Live Feeds in Marine Aquaculture

Live Feeds in
Marine Aquaculture

Live Feeds in Marine Aquaculture

Edited by

Josianne G. Støttrup, PhD
Danish Institute for Fisheries Research, Charlottenlund, Denmark

and

Lesley A. McEvoy, PhD
North Atlantic Fisheries College, Shetland Isles, UK

Blackwell
Science

Editorial Offices:
9600 Garsington Road, Oxford OX4 2DQ, UK
Tel: +44 (0)1865 776868
Blackwell Publishing, Inc., 350 Main Street,
Malden, MA 02148-5018, USA
Tel: +1 781 388 8250
Iowa State Press, a Blackwell Publishing
Company, 2121 State Avenue, Ames, Iowa
50014-8300, USA
Tel: +1 515 292 0140
Blackwell Publishing Asia Pty Ltd,
550 Swanston Street, Carlton South,
Victoria 3053, Australia
Tel: +61 (0)3 9347 0300
Blackwell Wissenschafts Verlag,
Kurfürstendamm 57, 10707 Berlin, Germany
Tel: +49 (0)30 32 79 060

First published 2003 by Blackwell Science Ltd

Library of Congress
Cataloging-in-Publication Data
is available

ISBN 0-632-05495-6

A catalogue record for this title is available from the British Library

Produced and set in Times by Gray Publishing, Tunbridge Wells, Kent
Printed and bound in Great Britain by MPG Books, Bodmin, Cornwall

For further information on Blackwell Science, visit our website:
www.blackwell-science.com

Contents

Foreword

In the preface the editors point out that marine aquaculture has shown an important evolution from a relatively modest operation to a mature bio-industry, both in its research and development as well as for the industry.

The maturation of the commercial ventures is seriously indebted to the huge progress in research and development in various disciplines relevant to aquaculture. Various indeed: if one feature can typify aquaculture research it is multidisciplinarity. Originally, the typical aquaculture researcher was a combination of a marine biologist, engineer, biochemist, physiologist, ecologist (at best), and a part-time plumber. Common knowledge on distinct topics was limited and progress was often achieved through sound, albeit empirical, experiments. Trial and error ruled, not necessarily in a one-to-one ratio. But as knowledge has broadened and deepened, aquaculture scientists became more specialized and fundamental research gradually came to underpin empirical findings.

With the drive for specialization it became harder for the individual scientist to keep track of all pertinent information as well as new developments in research fields other than his or her own. This explains and justifies the multiple initiatives to provide publications, like this book, offering comprehensive updates on a selected topic. To initiate such an initiative may well turn out to be a tedious job, but fortunately strong bonds were forged between leading research groups back in the days when the aquaculture '*who's who*' could still be printed on a single sheet of paper. And although some degree of (mostly healthy) competition exists, it is still rather easy to find enthusiastic and dedicated authorities willing to contribute to essential reviews of the state-of-the-art of, for instance, live feed technology.

As aquaculture developed, live feed has often been a bottleneck in the larviculture of many species of fish and shellfish, especially at times when upscaling from laboratory and pilot trials to large industrial units.

It is therefore a pleasure and an honor to introduce *Live Feed in Marine Aquaculture* wherein all its relevant issues are covered by representatives of some of world's finest aquaculture research groups.

Prof. Dr Patrick Sorgeloos

Preface

The past two decades have witnessed a dramatic expansion in the culture of marine finfish and crustaceans. Marine larviculture without live feed, or crustacean cultures without microalgae, are rarities in commercial aquaculture. The development of commercial formulated feeds remains today's upcoming challenge. In the meantime, the industry continues the struggle to produce stable quantities of high-quality live feeds. The different species used in marine aquaculture differ in their biology and culture requirements, providing ample challenges for the novice and requiring expertise in a commercial enterprise.

This book includes information on the biology and culture of copepods as well as of the better-known traditional live feeds such as rotifers and *Artemia*. Enrichment techniques for rotifers and *Artemia* have greatly improved their nutritional value for marine fish species and have contributed to the expansion of the industry. Nutritional defects, however, are still evident in some species and in other cases subtle differences such as decreased tolerance to low temperatures observed during the juvenile stages in marine fish are attributed to poor nutritional diets during the larval stages. With the increasing emphasis on fish welfare and the need to produce high quality fish both for the aquaculture industry and for stocking purposes, larval nutrition will continue to be a main focus area for research within marine aquaculture.

