/unit area of cultured bivalves to a level where dispersal processes are sufficient to minimise faecal and pseudofaecal accumulation on the seabed;
- regularly move rafts and longlines to new sites before there is excessive accumulation of wastes on the seabed below.

Table 23.3 Global production of table oyster species.

Oyster	Number of species	Production (10^6 t/year)	Number of countries producing
Pacific cupped oyster	1	4593	>30
Other cupped oyster species	11	116	24
Flat oyster species	5	ca. 6	19

Data from FAO Fishstat Plus (2009).

Cleaning the biofouling growths off the bivalves' shells and culture apparatus may also be a source of environmental problems where the biofouling debris is discarded into the adjacent water.

A different kind of environmental problem arises because bivalves are usually cultured in coastal regions where there are other users of the environment. As with other aquaculture operations in these regions, farms consisting of rafts, racks, longlines and floats may be perceived to be an environmental problem from an aesthetic, navigational or recreational viewpoint (section 4.4.4). On the other hand, there may be situations where bivalves can be used with positive environmental effects, such as removing particles and nutrients from pond effluent (Jones *et al*., 2001).

23.7 INDUSTRY REVIEWS

23.7.1 Table oysters

Oysters have long been regarded as a seafood delicacy and culture methods are long established (reviewed by Matthiessen, 2001).

By far the greatest proportion of global oyster production is from cupped oysters, with the Pacific oyster being pre-eminent (Table 23.3). The Pacific cupped oyster is farmed around the globe in more than 30 countries. As previously mentioned, its growth rates, favourable morphology for marketing and relative resistance to disease compared with most other table oyster species, and especially compared with flat oyster species, make it very attractive for culture. It has been widely introduced beyond its origins on the Pacific coasts of East Asia.

There are about 11 other species of cupped oyster that are cultured locally around the world. They may be 'boutique' industries supplying a market that prefers the local product, e.g. the Sydney rock oyster. Culture of five species of flat oysters, despite their relatively high value (>$3000/t versus $670/t) is minimal compared with cupped oysters (Table 23.3). Their particular propensity for diseases is a discouragement to farming.

Table oysters are cultured on the bottom, off the bottom (Fig. 23.13) and suspended from the surface. The spat may be obtained from natural settlement on culch, e.g. sticks, shells, tiles, etc., or they may be hatchery produced (Figs. 23.5 and 23.8). The spat may be left on culch or they may be removed. The simplest level of culture is with natural spat in areas of firm substrate. These oyster farms are usually fenced off, predators are controlled and other management measures undertaken. Harvesting is often by dredge. Stick culch with seed oysters may be placed horizontally on racks above the seabed or driven vertically into the seabed. Culchless oysters and oysters on small pieces of culch may be kept in trays or mesh containers on racks above the seabed or suspended from the surface from longlines or rafts.

Table oyster fisheries and culture industries have been characterised by great fluctuations in annual production. Factors causing these fluctuations are as follows:

- over-exploitation to the extent of reducing the reproductive output and subsequent recruitment where the aquaculture industry relies on natural spatfall;
- mass mortalities from various protozoan and viral diseases, apparently resulting from overstocking or introduced pathogens;
- mass mortalities from unusual environmental conditions, including temperature extremes, low salinities and red tides.

Heral (1990) provides an excellent example of wide fluctuations in annual oyster harvests from a culture industry. These data are for the French industry for over a century, 1865–1983. They result from factors described above.

The Sydney rock oyster industry in eastern Australia is another example of fluctuating fortunes. The industry commenced mainly in southern Queensland during the late nineteenth century with a simple system of oyster banks. The flourishing industry, however, crashed through

massive losses of oysters with QX disease (caused by a protozoan parasite, *Marteilia sydneyi* (section 10.5.1). QX continues to be a problem limiting the industry in southern Queensland and northern New South Wales. In southern New South Wales, the oysters suffer 'winter mortality' (caused by another protozoan parasite, *Mikrocytos roughleyi*). The Sydney rock oyster farms depend on natural spatfall and, as Pacific oysters spread north from Tasmania, the farms are now being 'contaminated' with unwelcome Pacific oysters.

The oysters of Pakistan are an example of precipitous declines in populations (Siddiqui, 2005), e.g. sizeable oyster reefs of *Crassostrea gryphoides* and *C. madrasensis* in the Hab River Delta completely disappeared in less than 20 years. Over-exploitation has been suggested, but it is likely that siltation from heavy soil erosion is a major factor in the demise of oyster populations along the Pakistani coastline. Recovery is likely to be slow in many locations, with the minimal levels of spatfall.

23.7.2 Mussels

Mussels do not usually attract prices as high as those obtained for table oysters. In some cases, as in parts of coastal China, mussels are cultured to use as food for other more valuable aquaculture organisms, such as shrimp. Furthermore, the low economic return per unit mussel usually renders hatchery production of spat and seed uneconomic, and almost all mussel industries are based on natural spatfall.

The relatively low economic value of mussels is compensated by their ease of culture and high productivity. The highest productivity of bivalve culture per unit farm area has been reported for marine mussels. These mussel farms operate in the western Galicia region of Spain in a series of sunken river valleys (rias). Primary productivity in the rias was found to average 10.5 µg organic carbon/L/h over the year owing to upwelling of cold nutrient-rich water and runoff from the adjacent hills (Figueras, 1989). This provides copious phytoplankton for mussel culture. Thus, a 20 × 20 m raft may produce 50+ t of mussels in 12–18 months and there may be six rafts/ha (Quayle and Newkirk, 1989). Meat yield is 30–50% of whole weight, so this farming system potentially yields meat at ca.100 t/ha/year without feed or fertiliser inputs. There is no low-input farming on land, such as grazing, that can even vaguely compare with this production rate. Maximum meat production from unsupplemented grazing is in the order of 2 t/ha/year.

Marine mussel (*Mytilus* species) production in Spain has varied from 158 200 to 247 800 t/year over the period 2000–2007 with a corresponding range of US$55.4–86.7 million. It is based on vertical ropes suspended from rafts. Seed mussels are attached to the ropes at ca.10 cm shell length and are grown to their marketable size, 80–100 cm shell length. This was often reached in less than a year in the past. Now it takes longer in the central regions of the rias, apparently because of the densities of mussels reducing phytoplankton levels.

As well as raft culture, mussels of both *Mytilus* and *Perna* species are grown in seabed culture, on vertical poles in the intertidal zone (Fig. 23.14) and in longline culture (Gosling, 2003) (Table 23.4).

Mussels are traditional cultured seafood in Europe and some parts of Asia. Despite this, there have been major changes in mussel production over recent decades. As shown in Tables 23.1 and 23.3, China now produces >400 000 t/year of marine mussels of several species, being by far the largest producer. Production in Europe is generally steady, with seabed-based industries being confronted with limited spat availability and farm sites. New Zealand markedly expanded its mussel production, including specialised products such as freezer packs of mussel in the half-shell. On the other hand, Thailand's production of green mussels was 50 000 t in 1971, but then declined precipitously owing, among other factors, to environmental deterioration and loss of farming areas to other purposes. The Thailand industry has now achieved consistent annual production of >250 000 t, but the history of mussel farming in Thailand is one of growth and decline, reflecting the kinds of problems faced by bivalve industries worldwide.

23.7.3 Scallops

Recently there have been two major publications on scallop farming: Hardy (2006) gives a comprehensive treatment of the various phases and aspects of culture, including business and marketing aspects; Shumway and Parsons (2006) give a broader coverage of the biology and ecology of scallops with some consideration of their culture.

The scallop's large adductor muscle is a highly valued seafood and they have been subject to heavy fishing in many regions. However, scallop fisheries are often unpredictable and over-exploited, so culture is an attractive alternative to fisheries. Despite the interest in culture, global aquaculture production of scallops is almost exclusively from China and Japan. They produced 1 165 000 and 247 000 t, respectively, of the yesso scallop (*Pecten yessoensis*) in 2007. This is about 97% of global scallop production and it is based on this one species. Interestingly,



Fig. 23.14 Mussel cultivation on poles, Lingreville, France. (Photograph by Féron Benjamin.)

Table 23.4 Details of some marine mussel mariculture industries. Blue mussel, *Mytilus edulis*. Chinese mussel, *Mytilus coruscus*. Green mussel, *Perna viridis*. Mediterranean mussel, *Mytilus galloprovincialis*. New Zealand mussel, *Perna canaliculus*.

Country	Mussel species	Main culture techniques
China	Blue, green, Chinese, etc.	Various
Spain	Blue	Raft
Italy	Mediterranean	Vertical poles
Thailand	Green	Vertical poles
France	Blue and Mediterranean	Vertical poles (‘bouchets’)
The Netherlands	Blue	Seabed
New Zealand	New Zealand	Longlines
Germany	Blue	Seabed
Philippines	Green	Vertical poles

at least in the past, the technology for culturing scallops contrasted between Japan and China in a way that one would not necessarily have predicted. The Japanese industry, which tends toward hatchery production as a rule, relied on collecting bountiful natural spat; while China had a large number of hatcheries (Cropp, 1989).

The yesso scallop is a cold-water species and most Japanese production is from northern Japan. While natural spatfall is used, it is not a casual operation. The reproductive state of scallop populations and progress of natural larval development are carefully monitored to time the deployment of spat collectors accurately in each region. The spat collectors (mesh bags with internal mesh) are hung from sub-surface longlines (e.g. Fig. 23.6). When the seed scallops are 10–15 mm they are removed from the collectors and placed in mesh nets (Fig. 23.12) suspended from longlines or rafts (section 23.4.4.3). Juvenile scallops, 30 mm, may then either be released to prepared regions of the seafloor or placed into lantern nets. The former culture method involves higher mortality from

predators if no meshes are used and it is essentially enhancing natural populations. The latter method is more expensive due to labour and equipment costs. Another less common culture method is 'ear hanging', whereby a small hole is drilled through the hinge 'ear' of the shells and the scallops are tied to supporting ropes (cf. Fig. 23.12). Harvest size (>100 mm shell length) is reached in 2–3 years (Cropp, 1989).

Scallops in general are cultured on the seabed at two scales. One is in small-scale enclosures; the other is on a large scale without enclosures (Hardy, 2006). Small-scale enclosures are tens of square metres in size, and require diving to maintain the equipment and harvest the scallops. Enclosure walls act as barriers to loose seaweed drifting along the seabed. They can become heavily fouled, reducing water flow through the enclosure and requiring clearing. Enclosures must be seeded with juvenile scallops of sufficient size to minimise predation by shell crushers. They are seeded with up to 12 scallops/m^2.

Large-scale culture operations involve areas of tens of square kilometres. The seabed may be initially dredged to remove predators such as starfish and large crabs. The area is seeded with millions of scallop spat of appropriate size. After the growing period to commercial size, 2+ years, the scallops are harvested by dredging. Thus, there is a 2+ year rotation of the farmed area. As noted above, there may be substantial mortality, >50%, but this is offset by the low cost of husbandry and equipment.

23.7.4 Clams and cockles

Some clams and cockles, like mussels, are the bases for large aquaculture industries that are often very simple and traditional in technology, e.g. the culture industries for blood cockles, *Anadara* species (Broom, 1985) (Table 23.1). They may consist of little more than collecting seed from areas of heavy natural settlement and then stocking the seed at appropriate densities, for example 10 million/ha, in defined farm areas where there are favourable conditions for growth and predator control. Notwithstanding technical differences, there is a major difference between the two bivalve groups in that clam and cockle aquaculture results in products that are more valuable than those from mussel aquaculture (Fig. 23.4).

Many clam industries are based on hatchery and nursery technology. For example, the hard clam or northern quahog (*Mercenaria mercenaria*) industry of eastern North America cultures the larvae and spat using similar hatchery methods and upweller systems to those employed in table oyster culture. Then the seed clams are transferred to the field at densities of 3000–4000/m^2 either into carefully prepared seabed areas, floating trays or trays on racks (Castagna and Kraeuter, 1981). After a year, when the juveniles reach about 25 mm shell length, they are spread out in the grow-out area. The considerably smaller Japanese carpet clam (*Ruditapes philippinarum*) is being cultured commercially in the USA on an increasing scale and it is based on hatchery production.

Aquaculture production of the hard clam (quahog, *Mercenaria mercenaria*) in the USA amounted to 27 000 t in 2007. This is a substantial quantity, but it is <1% of total global clam, cockle and arkshell production, which is derived from more than 25 species of cultured bivalves in this category.

With a great variety of species, growth rates and commercial sizes within this group, there is obviously a wide range of culture periods. Clams and cockles are, however, generally slow growers and periods of 2+ years to commercial size are common in the larger species. This adds to the necessity for low cost technology in the grow-out phase.

Reference has been made to the most unusual group of clams, the giant clams. These have been the target of much culture-oriented research in recent decades. Although the industry is in the initial phase of development, the technology and 'how to' culture manuals are available, for example Braley (1992) and Calumpong (1992).

23.7.5 Pearl oysters and pearl mussels

There is a recent publication on the biology of pearl oysters, and the processes and economics of cultured pearl production (Southgate and Lucas, 2008). There is the common idea that pearls are formed in an oyster, especially a pearl oyster, when a sand particle gets into its mantle cavity and causes irritation. In fact, such particles frequently enter the mantle cavity of bottom-dwelling bivalves and they are vigorously expelled. Pearls form when one or more epithelial cells from the outer epithelial layer of the mantle adjacent to the forming nacre layer are detached and transfer into the mantle's connective tissue (Taylor and Strack, 2008). Detached epithelial cells may be caused by damage from various sources in this narrow space between the mantle and the shell:

- a small intruder, such as a crab or gastropod;
- small pieces of broken shell or stone or the 'sand particle' lodged there;
- small embedded parasites.

The transferred epithelial cells multiply by cell division and may form a cyst within the connective tissue. The cyst

becomes a 'pearl sac' in which a pearl is secreted by the inner epithelial cells of the sac. There are three layers within a pearl, but they are the reverse of those in the shell (section 23.2.1) reflecting the sequence of their secretion. The periostracum layer is innermost, the prismatic layer is medial and the nacre layer is outermost, providing the lustrous surface of the pearl. Many bivalves produce natural 'pearls', for example it is a hazard of eating table oysters, and an enormous pearl was collected from a giant clam. However, only the mantle tissues of some bivalves secrete very lustrous nacre and are used in cultured pearl production. Interestingly, some gastropod molluscs also secrete a very lustrous nacre and these are used commercially (Chapter 24).

23.7.5.1 Pearl oysters

Before they declined or reached a level from which they could not recover, many pearl oyster stocks were intensely fished (Southgate *et al.*, 2008). The frequency of occurrence of natural pearls varies with the species and some stocks were fished for natural pearls. Other stocks were fished mainly for pearl shells, which were used for button manufacture and pearl inlay. Pearl culture methods for pearl oysters were developed and patented by several Japanese biologists early in the twentieth century. A spherical bead made from shell is used as the 'nucleus' for the cultured pearl. The bead, which must be of an appropriate size for the size of the oyster, is inserted into a pocket cut in the oyster's gonad (Fig. 23.15). Inserted with the bead is a small piece of outer mantle tissue removed from a donor oyster. Cells of the transplanted mantle tissue spread over the bead surface to make a pearl sac and this secretes successive layers of nacre onto the bead to make the cultured pearl. Thus, culture pearls are produced by host oysters supporting foreign cells that secrete the pearl (Taylor and Strack, 2008).

The period between bead insertion and harvest varies between about 9 months and 3 years, with the thickness of nacre and quality of pearl being directly related to this culture period. Only a low percentage of the inserted beads result in high value pearls. Losses occur from oysters that die from the insertion procedure, ejected beads and other processes that affect the shape and surface quality of the pearl (Taylor and Strack, 2008). After the harvest, the pearls must be graded before being presented for sale.

The cultured pearl industry has traditionally been dominated at all levels by the Japanese industry:

- technicians who inserted the beads;
- hatchery operators;
- managers of oyster farms;
- company owners or joint owners;
- pearl graders;
- pearl marketing.

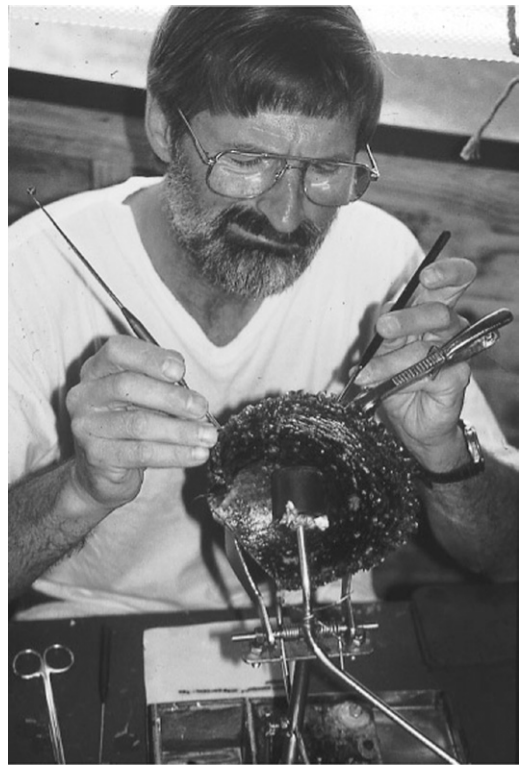


Fig. 23.15 Operating to insert a bead and piece of mantle tissue for cultured pearl production in a blacklip pearl oyster, Manihiki Atoll, Cook Islands.

The largest component of global pearl production has been from Japanese waters, based on the Akoya pearl oyster. The number of Japanese pearl farms peaked at >4000 in the 1960s and production of Akoya pearls peaked correspondingly at ca. 80 t/year at that time. The number of farms has since declined to ca. 1500 and pearl production to ca. 25 t/year. The decline in recent decades is due to factors such as coastal pollution, toxic phytoplankton blooms and disease. Crowding of farms beyond the capacity of the environment may be a common factor in these problems. On the other hand, cultured pearl production has been increasing in Australia, Indonesia and the Philippines (large silver-white pearls from *P. maxima*), in Polynesia (large black pearls from *P. margaritifera*) and China (Akoya pearls) (Tisdell and Poirine, 2008).

Similar techniques to those used for scallops in suspended culture are used for pearl oysters, e.g. rafts or long-lines with suspended nets or mesh frames, or 'ear hanging' on ropes (Fig. 23.11). The techniques vary with location and species. Again, the sources of oysters for bead insertion vary with location and species:

- by collection of appropriate-sized wild oysters from the field;
- from spat obtained from spat collectors and grown out in the field;
- by initial hatchery-rearing and then transfer to grow-out in the field.

23.7.5.2 Pearl mussels

Production of cultured pearls from freshwater mussels, *Hyriopsis cumingii*, is a very different process that is largely conducted in China. Pieces of donor tissue are used and the process requires a skilled technician, but there are three major differences from marine pearl culture:

1. There is no bead and the resulting pearls have no foreign nucleus.
2. The site of insertion is the recipient mussel's mantle, not the gonad (the mantle lobes are much thicker in these mussels than in pearl oysters).
3. Several donor mantle pieces may be inserted into the two mantle lobes of the host mussel.

The mussels with inserted tissue are cultured by suspension in freshwater tanks with flow-through or in freshwater environments. The mussels are suspended in groups: in netting baskets or netting bags or tied together via holes through their shells.

In the early stages of this industry, the resulting pearls were <6 mm diameter and irregular ('rice' or 'wrinkled' pearls). They were not very valuable individually, but their production was reliable and large numbers were produced per mussel. They were used in multi-strand necklaces. Furthermore, after the pearls have been harvested, the mussels could be returned to culture and their pearl sacs would produce several further crops of these nucleus-free pearls.

Recent technological improvements are resulting in larger and more spherical pearls which are considerably more valuable.

In China the people who engage in the culturing phase of pearl-producing mussels are often also engaged in agriculture and aquaculture of freshwater fish.

23.8 THE FUTURE OF BIVALVE AQUACULTURE

Aquaculture of bivalves originated in antiquity and has continued through the ages. With the general increases in global aquaculture production in recent decades there have been sustained increases in bivalve production. Mean annual rate of increase was 7.5% over the period 1987–2007. As with fish, crustaceans and seaweeds, China dominates bivalve production and has been the major influence on their increased aquaculture.

There is every reason to predict that bivalves will continue to be a major component of global aquaculture production:

- many species are regarded as premium seafoods and have high market demand;
- they have excellent nutritional properties;
- they filter feed and make no demands for artificial diets (except on a modest scale in the hatchery phase) nor on fish meal;
- they may be reared with relatively simple techniques;
- they feed very low in the food chains with an immediate step from phytoplankton to bivalves and thus capture a very high proportion of the energy of primary production;
- they can be cultured at very high densities in appropriate areas of high phytoplankton production and water quality conditions, and, without supplementary feeding, achieve levels of production/unit area or volume that are not vaguely matched by any other farmed aquatic or terrestrial animals.

A picture of vigorous growth of bivalve industries is not, however, completely honest: disease, pollution, biotoxins and availability of grow-out sites are limiting bivalve industries in some areas and regions. These ongoing problems must be dealt with by management and research.

Finally, here are some further predictions for the future of bivalve aquaculture:

1. Bivalve industries will increasingly develop hatchery production of spat, instead of dependence on the vagaries and limitations of natural spatfall.
2. Genetics and stock selection will be researched and developed from the current limited basis. Increasingly, there will be industries based on hatchery-produced seed stock that are reared from genetically selected broodstock to have particular traits that make them more commercially viable.
3. There will be technological developments in the grow-out phase to increase efficiency and make better use of available sites.

4. Bivalves will have a role to play in treating aquaculture effluents and the water of large recirculation systems by their filtering of suspended organic and inorganic particles, and by absorption of dissolved waste molecules.

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24
Gastropod Molluscs

Laura Castell

24.1 INTRODUCTION

Many marine gastropod molluscs are valued for their shells ('seashells') and there is a large trade in shells for the collectors' and tourist markets. Other marine gastropods are harvested for their meat or for both their shells and meat. More recent developments are the trade for the aquarium market, focusing on small individuals whose main function is to keep tanks clean by feeding on overgrowing algae, and the production of pearls, either naturally or cultured.

In most cases the interest in culturing gastropod species has resulted from the need to meet increasing demand for the product while facing overexploitation of natural stocks through fisheries. The fishery of marine gastropods accounts for almost 2% of the total fishery of marine molluscs. Most of these gastropod species are particularly susceptible to overfishing because generally they live in relatively shallow waters accessible to divers using mask and snorkel; and they move slowly and are easily targeted by an experienced diver. Thus, natural stocks have often been overfished in many places through commercial fisheries. In addition, wild juveniles are generally very difficult to find and, therefore, collecting juvenile wild stock from their natural habitat to grow under controlled conditions until harvest size is impractical. As such, the culture of these species began in earnest when techniques for larval rearing were developed.

Recent estimates of global aquaculture production of gastropods (abalone, conchs, 'sea snails' and freshwater snails) places production at more than 370 000 t, an increase of over 100-fold from estimates in 2002 with a corresponding increase in the value by 12.5 times to a present estimate of US$560 million[1]. The major change between 2002 came in 2003–2004 when China reported aquaculture production data on 'sea snails', together with the freshwater Chinese mystery snail (*Cipangopaludina chinensis*) and abalone. Global production in 2007 is summarised in Table 24.1. A large proportion is made up of the culture in China of 'sea snails', which include the gastropods *Rapana* spp. and *Babylonia* spp. (Fig. 24.1), and the mystery snail, a live-bearing freshwater inhabitant. Abalone, although third in weight of production ca. 38 000 t in 2007, is the most highly valued of the cultured gastropods with an associated value of ca. US$350 million, about 60% of the combined value for all gastropods.

The number of gastropod species being cultured around the world is increasing although for many of these species culturing is still in the early phases of development.

Apart from abalone and conchs, there is a distinct contrast between cultured gastropod species and the most important gastropods in terms of large-scale fisheries. The major fisheries in order of magnitude are as follows:

- whelks (family Buccinidae), which are fished in Europe, especially the British Isles, and the USA;
- strombs and conchs, which are fished in Mexico and the Caribbean;
- abalone (*Haliotis* species), which have a wide geographical distribution, but are fished mainly in the western Pacific region;
- the horned turban (*Turbo comutus*), which is fished in Japan and the Republic of Korea.

[1]All production data in this chapter for quantity and value from aquaculture and capture fisheries are from FAO Fishstat Plus (2009).

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Table 24.1 Global aquaculture production of gastropods in 2007 (FAO Fishstat Plus, 2009).

Group	Production (t)	Production (million US$)	Main producing countries
'Sea snails'	258 688	155.2	China
Chinese mystery snail	84 176	51.3	China
Abalone	31 760	352.4	China, Republic of Korea, South Africa
Strombs	1	ca. 0.01	Turks and Caicos Islands
Total	374 633	559	

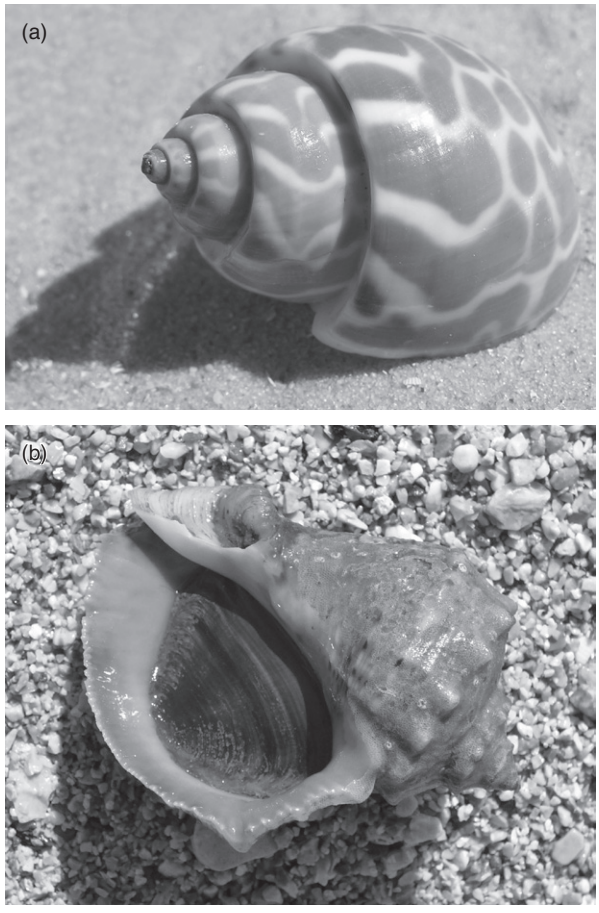


Fig. 24.1 Kinds of 'sea snails' used in Chinese aquaculture. (a) *Babylonia spirata*. (Photograph by Andrew Butko.) (b) *Rapana venosa*. (Photograph by George Chernilevsky.)

Other lesser fisheries are important for particular regions:

- the trochus or top shell (*Trochus niloticus*) in the Pacific Islands;
- loco or false abalone (*Concholepas concholepas*, which is not an abalone) on the coasts of Chile and Peru.

This chapter focuses on the culture of abalone (*Haliotis* spp.), which is challenging in that, whereas most animals in commercial aquaculture reach harvestable size within 4 months to 3 years of culture, most abalone take much longer. For example, Californian red abalone require up to 15 years to grow to minimum legal size of 197 mm in shell length (SL) (Tegner *et al*., 1992). Australian blacklip abalone take between 11 and 15 years to reach a size of 120 mm in Tasmanian waters, and this is still below the minimum legal size for harvest. The New Zealand abalone takes 3–5 years to reach 60–70 mm in size, whereas the legal size is 125 mm.

The range of gastropods cultured means that there is a range of culture methods which reflect variables such as the following:

- reproductive periodicity;
- spawing induction stimlus;
- type of larvae, lecithotrophic (non-feeding) or planktotrophic (requiring microalgae);
- settlement and metamorphosis substrate;
- food of early juveniles and through nursery phase;
- suitable size for grow-out;
- grow-out substrates;
- food for grow-out;
- protection required during grow-out;
- duration to marketable size.

These stages are typical of the aquaculture of many invertebrates. However, because these gastropods live on or in substrates and feed on them, there are particular issues in aspects such as settlement substrate, feeding during the nursery phase, and feeding and protection during grow-out.

Given the wide range of culture practices, techniques are highly variable depending on species and location. Detail of the culture techniques for the queen conch

(*Strombus gigas*) and topshell (*Trochus niloticus*) are included for comparison.

24.2 ABALONE

24.2.1 Commercial importance

There are over 90 species of abalone that occur along the west coast of North America, eastern and southern coasts of Asia, Pacific Islands, Australia, New Zealand, Africa and Europe. However, only some of these are commercially exploited (Table 24.2), and this is usually for the meat of their muscular foot. The beautiful nacre layer inside the shell of some species (Fig. 24.2), such as the paua (*Haliotis iris*) from New Zealand, is also used in handicraft industries. This nacre is the basis for pearl production. Thus, culture of pearls from abalone began in the 1960s, but it was in the late 1980s and early 1990s that abalone pearls entered the market to become a very lucrative activity. The pearls are characterised by unique colours. Abalone pearl farms have been developed in several countries, including New Zealand (*H. iris*), Korea and the USA.

Table 24.2 Commercially important species of abalone (*Haliotis* species).

Species	Common name	Locality
H. asinina[a]	Donkey's ear	Philippines, Thailand, tropical Australia
H. cocinea canariensis		Canary Islands
H. corrugata	Yellow, pink	USA, Mexico
H. cracherodii	Black	USA
H. discus	Kuro awabi	Japan
H. discus hannai	Ezo awabi, wrinkled	China, Japan, Korea, Chile (north)[b]
H. diversicolor	Colourful	China, Taiwan
H. gigantea		Japan
H. fulgens	Blue, green	USA, Mexico
H. iris	Paua, black foot	New Zealand
H. kamtschatkana	Northern, pinto	Canada
H. laevigata	Greenlip	Australia
H. madaka		Japan
H. mariae	Omani	Arabian Sea
H. midae	perlemoen	South Africa
H. roei	Roe's	Australia
H. rubra	blacklip	Australia
H. rufescens	Red	USA, Mexico, Chile[b]
H. tuberculata	Ormer	Europe, Israel[c]

[a]One of the fastest growing species of abalone.

[b]*H. rufescens* was introduced to Chile in the early 1980s. *H. discus hannai* was introduced to Chile in the late 1990s.

[c]*H. tuberculata* was introduced to Israel in the early 1990s.

Wild abalone stocks have consistently declined worldwide, reaching critically low levels in some areas, such as California and South Africa. Fisheries production peaked at 27,600 t in 1968 and then followed a steady decline to be <10 000 t/year in recent years. Paralleling this worldwide decline has been a continuous increase in aquaculture production to reach the current level. The latest statistics from the Food and Agriculture Organization of the United Nations (FAO) have 31 760 t in 2007, with China being the highest producer with 89% of global production, but some other countries, especially the Republic of Korea, are also increasing production (Fig. 24.3). The main consumers of abalone (wild and cultured) are China and Japan. Demand is still higher than supply, i.e. it is a demand-driven market (Chapter 12), so interest in the aquaculture industry remains very high.

The price of abalone meat is the highest among marine gastropods, a fact that is reflected in the worldwide interest on both its fishery and culture. There has been a steady increase in world abalone prices over the past 15 years. For example, prices for whole (in shell) wild abalone were over US$8/kg in California in the late 1980s and early 1990s (Tegner *et al*., 1992). However, some specialty dishes based on dried abalone and used for special occasions in Asia can fetch prices as high as $700/kg.

Although abalone culture has been practised for more than 60 years, it was during the 1970s that several important aspects of abalone culture were successfully investigated. Japan was probably the first country to culture juvenile abalone on a large scale; however, the late 1980s and early 1990s witnessed the development of abalone farms in several other countries such as Australia, Chile, China, Mexico, Republic of Korea, South Africa and USA. There are now farms in almost all countries with a history of commercial fisheries.

24.2.2 Biology

Hahn (1989) and Shepherd *et al.* (1992) are major references for the biology of abalone, and for their aquaculture at that time.

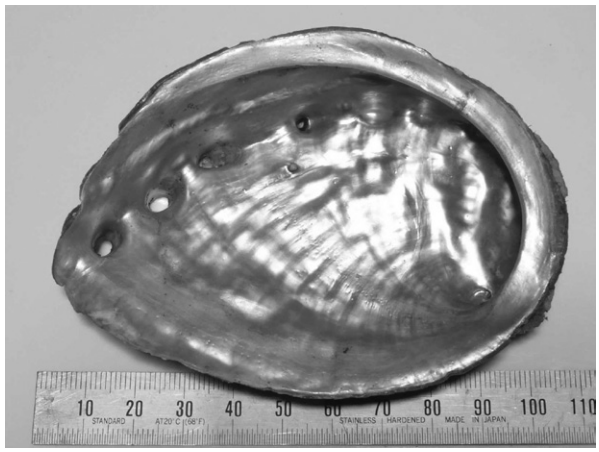


Fig. 24.2 Nacre of an abalone shell from Toba City, Japan. (Photograph by N. Yotarou.)

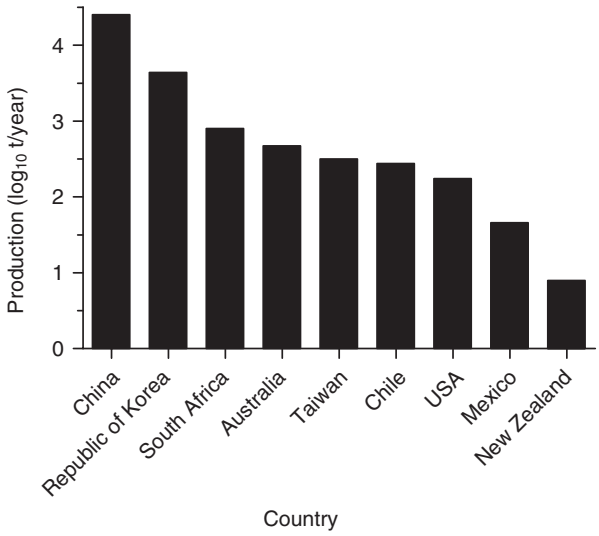


Fig. 24.3 Production of cultured abalone in 2007 in the nine countries with highest production (FAO Fishstat Plus, 2009). Note the $\log_{10}$ scale from ten thousands to unit levels.

Abalone live attached to rocks and boulders on rocky shores exposed to moderate to strong wave action. Some species are restricted to shallow waters, but most species are found to a depth of several tens of metres. Sexes are separate and sex can be determined by the colour of the gonad, which can be viewed by turning an abalone upside down and pushing the foot aside. The gonad develops on top of the digestive gland, showing a dark brown colour when immature, and turning green in females and cream-white in males as they mature.

Abalones are broadcast spawners; the eggs and sperm are shed freely into the surrounding water, where fertilisation occurs (Fig. 24.4). Typical of many marine invertebrate, spawning occurs during the warmer months of the year in many species. The tropical abalone *Haliotis asinina* offers the advantage of spawning all year round.

24.2.3 Culture techniques

24.2.3.1 Broodstock

There are the typical three options for obtaining mature broodstock:

1. Collect mature abalone during the spawning season.
2. Collect adults for conditioning before the spawning season.
3. Use farm-raised individuals.

As artificial diets for adults have developed, the second and third options are likely to become more popular.

Given adequate nutrition, water temperature seems to be the major environmental factor regulating the breeding cycle of abalone. Ambient temperature controls the rate of gonad development and therefore the timing of spawning. For example, daily increase in gonad index of *Haliotis discus* is greater as temperature increases. Furthermore, there is a positive relationship between the effective accumulative temperature, which is a measure of the total amount of time at a given temperature, and the percentage of abalone spawning. Thus, by keeping adults in good culturing conditions in terms of water quality and food availability, and at warmer than ambient temperatures, similar to those present during the spawning season, gonad development is accelerated (Fig. 24.5). Small changes in temperature can have an important effect on the results. For example, a recent study on wild-caught *Haliotis rubra* and *Haliotis laevigata* in Tasmania (Australia) showed higher success both in terms of gamete production and spawning rate when animals were kept at 16 °C instead of 18 °C and when they were held longer in the tanks. Adults are either fed on natural food, including seaweeds or on artificial feed. *H. asinina* fed either artificial diet or a combination of natural and artificial diet showed a higher reproductive performance in terms of spawning rate, fecundity and larval hatchability than those fed only natural diet, but they also showed slightly higher mortality.

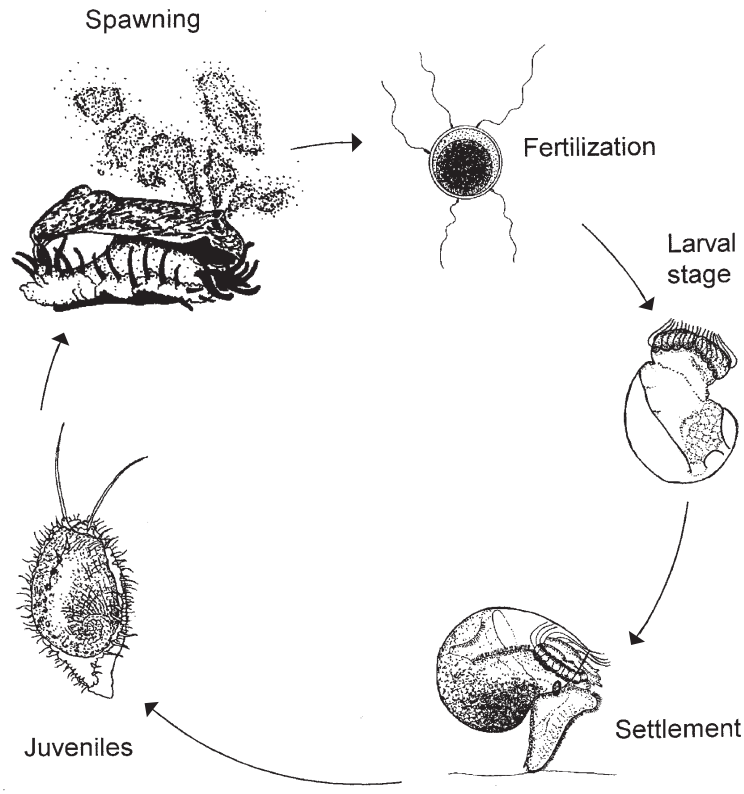


Fig. 24.4 Life cycle of abalone from fertilised egg to adult. (From Ann Fleming, with permission.)

24.2.3.2 Spawning induction

Ripe abalones often spawn spontaneously when collected, probably in response to handling stresses. Spawning can also be induced using a variety of methods. The three most commonly used are the addition of hydrogen peroxide (H_2O_2), (ultraviolet) light and increased water temperature:

1. H_2O_2 added to alkaline seawater induces gravid individuals to spawn. The peroxide stimulates the synthesis of a prostaglandin that is thought to trigger the spawning response.
2. Irradiation with ultraviolet light consists of passing water through a ultraviolet steriliser. The intensity of ultraviolet light required depends on the species, and the quality and temperature of the water. In Japan, an intensity of 2.42 W/L has been found to provide best results. Spawning response is generally observed within 3–8 h of exposure (Hahn, 1989; Fleming, 2000).
3. A sudden change in water temperature (lower or higher) may induce spawning in abalone; however, this 'thermal shock' method may result in variable spawning responses and immature gametes being spawned.

Desiccation or exposure to air is another induction method used in some farms. Generally, farms will combine two or three methods to induce spawning.

Released eggs are fertilised with a small volume of water containing sperm at a minimum density of 25 000/mL (Tong and Moss, 1992). After a maximum of 15 min, the eggs are repeatedly washed with filtered seawater and then transferred to clean hatching tanks.

24.2.3.3 Larval development

Larval development of abalone is illustrated in Fig. 24.4. The time between fertilisation and hatching varies between 10 and 24 h, depending on species and water temperature.

A few days after hatching, veliger larvae are collected and transferred to settlement tanks. Larval density varies between hatcheries and ranges from four to eight larvae/mL to 20–30 larvae/mL. Under favourable conditions,

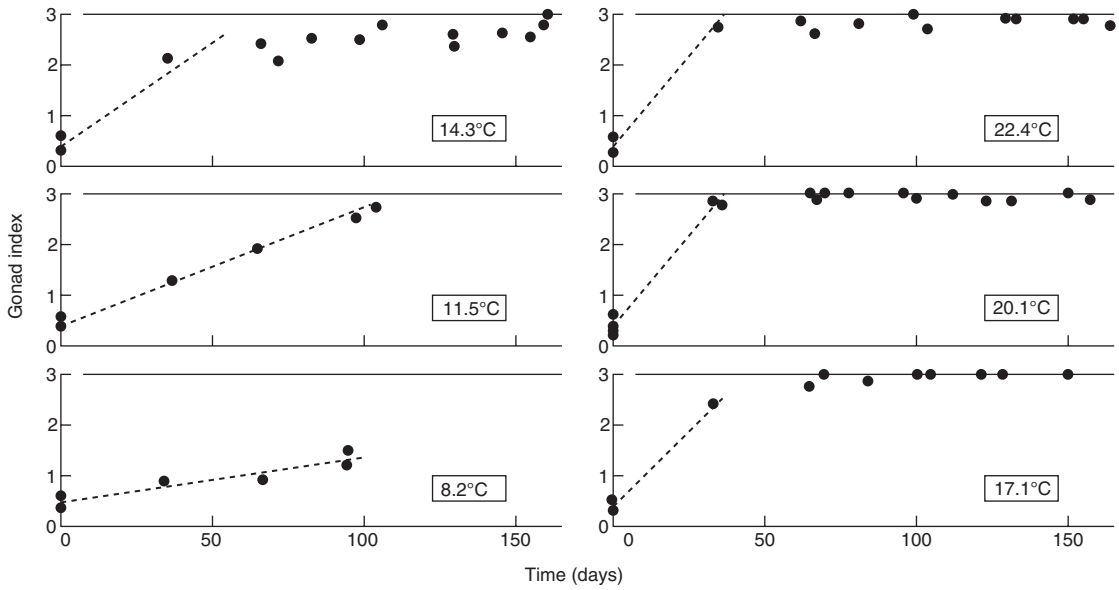


Fig. 24.5 Relationship between gonad development (expressed as gonad index) and water temperature in the abalone *Haliotis discus hannai* showing slower development at colder temperatures. (Reprinted from Uki and Kikuchi (1984) with permission from Elsevier Science.)

veliger larvae reach metamorphic competence at 3–8 days after fertilisation. Abalone larvae are lecithotrophic (section 6.3.4). As such, an exogenous food supply is not required; however, supplementing the water with dissolved organic matter (DOM) results in better growth. Larvae with sufficient yolk reserves and access to DOM in the water may extend their larval life; however, if they fail to settle after a certain period, they show low post-settlement survival (Kawamura *et al*., 1998).

24.2.3.4 Settlement and metamorphosis

The highest mortality occurs during this time, with survival typically ranging between 5 and 20%. This makes the period between settlement and the first few months one of the main milestones of abalone culture (Fleming, 2000) (Table 24.3).

Various natural and chemical substances have been shown to induce settlement and metamorphosis of abalone larvae:

- Encrusting coralline algae, microalgae (diatoms), bacterial films and are among the natural inducers.
- Pre-grazed settlement plates with abalone mucus. These are prepared by first allowing small juvenile abalone to graze on plates conditioned with diatoms before placing them in the settlement tanks.

Table 24.3 Example of survival rate at various stages of culture development of abalone.

Period of development	Survival rate (%)
Eggs to fertilisation	80–85
Fertilisation to larvae	80
Larvae to settlement	50
Settlement to 150 days	5–20
150 days to first year	80–90
First year to second year	90–95
Second year to 3–4 years	90–95

The period between settlement and the first few months is the most critical.

Modified from Fleming (2000) with permission from the FRDC Abalone Aquaculture Subprogram.

- The amino-acid neurotransmitter γ-aminobutyric acid (GABA) has been found to induce settlement and metamorphosis in several species of abalone.
- Monosodium glutamate, a cheaper alternative to some of the other chemical inducers, produced promising results for induction of *H. asinina* larvae (Stewart *et al*., 2008).

Good results in terms of growth and survival have been demonstrated with the use of pre-grazed settlement plates. These are prepared by first allowing small juvenile abalone to graze on plates conditioned with diatoms before placing them in the settlement tanks. This technique is now widely used in Japan (Takami *et al*., 1997). The bacteria present in the mucus left by juvenile abalone of the same species, combined with the mucus of the diatoms and the diatoms themselves, probably provide a good food source for the newly settled larvae as well as the older post-larvae.

Settling tanks are conditioned for several days to allow build-up of bacteria and diatoms on the tank walls. In some cases, tanks are inoculated with a diatom slurry (e.g. *Navicula* or *Nitzschia*) ca. 24 h before introducing the larvae. Conditioned settlement plates are also generally placed inside the tanks as they increase the settlement surface. The veligers are introduced into settling tanks at densities of 1000–2000/m^2 and left with minimal or no water flow for 1–4 days, until they have settled onto the plates or tank walls.

24.2.3.5 Juveniles

There is great variation in culture methods used for juvenile abalone. Very young abalone (2–5 mm SL), also referred to as post-larvae, show highly variable patterns of survival and growth, which are greatly influenced by the quality and quantity of their food. It is now clear that not all species of diatoms are beneficial and the specific diet of diatoms available to post-larval abalone is very important for controlling their growth and survival (Roberts *et al*., 1999). The different abilities of post-larvae to digest various diatom species are largely related to characteristics such as the diatom cell size, thickness and composition of the cell wall, and how hard they are attached to the substratum. The mechanism by which young abalone select their food remains an area of much-needed research.

Juvenile abalone are generally left in the settlement or nursery tanks until they reach a certain size and are then transferred to grow-out tanks, where they are left to grow until ready to sell. The size at which juveniles are transferred for grow-out culture varies. Their small size makes them especially vulnerable to handling so extending this period before transferring to nursery tanks can result in higher survival.

In China, 3–5 mm SL juveniles are moved from settlement plates to large punctured plastic plates (the holes improve water circulation). The plastic plates are supported in net-pans and placed in raceways until the juveniles reach up to 20 mm SL (Guo *et al*., 1999). In New Zealand, juveniles are left in the settlement tanks for 6–9 months, when they reach 10–15 mm SL (Tong and Moss, 1992), whereas in the USA juveniles are harvested at 10 mm. In Australia, juveniles are held in the settlement tanks until they reach 5–10 mm SL and then they are transferred to grow-out tanks (Fleming, 2000). In South Africa, juveniles are transferred at about 3–5 mm SL to an intermediate stage between settlement and grow-out. This stage consists of a small inverted cone sitting in a shallow tank. Animals hide under the cone and are fed algae, which is held down by the rim of the cone.