Filtering molluscs and penaeid shrimps require microalgal diets at least during some stage in their development. The development of mollusc culture is closely related to the quantity and quality of phytoplankton produced. In shrimp culture, despite the development of formulated diets, phytoplankton is still used in hatcheries to supplement the diet during the larval stages. Survival and growth in marine fish larvae can be improved by the addition of live cultures. Although their role is not fully understood, their positive effects are well documented.

This book provides the reader with the compiled information on most of the live feeds used in modern marine aquaculture. Although it may not be exhaustive, it will supply the basic information needed on the biology of the species and an introduction to the relevant literature. It will also serve as a practical guide, intended to provide the reader with a good overview on culture techniques for the different species involved and with substantial reference to related literature. Three chapters deal with the hatchery production, use and nutritional value of respectively *Artemia*, rotifers and copepods. A further chapter deals with the production, harvest and processing of *Artemia* from natural lakes. Two chapters on microalgae deal with their use and production in aquaculture providing the reader with a broad insight on the importance of phytoplankton in marine aquaculture, their production and nutritional value.

The book is intended for advanced undergraduates, postgraduates and researchers in the field of marine aquaculture. It may also be relevant to experimental researchers working on physiology, behaviour or energetics in these species, or to hatchery biologists who may wish to diversify or improve their culture methods.

Contributors

David A. Bengtson Professor and Graduate Program Director, Department of Fisheries, Animal and Veterinary Science, University of Rhode Island, Kingston, RI 02881, USA. Tel.: (1) 401 874 2668, fax: (1) 401 874 4017, e-mail: bengtson@uriacc.uri.edu

Chantal Cahu Senior research scientist, Laboratoire Nutrition, IFREMER, Centre de Brest, BP 70, 29280 Plouzané, France. Tel.: (33) 2 98 22 40 40, fax: (33) 2 98 22 45 45, e-mail: chantal.cahu@ifremer.fr

Pascal Divanach Head of Aquaculture Department, Institute of Marine Biology of Crete, PO Box 2214, Post of Poros, 71003 Iraklion, Crete, Greece. Tel.: (308) 81 24 15 43, e-mail: imbc@imbc.gr

Jean Dhont Researcher, Laboratory of Aquaculture & Artemia Reference Center, Ghent University, Rozier 44, 9000 Gent, Belgium. Tel.: (32) 9 264 3754, Fax: (32) 9 264 4193, e-mail: jean.dhont@rug.ac.be

Raymond Kaas Senior research scientist, Laboratoire Algae Biotechnology, IFREMER, Centre de Nantes, BP 21 105, 44 311 Nantes cedex 03, France. Tel.: (33) 2 40 37 41 09, fax: (33) 2 40 37 40 71, e-mail: raymond.kaas@ifremer.fr

Esther Lubzens Department of Marine Biology and Biotechnology, Israel Oceanographic and Limnological Research, National Institute of Oceanography, PO Box 8030, Haifa 31080, Israel. Tel.: (972) 4 8515202, fax: (972) 4 8511911, e-mail: esther@ocean.org.il

Jeanne Moal Senior research scientist, Laboratoire Invertebrate Physiology, IFREMER, Centre de Brest, BP 70, 29280 Plouzané, France. Tel.: (33) 2 98 22 40 40, fax: (33) 2 98 22 45 45, e-mail: jeanne.moal@ifremer.fr

Arnaud Muller-Feuga Head, Laboratoire Algae Biotechnology, IFREMER, Centre de Nantes, BP 21 105, 44 311 Nantes cedex 03, France. Tel.: (33) 2 40 37 42 20, fax: (33) 2 40 37 40 71, e-mail: amuller@ifremer.fr

René Robert Senior research scientist, Laboratoire Invertebrate Physiology, IFREMER, Centre de Brest, BP 70, 29280 Plouzané, France. Tel.: (33) 2 98 22 40 40, fax: (33) 2 98 22 45 45, e-mail: rene.robert@ifremer.fr

Jean Robin Senior research scientist, Laboratoire Nutrition, IFREMER, Centre de Brest, BP 70, 29280 Plouzané, France. Tel.: (33) 2 98 22 40 40, fax: (33) 2 98 22 45 45, e-mail: jean.robin@ifremer.fr

Josianne G. Støttrup Senior research scientist, Danish Institute for Fisheries Research, Department for Marine Ecology and Aquaculture, Charlottenlund Castle, DK-2920 Charlottenlund, Denmark. Tel.: (45) 3396 3394, fax: (45) 3396 3333, e-mail: jgs@dfu.min.dk