When juveniles are around 5–10 mm SL they start feeding on macroalgae (seaweeds). The supply of natural seaweeds depends on access to reliable sources throughout the year, and this can sometimes be a problem, especially as juvenile abalone can consume between 10 and 30% of their body weight per day (Hahn, 1989; Tong and Moss, 1992). Numerous species of macroalgae have been used to feed juvenile abalone, including species of *Macrocystis*, *Laminaria* and *Ecklonia*, although artificial feeds may be used (section 24.2.3.8).

24.2.3.6 Grow-out culture

Juveniles are grown to market size in farms using a variety of methods, including the following:

- long-line rafts;
- seacages (or barrels);
- land-based tanks;
- intertidal ponds;
- stock enhancement.

Factors such as the availability of land, the quality of surrounding coastal waters, and their degree of exposure to big swells and wave exposure determine the preferred culture method. Barrels and cages suspended in long-line rafts are the most common methods used in Mexico (barrels) and China (cages), although raceways stocked with plastic plates are also used in China (Guo *et al*., 1999). In Japan, a complex raft system holding tanks and seacages is used to grow juveniles up to 30 mm. Each raft can hold 400 000 juveniles between 15 and 30 mm SL (Hahn, 1989). A multiple-tier basket system has been used in Taiwan since the late 1990s. Abalone ca. 20 mm SL are placed inside baskets and the baskets are stacked in several (up to 14) tiers in indoor cement ponds (Fig. 24.6) (Chen and Lee, 1999). The tier systems (using baskets or raceways) represent strategies to use space more efficiently in places where land is limited. Land-based tanks and a system of barrels suspended in the ocean are commonly used in California and Chile (although sea barrels in Chile

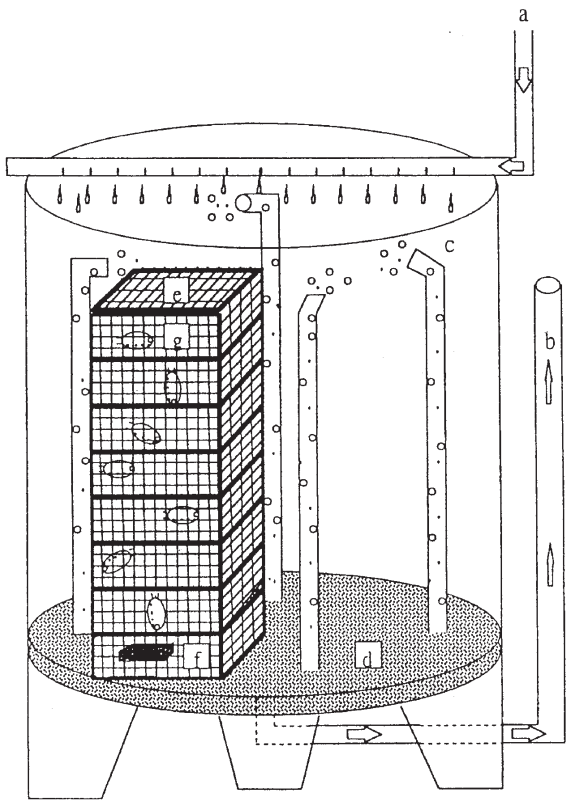


Fig. 24.6 Diagram of a multiple-tier basket system used to culture juvenile abalone (ca. 26 mm SL) in Taiwan. (a) Water inlet; (b) water outlet; (c) airlift; (d) gravel; (e) basket; (f) cement block; (g) abalone. (Reproduced from Chen and Lee (1999) with permission of Sandra Shumway/Journal of Shellfish Research.)

are used under strict conditions because abalone is not a native species), whereas land-based tanks are the preferred culture method in Australia and South Africa, where coastal waters are generally exposed to strong wave action, and have been the preferred choice in the south of China. Intertidal pond farming developed in China in the late 1990s. It consists of large 1- to 2-m-deep cement ponds where 2- to 2.5-cm juveniles are released and left for up to 20 months before harvesting.

There has been intensive research into the design of grow-out tanks to maximise survival and growth rates of abalone, while minimising the need for labour. Culture methods are evolving to suit the specific needs of each farm in terms of location and cultured species.

Growth rates of cultured abalone vary with species and culturing conditions (e.g. size, stocking density, temperature and salinity, percentage of oxygen saturation, water quality, food supply and culturing method). Maximising growth rates is one of the main driving forces in the development of culturing techniques. Growth rates for most species, typically temperate species, are generally up to a maximum of 30 mm/year in SL. Some examples are *H. laevigata* (20–30 mm/year), *H. rubra* (15–25 mm/year) (Fleming, 2000) and *H. iris* (ca. 20 mm/year) (Tong and Moss, 1992).

The fastest growth rates occur in the tropical abalone *H. asinina*, which can reach a size of 60 mm SL in 1 year (Capinpin *et al*., 1999). Although the culture of this species is at a relatively early stage, its fast growth rate, small commercial size and potential to spawn throughout most of the year give it great culturing potential.

24.2.3.7 Seaweed diets

Natural seaweeds are still mainly used in abalone culture and alternatives to collection from the wild remain an important area of research. Culturing methods have been developed, in some cases for many years, for some species of seaweed that are also used for other purposes (Chapter 13). For example, the alga *Gracilaria* has been commercially cultured in Taiwan since the early 1960s and now most of the produce is used to feed abalone (*Haliotis diversicolor supertexta*) (Chen and Lee, 1999).

Methods of feeding during grow-out vary considerably. In many regions, abalone are typically fed one main species of seaweed, generally the most abundant in their habitat or in the area surrounding the farm or one that is cultured for the purpose. A few other seaweed species and/or gel-based artificial feeds may used as needed if main supplies are low. For example, fresh kelp (*Laminaria* species) is one of the primary foods used in China and Japan (Guo *et al*., 1999; Hahn, 1989).

Abalone can feed on a variety of seaweed species and they may grow better when feeding on a mixture of diets given on a rotation of one species at a time (Simpson and Cook, 1998). There is also evidence that a combination of natural seaweed and artificial diets can result in faster growth than a single-seaweed diet (e.g. Chen and Lee, 1999). Even though many farms have established methods for feeding abalone with reasonable results, there is awareness that much more can be done in terms of increasing the productivity of abalone through the choice of diets.

Stocking densities in grow-out tanks play an important role on juvenile growth rates. It is not simply a matter of adding more food to the culturing system, because high densities can affect access to food even when this is available (Lloyd and Bates, 2008).

24.2.3.8 Artificial diets

Japan was probably the first country to develop and use artificial diets for abalone, but now most farms use artificial diets at some stage of their production. Despite their widespread use, commercial production of artificial diets was until recently mainly restricted to Japan and China, reflecting level of demand.

The development of artificial diets remains an area of intensive research, as there is great pressure to reduce manufacturing costs and improve diet performance. Besides the importance of the nutritional content of diets, aspects such as making them more attractive to the abalone and reducing the rate of decomposition and leaching of nutrients are also critical to the success of feeds. These are therefore receiving increased attention. There is also the additional factor that abalone change their diet as they grow and consequently different feeds need to be developed for small (<10 mm SL) and large juveniles. The various artificial diets are relatively similar in their compositions, with characteristically high protein and carbohydrate content, and low levels of lipid and fibre (Table 24.4). There is great scope for improvement in this area.

Besides the innate differences among species that make some abalone products more appealing to particular markets than others, factors such as diet, handling, transportation and processing methods can all influence several important factors reflecting the quality of the abalone product, including taste, texture, colour of the meat and the shell. There is still limited understanding of how these factors affect the quality of abalone, a fact that, when combined with the extensive range of the market in terms of species cultured and countries consuming the product, has made it difficult to decide on a standard set of characteristics to assess the quality of the abalone.

24.2.3.9 Diseases and pests

As the scale of abalone culture has increased, problems caused by deterioration of environmental parameters such as water quality and contamination, together with diseases and pests, have also intensified. The advent of diseases and pests that seriously affect abalone culture occurred in the mid-1990s. Two main types of infectious disease and two pests have been identified:

1. The first type of infection is caused by viruses, one of which, the withering syndrome, is a progressive debilitating disease that usually leads to the death of the abalone. It was initially found to affect only the black abalone (*Haliotis cracherodii*) in Californian farms, but has now been reported in several species, including the red (*H. rufescens*), pink (*H. corrugata*), white (*H. sorenseni*) and green (*H. fulgens*) abalone. The positive relationship between the intensity in the signs of the disease and the degree of infection by Rickettsiales-like prokaryote indicates that Rickettsiales-like prokaryote is responsible for this disease. A new type of viral disease known as abalone viral ganglioneuritis was discovered for first time in 2005 in wild broodstock, decimating a very large fraction of farmed and wild abalones in Australia. Abalone exposed to high temperatures appear to be at a greater risk of infection, a problem for aquaculture farms that in general do not have the facilities to reduce water temperature.
2. A bacterium (*Vibrio fluviales*) causes the pustule disease, which affects the Asian abalone (*H. discus hannai*), resulting in high mortality of abalone in hatcheries.
3. Infestation by a boring sabellid worm (*Terebrasabella heterouncinata*), was first discovered in the mid-1990s in Californian abalone farms as a result of its damaging effect on the abalone. The sabellid is endemic to South Africa and has since also been found to cause infestations in South Africa (Ruck and Cook, 1998). These pests bore tiny holes into abalone shells. Infected individuals show markedly reduced growth rates and meat yield, and they are generally weaker and more likely to die.
4. Another boring polychaete, the mudworm (*Polydora* species), has affected mainly sea-based farms in Tasmania, Australia, leading to mortalities of more than 60% of the stock. Removal of infected stock in combination with fast growing conditions and good water quality are successful measures to combat this pest (Fleming, 2000).

Table 24.4 Approximate mean proportions of the main nutritional components of artificial feeds for abalone (information collated from Fleming *et al.*, 1996).

Nutrient	Average (% dry matter)	Range of values (% dry matter)
Protein	30	20–50
Lipid	4	1.5–5.3
Carbohydrate	47	30–60

24.2.4 Challenges

The aquaculture of abalone continues to face several challenges, their importance varying with location and the

particular characteristics of farm systems. Among the most important are the following:

1. Improving growth rates, disease resistance and other favourable characteristics through stock selection programmes and use of modern genetics techniques (see, for example, Dunstan *et al.* 2007).
2. Reliability of constant and good quality food suply, both from natural sources and artificial feeds, remains an important constrain for many farms.
3. The occurrence of disease outbreaks that can have devastating effects on farms. Although research in this area has intensified, the prevention and treatment of diseases remain areas in much need to research.
4. For land-based farms, the availability of land in appropriate locations.
5. The establishment of an aquaculture farm requires an initial high capital investment and it can take several years for a farm to begin making net profits. From this point of view the culture of a fast-growing species such as *H. asinina* is very appealing.

24.3 CONCHS

24.3.1 Biology

Queen conchs (*Strombus gigas*) are found throughout the Caribbean, from Bermuda and the Bahamas in the north, to the Brazilian coast of South America. The natural habitat of the adults is shallow (ca. 8 m) seagrass beds that are protected from wave exposure and which contain sand patches and coral rubble covered with turf algae. They feed on seagrass, algae and drifting organic debris.

Queen conchs are dioecious and fertilisation is internal as the male copulates with the female. The female then lays egg masses within several weeks of copulation. Reproduction generally occurs during the warmer months of the year and lasts 6–8 months, depending on the locality.

24.3.2 Commercial importance

Queen conch meat is eaten throughout the Caribbean region and they are overfished, which has led to the interest in culturing them. Furthermore, they are collected for their attractive shells and, very recently, a method has been developed to produce cultured pearls from the conchs.

24.3.3 Culture

Dr Megan Davis-Hodgkins, Harbor Branch Oceanographic Institution, is an authority on conchs and has published several papers on their culture. Much of this information is contained in Davis (2000).

24.3.3.1 Broodstock

Queen conchs become sexually mature at 3–4 years of age and 180–260 mm SL (distance from the tip of the siphonal canal to apex of the spire). With the onset of sexual maturation, growth in shell length ceases and the pink flared-lip is formed by progressive thickening (Fig. 24.7).

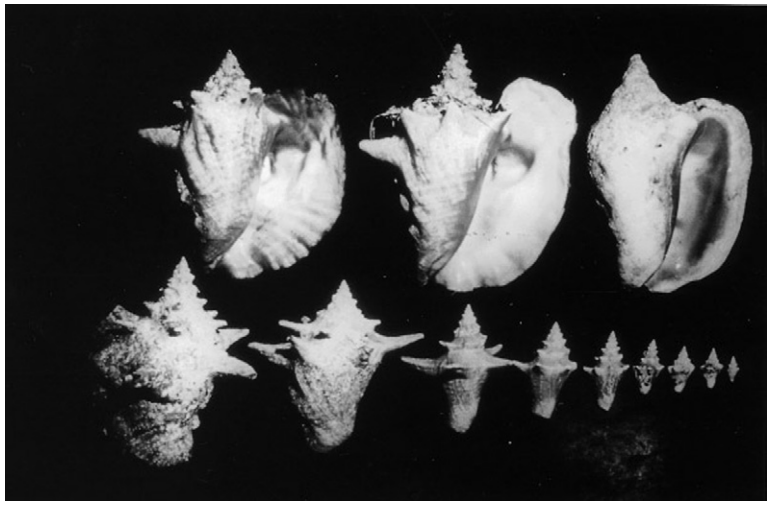


Fig. 24.7 Size progression of the queen conch *Strombus gigas* showing changes in shell form and development of the flared lip in adults. (Photograph by Katherine Orr.)

Before the spawning season, sexually mature conchs are collected and moved the broodstock to enclosed breeding sites (commonly known as 'egg farms').

24.3.3.2 Production and collection of egg masses

Egg mass production shows seasonal variation and is positively related to water temperature and weather conditions. The crescent-shaped egg masses are generally deposited in sandy substrates with some coral rubble covered with algae or seagrass. The average number of egg masses per female is in the order of one to four per month. An egg mass of ca. 100 g wet weight contains 400 000 eggs.

Fresh egg masses are collected from the 'egg farm' or field and transported to the hatchery in plastic bags filled with seawater. In the hatchery, egg masses are cleaned and disinfected with 0.5% sodium hypochlorite. They are left to hatch in flowing filtered water or in static water tanks with periodic water changes.

24.3.3.3 Larval rearing and settlement

Larvae start hatching 3–5 days after the egg mass is produced. Unlike abalone and trochus, which have brief periods of lecithotrophic larval development, conchs have a long period of planktotrophic larval development. This means that, compared with abalones and trochus, much more time and effort must be invested in rearing conch larvae, including the need for large-scale cultures of microalgae. Development of conch larvae to metamorphosis may take up to 40 days. Larval culture is one of the most difficult aspects of conch culture. Three critical developmental stages have been identified in veligers and these are related to nutrition:

1. at 3–5 days after hatching, when food becomes increasingly important;
2. around 6–11 days when yolk reserves are exhausted;
3. at the time of metamorphic competence.

Conch veligers are fed microalgae. The flagellate *Isochrysis* species and the diatom *Chaetoceros gracilis* are among the most commonly used species.

Metamorphosis can be induced using the following:

- water-soluble extracts of a natural inducer, such as *Laurencia*;
- the red alga, *Laurencia* species;
- neuroactive compounds such as KCl and H_2O_2.

24.3.3.4 Juveniles

Newly metamorphosed conchs or post-larvae (3–4 mm SL) are placed in sand trays at densities of 3200 conchs/m^2. These trays have a window screen sewn to the bottom to allow water circulation, and they are filled with a layer of 1-cm-deep clean sand. The trays are placed in fibreglass tanks and cleaned periodically.

Conch post-larvae graze on benthic algae. However, fast-growing planktonic algae, such as the diatom *C. gracilis*, can be used as a food source for conch of 1–4 mm SL after flocculation

After reaching a manageable size (12–15 mm SL), juveniles are cultured using a variety of methods, including shaded ponds and raceways. Densities need to be reduced as juveniles grow, from 275 conchs/m^2 for 12–15 mm SL to 30–50 conch/m^2 for conchs of 60 mm SL.

Artificial feeds for juveniles larger than 5 mm SL became available in the late 1990s. Two types of artificial food are used to feed juvenile conchs: a gel-based diet and a pelleted conch chow. They are now used in some farms and support growth at least comparable to that observed using natural diets.

24.3.3.5 Grow-out

Sea enclosures can be used to grow the larger juveniles, from 9 to 16 cm in SL over 1 year. Protection from predators using appropriate fencing material and traps is needed. Densities in these enclosures are kept at natural levels (one to two conchs /m^2) and groups must be rotated around several enclosures to avoid overgrazing.

24.4 TROCHUS

24.4.1 Biology

Trochus is a herbivore that grazes on the algal turfs covering coral rock. It inhabits the intertidal and subtidal zones of coral reefs exposed to wave action and is generally found at depths shallower than 10 m. Juveniles are are very cryptic and most frequently found on the intertidal reef flat.

24.4.2 Commercial importance

Trochus has been a major source of family income and an export product in some Pacific islands. The pearly nacre inside the shell is used to make 'pearl' buttons and handicrafts, whereas the meat of the muscular foot is eaten fresh or dried (Fig. 24.8). Adult trochus have been introduced to several Pacific islands, with some successes, for example the Cook Islands. The fishery declined with the introduction of synthetic buttons, but has recovered with a return in fashion to 'natural' buttons.

Lee and Lynch (1997) is a major source of information on the biology and aquaculture of trochus.

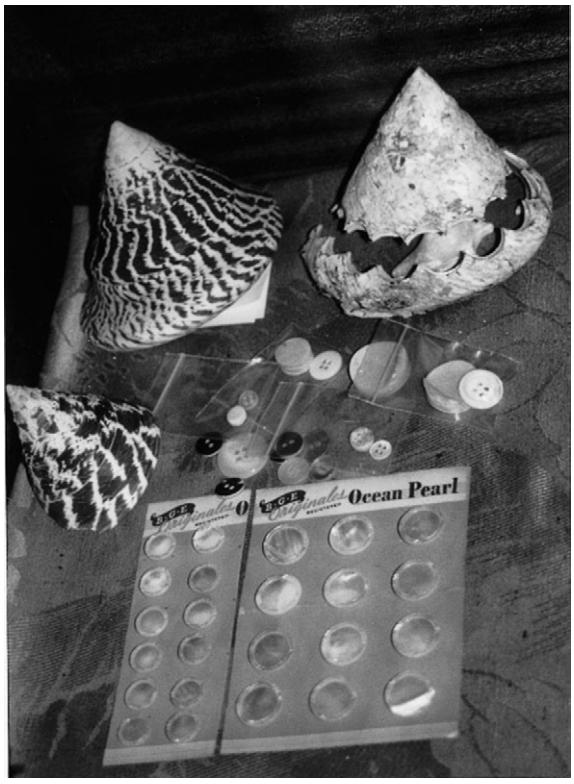


Fig. 24.8 Shells of *Trochus niloticus* showing the characteristic pattern and their use in manufacturing buttons. (Photograph by John S. Lucas.)

24.4.3 Culture methods

24.4.3.1 Broodstock and spawning

Trochus are dioecious. However, to determine sex, the animal must be pushed back into its shell and extrusion of some gametes may occur from gravid individuals.

Although trochus may have periods of more intense spawning in the wild, it may be possible to obtain a successful spawning throughout most of the year (Nash, 1993), with the exception of periods of lowest mean gonad index (gonad size/body size).

Males held in tanks may spawn on successive nights. Females, however, have not been observed to spawn more than once within a month. Furthermore, the proportion of males spawning in tanks is generally much larger than the proportion of females.

For spawning, trochus broodstock are best collected from the wild a few days before the new moon, minimising the need for long stays in tanks. Most trochus larger than 80 mm SD are reproductively mature. Between 20 and 50 individuals are needed for small-scale production and, at a larger scale, 100 adults may be used. The newly collected broodstock are kept in tanks with static and strongly aerated seawater. Spontaneous spawning generally occurs at any time during the afternoon or evening. Ultraviolet-irradiated water and elevated water temperature may be used for spawning induction.

Males generally spawn first, releasing white aggregates of sperm. The quantities of sperm released can be very large and may rapidly cloud the water. Females release up to one million eggs per spawning event.

The eggs, ca. 180 µm in diameter, are siphoned from the spawning tank and then cleaned of excess sperm and mucus by repeated washing with filtered seawater. They are then transferred into containers filled with filtered (1–10 µm) seawater, preferably at densities <1/mL.

The eggs can be reared in the following:

- static water with light aeration;
- small containers lined with ca. 100-µm mesh and partly immersed in a larger container or tank supplied with gently flowing filtered water.

24.4.3.2 Larvae

Larval development of trochus is outlined in Fig. 24.9. Trochus larvae hatch as trochophores, 10–24 h after fertilisation. Hatched larvae can be left in the container until they are competent to metamorphose. Feeding during larval development is unnecessary, as trochus larvae, like abalone larvae, are lecithotrophic.

24.4.3.3 Settlement and metamorphosis

Veligers are ready to settle after ca. 3 days at 27–30 °C. Well-developed veligers have dark pigment spots or ‘eyes’, a large propodium and a reduced velum. They spend increasingly long periods on the tank bottom with frequent but short bursts of swimming activity. At this stage the larvae are transferred to the juvenile rearing tanks, which have been conditioned for 2 or 3 weeks to allow a film of algae and bacteria to develop on the walls of the tank. Water is kept static with gentle aeration for a few days, until no swimming larvae can be seen. The algal surface serves to induce metamorphosis and is utilised as a food source for newly settled individuals.

24.4.3.4 Juveniles

The nursery tanks where larvae settle are commonly used to grow juveniles, with increased water flow and continuous replacement of conditioned plates, minimising the

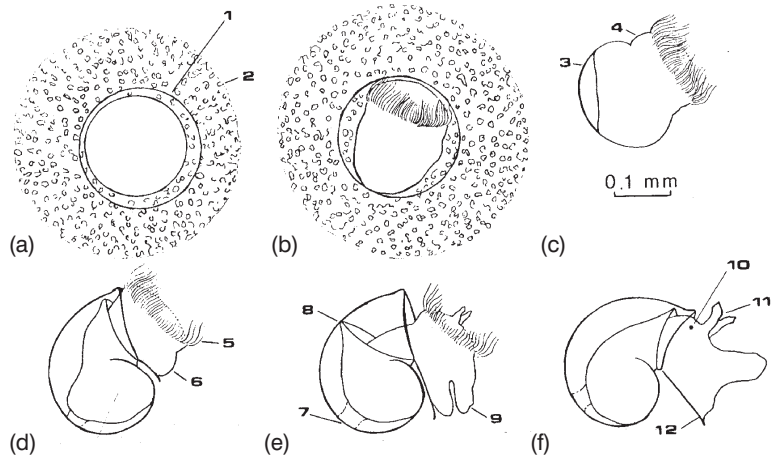


Fig. 24.9 Larval development of *Trochus niloticus*. (a) Fertilised egg; time (*t*) = 3 min after fertilisation. (b) Trochophore just before hatching; *t* = 11.5 h. (c) Pretorsional trochophore with embryonic shell growth; *t* = 14 h. (d) Veliger with complete larval shell, after 90° torsion; *t* = 20 h. (e) Veliger with reduced velum, approaching metamorphic competence; *t* = 65 h. (f) Metamorphosed juvenile; *t* = 73 h. (1) Vitelline membrane; (2) jelly layer; (3) embryonic shell; (4) foot rudiment; (5) velum; (6) foot; (7) attachment site of larval retractor muscle; (8) mantle margin; (9) propodium; (10) eye; (11) cephalic tentacles; (12) operculum. (Reproduced from Heslinga, (1981) with permission. Courtesy of George Davis, Department of Microbiology and Tropical Medicine.)

need for handling small juveniles. The juveniles graze on the algae-covered surfaces within the rearing tank. As juveniles grow, their food intake increases; for example, Dwiono *et al*. (1997) found that 20 000 individuals of 4–7 mm SD would graze out the algae growing on the tank surface (6.5 m^2) in just 1 week. Continuous replacement of algal plates is therefore necessary. Plates are conditioned by suspending them in the sea for 1 or 2 weeks or by inoculating them with cultures of diatoms. An artificial diet is yet to be developed for trochus. The problem is that trochus, unlike abalone and conchs, only feed by grazing on algae on hard surfaces.