Gilbert Van Stappen Researcher, Laboratory of Aquaculture & Artemia Reference Center, Ghent University, Rozier 44, B-9000 Ghent, Belgium. Tel.: (32) 9 264 37 54, fax: (32) 9 264 41 93, e-mail: gilbert.vanstappen@rug.ac.be

Oded Zmora National Center for Mariculture, Israel Oceanographic and Limnological Research Ltd, PO Box 1212, Eilat 88112, Israel. Tel.: (972) 7 6361442, fax: 972 7 6375761, e-mail: zmora@agri.huji.ac.il

Abbreviations

AA	Amino acids
ARA	Arachidonic acid; 20:4n-6
AscA	Ascorbic acid
AscAS	Ascorbic acid-2-sulfate
ATP	Adenosine triphosphate
AWL	Air-water lift system
BOD	Biochemical oxygen demand
DHA	Docosahexaenoic acid; 22:6n-3
DPH	Days post-hatching
DPF	Days post-(first) feeding
DPPC	Dipalmitoyl phosphatidylcholine
DW	Dry weight
EEZ	Exclusive economic zones
EFA	Essential fatty acids
EPA	Eicosapentaenoic acid; 20:5n-3
ESD	Equivalent spherical diameter
FAO	United Nations Food and Agriculture Organisation
FCE	Food conversion efficiency
FW	Weight after preservation in buffered saline formaldehyde
GMO	Genetically modified organisms
GSL	Great Salt Lake
HUFA	Highly unsaturated fatty acids with 20–22 carbon atoms and more than three double bonds
ILL	Incipient limiting level
ISA	International Study on *Artemia*
LC_{50}	Lethal concentration for 50% of the sampled population
LNA	Linolenic acid; 18:3n-3
L-type	Large type
PAR	Photosynthetically active radiation
PL	Phospholipid
PLa	Post-larvae
PUFA	Polyunsaturated fatty acids with more than one double bond
SCP	Single cell proteins
SFB	San Francisco Bay
SGR	Specific growth rate
SL	Standard length
S-type	Small type
TAG	Triacylglycerols
WW	Wet weight

Chapter 1

Status of Marine Aquaculture in Relation to Live Prey: Past, Present and Future

David A. Bengtson

1.1 A Historical Perspective

It is difficult to determine exactly where and when marine aquaculture began. Milkfish culture has been conducted in Asia for centuries, based on the capture of fry from the wild (Pamplona & Mateo 1985; Liao 1991), so that modern rearing methods and live feed in the hatchery were not required. The efforts to repopulate the seas of Europe and North America in the late 1800s may provide a more useful starting point for a brief historical review of the modern methods. In response to the fishery crisis at that time, 'hatcheries' were constructed in several countries for the purpose of providing fertilised eggs, developing embryos and larvae for distribution back into the ocean. The hope was that these would thrive and be recruited into the commercial fisheries. Given the knowledge of freshwater fish culture in Europe and the Americas, especially of salmonid culture, which had been rapidly developing since the mid-1800s and the attendant propagation, transportation and introduction of salmonid populations (Stickney 1996), this was not an unreasonable hope for the times. By the 1890s, Britain, France, Canada and the USA all had fish hatcheries devoted to the propagation of commercially important species, such as cod (*Gadas morhua*), haddock (*Melanogrammus aeglefinus*), turbot (*Scophthalmus maximus = Psetta maxima*), winter flounder (*Pleuronectes americanus*) and lobster (*Homarus* sp.). The prevailing practice was to obtain gravid adults of a given species, strip them of their gametes for purposes of controlled fertilisation, sometimes on-board ship (some of the hatcheries were in fact ships), sometimes on shore, and maintain them no longer than the prolarva stage prior to release back to the ocean. The reason for the release at such an early stage of development was simple: there was no convenient live feed with which to provide them for their postlarval survival and growth. Cod larvae were raised in concrete ponds in Flødevigen, Norway, in the 1880s on a diet of natural zooplankton and in the absence of predators (Rognerud 1887), but apparently the results of this 'experiment' were interpreted to mean that the larvae should survive in nature, not that juveniles could be reared for release. It is only with the benefit of hindsight that we know that these ocean stocking efforts were doomed to fail, owing to the high mortality rates of fish early life-stages in the oceans. Nevertheless, many of these programmes were sustained for decades until the lack of evidence of any success from them became apparent. We will never know whether earlier discovery of easily culturable live feeds would have allowed hatchery culture of these species to a later stage when they might have had better chances of oceanic survival. Indeed, the field of stock enhancement might have been advanced by several decades had convenient live feeds been available in the late 1800s.