Young juveniles grow at 1–2 mm/month and 30 mm SD can be reached in ca. 1 year (Nash, 1993). The grazing substratum has an important effect on growth. Other factors such as culture density and husbandry techniques affect juvenile growth rate. The fastest growth rate of cultured trochus was reported by Heslinga (1981), who found that juveniles reached an average 60 mm in SD in 1 year when growing at low densities in concrete raceways at 27–30 °C.

24.4.3.5 Grow-out

Although juveniles up to 30 mm in SD can be reared in tanks, labour requirements to sustain their heavy feeding on algae-coated surfaces and the running costs of large onshore aquaculture facilities are limiting when attempting to rearing on a commercial basis. Grow-out culture has been tried using floating cages made of plastic mesh. Cages fixed to the reef flat have been tried in eastern Indonesia and more recently in Vanuatu. In Indonesia, cages were made of 2-mm fishing net and concrete brick walls, and were stocked with 8- to 15-mm SD juveniles,which grew faster than tank-reared juveniles (Dwiono *et al*., 1997). In Vanuatu, juveniles were cultured in three types of cage: steel and plastic cages fixed to the reef, and plastic floating cages. The best results for growth were obtained with the reef-based cages, but there was higher recovery of individuals in the floating cages.

24.5 STOCK ENHANCEMENT

In response to overfishing and dwindling stocks, there have been attempts at restocking natural populations with small juveniles of abalone, conchs and trochus obtained from culturing. The success of these attempts has been highly variable and questionable. Many factors seem to affect the success of these releases: factors such as juvenile size, habitat characteristics, predator types and their abundances, and handling techniques. Size seems to be a major factor and, although it is desirable to release large individuals, this requires the expense of keeping them in

Table 24.5 Comparison of development and culture methods for some gastropods treated in this chapter. The phases that are the greatest hindrance to their commercial culture are indicated in bold.

	Abalone species	Queen conch	Trochus
Sexes	Dioecious	Dioecious	Dioecious
Size at maturity	Varies with species		50–90 mm shell depth
Reproductive seasonality	Usually summer spawning	Warmer months	Warmer months
Broodstock	Usually from field	Egg masses collected from field	Collected from field
Broodstock conditioning	Food and temperature	n/a	Collected just before spawning
Spawning induction	Peroxide; thermal shock; ultraviolet irradiated water	n/a	Largely spontaneous
Larval development	10–24 h	**Up to 40 days**	ca. 3 days
Larval nutrition	Lecithotrophic	**Planktotrophic; fed with microalgae**	Lecithotrophic
Initial shell length	2–5 mm	3–4 mm	2 mm
Early nursery facility	Settlement tank	Sand trays in tanks	Settlement tank
Early nursery period	Up to 9 months	5+ months	1 year
Early nursery food	Diatoms on tank and other surfaces	Benthic algae in sandy substrate	Algae on surfaces of conditioned plates
Late nursery size	10–15 mm SL	12–15 mm SL	30 mm
Late nursery facilities	Raceways, tanks	Ponds and raceways with sandy substrate	Raceways
Late nursery food	Macroalgae; manufactured feeds	Gel-based and pelleted manufactured feeds	**Algae on surfaces of conditioned plates**
Grow-out culture facilities	Longline rafts, baskets and seacages; tanks; intertidal ponds	Protective mesh enclosures on sandy substrates	**Experimental: floating cages and cages fixed to reefs**
Grow-out food	Macroalgae; manufactured feed	From grazing in their normal habitat	Grazing on algae coating hard substrates
Diseases and pests	Virus and *Vibrio* infections; boring polychaete worms	No data	No data
Period to good commercial size	**4+ years for most species**	No data	No data
Genetic manipulation	Hybridisation; triploidy	X Life cycle not closed?	X Life cycle not closed

prtected nursery-stage facilities for several years. Despite the variability in results, mass stock enhancement with juvenile abalone is commonly practised in Japan, as it is with other shellfish and fish.

Introductions are another story. Since the 1920s, groups of adult trochus have been introduced to various Pacific islands. Not all of these introductions have apparently been successful, but there are now good populations of trochus in the Cook Islands and Tonga.

24.6 CONCLUSION

There are 60 000–80 000 gastropods species; obviously they represent a huge range of morphology, biology, habitats and development patterns. Abalone, conch and trochus reflect some of this variation in their different habitats, diets, and modes and periods of development. These obviously influence the types of culture required and present different problems to be overcome in their commercial aquaculture (Table 24.5). In the case of the abalone, it is

the long period required to reach their large adult size: a period that involves far too many years before there is a positive cash flow in establishing a farm. This is being overcome by marketing the abalone at 'cocktail' size. There is another opportunity to be realised with the small species *H. asinina*, which grows rapidly in tropical waters to reach 'cocktail' size in about a year. Conch and trochus are still at very early stages of commercial culture. The long period of planktotrophic development of the conch is the most demanding phase of culture. But this should not be a long-term problem, because similar techniques for rearing planktotrophic bivalve larvae are well developed. The food of trochus and its mode of feeding are a major problem. As they get larger in the nursery facility, they require increasingly large amounts of algae-conditioned surfaces. These are labour intensive to provide and there is increasing pressure to get the trochus into the field and grow-out phase. However, they are still very vulnerable to predation in their natural habitat after a year of nursery phase growth. Large surface areas that are protected against predators are required and as yet no satisfactory techniques have been devised.

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25
Ornamentals

Daniel Knop (marine) and Jonathan Moorhead (freshwater)

25.1 INTRODUCTION

The maintenance of aquatic organisms for aesthetic and ornamental purposes has been practised for almost 1000 years. It finds its origins in ancient China during the Sung Dynasty (ca. 960–1279 AD), with the goldfish, *Carassius auratus* (Balon, 2004). This species was kept in outdoor 'ponds of mercy' or as a part of a manicured, ornamental garden, and has remained popular since the introduction of the indoor aquarium in 1548. Establishing rapid and reliable transportation and improving shipping techniques have allowed the aquarium hobby to reach world popularity. The market for, and trade of, ornamental species in the past 25–30 years has grown significantly (Wittington and Chong, 2007). The past 15 years in particular have seen developments in nutrition, responsible captive care and life-support systems, making aquarium care easier and more successful, especially in maintaining marine organisms. This has boosted the popularity of the aquarium hobby, and has contributed significantly to recent trade and industry growth.

25.2 THE AQUATIC ORNAMENTAL INDUSTRY

The trade of aquatic organisms for home and commercial aquariums, and water gardens, along with associated equipment, furniture, accessories and literature, has become a multi-billion dollar industry known as the aquarium or aquatic-ornamental industry (AOI) (Prang, 2007). The most recent estimates of the value of the AOI are in the vicinity of US$15 billion per annum (Prang, 2007).

Among a myriad of aquatic organisms often traded, freshwater and marine fish species are the most popular, and, aside from aquarium supplies, form the primary product of the AOI (Lecchini *et al*., 2006). On a unit weight basis, ornamental fish are the most valuable fisheries commodity in the world (Hardy, 2003).

Maintenance of comprehensive and accurate trade statistics that define trends in AOI trade activities are limited and variable. This is largely due to the complexity of the industry, incomplete or misclassifications of aquatic species, differences in freight stocking densities, and sporadic or absent record keeping displayed by importing and exporting countries. However, annual world retail trade values, for aquarium fish alone, have been estimated at nearly US$1 billion to more than US$3 billion (Olivier, 2003).

25.3 TRADE IN ORNAMENTAL FISH

Export of ornamental fish makes up about 1% of the total world trade in fish, with an export value of just under US$200 million (Olivier, 2003). Among the 100 or more countries known to be involved in exporting ornamental fish, 11 provide a little over 75% of the total export value, of which Asian countries dominate (Fig. 25.1). Singapore provides about 24% of the total export value, followed by Hong Kong (9.4%), Indonesia (7.0%) and Malaysia (6.3%). Although Singapore does propagate fish locally, its position as the largest exporter comes from being the central 'hub' of ornamental fish trade for the Asia region (Olivier, 2003; Prang, 2007). Low import duties in Singapore allow Singapore-based importers and exporters to import fish from other Asian countries and re-export cheaply (either immediately or after a period of reconditioning) to more than 60 countries around the world (Olivier, 2003).

The aquarium hobby is practised mainly in industrialised countries and the leading importers of ornamental fish

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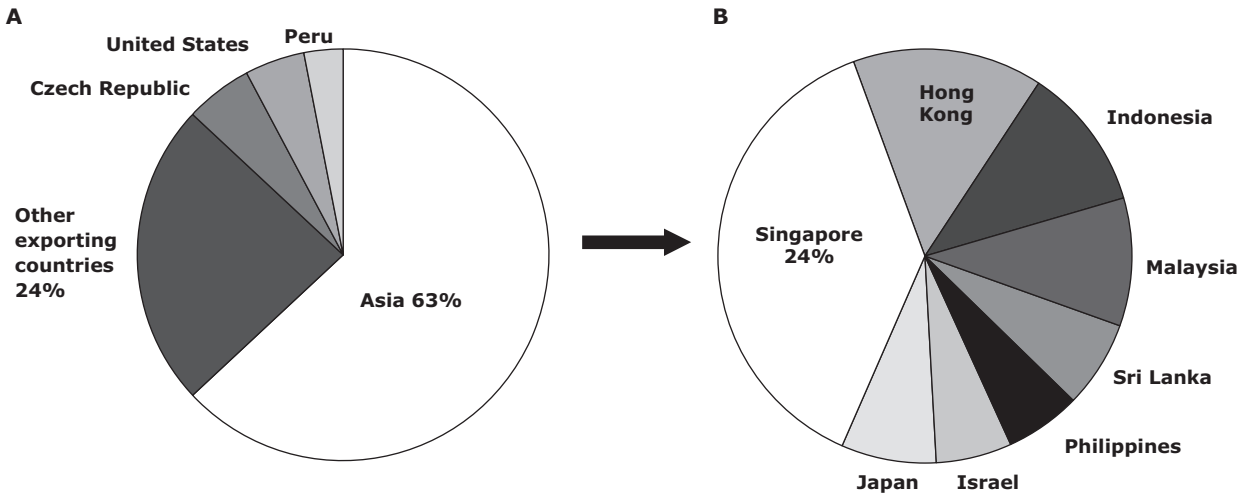


Fig. 25.1 The share, in value, of the world trade in ornamental fish for the major exporting countries (left pie chart), with countries of Asia displayed separately (right pie chart). (Adapted from Olivier, 2003.)

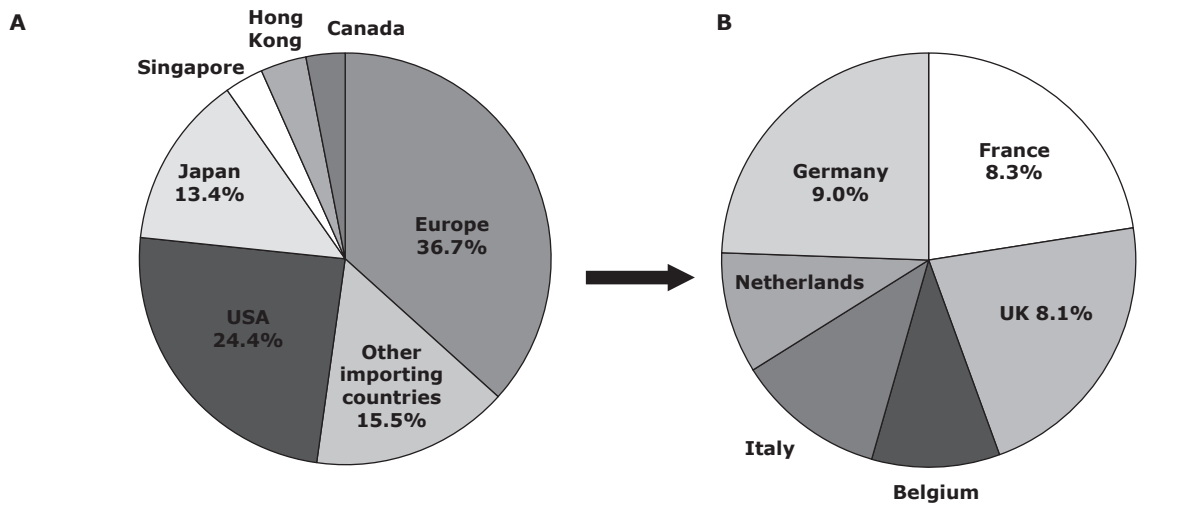


Fig. 25.2 The share, in value, of world trade in ornamental fish for the major importing countries (left pie chart), with countries of Europe displayed separately (right pie chart). (Adapted from Olivier, 2003.)

reflect this trend (Olivier, 2003; Prang, 2007). The USA leads the world in ornamental fish imports, followed by Japan and countries in Europe (Fig. 25.2). The major importing countries currently source most of their ornamental fish from Asian countries, in particular Singapore (Olivier, 2003).

Trends in imports and exports can vary from year to year depending on factors including the economic situation in the importing/exporting country, the popularity of the aquarium hobby in competition with other activities (e.g. information technology), disease outbreaks, and major weather phenomena such as El Niño (Olivier, 2003;

Prang, 2007). Trade trends have also recently been affected by a shift in sourcing ornamental fish from regions closer to the consumer. Countries such as Thailand, Indonesia, Singapore, China (including Hong Kong), Malaysia and Japan have long specialised in breeding and propagating freshwater ornamental fish. However, European importers for example are increasingly turning to producers closer to their markets, such as the Czech Republic, Spain, Israel, Belgium and the Netherlands for supplies of freshwater ornamental fish, in order to reduce transport costs and make higher on-sale profit.

25.4 COMPARING THE FRESHWATER AND MARINE ORNAMENTAL FISH TRADES

Freshwater ornamental fish and their marine counterparts differ significantly when it comes to the volume and value of specimens traded and the methods by which they are sourced. Of the 4000–5000 fish species kept in aquaria worldwide, approximately 1500 to 1600 are commonly traded in the ornamental market Olivier, 2003. Roughly half or more (ca. 750 species) are from freshwater, whereas the others come from marine sources (Olivier, 2003; Wittington and Chong 2007). By value, freshwater fish dominate the market, making up approximately 90% of sales (Olivier, 2003; Wittington and Chong, 2007). In volume, however, these values would be much higher for freshwater fish, owing to the stronger unit value of marine ornamental fish sales.

Of the portion of freshwater fish traded, it is estimated that 90–96% are captive reared, with the remainder collected from the wild. In contrast it is estimated that between 90 and 99% of all marine ornamental fish traded are wild caught.

Even though most freshwater fish within the ornamental trade are reared in captive breeding facilities, collection from the wild is still a large-scale sector involving millions of fishes from a large range of habitats. The main areas from which wild caught freshwater fish originate are South America, including Columbia, Peru and Brazil, south-east Asia, particularly Thailand and Indonesia, and Africa, including Congo, Nigeria and Malawi. The socio-economic importance of these fisheries can be very high (Chao and Prang, 1997). Fish originating from South America, in particular, are almost exclusively wild caught, and originate mainly from the Amazon basin. In some cases wild capture is seen to be a threat to wild species, and has been blamed for local extinctions in areas such as south-east Asia and local declines in the Brazilian Amazon. However, there are also ornamental fish species that have been conserved through the ornamental trade, via successful captive breeding programmes (Ng and Tan, 1997).

Marine aquarium fish are known to originate mainly from the tropical archipelagos of Indonesia, as well as the Philippines, Sri Lanka, the Maldives, the Caribbean, regions of the Red Sea, and central Pacific islands such as Hawaii. Like that of freshwater species, wild capture of marine ornamental fish has also been blamed for localised declines in several popular ornamental species. This is an ongoing conservation concern, particularly considering that at least one-third of coral-reef fish species are collected from poorly managed fisheries, in developing countries such as the Philippines and Indonesia, where destructive capture methods are widely used.

Despite the dominance of freshwater fish in worldwide trade activities, the popularity of marine aquaria is growing and stands to be a major growth sector in the AOI in the future. This is particularly true as interest grows in the value of marine ornamental aquaculture, for the ornamental trade, and as a part of the evolution of effective coral-reef management plans. Such interest lends great potential to the growth of the captive breeding industry for marine ornamental fish.

25.5 TROPICAL MARINE ORNAMENTALS

The past 100 years, or so, have seen the marine aquarium hobby develop rapidly as a result of increasing knowledge about marine organisms and general technical improvements that led to innovative aquarium technology. About 100 years ago it was difficult to maintain high water temperatures, so aquarists in Europe mostly concentrated on fish and invertebrates, without symbiotic zooxanthellae, from the temperate Mediterranean Sea. During the 1960s, the marine aquarium hobby focused mainly on tropical reef fish which were kept in ‘sterile’ tanks decorated with coral skeletons regularly bleached for cleaning. During the 1980s, in Europe a tremendous shift began to convert the average marine aquarium from a fish tank into a coral reef tank. Living corals started to be the centre of attraction, and coral fish were mainly playing the role of complementary organisms for the ‘little coral reef in the living room’, excluding all those coral fish that prey on corals. In the USA this is different; though the same coral-oriented type of aquarist is found, most of the marine aquarists in the USA are ‘fishkeepers’ which sometimes add a few corals or other invertebrates to the tank but mainly set priorities for fish, whereas the average European marine aquarist is mainly coral-oriented. Also, the USA has a higher percentage of marine aquarium keepers among all aquarium hobbyists than Europe.

During the 1980s, aquarium-keeping of soft corals (subclass Octocorallia) started to be successful enough to

populate new aquaria with only fragments that grew into huge coral populations. During the 1990s the same started with stony corals (order Scleractinia). The growing knowledge of coral ecology was paired with increasing use of commonly available technology converted into technical devices for maintaining water quality and illuminating the corals containing symbiotic dinoflagellate algae (or zooxanthellae) in their tissues. Since the 1980s hobbyists have mainly tried to modify aquarium conditions according to the needs of the zooxanthellate marine invertebrates. This development has led to much advancement and it is far from being completed at present; a huge development is foreseeable for the near and far future, giving great dynamics to the marine aquarium hobby. Because these technical improvements strongly influence the species kept in aquaria, the list of organisms demanded by the trade is undergoing a constant shift, whereas in the freshwater hobby the demand for species has been much more constant over decades. This is a general problem of mariculture for the hobby aquarium market; it requires close communication between the aquarium industry/retailers/aquarists and the mariculture side.

The future of the marine aquarium hobby is likely to improve in the fields of sexual propagation of corals, successful keeping of non-zooxanthellate octocorals (deep-water gorgonians, dendronephthiids) and other filter-feeders (sponges, sea squirts, etc.), so the present stony-coral-oriented demand for aquarium species will probably still experience a further shift in the future and is far from being constant.

There seems to be a tremendous difference in the general way the marine aquarium hobby is being practised in industrialised countries and developing countries. In rich and industrialised countries (e.g. USA, central Europe, Japan) the technical equipment is readily available and relatively cheap, while the livestock (living corals, fish, live rock) needs to be imported and therefore is the most expensive part of the hobby. Consequently all necessary technical equipment is being installed to grow tiny coral fragments into large colonies, resulting in propagation of corals by private hobbyists that spread available species across the market. In developing tropical countries with coral reefs (e.g. Philippines, Indonesia) the situation is very different; aquarium technology needs to be imported and consequently is very expensive, often unaffordable, while corals and fish are readily available and commonly traded for low prices. Here, replacing perished tank inhabitants is much cheaper than installing appropriate aquarium technology, and consequently tanks are often stocked with organisms, regardless of their aquarium suitability, and they are replaced at regular intervals like cut flowers.

Future sustainability of the marine aquarium industry will increasingly rely on culture of aquarium species. The following sections describe the husbandry and culture of the major taxa used by the industry.

25.6 AQUACULTURE OF CORAL REEF FISH

As with most other marine fish, culturing ornamental marine fish depends on obtaining large numbers of eggs, developing them into larvae and raising the larvae into juveniles. Although much success has been achieved in this field, the number of species that can be routinely bred in numbers justifying commercial operations is limited.

25.6.1 Major species

The first successful breeding attempts were made with clownfish (*Amphiprion* species), followed by dottybacks (Pseudochromids) and some gobies (*Gobiosoma* species), and those three groups represent the range of species regularly available in trade. Some others, like the Banggai cardinal fish (*Pterapogon kauderni*), are not difficult to breed and are regularly produced by hobbyists, but their small number of juveniles does not support commercial breeding.

Captive-bred species of anemone fish or 'clownfish' dominate the commercial trade in coral reef fish, constituting 15 out of the 29 most common species (Table 25.1 and Fig. 25.3). The small number of genera is also notable. There are another 29 captive-bred species that occasionally occur in the trade. These include another three *Amphiprion* species, four species of *Cryptocentrus* (gobies) and four species of *Meiacanthus* (blennies). The development of

Table 25.1 Captive-bred species of tropical marine fish that regularly occur in the ornamental trade (revised from Brons, 2002).

Genus	Common name	Number of species
Amphiprion	Clownfish	14
Premnas	Clownfish	1
Gobiosoma	Gobies	4
Pseudochromis	Dottybacks	6
Hippocampus	Sea horses	2
Pomacanthus	Angel fish	1
Chromileptus	Grouper	1
Total		29



Fig. 25.3 Tomato clownfish, *Amphiprion frenatus*. (Photograph by Brian Gratwicke.)

captive breeding techniques for marine ornamental fish and the major species on which this research has focused, was recently reviewed by Moorhead and Zeng (2010).

25.6.2 Main centres of production

Ornamental marine fish are usually either cultured in their country of natural occurrence, or in the country in which they will be marketed. Obviously the former requires transportation of the fish to reach the retail market, which adds to their price. Wild-caught specimens need to be transported the same way, but their catching is far less expensive than breeding. Labour for operating a coral fish farm may be cheap in these tropical countries of origin, but transportation fees exceed this advantage by far. Captivity-raised coral fish that are bred in a tropical country are produced with much higher effort and time investment and therefore require higher price than wild collected specimen. When shipped to the country of marketing, their price necessarily exceeds that of wild-caught specimens and is in no way competitive. Breeding coral fish in the country of marketing leads to more competitive retail prices because it skips intercontinental transportation and therefore avoids significant shipping costs. This way the retail price can in some cases compete with that of wild caught coral fish being treated with high-quality procedures following the Marine Aquarium Council certification system. Consequently, many attempts to breed ornamental marine fish in tropical countries have failed from the lack of competitive prices and will continue to do so as long as wild collection of the same species is legal and not regulated in ways that increase the retail price.

The main centres of coral fish production are either located in the countries of origin (e.g. 'Aqualife Research' in the Bahamas and 'C-Quest' in Puerto Rico), in the main markets, which are in central Europe (e.g. 'Tropical Marine Centre' in the UK, 'Brons Mariculture' in the Netherlands, 'Marin Fischzucht' and 'Pro-Marin' in Germany) or the USA (e.g. ORA Farms and Dynasty Marine Associates, Florida). The main market for ornamental marine fish is the USA, followed by Japan and Central Europe (Germany, UK, Italy, France, Netherlands).

25.6.3 International statistics of quantity and value

Wabnitz *et al*. (2003) reported that only 1–2% of all traded ornamental marine fish are captive-bred. Following their numbers derived from GMAD stating traded coral fish during 1997–2002, around 4 300 000 coral fish have been imported to the marketing countries worldwide during these 6 years (importers' data). Based on this, the total number of captive bred coral fish can be estimated to be between 430 000 (1%) and 860 000 (2%). This is equivalent to an annual production of ca. 7166–14 333 coral fish.

25.6.4 General culture techniques for coral reef fish

25.6.4.1 Broodstock and spawning

Methods used for large-scale culture of marine fish for human consumption are covered in detail in Chapter 18. Culture techniques for ornamental reef fishes are fundamentally similar but on a smaller scale. The following account highlights aspects that are specific to hatchery production of ornamental species.

Keeping broodstock to produce eggs is a costly part of breeding marine ornamentals and, for some species, commercial breeding fails at this point because the number of offspring is too small to compensate for the expense of keeping broodstock. *Pterapogon kauderni* and other mouth-breeding species are a good example. Although a pair of clownfish will produce several hundred eggs, sometimes even 2000 or 3000, a pair of *Pterapogon kauderni* will produce just 15–20 juveniles. For the amateur hobbyist this is not a problem, but it is a constraint for commercial fish breeding.

Success relies on excellent broodstock nutrition and a stress-free environment. This is achieved for some species by keeping just a pair of the broodstock in a small tank. Artificial breeding cavities help in many cases (Fig. 25.4). Usually, the parental fish are needed to maintain the egg mass until the larvae hatch. Egg masses can be taken out of the broodstock tank the evening before expected hatching, but some breeders have more success in collecting the larvae in the broodstock tank after hatching, usually with



Fig. 25.4 Pair of blue devil damselfish (*Chrysiptera cyanea*) maintaining egg mass in terracotta shelter.

the help of a larvae trap where larvae are attracted to a light source (Mai, 2008).

Rearing systems for coral fish breeding try to create environmental conditions required by the larvae during their planktonic development. One of the main problems is to keep larvae and their food in suspension without physical damage to the larvae. Another is maintaining the water quality in the required range despite the heavy feeding. To achieve a high food density it is necessary to work with small volumes of water, but maintaining water quality requires a large water volume, which is contradictory. This has led to the development of various innovative systems for larval culture including small larval culture tanks, with a flow-through water supply, that are attached to a bigger system for water maintenance, and small basket-like containers (10–18 L) placed in a larger tank with 300–500 L (Holt, 2003). Success requires creative designs, sometimes inventing a new method in order to be successful with a certain fish species.

Other environmental factors important to larvae rearing are temperature, salinity, pH, sufficiently high oxygen concentration, low nutrient levels, and an appropriate day/night cycle that can vary from 12/12 hours (as is typical for tropical coral reefs) to 16/8 or even 24/0. The food organisms added to the culture system may change the water conditions, so the rearing system must focus on maintaining the stability of water quality parameters.

25.6.4.2 Larval foods

The various foods used for fish larvae are covered in detail in section 9.4 and their use in feeding fish larvae is covered in detail in section 18.6. The reader is referred to these sections for background information. Feeding the larvae of coral reef fish, and many other fish, is most difficult as the larvae exhaust endogenous nutrient stores (egg yolk) and begin to feed exogenously. The size of their mouth opening limits the food size, so prey items must be of a suitable size. Under natural conditions, they feed on micro-zooplankton like mollusc larvae, protozoans (ciliates, foraminiferans), and copepod eggs and nauplii. The size of food particles found in the guts of small coral fish larvae is mostly below 60 µm, with a general size range between 3 and 100 µm (Holt, 2003). For the developing larvae there is a need for essential nutrients in their prey, particularly the highly unsaturated fatty acids (see sections 8.3.3 and 18.6). Copepod eggs, nauplii and copepedites are known to be high in highly unsaturated fatty acids (section 9.4.8), but they are not extensively used for raising larvae because their culture is difficult to perform on a reliable basis (section 9.4.8). Marine ornamental larviculture relies mainly on live foods that are easy to culture (e.g. rotifers and brine shrimp) (section 9.4 and Figs 9.5 and 9.6). However, the larvae of some marine ornamentals, such as sea horses, have proved to be impossible to raise without inclusion of copepods in their diet during the first few days of feeding (Payne and Rippingale, 2000).

The most commonly used food items for raising coral fish larvae are rotifers and brine shrimp (see sections 9.4 and 18.6), though the nutritional content of the latter is far less than ideal. At a certain stage of larval development, the shift from rotifers to bigger prey items is necessary, and this is the time when brine shrimp nauplii will usually be used (Fig. 9.9). Brine shrimp nauplii are hatched from dried cysts (section 9.6), and if not enriched, they must be fed immediately before they lose nutritional value when they consume their natural egg yolk food storage. Most brine shrimp nauplii are enriched with nutritive solution concentrates like 'Selco'® before feeding (see section 9.4.7).

25.7 AQUACULTURE OF MARINE INVERTEBRATES

25.7.1 Stony corals (order Scleractinia)

During the 1980s soft corals were in high demand in the aquarium market, whereas stony corals could not yet be grown in tanks and there was no market, except for some scleractinians with large polys (e.g. *Tubastrea* species). These, in contrast to staghorn corals, die very slow when kept under inadequate aquarium conditions and therefore provide some suitability for the aquarium. Recently, the market has shifted towards stony corals, so the prevalence



Fig. 25.5 Cultured *Acropora* in Tonga. Pieces of coral are glued to a base and can be cultured in raceways or tanks with shallow running sea water, or in the ocean. Appropriate levels of illumination may be achieved in tanks using overhead shading, and algal overgrowth of the corals can be prevented using herbivorous fish. (Photograph by Antoine Teitelbaum.)



Fig. 25.6 Coral seedlings growing on a mesh platform in the ocean in Tonga. (Photograph by Antoine Teitelbaum.).

of soft corals in aquaria has been reduced remarkably. There continues to be a market for soft corals (see section 25.7.2), but demand and pricing levels cannot be compared to those of the 1980s and the early 1990s.

The staghorn corals (e.g. *Acropora* and *Montipora* species) are the main focus of coral reef aquarists, especially the most colourful specimens (Figs 25.5 and 25.6). Those are followed in importance by the pocilloporid corals (e.g. *Pocillopora* and *Stylophora* species). Many other coral genera are also in demand, but to a much lesser extent than these. Apart from aquarium durability, i.e. the coral will not only survive in aquaria, but also grow and allow captive propagation, the main attraction is the coloration. The difficult part is to create aquarium conditions (illumination, trace element supply, nutrient levels) that maintain the colour pigmentation. This is quite difficult, and after weeks or months many corals lose colourful pigmentation and coral tissues become brown, reflecting the colour of the zooxanthellae (symbiotic algae) in their tissues. This occurs because the density of zooxanthellae increases in response to high nutrient levels in the tank water.

The aquarium market has demand for any zooxanthellate coral species with strong colour pigmentation. But the corals' adaptation to different natural habitats and environmental conditions has led to different survival strategies among the coral families, also affecting growth and therefore the ability to replace lost colony parts. Corals species in shallow parts of the reef (e.g. reef top, lagoon) experience drastic environmental changes like varying water temperatures and salinities, some even fall dry during low ebb tides, being exposed to rain or hot sunshine. These species usually have an ability to grow fast and replace lost portions of a colony. Damage during low ebb tides can be dramatic, but after a few months it has been fully compensated for by the fast growth. These corals are mostly tolerant to slightly unsuitable conditions and survive proper handling and transportation as well as farm propagation (Fig. 25.6), and their rapid growth make them ideal for captive propagation.

Corals adapted to deeper portions of the reef where they are not exposed to drastic changes of conditions are usually much less tolerant to environmental changes. Instead of growing quickly, their survival strategy includes the ability to photosynthesise at lower light intensities with reduced spectral composition, at the expense of growth rate. These genera usually grow remarkably slower than shallow-water species and develop a denser skeleton. Their ability to re-grow lost portions of a colony is strongly limited, and consequently their potential for economically profitable captive propagation is very small, though aquarists have successfully developed captive propagation methods. Member States of the European Union have temporarily banned the importation of certain species of the genera *Blastomussa*, *Catalaphyllia*, *Cynarina*, *Euphyllia*,

Trachyphyllia, *Hydnophora*, *Goniopora*, and *Plerogyra* originating from several locations.

25.7.2 Soft corals (subclass Octocorallia)

Although the fragmentation of stony corals requires little skill and experience and is easy to teach and perform, fragmenting soft corals is much more difficult if the growth form of the respective coral species requires fragmentation and re-attachment to a substrate. At the same time, the market demand for soft corals in general is smaller than that for stony corals, and the retail pricing range per specimen is much lower. This limits their potential for mariculture, though many species are absolutely suitable for the mariculture methods and transportation as well as aquarium husbandry.

Generally all zooxanthellate octocorals are marketable in the aquarium industry. But some genera are in higher demand by the aquarist and at the same time more difficult to keep. So, despite the fact that they do grow and propagate asexually in reef tanks, in some aquaria the population decreases after a while and does not prove to be constant over many years, because of varying conditions, for example trace-element availability, high water temperatures and other factors. This creates a constant demand in the aquarium market. *Xenia* and *Heteroxenia* are two of those genera that generally have a high market demand. The related *Cespitularia* is also in high demand but because it is extremely sensitive to shipping, it is rarely found in the aquarium market. Improving shipping methods may open doors to its mariculture. Some other octocorals have a high demand because of their beautiful coloration and/or rare occurrence, like the strongly green fluorescent *Clavularia* species (*C. viridis*), as well as *Knopia* with its green fluorescent oral disc. All of these are extremely easy to propagate asexually because they just overgrow available substrate and do not require any fragmentation. In nature, *Knopia* grows in deeper water (around 15 m), and it can be difficult for it to adapt to the strong illumination usually found in reef aquaria; consequently it makes sense to keep mariculture populations adapted to shallow water conditions because their survival in aquaria is much higher than that of wild harvested specimens. But aside from these, all genera and species of the families Xeniidae and Clavulariidae have a market potential and are suitable for mariculture.

The family Alcyoniidae probably contains the largest number of zooxanthellate and aquarium-suitable species. *Sarcphyton*, *Lobophytum*, *Sinularia*, *Klyxum*, *Rhytisma*, *Cladiella* are all are suitable and marketable to the same extent, with *Cladiella* and *Klyxum* being somewhat more problematical for tissue infection, which makes substrate re-attachment more difficult.

The family Nephtheidae is another containing genera suitable for mariculture: *Nephthea*, *Litophyton* and *Capnella*. Their propagation is relatively easy and their market demand is relatively low but quite constant. With some very 'fashionable' stony corals it may be possible to produce and sell many specimens from the same coral species, for example blue *Acropora echinata*, but with Octocorals this will generally not work, except for a few species with constant market demand (e.g. *Heteroxenia* or *Knopia*). Mariculture of octocorals requires species diversity.

Zooxanthellate gorgonians (e.g. family Gorgoniidae, genera *Gorgonia*, *Pseudopterogorgia*; family Plexauridae, genera *Plexaura*, *Pseudoplexaura*, *Eunicea*) are another group with high mariculture potential. These genera occur in the Caribbean and have high market demand; even though their asexual propagation requires fragmentation, it is extremely simple because gorgonians have a solid and firm skeleton that can easily be attached to substrate. Related Indo-Pacific families include species with encrusting growth that are extremely easy to mariculture (e.g. family Anthothelidae, genus *Erythropodium*; family Briareidae, genus *Briareum*).

25.7.3 Sea anemones (order Actiniaria)

Sea anemones generally have a high market potential because most of the aquarium-durable zooxanthellate species are host anemones for clownfish. However, because of their different nutritional requirements, their aquarium survival is species dependent, and some sea anemone species are difficult to keep when kept together with octocorals or Scleractinia. One species stands out from the others because of its high aquarium suitability and the tendency to propagate asexually and even tolerate fragmentation (Knop, 2002): *Entacmaea quadricolor*. This species can easily be kept together with corals, is accepted as the host anemone by most clownfish species, and in contrast to the other three available colour morphs, the red one has very high market demand. Therefore it has great potential for mass production for the aquarium market. Other host anemones have occasionally shown asexual propagation in captivity but it is still far from being reproducible.

25.7.4 Zoanthid anemones (order Zoanthiniaria)

Zoanthid anemones of the order Zoanthiniaria can grow rapidly under ideal conditions (a monoculture tank with only one species). The species *Zoanthus sociatus* has many

beautifully coloured morphs that are in very high demand in the USA and sell for high prices. They seem to be an ideal species addition for an existing mariculture project as they do not require any active intervention; they simply overgrow available substrate.

25.7.5 Disc anemones (order Corallimorpharia)

Corallimorphs of the families Discocomatidae, Ricordeidae and Corallimorphidae can develop rapid growth under ideal conditions (a monoculture tank with only one species). *Ricordea florida* doubtlessly is the most difficult one; however, cultivation and asexual propagation are possible, and because the demand for this extraordinarily colourful species with three different colour morphs is good and aquarists pay extremely high prices for them, it is a very promising species for mariculture.

25.7.6 Decapod crustacea

25.7.6.1 Shrimps

In contrast to fish and corals, shrimps play a minor role in marine aquaria, but they are still the most popular free-living invertebrates. They are usually present in many tanks as cleaner shrimps, scavengers or for pest control, and sometimes as commensal shrimps in cnidarians. Most research into breeding has been done with the genera *Lysmata* and *Stenopus* (see, for example, Calado (2008) and Fig. 25.7). Breeding techniques are known for several other genera, but commercially successful breeding has been developed for relatively few (Fig. 25.8).

Most traded ornamental shrimps are found in the families Hippolytidae, Stenopodidae, Rhynchocinetidae and Palaemonidae, but also Alpheidae and Gnathophyllidae. Breeding techniques have been developed for some species of these families.

The main production of ornamental shrimps takes place in the countries of their marketing and focuses on the major species in trade (Europe: Tropical Marine Center, UK, *Lysmata debelius*, *L. amboinensis*, *L. californica*; USA: Oceans, Reefs and Aquariums, Florida, *L. rathbunae*).

The main markets for ornamental shrimps are identical with the centres of the marine aquarium hobby, mainly the USA and central Europe, especially Germany, UK, Netherlands, France, Italy, and to much smaller extent Switzerland, Austria, Norway, Sweden and Denmark. The largest market in Asia is Japan.

The USA dominates the global marine ornamentals market with a two-thirds share; it imports invertebrates that originate from 18 countries and include 310 species from 209 genera. Among crustaceans, the 'top sellers' in the USA are *Stenopus hispidus* (4.6% of the invertebrate imports), *Lysmata amboinensis* (1.1%) and *Lysmata californica* (0.9%) (Global Marine Aquarium Database, 2008). Most of those are wild collected, although all three species have been successfully bred in captivity. Therefore it would be very helpful to improve breeding methods through

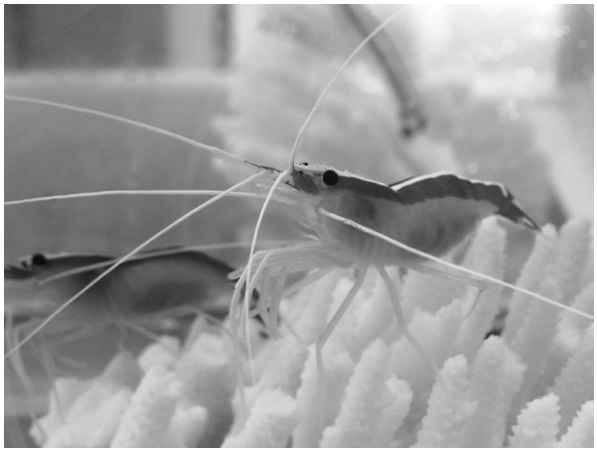


Fig. 25.7 The popular and cultured aquarium shrimp *Lysmata amboinensis*. (Photograph by Matt Salmon, Australian Institute of Marine Science.)

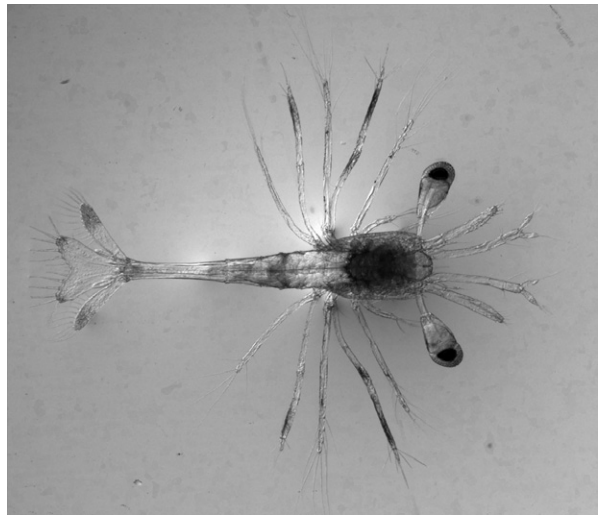


Fig. 25.8 Cultured late stage larva of *Lysmata amboinensis*. (Photograph by Matt Salmon, Australian Institute of Marine Science.)

research to avoid the present bottlenecks in larval husbandry that makes commercial breeding less profitable.

In contrast to fish and cnidarians, decapods (including hermit crabs, spider crabs, porcelain crabs) represent a small percentage of aquarium inhabitants. Their wild harvest alone does not represent a serious pressure on natural reefs. However, any breeding of marine ornamentals is an important step towards making the aquarium hobby independent of natural resources. Furthermore, farm-raised decapods in general have the advantage of being better adapted to captive aquarium conditions than wild-caught specimens and are more free of pathogens. Thus they command higher market value.

25.7.7 Molluscs

25.7.7.1 Giant clams

Giant clams (family Tridacnidae) are a minor group of aquarium inhabitants compared with fish or corals by biomass or total value. However, they are extremely popular and sell for very high prices, so that giant clam culture has a potentially huge market. Giant clam farming activities are concentrated in the Pacific island region (Fig. 25.9). The location of the clam farms producing for the aquarium market is strategically beneficial for shipping to the USA and Japan, with long shipping durations to Europe. This leads to most farm-raised clams entering the USA and Japan, and only small numbers being shipped to Europe. Commercial giant clam farming in Asia focusing on exporting to Central Europe would therefore meet a huge demand in the marine aquarium market. Comprehensive culture manuals are available for giant clams (see, for example, Braley, 1992).



Fig. 25.9 Cultured giant clams (*Tridacna crocea*) in tanks/raceway culture in Vanuatu. (Photograph by Antoine Teitelbaum.)

25.7.7.2 Gastropods and nudibranchs

Some gastropod snails and nudibranchs are used by marine aquarists because their feeding habits help manage problems relating to pest control. These include snails of the genera *Trochus* (section 24.4) and *Turbo* (herbivores feeding on algae) and the nudibranch *Berghia verrucicornis* (feeding on small sea anemones of the genus *Aiptasia*). Herbivorous snails are cultivated by some giant clam farms in the Pacific Islands (Fiji, Marshall Islands, Palau, Solomon Islands, Tonga, Vanuatu). *Berghia verrucicornis* is being cultured on a small scale by some hobbyists and offered in the market commercially. Culture methods for *Trochus* (Chapter 24), *Turbo* and *B. verrucicornis* are well established and they present considerable potential for cultivation for the marine ornamental industry.

25.8 AQUACULTURE OF LIVE ROCK

25.8.1 Use of 'live rock' in coral reef aquaria

Contrasting with the use of dead coral skeletons for decoration of marine aquaria that was common during the 1960s in the USA and central Europe, modern coral reef aquaria containing live corals are usually decorated with 'live rock'. Apart from the aesthetic properties of this natural looking material, live rock functions as ecological foundation within the tank system because it has the advantage of carrying a wide range of marine organisms, so increasing the species diversity of a marine aquarium. Countless creatures enter the tank as hitch-hikers in the live rock, and bacteria on the surface and deep in the rocks fulfil important functions in maintaining water quality (nitrification and de-nitrification). Therefore, live rock is heavily traded in all markets with marine aquarium hobbyists.

Live rock can be defined as 'any type of rock encrusted with, and containing within its orifices, a wide variety of marine organisms including algae and colourful sessile invertebrates' (Livengood and Chapman, 2007). It can contain annelids (e.g. bristleworms, serpulids), a wide range of crustaceans (e.g. shrimps, crabs), molluscs (e.g. gastropods, vermetids), tunicates, sponges, bryozoans, hard and soft corals, and various marine algae including calcareous algae (e.g. *Mesophyllum*).

25.8.2 Main centres of production

The artificial production of live rock for marine aquaria follows two models: it is either produced in the country of marketing, namely the USA, or it is farmed in countries

harvesting and exporting natural live rock where it represents an alternative to wild collection. Currently, the main centres of artificial live rock production are Florida (USA) and Fiji (Walt Smith International). Generally, producing live rock in a tropical country with coral reefs ensures higher species diversity than production in the marketing country can do. Culturing in the marketing country has the advantage of eliminating intercontinental transportation, which saves freight cost and reduces the retail price remarkably. In southern Florida, inland culture in tanks is being practised as well as culture in shallow coastal waters, whereas in Fiji it is done as marine culture and is practised in raceways and natural marine environments in Tonga (Fig. 25.10).

25.8.3 Production statistics for live rock

The main markets for live rock are identical to those for live corals, namely the USA, Canada and central Europe, especially Germany, France, Italy and the UK. Japan also has a well-developed reef aquarium hobby, and a reasonable share of the live rock used in aquaria originates from Japanese coral reefs in Okinawa.

Lindsay *et al*. (2004) consider the Pacific islands as the major supplier of live rock and state that 95% of all Pacific live rock has been imported to the USA. They estimate that between 1992 and 2000, over 14 million kg of live rock was exported from the Pacific, and furthermore state that the live rock exports from Fiji and Tonga doubled each consecutive year from 1992 to 1997 to reach 650 000 tonnes in 2003. Cultured live rock seems to represent only a minor share of total live rock sales in marketing countries.

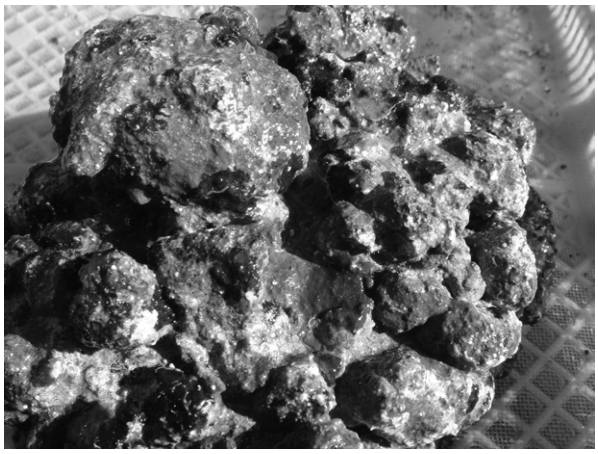


Fig. 25.10 Cultured live rock from Tonga. Irregular lumps of cement-based substrate are cultured in a raceway/tank or in the ocean to develop desirable epibiota. (Photograph by Antoine Teitelbaum.)