Just as many of the ocean stocking programmes of the late 1800s and early 1900s were being phased out, two developments occurred half a world apart that paved the way for much of the development of modern marine aquaculture. First, nauplii of the brine shrimp, *Artemia*, were found to be a good food for raising both freshwater and some marine larval fish (Seale 1933; Gross 1937; Rollefsen 1939). This allowed the culture of at least some fish species (those with mouths large enough to ingest *Artemia* nauplii as a first food). The use of *Artemia* nauplii as a convenient live feed, not only for fish, but also (and especially) for crustaceans, has perhaps done as much for the explosion of marine aquaculture in the late 1900s as any other development. Secondly, in the 1930s, Japanese researchers, beginning with Dr M. Fujinaga, began research on the culture of the kuruma prawn, *Penaeus japonicus*, which subsequently led to the development of the shrimp industry that we know today (Liao & Chien 1994). That research, interrupted unfortunately by World War II, continued through the 1960s, when commercial culture of *P. japonicus* was finally achieved.

Meanwhile, techniques were developed in the 1920s and 1930s that led to the development of molluscan hatcheries. Oyster culture, which has been known since Roman times, expanded in Japan in the seventeenth century with the finding that oyster larvae would settle on bamboo stakes, and expanded further in Europe, North America and Australia in the nineteenth century based on bottom culture (Bardach *et al*. 1972). Similarly, clam culture has been known in Japan and mussel culture has been known in France for several hundred years (Bardach *et al*. 1972). However, molluscan culture always relied on the settling of larvae from the natural zooplankton (and still does in many areas). Wells (1920) used a milk clarifier to retain oyster larvae while their water was being changed. Although hatchery spawning of oysters had been demonstrated as early as 1879, no one had been able successfully to change oyster culture water, and therefore replenish the algal food, without losing the larvae (Wells 1920). Wells (1927) then went on to raise clam larvae as well. Spawning and successful larval culture of mussels was not achieved until the early 1950s (Loosanoff & Davis 1963). Investigations of algal feeds for the rearing of molluscan larvae took place in the 1930s at both the Conwy, Wales, Fisheries Experiment Station (Walne 1974) and the Milford, USA, Bureau of Commercial Fisheries Biological Laboratory (Loosanoff & Davis 1963). Fertilisation of large tanks of filtered seawater to induce mixed phytoplankton blooms as food for molluscan larvae was carried out continuously beginning in 1938 (Loosanoff & Davis 1963), despite the contention that 'large-scale cultivation of microalgae … was probably first considered seriously in Germany during World War II' (Becker 1994). Decades of work at the Conwy and Milford laboratories paved the way for hatchery production of molluscs for commercial aquaculture in which natural settling of larvae was either impossible or undesirable.

The culture of algae seems to have its origins in the late 1800s and was enabled by the methods developed by bacteriologists (Bold 1950). Marine algal culture lagged behind its freshwater counterpart, which successfully used uncomplicated media (Pringsheim 1924; Schreiber 1927) in the early 1900s. A significant advance in marine algal culture was reported by Gross (1937), who tried to culture diatoms and dinoflagellates, but whose attention was drawn to 'nannoplankton flagellates, most of them probably unknown systematically' of about 2–10 μm in size. He was able to culture these and use them as feed for harpacticoid copepods over three copepod generations. He summarised his work by writing 'All these experiments led me to the conclusion that the autotroph nannoplankton flagellates are of great importance in the food economy of the sea.' Little did he know that they would also be of great importance in aquaculture. Methods for marine algal culture continued to advance during the middle of the twentieth century with the development of artificial media (Provasoli *et al.* 1957) and the development of 'f' medium for the enrichment of seawater (Guillard & Ryther 1962). Improved methods for monospecific algal cultures allowed expansion of hatcheries for molluscan aquaculture and enabled culture of live invertebrates as feed for larval fish and crustaceans.

Another extraordinarily important advance was made in the 1960s, when Japanese researchers discovered that rotifers, *Brachionus plicatilis*, previously considered a pest in culture ponds, could be used as a first food for larvae of both freshwater and marine fish species (Hirata 1979). This advance clearly allowed the culture of many more species whose larvae hatched at such a small size that their mouth gapes were insufficient for the ingestion of the larger *Artemia* prey. In retrospect, considering the large number of commercially important marine fish species that have been brought into culture and that rely on rotifers as first food in culture facilities, the debt to those initial Japanese culturists is profound.