25.8.4 General culture techniques for live rock

Production of artificial live rock generally uses a hard substrate that will be exposed to a species-rich marine community, allowing the organisms to become established on it. This can take place near a tropical reef, marine life communities in subtropical waters, or in land-based tanks of suitable size. Naturally, the quality of the artificial live rock increases with the number of species it has been exposed to, provided they belong to those surviving in tropical marine aquaria.

Two different concepts are practised to obtain the substrate: the use of natural dry rock, or the production of artificial materials. Generally both work the same way. The artificial material currently used for live rock production is mainly cement-based. An alternative not yet in use for artificial live rock production (up to now only common for producing 'dry' reef tank decorations) would be a porous ceramic material that can also contain a reasonable portion of calcium carbonate. This would be more lightweight than concrete cement, which is an advantage when shipping costs are considered.

In terms of quality, the most important factor is the porosity of the material: the more indentions and large, deep pores that artificial live rock has, then the more inner surface it offers, and the greater the chance of establishing a huge variety of marine organisms in it. Also, with a very porous structure the artificial live rock has less weight, which saves freight cost. Natural live rock is very porous and lightweight, and artificial live rock can only compete if it has comparable porosity, weight and settlement of living organisms. Therefore it would be advisable to develop ways of creating porosity in the artificial live rock material (e.g. by mixing it with organic materials that disappear after hardening, like cooked rice, especially with the use of ceramic materials that need to be treated with high heat in an oven which burns organic materials).

25.9 CULTURE VERSUS FIELD COLLECTION OF MARINE ORNAMENTALS

25.9.1 Fish

Global trade of marine ornamental fish species includes more than 1200 species that originate from 45 countries (Bruckner, 2001). This extraction of coral fish from natural ecosystems is ambivalent as it has both negative and positive effects. The harvest puts pressure on the reefs, whereas the income it generates creates livelihood for

coastal communities. Simple solutions like a total prohibition of wild collection can easily develop adverse effects with bigger negative impact to natural ecosystems than the coral fish collection currently has: after prohibition, coastal citizens of tropical countries may replace the lost income by other, more destructive coral-reef fishing practices. Coral fish collection performed in a responsible manner can represent an environmentally oriented means of generating income for coastal communities; furthermore it can encourage those communities to protect the reefs as their source of income (Fig. 25.11).

However, the marine aquarium hobby is growing worldwide, and the growing demand for ornamental marine fish can hardly be satisfied in an environmentally friendly and sustainable manner. Farm-raising of coral fish will be necessary to supply this increasing market demand. This will require improved farming technology and an increase in the number of captive-bred species. Capture of coral fish larvae in the wild for subsequent culture and supply to aquarium market is now being practised (see, for example, Bell *et al*., 2009), which may provide an opportunity to reduce the harvest pressure on adult coral fish.

25.9.2 Live rock

Artificial live rock can technically replace natural live rock in marine reef aquaria, if it has the same quality. Because coral husbandry in aquaria is generally increasing, the demand for live rock is growing, and to reduce harvesting pressures from coral reefs, the advantage of cultivating live rock against wild collection is self-evident. However, in practice, cultivated live rock has not yet started to compete seriously with wild-harvested live rock in aquarium markets. One of the main reasons seems to be that artificial live rock substrates often are too solid and heavy, which increases shipping price and greatly reduces settlement of living organisms. Strategies for improving the quality by enhancing porosity will be necessary to increase competition against wild-collected live rock, and based on the assumption that the reef aquarium hobby will continue to grow during the coming decades, this will offer potential for successful mariculture, especially if performed with farm culture of corals.

25.10 TROPICAL FRESHWATER ORNAMENTALS

Most freshwater ornamental fish originate from breeding and rearing businesses. The type and size of the business varies greatly and often reflects the socio-economic environment in which the business is found. Within Asia and South America, farms can vary in size from small backyard setups to large pond farms, but they are generally low-technology ventures (Olivier, 2003). In Singapore, businesses are small and often family owned and operated,



Fig. 25.11 Wild collected coral fish at an export station in Vanuatu.

or they are grouped into agrotechnology parks by government agencies (Olivier, 2003). In the poorer areas of the Philippines, basic fish-rearing businesses operate even in areas where sanitation and general living conditions are poor (Olivier, 2003). In developed countries such as the USA, captive culture tends to occur at a more intensive level by high-capacity industrial breeding companies, and in Europe the ornamental fish trade is sustained by a few intensive commercial businesses and various individuals or groups of amateur and professional breeders who sell to wholesalers and retailers, or directly to the public (Olivier, 2003). In many instances, the key to success for breeders, in a marketing sense, is variety. Breeders who are able to provide a larger variety of captive-reared species and variants of species will often be able to sell a larger number of fish.

Exporters generally buy fish directly from farmers and fishers or from a wholesaler. The facilities of an exporter are often large, holding many containers, aquaria and concrete holding tanks to house incoming fish. It is here that fish are quarantined and acclimatised to captive conditions for periods of days to months, before being shipped to importing countries (Olivier, 2003).

On the receiving end of shipped consignments are the importers. Like the exporters, importers also require large infrastructure, as they are responsible for quarantining and acclimatising new arrivals to captive life, introducing them to formulated foods, and variations in captive water conditions. These companies often work simultaneously as an importer/exporter, particularly in trading 'hub' regions such as Singapore, the USA and the Netherlands (Olivier, 2003). Consignments in these areas are shipped in, then bulked together and re-exported worldwide.

Studies of fish shipment have shown that, in addition to ensuring good water quality of shipment water, post-shipment mortality can be much reduced with appropriate pre-shipment treatment, and post-shipment recovery techniques for fish, through health and nutritional prophylaxis and the use of pro-biotics (Lim *et al*., 2003).

25.11 COMMONLY TRADED FRESHWATER SPECIES

The AOI by its very nature supplies a luxury item and tends to target a select group of end users. In supplying a product that creates an aesthetically pleasing 'mini-biotope', the AOI is highly selective in the species that are collected, cultured and traded (Lecchini *et al.*, 2006). Species that display vibrant coloration, unique and interesting behaviour and habits, or some degree of novelty, in addition to (but not limited by) their suitability for aquaria, are key criteria dictating the species traded and the level of their exploitation (Lecchini *et al*., 2006; Sinha and Asimi, 2007).

There are approximately 500 varieties of tropical freshwater fish and 10 varieties of goldfish that are commonly stocked by aquarium dealers (Fig. 25.12). Families that dominate trade include the Poeciliidae, Characidae, Cichlidae, Osphronemidae, Helostomatidae and the Cyprinidae (Ng and Tan, 1997).

25.11.1 Poeciliidae

The Poeciliidae is a large family indigenous to North, Central and South America (Rowley *et al.*, 2005). From this family, the guppy, *Poecilia reticulata*, is probably the best known of all tropical ornamental fish. With the help of the aquarium trade, many members of this family have established non-indigenous populations around the world (Rowley *et al.*, 2005). Poeciliidae are commonly referred to as 'live-bearers', as many use internal fertilisation and internal egg incubation, giving birth to live young after a given gestation period. Some within this family, however, are also egg-scattering. Among those that bear live young, there are varying degrees of maternal support given to developing larvae. Some are strictly lecithotrophic, where there is sufficient yolk to support complete embryonic and fetal development. Other species are matrotrophic, where minimal yolk is provided, with most nutrients being provided through a placenta or similar structure.

Fish of this family belong to two genera: *Poecilia* which includes the guppies and mollys, and *Xiphophorus* which includes the platies, swordtails and varieties. There are multiple varieties currently available in the ornamental trade, largely because of the ease with which hybridisation between the genera can occur. The diet of poeciliids generally consists of algae, insects and crustaceans.

25.11.2 Characidae and related families

The 'characins' are composed of nearly 900 species in approximately 170 genera originating from Central and South America and Africa. The most well-known species, to the aquarium trade, are the neon tetra (*Paracheirodon innesi*) and the cardinal tetra (*Paracheirodon axelrodi*), both of which are intensely commercialised species in the ornamental trade. Together, the guppy and the neon tetra represent more than 25% of the world market in volume and more than 14% in value (Olivier, 2003). The common breeding strategy for species of this family is to broadcast eggs and sperm over various types of structure, with no further parental care. Their diet tends to be quite varied and may include leaves, nuts, insects crustaceans, fish, micro-crustaceans and other vertebrates.

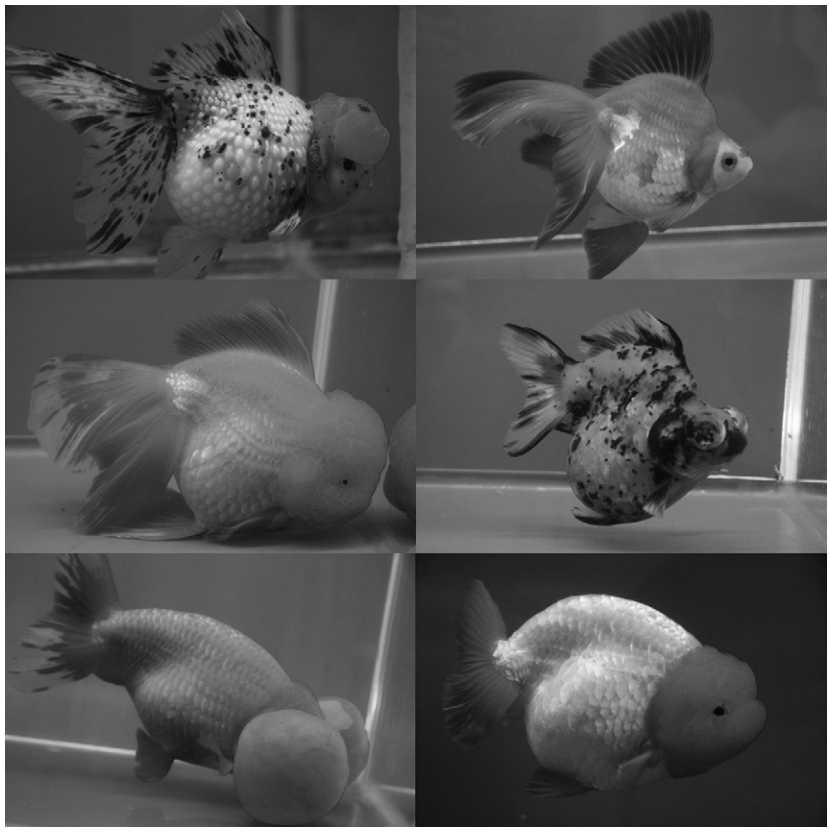


Fig. 25.12 Six breeds of goldfish that were shown at the twentieth Pramong Nomklao fish show, Thailand. (Photograph by Melanochromis.)

25.11.3 Cichlidae

Otherwise referred to as cichlids, members of the family Cichlidae have a general distribution throughout Africa and outlying islands, along the southwestern Asian coast, and Mexico, Central and South America, and the West Indies in the New World (Goldstein, 1988). There are approximately 1300 to 2000 cichlid species in about 105 genera, the greatest diversity of which may be seen in east Africa's Lake Malawi, Lake Tanganyika and Lake Victoria. Species common to the aquarium trade include the angelfish (*Pterophyllum scalare*), discus (*Symphysodon* spp.) (Fig. 25.13) and the oscar (*Astronotus ocellatus*) from South America, the Dempsey (*Archocentrus octofasciatum*) from Central America, and species from lake Malawi, Africa. All cichlids display some level of parental care and many species aggressively defending territories. Many cichlids are substrate spawners and display bi-parental care of eggs in a nest, whereas others display maternal mouth brooding behaviour. Cichlids exploit various dietary sources and vary greatly in dietary requirements: some are diet specialists whereas others are generalist feeders and their diets may include plants, algae, invertebrates and fish.

25.11.4 Osphronemidae and Helostomatidae

Collectively these families are referred to as gouramis, but they also contain the very popular Siamese fighting fish (*Betta splendens*) and the paradise fish (*Macropodus opercularis*). Within these two closely related families, most aquarium species come from the Osphronemidae; however, the commonly traded kissing gourami (*Helostoma temmincki*) belongs to Helostomatidae. These families are also commonly named 'labyrinth fish', which refers to a modified gill used to breath atmospheric oxygen. In addition to the kissing gourami and the Siamese fighting fish, gouramis belonging to the genus *Trichogaster*, such as the three-spot gourami (*Trichogaster trichopterus*) and the genus *Colisia*,

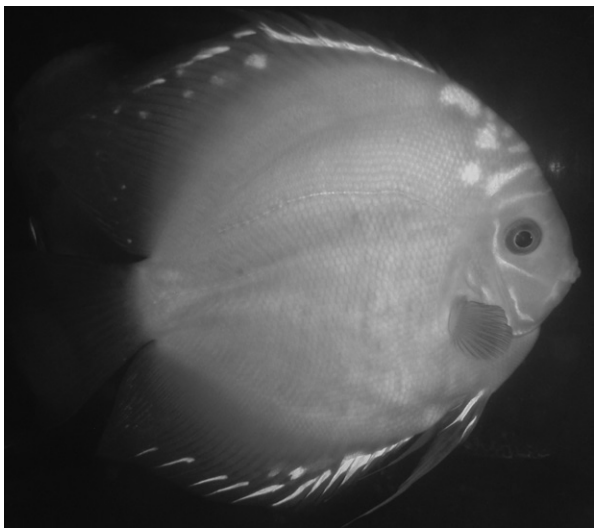


Fig. 25.13 Discus, *Symphysodon aequifasciatus*. (Photograph by Anneli Salo.)

particularly the dwarf gourami (*Colisa lalia*), are most frequently found in the aquarium trade. Many labyrinth fish spawn in bubble nests, constructed and maintained by the male, who also cares for the eggs and fry. Some are also known to be mouth brooders. The female kissing gourami broadcasts buoyant eggs for her partner to fertilise. The diet of labyrinth fish includes primarily insects and crustaceans; however, algae forms a large part of the diet of the kissing gourami.

25.11.5 Cyprinidae

The family Cyprinidae, better known as minnows or carp, are the largest family of fishes. There are over 2000 species from about 210 genera, originating from Africa, Asia, Europe and North America, with the greatest diversity being found in south-east Asia. The most commonly traded species on the ornamental market include the temperate goldfish and koi (*Cyprinus carpio*), and tropical species such as barbs (*Barbus*, *Capoeta* and *Puntius*) from Africa and Asia, danios (*Brachydanio* and *Danio*) from southern Asia, rasboras (*Rasbora*) from southern and south-east Asia, and freshwater sharks (including *Epalzeorhynchus* and *Labeo*), which originate from south-east Asia. Cyprinidae are commonly broadcast spawners, with groups or aggregations dispersing eggs and sperm over gravel, plants and woody debris with no further parental care. Like the cichlids, feeding strategies within this family are diverse and appropriate foods may include algae, macrophytes, zooplankton, insects, crustaceans and other fish.

25.12 AQUACULTURE OF FRESHWATER ORNAMENTAL SPECIES

Culture of freshwater ornamental fish is a well-established industry traditionally characterised by small-scale and/or family owned operations, particularly in Asian countries. However, in the past 25 years, seemingly in step with advances in food-fish aquaculture (Chapter 18), ornamental aquaculture, particularly in industrialised countries, has progressed considerably. Larger culture businesses have emerged and there is a general trend towards more intensive culture techniques to maximise production and to minimise fish loss and water use. Several technologies and techniques commonly used in food-fish aquaculture, particularly for the culture, preparation and nutrient manipulation of live feeds, are used or have been adapted for the production of freshwater ornamental fish, and there is potential for many more to be adapted (Lim *et al.*, 2003). The most researched ornamental species include the guppy, swordtail (*Xiphophorus helleri*), angelfish, dwarf gourami and goldfish.

In contrast to the traditional single-species farms that characterise food-fish aquaculture, aquarium fish farmers can individually culture many different species and varieties of fish that vary not only in appearance and value, but also water quality requirements, nutrition and mode of reproduction. Ornamental aquaculture therefore can often demand a higher level of knowledge, expertise and management ability than other types of aquaculture (Watson and Shireman, 1996).

The main producers of captive reared freshwater ornamental fish include the USA (mainly Florida), Thailand, Singapore, Indonesia, Hong Kong and Malaysia, as well as the Czech Republic, which is an important supplier to the European market (Watson and Shireman, 1996). The most common captive reared freshwater ornamental fish belong to 11 families (Table 25.2).

25.12.1 General culture techniques

Freshwater ornamental fish can be cultured in ponds, pond cages, raceways and enclosed tanks and aquaria. Depending on the culture methods and techniques used, yields at harvest time may vary greatly.

In general, facilities for the production of aquarium fish are small compared with food-fish production facilities, with lower stocking densities during larval rearing and grow-out. However, production can resemble semi-intensive food fish production, with stocking densities of some ornamental fish species matching that of food species. For example, stocking densities of tiger barb and swordtail during grow-out can be up to 10 000 fish/m^3,

Table 25.2 The common names of the most frequently reared freshwater ornamental fish grouped by their associated family.

Family	Common names
Adrianichthyidae	Medakas
Aplocheilidae	Panchax
Callichthyidae	Corydoras and other armoured catfish
Characidae	Tetras
Cichlidae	Angelfish, oscars, ram cichlids and African cichlids
Cyprinidae	Goldfish, koi, danios, barbs, rasboras
Fundulidae	Topminnows and North American killifishes
Hemiramphidae	Halfbeaks
Nothobranchiidae	African killifishes
Osphronemidae	Gouramis and Siamese fighting fish
Poeciliidae	Guppies, mollies, platies and swordtails

compared with the larval stages of food fish such as Atlantic cod (*Gadus morhua*) and barramundi (*Lates calcarifer*), which reach maxima of 30 000–55 000 fish/m^3 (Tlusty, 2002).

25.12.2 Pond culture

Pond culture of ornamental fish is globally the most popular technique for commercial production (Fig. 25.14). Ponds can vary in size, type (earthen or lined) and cost. In Florida, for example, most ponds are water-table ponds constructed on a sandy loam, and have an average size of roughly 8 m × 24 m (Watson and Shireman, 1996). In China, farms are often located on or near lakes or rivers, with ponds as large as 20 m × 60 m to several acres in size (Willis and O'Sullivan, 2007). Fish are generally produced using an extensive culture method, with low stocking densities (Lim *et al.*, 2003). Ponds are dried, cleared of weeds and then filled, and depending on the farm may also be sterilised (often using hydrated lime) and fertilised to promote primary productivity and healthy zooplankton growth (section 9.9). Natural production in the ponds may partly or completely satisfy the feeding requirements of



Fig. 25.14 A pond-based culture system for ornamental fish in Singapore. Utilising net enclosures allows stocking of multiple species or different cohorts or sizes of fish within the same system, and easy inspection and harvesting.

larvae and juveniles; however, some degree of supplemental feeding may occur.

When a pond is ready to be harvested, the most common harvesting methods are traps or seine nets. The least stressful and damaging capture method will ensure good survival and minimise damage to fish, which directly relates to sale quality. Catchment sumps in ponds are also used to concentrate fish when the pond is drained, making harvesting easier (Willis and O'Sullivan, 2007). After harvest from ponds, fish are often kept in holding tanks with high-quality water and treated for parasites and disease, inspected and sorted, and purged before transport (Tamaru *et al.*, 2001).

Cage or net culture within ponds (Fig. 25.14) enables farmers to benefit from the natural productivity of ponds, harvest more easily, culture multiple species or cohorts of different size or age in the one pond, or confine broodstock, while allowing fry to swim freely into the open pond (Tamaru *et al.*, 2001). Additional labour is needed to control biofouling on cages, however, particularly for producers with large ponds. Cage culture increases their ability to diversify production, which is an important advantage in ornamental aquaculture.

Pond culture is one of the cheapest methods for producing ornamental fish, owing to the relatively low level of labour required for maintenance, and low inputs. Until recently pond culture has been the only effective method of rearing small mouth larvae such as those of the dwarf gourami (Lim *et al.*, 2003). However, there is little control over the environmental parameters that influence ponds, and there are inherent difficulties in controlling water quality (Lim *et al.*, 2003). The use of ponds is therefore limited to areas with satisfactory soil conditions, environmental parameters within the tolerance range of the species being farmed, and a source of good quality water in large volumes. Additionally, with increasing latitude, farming regions are subject to more varied environmental conditions with changes in season. Fish that respond to environmental cues as a part of their reproductive cycle may cease breeding for parts of the year, limiting farm production. For example, goldfish pond farms in China are restricted to two spawning seasons per year, in autumn and spring (Willis and O'Sullivan, 2007). Goldfish fry are also very sensitive to temperature change in the first few weeks after hatching, which can result in heavy losses of fry raised in ponds. Growth rates also decrease as temperature drops. It follows that pond culture of tropical species is largely restricted to tropical zones such as south-east Asia and Florida.

Ponds are also more susceptible to the entry of pests, such as weeds, parasites, pathogens and predators, which can reduce production output, decrease fish quality and make harvest difficult (Tamaru *et al.*, 2001).

25.12.3 Large tank and raceway culture

Systems of large tanks and raceways are also commonly used for culture of freshwater ornamental fish. These structures can be constructed of plywood lined with canvas, high-density polyethylene or concrete, and are kept outside or under shelter (Olivier and Kaiser, 1997). Tanks and raceways may be fertilised in much the same way as ponds, or seeded with water from ponds with algal blooms. However, stocking densities tend to be higher, with higher levels of supplemental feeding. These systems may operate as static, flow-through, semi-recirculating or recirculating systems with their own biological filtration systems (Thorne and Hickton, 1999). Like ponds, however, farms with large tanks and raceways tend to be confined to areas that suit the environmental tolerances of the culture species, and may be subject to variable production in response to environmental seasonality.

25.12.4 Aquarium culture and indoor recirculating culture systems

Small tanks including glass aquaria are also used to culture freshwater ornamental fish. Many of the problems experienced in outdoor ponds and tanks can be avoided with aquarium culture. Using many small vessels also allows the operator to diversify and produce a variety of aquarium species. Aquarium and intensive recirculating systems are generally kept in insulated rooms, which allow maximum control of environmental conditions, disease, feeding and stocking densities. They allow year-round production, and make it possible to locate farms close to markets (Halachmi, 2006). However, like any intensive recirculating system, operating costs and labour needs are relatively high owing to the need for more technology and greater infrastructure. This technique also has associated management problems including the effect of high-density culture on water quality, growth and filial cannibalism (Olivier and Kaiser, 1997).

Intensive indoor production is commonly used to raise fish that are difficult to breed, or have specific breeding requirements, and that fetch a higher market price (Thorne and Hickton, 1999). Pond farmers can also use aquarium and indoor systems to achieve year-round spawning of broodstock before stocking ponds for grow-out; they may also collect young from outdoor breeding ponds and rear them indoors.

Fish such as discus are primarily bred, raised and kept in indoor aquarium systems as they tend to form exclusive

breeding pairs and are more demanding with respect to water quality and feeding.

25.12.5 Reproduction and spawning

In both freshwater and marine ornamental aquaculture, breeding and rearing still relies primarily on broodstock sourced from the wild. However, with continued progress in the domestication of ornamental species, reliance on wild breeders will decrease. Freshwater ornamental fish can be broadly divided into two main categories: live-bearers and egg-layers.