The 1960s saw widespread interest in the culture of commercially important marine fish species, first from a research perspective. In Japan, efforts were made to culture larvae of red sea bream, flounder and puffer fish, among others. In Britain, the White Fish Authority engaged in activities particularly in the area of flatfish culture (Shelbourne 1964) that ultimately led to the first commercial production of turbot in 1976 (Person-Le Ruyet *et al.* 1991). In France, research conducted primarily in the 1970s led to the development of the French sea bass and turbot industries in the 1980s (Person-LeRuyet *et al.* 1991; Coves *et al.* 1991). In many countries, including the USA, interest in larval fish biology from a fisheries perspective caused many laboratories to begin rearing larval fish on field-collected zooplankton, sometimes supplemented with rotifers and *Artemia* nauplii (e.g. Houde 1972), in order to conduct fisheries research (e.g. Laurence *et al.* 1981). Norwegian scientists, using some pertinent results from the cod-spawning and restocking efforts 100 years earlier, began pond culture of cod larvae using natural zooplankton in the mid-1970s, and followed that with a major research programme on halibut culture beginning in the 1980s. Many of the above efforts documented the difficulty of rearing the extremely delicate marine larvae through the first-feeding stages and on to metamorphosis and subsequent grow-out (e.g. Jones 1972). Clearly, early efforts at rearing larval marine fish, whether using natural zooplankton, rotifers or *Artemia*, were fraught with difficulties, to the point that the famous Kyoto conference in 1976 declared larval rearing a major bottleneck in marine aquaculture (Pillay 1979).

As the 1970s saw the beginning of commercial production of several marine finfish and penaeid shrimp species, this decade is also noteworthy for the discovery that live feeds vary significantly in quality. From early reports suspecting pollutants (Bookhout & Costlow 1970) to later, more definitive, studies (Watanabe *et al.* 1978; and several papers from the International Study on *Artemia*: see Persoone *et al.* 1980), it became very obvious that different geographical strains of brine shrimp differed in their ability to support good survival and growth of marine larvae. The finding that the differences were due primarily to fatty acid profiles led to productive collaborations between aquaculturists and biochemists that have resulted in literally hundreds of publications on the subject, as well as commercial products that have played no small role in the development of marine aquaculture. The lessons learned from brine shrimp were also shown to apply to rotifers (Lubzens *et al.* 1984), to be intimately connected to algal food supply (Léger *et al.* 1986) and to explain much of the high quality of natural zooplankton as a food item. The necessity for aquaculturists to understand in detail the physiology and biochemistry of the organisms that they raise has contributed much to making marine aquaculture a sophisticated industry.

One event of the 1970s played a major role in the development of marine aquaculture, especially stock enhancement: the establishment of exclusive economic zones (EEZs). This event convinced the Japanese that they needed to become self-sufficient in seafood production, because they could no longer fish at will in the coastal waters of many nations and because they saw that an interruption of supplies on an international scale was a real possibility (Sproul & Tominaga 1992). The Japanese government responded by embarking on a massive research and hatchery-building campaign (Davy 1990, 1991). National and prefectural hatcheries now produce millions of fish, prawns and crabs for release into Japanese waters each year through the efforts of the national and prefectural Sea Farming Associations. As part of this effort, Japanese researchers have often led the way in marine aquaculture research and the practical applications of that research can be seen around the world. Investigations into the improvement of live feed, especially rotifers and *Artemia*, have certainly been a major contribution of the Japanese research programme.

The last quarter of the twentieth century saw the explosion of marine aquaculture, both shrimp and fish. The aforementioned Japanese work on kuruma prawn led ultimately to the culture of numerous penaeid species around the world. Although postlarval shrimp for stocking into grow-out ponds were for years collected from the wild, the recent trend has been toward hatchery production, which is heavily dependent on microalgae and *Artemia* nauplii as larval feeds. Japanese research on red sea bream (*Pagrus major*) similarly led to the development of that species for commercial aquaculture in Japan as well as for stock enhancement. Research on other fish species in various areas of the world has led to large-scale aquaculture production of gilthead sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*) in the Mediterranean region, Asian sea bass (*Lates calcarifer*) in the Indo-West Pacific region, turbot in western Europe and olive flounder (*Paralichthys olivaceus*) in east Asia, among other species, all dependent on live feed in the hatchery stage. Although commercial aquaculture of these species has become well established, a variety of species is still undergoing commercial growing pains, for example, Atlantic halibut (*Hippoglossus hippoglossus*), several groupers (*Epinephelus* spp.) and cod; again, all require live feed in the hatchery. Indeed, of all the marine fish species in production or in the research and development pipeline, it seems that only the wolfish species (*Anarhichas* spp.) can be routinely fed formulated diets directly upon hatching. It appears likely that live feed will be required well into the future, not only for the established and nearly established species, but also for the plethora of new bream, sciaenid, flounder and sole species currently poised to make their debut appearances on the world's commercial aquaculture stage.