25.12.5.1 Live-bearers

Those fish classified as live-bearers encompass almost 1000 species (Tamaru *et al.*, 2001). Commercial culture of live-bearers, including breeding and grow-out, generally occurs in outdoor ponds where adults are stocked into ponds and allowed to breed freely for 6–7 months before they are harvested and graded (Tamaru *et al.*, 2001). Live-bearers are not monogamous and so are stocked in breeding tanks as groups usually with male : female ratios varying between 1 : 1 to 1 : 5 (Tamaru *et al.*, 2001). Stocking densities in ponds can vary greatly, with anywhere between 50 and 1000 of the best broodstock specimens being released into a single pond.

25.12.5.2 Egg-layers

The main groups of egg layers are barbs, tetras, gouramis, danios and cichlids. Although some egg-layers can be cultured exclusively in ponds (much like live-bearers), most require a hatchery for broodstock conditioning, pairing, breeding and/or larval rearing. Egg-layers are generally classified as egg scatterers, substrate spawners, bubble-nest builders or mouth brooders.

Egg scatterers usually spread their eggs around weeds or onto gravel and include species such as tetras, rasboras and danios. Substrate spawners lay eggs that are sticky and attach to suitable substrates including plants, rocks or timber, or artificial structures such as bottle brushes, rope, terracotta tiles, plastic pipes or other materials (Willis and O'Sullivan, 2007). Some substrate spawners, particularly cichlids, show parental egg care, whereas others do not. Substrate spawning species include catfish, cichlids, barbs, rainbowfish and killifish. Bubble nest builders are largely gouramis and Siamese fighting fish. The male constructs and maintains the nest, and guards and cares for the eggs and fry. Mouth brooding fish take newly spawned and fertilised eggs into their mouth and retain them until they hatch. Depending on species, the brooder can be male or female. The mouth-brooding parent will feed sparingly, if at all, during the brooding period. Members of the family Osteoglossidae, which include arowanas, saratoga and some cichlids, are mouth brooders (Thorne and Hickton, 1999).

Some species, particularly cichlids, form strong mating pairs for life and are held together in individual tanks that spawn regularly. Most of the other egg-layers, however, are paired up at random in spawning groups or farmer-prescribed pairs. Commercial production techniques for egg layers are commonly closely guarded secrets, known only to the producer.

25.12.6 Broodstock conditioning and spawning induction

With the variety and diversity of fish species that can be cultured in the ornamental industry comes a diversity of methods and techniques required to condition the broodstock of different species for gamete release. In basic terms, final oocyte maturation with ovulation in female teleosts requires a surge in the secretion of gonadotrophin by the pituitary gland (see sections 6.2.1 and 18.5.6). For live-bearers and many egg layers, manipulations of sex ratios, optimum nutrition and feed rations, maintenance of optimum water quality parameters and manipulation of physical conditions such as water temperature and photoperiod, are enough to encourage final oocyte maturation and elicit ovulation (Kaiser *et al.*, 2003).

However, for some species of freshwater fish there has been limited or no success in eliciting final oocyte maturation followed by ovulation. The freshwater red-tailed black (*Labeo bicolour*) and rainbow sharks (*Epalzeorhyncus frenatus*) are examples of species with an inability to reach final oocyte maturation and/or ovulation under captive conditions. Information generated in food fish aquaculture, however, has aided the propagation of these species through the use of hormone-induced spawning techniques (see sections 6.2.1 and 18.5.6). Hormone technologies have also helped farmers achieve year-round production of seasonally spawning species of freshwater ornamental fish. Future applications of hormones may facilitate production of mono-sex populations, which may be particularly useful for species that are highly sexually dimorphic and fetch different prices depending on the sex of the fish (Shim *et al.*, 1987)

25.12.7 Foods and nutrition

Compared with the food-fish industry, the development of products, techniques and technologies relating to aquaculture feed formulation, feeding and nutrition of ornamental fish has lagged. Despite the economic importance of orna-

mental fish production, comparatively few data are available on the nutritional requirements of many ornamental fish. Most feeds commonly used for ornamental aquaculture are not specifically designed for ornamental fish, but rather for food-fish species (see Tamaru *et al*., 2001). Diets that are designed for ornamental fish are generally packaged for hobbyists, making them economically unsuitable for large-scale farm use. Particularly in developing countries, broodstock, larval and grow-out foods can be basic. For example, adult Asian Arowana (*Scleropages formosus*) reared in ponds in Singapore are fed frogs, small fish and beef, pork or chicken as a conditioning diet for reproduction. Goldfish culture in China commonly relies on natural assemblages of algae and zooplankton established in fertilised ponds for larvae, wholemeal flour and dried fish for fry, and locally produced pelleted diets consisting of various meat and flour products for adults (see Willis and O'Sullivan, 2007).

However, in the past 15 years, feed and nutrition studies directed towards ornamental fish have become more frequent and have achieved much in integrating techniques and technologies established in food-fish aquaculture. Food type, quality, quantity, composition, ration size and feeding frequency are among the most important factors shown to affect growth and reproduction in ornamental fish, and it is important to optimise these factors to reduce undue cost and wastage (James and Sampath 2004a,b). Past research has focused on the development of live food protocols and investigating some specific nutrients in formulated feeds for ornamental fish.

25.12.7.1 Live foods

Live foods are commonly used for all stages of ornamental fish aquaculture and in particular for larval rearing. In general, it is thought that the industrial development of freshwater ornamental fish culture has been hampered by the lack of suitable live foods in adequate volumes for feeding fish at various production stages (Lim *et al*., 2003; James and Sampath, 2004b). The most common traditionally used live foods are macro-zooplankton and worms including *Moina*, *Tubifex*, bloodworms, mosquito larvae, *Daphnia* and in some instances brine shrimp, *Artemia*, nauplii (Dhert *et al.*, 1997; Garciá Ulloa and Gómez Romero, 2005; Velu and Munuswamy, 2003). To avoid the use of potentially contaminated *Moina*, brought about by the techniques used to culture it, there has been a recent shift to the cleaner *Artemia*.

All stages of *Artemia* life cycle (see section 9.4.3 and Fig. 9.6) can be used to culture freshwater ornamental fish. For example, *Artemia* nauplii have been successfully used as a food for species such as the guppy (for both fry and adults) and the brown discus, *Symphosodon aeuifasciata axelrodi*, with good growth and survival (Lim *et al.*, 2003). They are a smaller size than *Moina*, which makes them better suited to feeding young fish (Lim *et al.*, 2003). On-grown *Artemia* have also shown potential as a grow-out feed for juvenile discus, producing faster satiation, faster growth rates and good survival compared with *Moina* and frozen bloodworms (Lim *et al.*, 2003).

Considering the non-selective feeding nature of *Artemia*, there is a high potential to manipulate their nutritional composition for both grow-out and broodstock conditioning through enrichment (Dhert *et al.*, 1997; Lim *et al.*, 2003; see also section 9.4.7). *Artemia* may also be used to deliver hormones for spawning induction or vitamins and immunostimulants during prophylactic treatments (Lim *et al.*, 2003). There are major drawbacks to feeding *Artemia* to freshwater fish, however: first, their price, and secondly, nauplii survive only 30–60 minutes in freshwater before dying and must be replenished every 2–3 hours. This can be managed by hatching large quantities of *Artemia* nauplii and using cold-storage techniques to have an easily accessible, continuous supply (Lim *et al.* 2003).

An alternative to *Artemia* nauplii is decapsulated *Artemia* cysts, a technique developed for food fish and shrimp hatcheries (Dhert, *et al*., 1997; Velu and Munuswamy, 2003). Unlike many marine fish species, freshwater fish are able to digest the embryonic membrane of decapsulated *Artemia* cysts (section 9.4.5), which enables them to access a food with good nutritional content and higher energy than *Artemia* nauplii (Lim *et al.*, 2003). Decapsulted *Artemia* cysts have supported good growth, survival and stress resistance in freshwater fish larvae including those of goldfish, common carp, barbell (*Barbus barbus*) and grass carp (*Ctenopharyngodon idella*) and fry of guppies, mollies, platies, swordtails and black neon tetras (*Hyphessobrycon herbertaxelrodi*), often outperforming control diets including *Artemia* nauplii and *Moina* (Lim *et al.*, 2003; Velu and Munuswamy, 2003). The use of decapsulated freshwater fairy shrimp, *Streptocephalus dichotomous*, cysts has also shown promise in rearing angelfish larvae (Velu and Munuswamy, 2003)

There are, however, species of freshwater ornamental fish whose larvae are too small to ingest *Artemia* nauplii or decapsulated cysts (Arimoro, 2006; Dhert *et al.*, 1997). In the past, the culture of fish with small mouths has been a major bottleneck to ornamental aquaculture, with the lack of appropriate foods for these species hindering their production (Arimoro, 2006; Lim *et al.*, 2003; Velu and Munuswamy, 2003). Traditional practices for rearing

small-mouthed larvae of ornamental fish have relied upon natural plankton blooms in fertilised ponds, milk powder, powdered feeds or cooked egg yolk suspension (Dhert *et al.*, 1997). Such practices are highly polluting, limit stocking density and often yield low survival. However, the marine rotifer *Brachionus plicatilis* (see section 9.4.1 and Fig. 9.5) has been used successfully for rearing larvae of several freshwater fishes including the Japanese ornamental koi, *Cyprinus carpio* (Lim *et al.*, 2003). Like *Artemia* nauplii, though, the marine rotifer quickly sinks in freshwater, living only 2 hours.

Attention therefore has turned to the freshwater rotifer *Brachionus calicyflorus* as a potential food for freshwater fish larvae. This species can be continuously cultured, nutritionally enriched, and displays variance in sizes between strains, making them suitable for fry of various sizes and nutritional requirements (Arimoro, 2006). Freshwater rotifers significantly improved larval growth and survival of the dwarf gourami and the white cloud mountain minnow (*Tanichthys albanubes*) compared with the conventional food of egg yolk (Lim *et al.* 2003). In combination with *Artemia* nauplii, *B. calicyflorus* has proved useful in raising discus larvae in the absence of their parents, eliminating the risk of parental cannibalism and shortening the brooding interval of spawners (see Dhert *et al.*, 1997; Lim *et al.*, 2003). Other small-mouth larvae from the gourami, tetra and rainbowfish families also stand to benefit from feeding on freshwater rotifers, particularly as techniques for the culture of this food organism are better established (Arimoro, 2006; Dhert *et al.*, 1997).

25.12.8 Nutrition

Although ornamental fish are traditionally fed live foods, this practice produces variable results, because live foods are often nutritionally deficient and can act as vehicles for disease transmission if not stored and disinfected properly. There is growing emphasis on supplementing or completely replacing live foods with inert diets in ornamental fish culture (James and Sampath 2004a,b), and the major advantage of using inert feeds is the ability to deliver balanced nutrition of known quality, in known quantities.

Broadly speaking, it is common practice for diets of food-fish species to be used for ornamental culture, and often the diet used for grow-out is also used for conditioning fish for reproduction (Garciá Ulloa and Gómez Romero, 2005). If a specific diet exists for an ornamental fish, its formulation is often based on extrapolation of results of feeding trials performed with food fishes. Considering that the nutritional requirements of fish can vary between species and stages within an individual's life cycle, it appears obvious that, to accommodate the needs of ornamental fish and various stages during their life cycle, specific diets for ornamental fish must be developed. However, research directed towards nutrition in ornamental aquaculture is progressing slowly.

Studies directed towards protein nutrition in freshwater ornamental fish have shown protein requirement to be similar to those of food fishes classified in the same trophic category. For grow-out this can range from 30% dietary protein for omnivorous goldfish to 40–50% for the carnivorous discus and angelfish. A clear example of the need for further research to optimise nutrition in ornamental fish feeds and reduce cost and waste is shown in research with the swordtail. The previously recommended dietary protein level for swordtails and other live-bearers was anywhere from 28 to 50%. However, Chong *et al*. (2004) showed that food protein content below 40% had negative effects on reproductive performance.

The dietary fatty-acid requirements of freshwater ornamental fish are poorly understood although it is commonly accepted that they require either dietary linoleic acid (18:2*n*–6), or linolenic acid (18:3*n*–3), or both, whereas marine species require longer chain eicosapentanoic acid (20:5*n*–3) and docosahexaenoic acid (22:6*n*–3). Unlike freshwater fish, marine species cannot synthesise these highly unsaturated fatty acids from linolenic acid and linoleic acid (see section 8.3.3). However, dietary inclusions of highly unsaturated fatty acids (with a balanced ratio of *n*–3 and *n*–6 unsaturated fatty acids) in conditioning diets for zebrafish, *Danio rerio*, broodstock has shown to improve reproductive performance (Jaya-Ram *et al*., 2008). More specific investigation of dietary inclusion of docosahexaenoic acid in broodstock diets of freshwater ornamental species, including the common carp, has shown it to improve egg hatchability (Sales and Janssens, 2003). Furthermore, phospholipids that are commonly synthesised by juvenile and adult fish in adequate amounts from other dietary constituents have been shown to be essential in fast-growing carp larvae, *Cyprinus carpio*, which appear to use the nutrient faster than they are able to synthesise it (Fontagnè *et al.*, 1998).

There has been recent research interest in the ornamental aquaculture industry regarding dietary inclusion of carotenoids. Carotenoids contribute to the colour of the flesh, the exoskeleton of aquatic animals and are the primary source of colour in the skin of fish. They can also play important functions as pro-vitamin A, antioxidants, immunoregulators and are suggested to have a function in reproduction and disease resistance. Considering that skin colouration

is a major characteristic by which ornamental fish are valued, nutrients that enhance skin pigmentation are especially important in ornamental aquaculture. Fish are unable to synthesise carotenoids *de novo*, and therefore rely on an adequate dietary supply to achieve their natural pigmentation. The primary sources of carotenoids are microalgae, phytoplankton and higher plants. Recent studies on ornamental fish have shown that alfalfa meal, microalgae (such as *Hematococcus pluvialis*), paprika and china-rose (*Hibiscus rosasinensis*) are all sources of carotenoids that have a positive effect on pigmentation when included in the diet (see, for example, Sinha and Asimi 2007).

There have been a few studies investigating the dietary requirements of particular species, but the dietary requirements of vitamins, minerals and carbohydrates are largely unknown for many other ornamental species.

Overall, in commercial aquaculture, there is a lack of high-quality formulated foods that satisfy the nutrient requirements and ingredient digestibility of any ornamental fish, to promote optimum growth and lower feed waste and nutrient loss (Chong *et al.*, 2003; James and Sampath, 2004b). It comes as no surprise, therefore, that there are numerous problems with the sole use of inert feeds in ornamental aquaculture, and that live feeds are considered superior to inert feeds in all aspects of ornamental aquaculture, including broodstock conditioning, larval rearing and grow-out (see, for example, Velu and Munuswamy 2003).

There is considerable need for research into the nutritional requirements of freshwater ornamental fish, which in turn will allow formulation of more appropriate foods to satisfy their specific dietary requirements.

25.13 PRODUCTION AND MARKETING GOALS

Although food fish are primarily marketed on the basis of mass, ornamental fish are sold according to number. Therefore, the goal of production in ornamental aquaculture is to produce the greatest number of fish of a given size with consistently low size variation, within the shortest possible time (Olivier and Kaiser, 1997).

The major criteria for selection of ornamental fish destined for market are as follows:

- size;
- quality in coloration;
- finnage;
- absence of deformity or damage;
- even sex ratios.

Fish that do not meet these criteria are commonly discarded (Tamaru *et al.*, 2001; Watson and Shireman, 1996). For live-bearers the number of discarded fish can exceed half the total yield from a pond, particularly in varieties that have more exaggerated features such as high fins or lyre-tails. Higher yields of female live-bearers results in even greater discard rates, as the market often demands an even sex ratio (Watson and Shireman, 1996).

Although most sizes harvested at the end of grow-out periods are marketable, the estimated farm-gate price per fish will vary depending upon body size, which can often determine whether a certain size is economically viable to sell (Tamaru *et al.*, 2001). For example, the smallest marketable size for goldfish tends to be 5.0 cm whereas the largest is 25–30 cm. Acceptable market size for dwarf gouramis is greater than 5.0 cm and for swordtails this ranges from 2.5 cm total length (denoted as 'small') to 7.6 cm total length (denoted as 'large').

25.14 THE FUTURE OF THE ORNAMENTAL INDUSTRY

The ornamental industry is a growing industry with worldwide standing. There is potential for environmentally stable exploitation of wild stocks and for the development of commercial rearing facilities as research continues and advances are made in technologies for life-support systems. Although a fraction of the world's freshwater fish species are commonly kept and traded, the aquarium hobby thrives on novelty, making the industry dynamic. There is a long way to go in the development of optimal culture methods and techniques for current commonly traded and cultured species. However, with advances in aquaculture technologies there is significant potential for the continued domestication and culture of ornamental species, particularly marine ornamental species, and the development of new strains of various colours and morphological features.

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26
The Next 20 Years[1]

Rohana Subasinghe and Nathanael Hishamunda

26.1 INTRODUCTION

World per capita consumption of fish and fishery products has risen steadily over recent decades from an average of 11.5 kg during the 1970s, 12.5 kg in the 1980s to 14.4 kg in the 1990s. In 2007, capture fisheries and aquaculture supplied the world with over 113.7 million t of food fish[2], providing an apparent per capita supply of 17.1 kg (live weight equivalent), which is among the highest on record (Fig. 26.1). Global production of fish[3] from aquaculture has grown rapidly during the past four decades, contributing significant quantities to the world's supply of fish for human consumption. Aquaculture currently accounts for nearly half (44.3%) of the world's food fish (Fig. 26.2). With its continued growth, it is expected that aquaculture will in the near future produce more fish for direct human consumption than capture fisheries[4].

In 2007, the world consumed 113.7 million t of fish, of which 50.3 million t originated from aquaculture. The Earth's population is forecast to reach 8.32 billion in 2030. If capture fisheries production and the non-food uses of fish remain constant, aquaculture needs to produce over 80 million t by 2030 to maintain the current per capita consumption. That is, 20 years from now, aquaculture will need to produce an additional 30 million t of fish per year compared with current annual production.

[1]This chapter draws largely from both published and unpublished data, and information from FAO Fisheries and Aquaculture Department.

[2]'Food fish' in this chapter refers only to fish, crustaceans and molluscs destined for human consumption.

[3]In this chapter, unless otherwise stated, 'fish' refers to fish, crustaceans and molluscs.

[4]All figures quoted in this chapter are based on FAO statistical data or data derived through FAO activities.

Assuming a sustained demand for fish (the world is prepared to pay for fish as a desirable food product), the question remains whether the sector can grow fast enough, and sustain the projected demand for food fish, while ensuring consumer protection, maintaining environmental integrity and achieving social responsibility.

26.2 RECENT TRENDS IN AQUACULTURE DEVELOPMENT AND MAJOR CHALLENGES

A recent study conducted by the Food and Agriculture Organization of the United Nations (FAO) shows six major development trends in aquaculture, which include the following:

1. a continuing intensification of aquaculture production resulting mainly from the increasing unavailability of aquaculture sites and the growing restrictions on using non-agricultural land for aquaculture;
2. a continuing diversification of species use, particularly high-value marine species in regions and countries where aquaculture is well established;
3. a continuing diversification of production systems and practices, including integration of aquaculture into existing farming systems;
4. an increasing influence of markets, trade and consumers in prompting producers and processors to pay more attention to environmental concerns, food quality and safety, and moving towards greater value adding and development of processed products for exports
5. an enhanced regulation and improved governance of the sector, with strong emphasis on self-regulation, especially through farmer associations;

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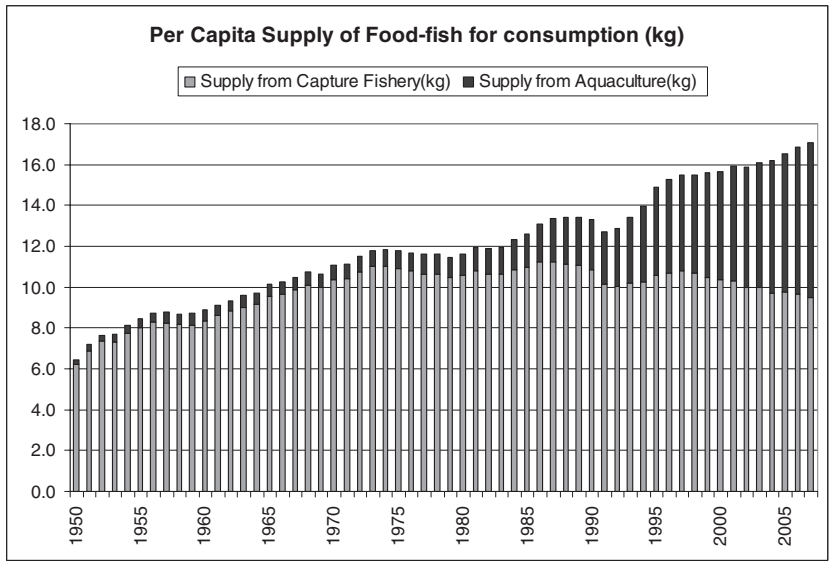


Fig. 26.1 Per capita supply of food fish (kg) from capture fisheries and aquaculture for human consumption, 1950–2007.

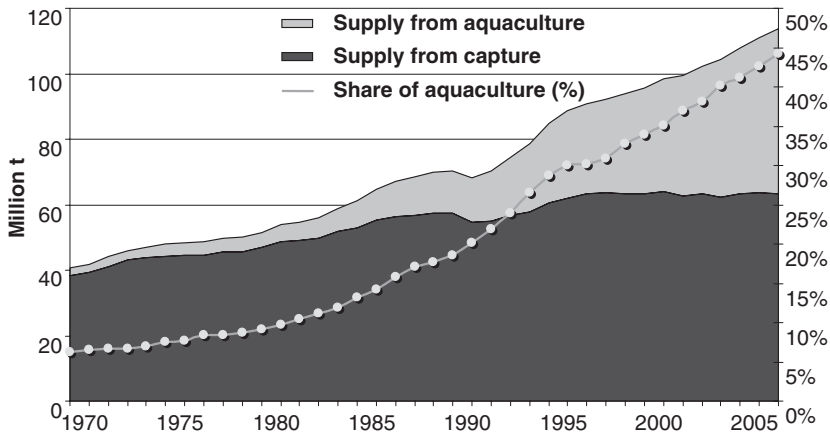


Fig. 26.2 Contribution of aquaculture to the global food fish supply as quantity (million t) and percentage.

6. increasing attention on better management of the aquaculture sector through production efficiency, economic sustainability and overall competitiveness.

The study noted, however, that these trends do not necessarily apply equally to all the regions because of differences within and between regions in the developmental stage of aquaculture (Figs 26.3 and 26.4). The situation is particularly different for sub-Saharan Africa.

The main challenges for the future will be for policy makers and development agents to create an enabling environment for aquaculture to develop substantially enough to meet the demand for fish. The major enabling factors that were identified include the following:



Fig. 26.3 Barramundi cage farms within an estuary in Malaysia.



Fig. 26.4 Atlantic salmon farm in a fjord, Norway.

- national and international market developments and access to markets;
- changing population and demography, seafood consumption habits and patterns;
- consumer preference, and increasing consumers' purchasing power;
- technology development and improvement in management systems;
- access to sufficient services and quality inputs;
- adoption of environmental management practices for protection and sustainable use of aquatic resources;
- access to land and water resources and adequate physical infrastructure;
- ensuring food safety;
- skills development and capacity enhancement;
- efficient communication and knowledge transfer.