One of the more interesting controversies in the live feed area is the view that natural or cultured copepods are necessary for at least some species, as opposed to the view that rotifers and *Artemia* nauplii are quite sufficient. The former view seems to come from the

Nordic countries, particularly Norway and Denmark. A valuable result of this controversy has been the extensive biochemical analyses of natural zooplankton to determine whether particular nutritional ingredients are present there, but lacking in rotifers and *Artemia*. Van der Meeren and Naas (1997) provide an excellent review of larval fish rearing in large, enclosed systems primarily through the use of natural zooplankton. Norwegian scientists have pioneered this field of larviculture in ponds and natural inlets that have been closed off from the sea. The procedure requires either the filtration of incoming water or the use of rotenone to kill any predators. The enclosures can be fertilised to increase the phytoplankton productivity within, so that large populations of copepods are available on which the larvae can feed. Alternatively, larvae can be reared in a bag enclosure and provided with additional zooplankton that has been collected from the adjacent waters by the use of a plankton wheel (see van der Meeren & Naas 1997, p. 373). Cod have been raised successfully in Norwegian lagoons using these methods and halibut have been raised there in bags. A company in Denmark produces turbot larvae in large concrete tanks in which zooplankton 'blooms' are induced. In addition, at least one of the tanks is devoted exclusively to extensive copepod culture and used to feed the larval fish tanks if live prey levels therein fall too low. While larvae of all these species grow extremely well on the natural zooplankton, the production at such facilities is, almost by definition in the temperate zone, seasonal and not amenable to more intensive production methods in which juveniles must be produced year-round. However, some species produce larvae with mouths that are too small to ingest even rotifers at first feeding and an alternative live prey, such as copepod nauplii, would be necessary to culture such species. Furthermore, it is well known that the nutritional value of copepods is better than that of the convenient live feeds such as rotifers and brine shrimp. Thus, abundant rationale exists for research on the mass production of copepods. Research efforts into rearing copepods in intensive indoor systems have shown some promise, but commercial-scale production has not been achieved (see review by Støttrup 2000).

Oddly, the use of live prey in hatcheries may be strongly related to one of the banes of the marine larviculturist, disease. As beneficial, indeed critical, as rotifers have been to the development of marine fish larviculture, it has been known for some time that rotifer cultures fed to a tank of fish can also carry pathogenic bacteria, such as *Vibrio* spp., that can lead to subsequent disease problems (Gatesoupe 1982). In a similar way, bacteria from *Artemia* hatching water, if the *Artemia* cysts have not been decapsulated or otherwise disinfected, can introduce to the fish tanks xenobiotics from wherever in the world the *Artemia* cysts originated. Disease has become a major consideration in hatcheries and the microbial ecology of hatchery tanks has become an area of intense research which one hopes will lead to more predictable hatchery outputs (Vadstein *et al*. 1993; Vadstein 1997). Live feeds thus have both good and bad aspects, and one challenge of the future is to minimize the bad aspects.

1.2 Marine Aquaculture Today and in the Future

At the time of writing, the United Nations Food and Agriculture Organisation (FAO) had just released its preliminary estimates for fisheries and aquaculture statistics from 1999, which indicate that world aquaculture production was 32.9 million tonnes (19.8 million t from freshwater, 13.1 million t from marine) (FAO 2000). Thus, aquaculture makes up more than 35% of the total 92.6 million tonnes of fisheries products consumed by humans. Marine aquaculture has been growing by about 0.9 million tonnes per year in recent years, while the growth of freshwater aquaculture has been closer to 1.1 million tonnes per year. In 1998, the last year for which full statistics are available, aquaculture production of purely marine fish was 781,000 t, thus lagging behind crustaceans, mostly shrimp (1,564,000 t), and diadromous fish, mostly salmonids (1,909,000 t), and far behind freshwater fish, mostly carp (17,355,000 t) (FAO 2000).

The major research endeavours in marine hatchery aquaculture today can be divided into three broad categories: improving reliability of production for existing species, development of culture methods for new species, and maximising the survival probability in the wild for hatchery-reared fish in stock enhancement programmes. Production reliability is being improved by several strategies. Selective breeding programmes for both fast growth and disease resistance should result in improved hatchery production in future years and those for improved flesh quality should ultimately yield a better product going to market. Improved management of microbial ecology in hatchery tanks through better husbandry, use of probiotics, etc., should also help production reliability. Development of vaccines, delivered by injection to older juveniles and by immersion to younger juveniles, should likewise aid in the minimisation of disease problems. Finally, the search for replacements for live feed proceeds apace as the world-wide availability of *Artemia* remains a question (see below) and the culture of algae and rotifers continues to be a labour-intensive requirement for marine hatcheries. The development of culture methods for new species tends to demonstrate the similarity of the requirements for raising different marine fish species, rather than differences between them. The research in this area generally involves the fine-tuning of widely accepted principles and procedures for application to the new species in question; if the culture of a species requires more than fine-tuning, its commercial development can be slowed or impaired (as in the case of Atlantic halibut). For example, if live feed other than rotifers and *Artemia* are required, the development of a new species is immediately hindered. The maximisation of survival of hatchery fish in the wild has been primarily the province of Japanese researchers (e.g. Tsukamoto *et al.* 1989; Yamashita *et al.* 1994), but their methods have more recently been adapted by others (e.g. Leber *et al.* 1997; Otterå *et al.* 1999). The basis of this area of research is the production of very high-quality juveniles from hatcheries (using only first-generation broodstock to maintain genetic integrity with the natural population), the identification of optimal release strategies (fish size, season, release site) and the use of conditioning methods, both in the hatchery and in the wild, to allow the fish to make the transition from hatchery to natural environment with maximum likelihood of survival. The fish are generally released as juveniles and therefore well adapted to formulated diets, but clearly the use of high-quality live feed is necessary earlier in the hatchery to produce the high-quality juveniles needed for release.

As we proceed into the future, a few big questions dominate the landscape. The overriding one is 'How do we make aquaculture sustainable?' The environmental consequences of the explosion of marine aquaculture in the last quarter of the twentieth century have become a major international concern within the past decade. From shrimp farming in mangrove areas to organic enrichment from salmon net-pen culture, the ecological insults brought about by marine aquaculture are trumpeted to the world's consumers by environmental groups. The global aquaculture industry is responding (Boyd 1999; SSFA & NAFC 2000) and there is cause for optimism that improved practices will be the norm in the future.

A second major question is 'What will we feed aquacultured organisms in the future?' This question applies both to hatchery-reared fish and crustaceans and to those in grow-out operations. At the hatchery level, the industry is currently undergoing a kind of crisis in *Artemia* cyst availability. This is due in large part to recent poor harvests from the traditionally productive Great Salt Lake, Utah, USA, along with increased regulation of harvests from those waters. The identification of new sources of *Artemia* cysts for harvest, for example in Asian countries that once belonged to the Soviet Union, allow some hope that this crisis will soon fade. Recalling that the last *Artemia* crisis in the mid-1970s led to the discovery of new geographical strains and focused research on *Artemia* cyst quality, one wonders what the current crisis will yield. A renaissance in research on formulated diets to replace *Artemia* is already underway (Kolkovsky *et al.* 1997; Yúfera *et al.* 1999) and one hopes that the results will be more commercialisable than those from the flurry of research on microdiets that arose from the last *Artemia* crisis. In a manner similar to the *Artemia* crises, periodic shortages of fish meal world-wide (usually due to climatic conditions off western South America) bring about intensive research into fish meal replacements. Recently, however, the aquaculture industry, as well as environmental groups, have questioned whether the projected growth of the industry over the next 30 years is possible in the light of fish meal availability even in the best of times (Naylor *et al.* 2000). It appears that partial or complete replacement of fish meal in the formulation of diets for some species will be necessary or desirable if the industry hopes to grow to the degree necessary. While this is a question primarily for grow-out producers, the ramifications will certainly be felt all the way back to the hatchery phase of the industry (Will we no longer grow species that require fish meal? Should we select for individuals that have minimal fish meal requirements?). One final major question concerns the role that biotechnology will play in aquaculture. Clearly, the biotechnology industry is already playing a role in products for the prevention, diagnosis and treatment of disease. Genome mapping is beginning for some of the major aquaculture species (M. Gomez-Chiarri, personal communication), but the question of whether genetically modified organisms (GMO) will be allowed in the marketplace is still a question for regulators. An even greater question is whether consumers will accept such products. It is likely that the answers to those questions will become apparent with GM products from terrestrial agriculture before aquaculture will address them in a major way.

1.3 The Status of Larviculture and Live Feed Usage

It may be useful in this introductory chapter to describe the status of marine finfish and crustacean larviculture and live prey usage in different regions of the world, so that the reader receives a broad overview on a global scale. The review will be presented continent by continent, in alphabetical order, based on production figures supplied by FAO for the calendar year 1997 (FAO 1999) and various articles as cited.