26.3 AQUACULTURE DEVELOPMENT SLOWS DOWN, BUT IT CONTINUES TO GROW

The growth of aquaculture (in volume) has already begun to slow down. From a yearly average of 11.8% in 1985–1995 it has decreased to 7.1% during the following decade, and the average rate of growth during 2004–2006 was 6.1% in volume and 11% in value. Will the current growth rate maintain its course to produce the additional 30 million t of food fish a year needed to maintain the current per capita consumption of fish by the year 2030? The 2004–2006 growth rate of around 6.1% a year could very well falter; the sector may encounter numerous constraints in the coming decades.

26.3.1 Trends in species, consumption and trade

World aquaculture production is dominated by species at the lower end of the food chain. Carp and shellfish account for a significant share (>70%) of species cultivated in developing countries for human consumption. However, in response to a ready market for species at the higher end of the food chain in both developed and developing countries, their production in recent years has been growing rapidly compared with that of species at the lower end of the food chain. The demand for fish as a healthy and nutritious food commodity is increasing, even in the developing world. Personal disposable income of the three main populations in the developing world, China, India and Indonesia, is steadily growing even as their populations increase. The demand for low-value species for national consumption is currently met primarily through national production. This, however, may not be the case in the coming decades. Low-value fish may continue to be farmed and supplied for local consumption in regions and countries where the cost of production is low and production conditions are better; while nationally produced high-value fish may enter the global market.

Globally, fish is an important export commodity. The value of world exports of fish and fishery products grew by 9.5% in 2006 to equal US$86 billion and by nearly 7% in 2007 to equal US$92 billion. The proportion of world fish production (144 million t) that was traded internationally in 2006 represents 37% of the total, or 54 million t. Developing countries account for approximately 50% of all fish exports with a record net export revenue of US$25 billion (exports minus imports) (Table 1.3). World imports reached US$96 billion in 2007, but were mostly by developed countries, responsible for 80% of all imports by value. This share, however, is falling over time as developing countries are importing more and more, both as raw-material for their processing industry and for domestic consumption.

Through trade, globalisation is increasingly playing an important role in aquaculture development. Its requirements are two-fold:

1. strengthening of national, inter-provincial or inter-state, as well as regional and international biosecurity, and food safety measures;
2. enhancing the ability, through training, legislation, codes of practice, certification, traceability schemes of governments and producers, to comply with trade and market access requirements for safe and quality products.

These requirements are creating a considerable drive for importing and exporting countries to harmonise standards collectively as well as address issues such as the certification of products and processes. Further global cooperation and harmonisation of standards for aquaculture production and trade will be important to support the increasingly globalised aquaculture sector.

As a consequence, policymakers emphasise the need for better governance of the sector. They are aware that policies can be much more effective if producers participate in planning, decision making, management and regulation processes. Such recognition has led some governments to build upon existing national capacities to assist producers and processors to comply with mandatory food safety regulations, while empowering farmers and their associations for greater self-regulation. However, this is not always the case.

26.3.2 Small-scale producers and market access

Aquaculture is an income generating activity, with significant potential for creation of employment, poverty reduction, community development and food security. It has two distinct facets in terms of production: producing fish for domestic markets (Figs 26.5 and 26.6) and for international markets (Fig. 1.12). Producing for domestic markets directly improves national food security whereas producing for international markets creates employment, provides income and brings foreign exchange, thereby indirectly contributing to food security.

Commercial producers of aquatic food range from large numbers of small-scale producers to a small number of large-scale producers. Large commercial producers are generally better organised, often vertically integrated along the supply chain, and they use capital intensive production systems (Fig. 26.7a, b). They have advantages



Fig. 26.5 Drying milkfish fillets, Philippines.

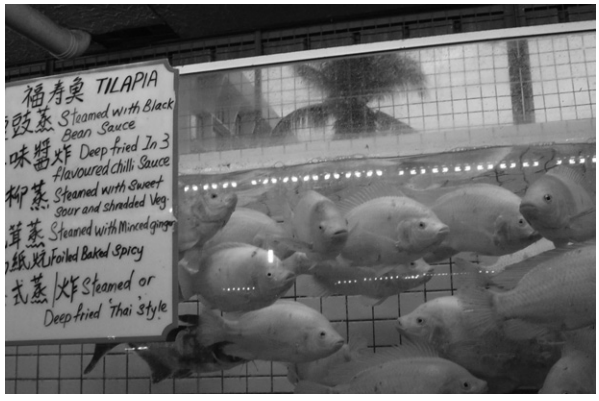


Fig. 26.6 Live tilapia for sale in restaurant, Malaysia.

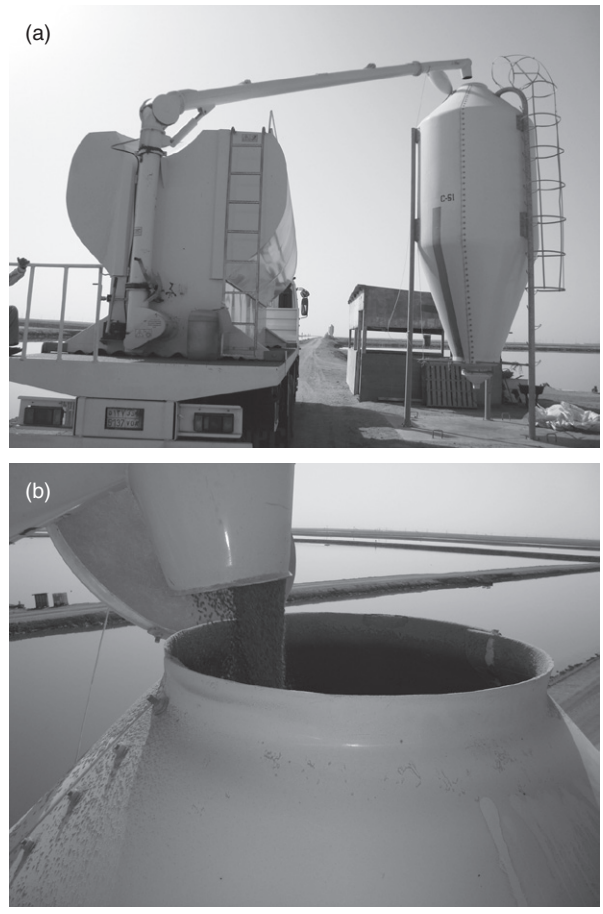


Fig. 26.7 Equipment for managing large quantities of feed in a capital-intensive marine shrimp farm, Saudi Arabia. (a) Bulk feed truck and pond-side hopper. (b) Discharging feed pellets into a pond-side hopper for subsequent supply to the pond. (Photographs by NPC.)

of scale and are able to compete effectively on international markets. Small producers tend to be less organised, not vertically integrated, rely heavily on service providers and are capital extensive, thus, by and large, they continue to cater for local markets. However, with as much as 80% of production in many Asian countries deriving from small-scale producers, it is also evident that a large part of this production, especially shrimp and marine finfish, enters into international trade. This happens through the use of clusters, common collection points and through the activities of buyers who supply domestic processors for exports.

Small-scale producers may find it difficult to enter directly into international markets, mainly because international corporate buyers need a regular supply of quality and safe products in large quantities. This, however, does not imply that the products from small-scale farms are of sub-optimal quality; however, it indicates that these farms are unable to produce regular supplies of large quantities and comply with fish quality, safety standards and requirements of the international markets. They can, however, achieve this by organising into groups/clusters (often called producer organisations) which work in a more coordinated manner for export purposes. The growing concen-

tration of distribution at the retail level through large international supermarket and hypermarket chains, as well as the emerging consolidation on the processing side, has created the need for new innovative structures at the producer level through networks of some sort and coordinated commercial activities. These will be of paramount importance in development.

For some aquatic commodities and in some countries, the number of mainstream commercial production units is decreasing but their size is increasing. However, as the sector matures and attracts foreign investment, it appears that large enterprises (including multinationals) gradually take the upper hand. If this situation happens in other commodities that are predominantly produced by small-scale producers and internationally traded, or have potential for international trade, there is a need to assist those small-scale producers to become more competitive in the market. Otherwise, they may lose household income and thus may face food insecurity.

In the same manner, small-scale aquaculture producers should be encouraged to explore new organisational structures and innovative commercial arrangements to exploit the opportunities offered by globalisation and to enter international and regional markets. This has already been demonstrated in many developing countries, particularly in Asia, and not only for high-valued species. In fact, there is also growing regional trade in so-called low-value farmed fish species.

26.3.3 Corporate social responsibility

In aquaculture production, as in other agriculture commodities, the choice of crop should be driven by the market. Commodities such as pangasid catfish, tilapia, etc., grown in Asia and Latin America, have been able to penetrate Western markets mainly based on their low prices. Perhaps another way forward for such small-scale operators to gain or maintain market access could be to produce for niche markets. A shift from a production driven approach to a market driven approach with emphasis on the whole supply chain from producer to consumer should be explored. Assisting small-scale farmers to gain market access is increasingly considered as an important corporate social responsibility of all involved in the market chain. Making this a significant reality is a challenge.

26.4 MARINE RESOURCES AND AQUAFEEDS

Some types of aquaculture rely on wild caught seed and broodstock. Although wild caught shrimp seed and broodstock are phasing out, but still present (Figs 21.6 and 26.8), in some countries there remains a heavy reliance on wild caught seed of some marine and some freshwater species. Continuing to rely on wild caught seed is a concern for the future of the industry and adequate measures are needed to rectify this shortfall.



Fig. 26.8 Marketing shrimp broodstock, Bangladesh.

There is a concern that the available marine feed resources (fishmeal and fish oil) may not be sufficient to meet the demand of projected aquaculture production. About 23.13 million t of compound aquafeeds were produced in 2005 of which ca. 42% was consumed by aquaculture. The aquaculture sector consumed ca. 3.06 million t or 56% of world fishmeal production and 0.78 million t or 87% of total fish oil production in 2006, with over 50% of fish oil going into salmonid diets. The amount of fishmeal and fish oil used within aquafeeds has grown over threefold between 1992 and 2006. This increase has been possible as the poultry sector gradually reduced its reliance on fishmeal.

Besides fishmeal or fishmeal based diets, ca. 5 million to 6 million t of low-value/trash fish are used as direct feed in aquaculture production worldwide. A recent estimate places the Asian use of trash fish as fish feed at about 1.6 million to 2.8 million t/year. In addition, large amounts, yet to be quantified, are also used by the pet food industry and the fur animal sector. Fishmeal and fish oil production have remained static over the past decade and a significant increase is not anticipated in the foreseeable future. There is evidence that the fishmeal usage in the animal production sector, particularly poultry, will continue to decrease in the coming years. Furthermore, it is expected that the proportion of fishmeal and fish oil use in aquafeed will also be reduced substantially by the increasing use of



Fig. 26.9 Salmon farming in seacages, Chile.

vegetable-based protein and oil as well as greater efficiencies in feeding through better feed management.

However, the world prices of fishmeal, fish oil and other feed ingredients are rising. The price of fishmeal increased from around US$500/t in 2000 to around US$1200/t in 2008. The price of fish oil also increased from US$300/t in 2000 to US$1,800/t in 2008. The average price of other feed ingredients, particularly cereal grains and soya bean, also rose by 30–130% during 2007. These price developments are bound to affect aquaculture production. Although the tilapia and catfish farming sectors use low levels of dietary fishmeal and fish oil, varying between 2 and 7% for fishmeal and 1% for fish oil, the increasing prices of other feed ingredients, particularly the grains, will have a major impact on the feed price (Fig. 26.9).

Increasing prices of fishmeal, fish oil, grains and other feed ingredients, and fuel and energy will certainly affect the cost of aquaculture production. With such a situation, can aquaculture farms be economically viable or sustainable? Sustainability remains a concern, however, when the demand for aquaculture products is outstripping the supply, and prices soar so that even inefficient farms might make money.

It is important to note that, over the past 4 years, the price of compound feeds in the salmon sector has only increased by around 15%. This is because the sector is highly organised, benefitting from continued research on salmonid physiology which helped in substituting fishmeal and fish oil adequately to keep the price down while maintaining the feed quality (Fig. 26.9).

26.5 ENVIRONMENTAL AND SOCIAL ASPECTS

The environmental impacts of aquaculture development have received a high degree of attention over the past three decades, typically in cases where societal benefits were negatively affected by unregulated aquaculture development, although these claims may not always be supported by hard evidence. With the increasing demand for environmental protection and services in a situation of diminishing land, water and feed resources, this attention is likely to become more pronounced in the coming decades. With weak or improper regulations for the allocation and use of natural resources, conflicts can arise between resource users. Invariably, less influential and disadvantaged stakeholders are denied access to such resources. Unregulated or improperly regulated aquaculture development also results in a high discounting rate on the use of natural resources capacity and, therefore, encourages practices that exploit them beyond renewal.

The main concerns articulated by the general public and environmentally conscious community groups will

continue to be echoed. These concerns include the following:

- sustainability of resources used in aquaculture;
- impact of aquaculture on aquatic biodiversity;
- sustainability of fish feed practices;
- use of alien species in aquaculture;
- escapees and consequent effects on wild populations;
- release of organic matter into natural waters;
- discharge of antibacterial and chemical residues into natural environment;
- over fishing of wild species for aquaculture;
- weakness in spatial planning and competition with other activities, etc.

They raise the question of whether the true environmental cost of certain aquaculture practices is adequately known. As a result of strong public scrutiny on the environmental impacts of some types of aquaculture development, significant progress in addressing many of the key concerns in the environmental management of aquaculture has been made. Public pressure and continued commercial necessity have led the aquaculture sector to improve management, reduce its environmental impacts and encourage governments to increasingly recognise that aquaculture, when well planned and well managed, can yield broad societal benefits with minimum concomitant environmental degradation.

However, the image of aquaculture, at least among the environmental lobby groups, is still sullied. Because the aquaculture sector still causes negative environmental and social impacts, at least in some production systems and practices, this image remains. Needless to say that all food production systems have an environmental impact (section 4.1). The question is to what degree a food production sector should perturb the environmental processes. Recent progress leaves no room for complacency. Continuing improvements, policy interventions and investments are required to ensure a higher degree of environmental sustainability and economic viability in the sector as pressure on the natural resource base and public awareness of environmental issues are reaching unprecedented levels. An ecosystem approach to aquaculture development and management can help reconcile the human and environmental objectives of sustainability.

Aquaculture can make a positive contribution to environmental restoration and can help reduce the negative impacts of other human activities, such as fishing. Multitrophic aquaculture (growing fish, molluscs and seaweeds together in the same environment) is one such opportunity. Such systems not only contribute to environmental restoration, but also help in increasing production and profit. Inclusion of molluscs in marine and coastal production systems also improves carbon sequestration.

Aquaculture, as a source of food, contributes to global food security and poverty alleviation, and has a significant social responsibility. The sector should operate in a socially responsible, environmentally sustainable, and economically feasible manner, contributing to meeting millennium development goals. Is this the case at present? Although many regional and global assessments have been conducted by several agencies including FAO, an objective assessment of the sector's contribution to poverty alleviation and food security, social benefits and contribution to meeting the millennium development goals is still required.

26.6 DIVERSIFICATION AND EXPANSION

It is natural that successful fish farmers wish to expand and/or diversify their operations. Also there will be newcomers to the industry. The question is whether there will be enough suitable land, fresh or coastal waters for fish farming, globally. There are regional and national differences, and some regions and/or countries will have adequate land and water resources while others may not. Thus, many countries are seriously considering expanding aquaculture production into marine waters (open ocean aquaculture) (Figs 1.6, 26.10). The issues are as follows:



Fig. 26.10 A large partially submerged seacage being deployed in the Gulf of Mexico. The cage will be submerged until only the very uppermost portion is above the ocean surface (photograph by Dr Tim Reid, with permission for use by the Gulf of Mexico Offshore Aquaculture Consortium).

- Are we ready for such a move?
- Do we have adequate knowledge on the costs and benefits of such a move?
- Do we have adequate laws and regulations to protect ocean environments from the pollutants and environmental threats arising from such production activities?
- Where such systems exist, are there enough resources to implement them?
- Will open ocean aquaculture be economically feasible?

It is imperative that these issues are urgently addressed and research carried out so that the potential negative impacts of open aquaculture can be minimised and its socio-economic benefits maximised.

Aquaculture moves forward with improved technology, farmer innovativeness and resilience. Over the past few decades aquaculture technology has advanced tremendously, improving productivity, reducing environmental impacts, increasing profitability and enhancing sustainability. Important improvements in technologies as applied to aquaculture include: recirculation technologies, aquaponic systems, submergible cage culture systems, feed systems, genetic selection and manipulation, disease control and health management, quality assurance and safety of products, good seed and feed, and information management.

Industrialised production systems are continuously developed with increased vertical integration and systems approach to aquaculture. There will be new species, regularly added into the list of cultured species. Therefore, there is a continuing need for technological improvements, innovations, and appropriate research. The challenge will be to make the innovations useful and available to the small-scale farming sector, which supplies over 80% of the global aquaculture production. Genetically modified organisms is a controversial issue in agriculture. Significant research has gone into producing genetically modified fish and transgenic aquatics, and some are ready to be placed on the market (section 7.8.1.1). The global debate on their risks to human and environmental health will continue and it is unlikely that genetically modified fish will make a significant contribution to meeting the future demand for fish in the short term.

During the past two decades severe production losses in some species owing to disease were experienced, making disease one of the most significant constraints to aquaculture development (section 10.1). While we tackle and manage existing diseases, new pathogens and diseases will emerge. Continuing research into all aspects of health management and concerted efforts on reducing trans-boundary spread of pathogens is a must for maintaining sectoral sustainability.

Food safety and animal health and welfare are two issues that will continue to attract international policy, regulatory and market attention during trans-boundary trading and movement. For those issues, the global governance instruments exist: the Codex Alimentarius and World Organisation for Animal Health (OIE), which are recognised by the World Trade Organization Sanitary and Phytosanitary Agreement (WTO/SPS) as facilitators and regulators of responsible international trading. However, are the standards set by such instruments fully met and complied with the producers and traders? Is the process of setting standards fair and adequately participatory or consultative, and is there sufficient room for developing countries to participate in a standard setting process? There is a need to look at the effectiveness and efficiency, and identify necessary improvements, of such global governance instruments.

26.7 COMMUNICATION AND NETWORKS

In recent years, the demand for reliable and timely information on the status and trends of aquaculture has greatly increased. The demand stems from the need to formulate sound policies and development plans, and implement and monitor them. The demand also stems from the need to respond to reporting requirements of international agreements and to public demand for transparency and accountability. There have been many attempts to improve the information base on aquaculture, globally. In Asia, the base for the improvement of information was made possible through more formal networking amongst countries and institutions. There is great need to establish more networks in other parts of the world. As globalisation proceeds, with increasing flows of products, services and investment across regions, the need for more communication between regions will also increase. New technologies would also enhance and facilitate such progress.

Many networks of producer associations and groups assisted by the private sector as well as donor and development agencies do exist and have contributed significantly to the sector development. From aquaculture self-help groups, including women's groups in poor villages in Asia, to the more formal regional and international associations with their headquarters in Europe and the USA, producer groups have increasingly been playing a major role in global aquaculture development. The challenge is to establish and empower more and more producer groups so that the aquaculture sector can be better managed by the true owners themselves.

26.8 AQUACULTURE INSURANCE

The rapidly increasing production processes involved in aquaculture development (e.g. intensification, use of submergible cages, recirculation systems, aquaponics, etc.) can significantly increase the vulnerability of farms to disease outbreaks, storm damage and other risks. Thus, recently, there has been an increasing demand for insurance schemes to share and cover the risks involved. Aquaculture insurance is a risk management tool and demand for aquaculture insurance schemes is now at an all time high. There are about 8000 aquaculture insurance policies in force worldwide, but these are unevenly distributed across the sector, with few or none in regions such as sub-Saharan Africa, south America and large parts of Asia. Insurance will be an issue requiring due attention in the future by stakeholders, particularly the entrepreneurs, investors and governments. It remains a challenge of applying such financial risk management strategies to small-scale farmers.

26.9 UNEXPLORED OPPORTUNITIES

The aquaculture sector may benefit from tapping some unexplored opportunities. Open water and offshore mariculture are seen as key unexplored opportunities for producing aquatic food for the future. This would need to be supported by appropriate policy and planning, including open water zoning, legal and management frameworks. Development of genetically-improved aquatic organisms ('designer fish') with special characteristics (e.g. market requirements, environmental characteristics, and disease control and production efficiency) will take place as acceptability and public perception for such products are improving and/or changing.

More efficient feeds tailored for specific needs ('designer feeds') will be developed to improve resource use efficiency in the aquafeed sector. Use of vegetable proteins and lipids to replace fishmeal and fish oil will be further considered (section 8.8) and feeds to suit specific dietary requirements of fish and crustaceans, that are economical to use in semi-industrial or commercial farming, will be developed. As fishmeal use is likely to increase for tropical omnivorous species (e.g. carp, tilapia and milkfish) and shrimp in Asia, as these industries expand, the great opportunity and challenge for aquaculture will be the reduction of fishmeal usage for omnivorous species.

Increased attention to recreational fisheries, eco-tourism and ornamental fish production will widen the horizon of aquaculture's contribution to the society. Non-food uses of aquaculture products, including development of innovative re-use technologies for by-products and waste material from aquaculture products (e.g. salmon skin, seaweed washings, etc.) show potential for future investment.

Marine bio-active compounds, neutraceuticals, natural products, etc., from marine organisms, algae in particular, appear to play a significant role in pharmaceutical industry.

Pet foods presently use a large amount of wild caught fish, and commitments have been made by the industry to eliminate use of such marine resources. Aquaculture could provide an alternative source, opening a significant new market.

26.10 CONCLUSIONS

Aquaculture production is growing and contributing in increasing volumes to national and international demand for aquatic products. This growing sector, however, needs investment in market services, infrastructure information systems, processing, reliable year-round supply capacity and marketing systems. While doing so, it will be necessary to address the challenges in many markets to environment and sustainability concerns. Issues such as carbon neutrality and energy requirements for food production are receiving more attention. There is a drive towards integrating environmental costs into the value chain and markets. Will this be possible? Who will pay?

Consumer demands for quality, safety and value for price of aquatic products are on the increase and will continue in the coming years. These demands include product consistency, reliability of supply, food safety, product choice and, more recently, sustainability of production and social equity. Some of these demands are leading to the need for new standards, and interest in labelling and certification of aquaculture systems and products. Harmonised standards and responsible certification systems are becoming an important concern.

Access to capital and investments are a must to sustainable development of the aquaculture sector. However, if the current global economic crisis continues for some time, the commercial expansion of the sector may be retarded. Adding to the economic crisis, the prices of feed ingredients and fuel are also increasing, and, as a consequence, the cost of production is increasing. Some farmers are finding it difficult to continue farming certain species, whereas others are even converting fish farms to rice paddies. The long-term effect of this situation is unclear and unpredictable; studies are needed in this area.

Another constraint to aquaculture development could be the unpredictable and uncharacterised impact of climate change. Climate change presents unquantifiable threats of temperature increase, weather and water supply. The past 5 years have seen unprecedented impacts on the sector

from natural disasters: aquaculture in Aceh (Indonesia), Bangladesh, China and Myanmar have all suffered from severe natural disasters. There is a need for the aquaculture sector to join other economic sectors in preparing to address the potential impacts of global warming, and strengthen the adaptive capacity of aquaculture farmers, particularly the more vulnerable small-scale farmers.

A counterpoint to the rapid development of global aquaculture is its limited development in sub-Saharan Africa. There is a strong belief that Africa has the full resource potential for aquaculture growth. Although aquaculture is slowly finding its niche in many countries in Africa, the overall contribution could be improved considerably, making Africa a high-priority region for aquaculture development.

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