1.3.1 Africa

Africa's marine finfish and crustacean production comes largely from countries bordering the Mediterranean. Egypt is the leading producer, with over 16,000 t of mullet production, but these are grown from wild-captured fry, so no live feed is used (Wassef 2000). Egypt also produces more than 2000 t each of sea bass and seabream; the fry are mostly collected from the wild, but hatchery production is expanding, therefore requiring the use of rotifers and *Artemia* (Wassef 2000). Morocco and Tunisia also produce hundreds of tonnes each of sea bass and sea bream, so also require hatchery production using rotifers and *Artemia* (Romdhane 1992). Madagascar and the Seychelles Islands produce significant quantities of shrimp, *Penaeus monodon*, and South Africa has a small production of *Penaeus indicus* and *P. japonicus*, all of which require algae and *Artemia* as live feeds in the hatchery.

1.3.2 Asia

Moving out of Africa and proceeding through Asia from west to east, one finds that Israel and Turkey, like Egypt, produce significant quantities of sea bass and sea bream, all apparently from hatchery production and requiring rotifers and *Artemia*. Iran and Saudi Arabia both report production of hundreds of tonnes of penaeid shrimp, requiring the use of algae and *Artemia* in hatcheries. A small amount of marine finfish culture is reported from Kuwait and Qatar.

Penaeid shrimp culture dominates the mariculture of Pakistan (ca. 50 t), India (>50,000 t), Sri Lanka (ca. 5000 t), Bangladesh (>50,000 t), Myanmar (ca. 8 t) and Vietnam (ca. 80,000 t). Although some extensive culture using wild-caught shrimp still exists in India and Vietnam (Binh & Lin 1995; Shetty & Satyanarayana Rao 1996), the majority of the above production appears to rely on hatchery production using the normal methods with algae and *Artemia* (Shetty & Satyanarayana Rao 1996; Nien & Lin 1996).

Thailand is the world's largest shrimp producer (FAO 2000), based primarily on *P. monodon*, with production of >200,000 t in 1997 (FAO 1999). Since this production is almost exclusively hatchery based, use of algae and *Artemia* is extremely heavy. Thailand also has significant production of Asian sea bass, *L. calcarifer* (>4000 t), grouper, *Epinephelus* spp. (ca. 800 t), and threadfin, *Eleutheronema tetradactylum* (ca. 400 t), requiring hatchery usage of rotifers and *Artemia*. The Philippines, Indonesia and Malaysia are somewhat similar to Thailand, having predominantly shrimp culture with *P. monodon* as the major species (although with substantial culture of *Penaeus merguiensis* and *Metapenaeus* spp. as well), but also exhibiting increasing production levels of finfish, *L. calcarifer* in the case of Indonesia and a variety of species (e.g. snappers, basses, rabbitfish, groupers) in the case of the Philippines and Malaysia. Thus, these countries also have significant hatchery production of both shrimp (using algae and *Artemia*) and fish (using rotifers and *Artemia*). It should be pointed out that both Indonesia and the Philippines are predominated by milkfish culture, but that industry still depends largely on capture of fry from the wild and therefore does not require live feed for larviculture. Singapore, Hong Kong and Taiwan are similar to each other in having their fish and crustacean mariculture activities dominated by finfish culture, with relatively little, if any, penaeid shrimp culture. They all culture Asian sea bass, groupers and snappers to greater or lesser degrees, and Hong Kong produces significant quantities (ca. 800 t) of silver bream, *Rhabdosargus sarba*, but Taiwan produces a wide variety of marine finfish, including over 4000 t of black sea bream, *Acanthopagrus schlegeli*, and ca. 400 t of red sea bream, *Pagrus major*, among many others. Culture of these high-value species is quite industrialised, with significant hatchery production relying on the standard formula of rotifers and *Artemia*.

The People's Republic of China is the world's largest aquaculture producer, responsible for a remarkable two-thirds of all aquaculture production globally. Their marine finfish and crustacean production is a fairly minor component of their total production, but still dwarfs that of most other countries. Shrimp production, mostly *Penaeus chinensis*, still exceeds 100,000 t per year despite problems with disease epidemics in the 1990s. Cen and Zhang (1998) state that all shrimp seed for production now comes from 'a controlled environment', rather than being collected from the wild. It is impossible to determine from FAO statistics the production of individual marine fish species in China, but Cen and Zhang (1998) report 145,000 t of production in 1995 (which had apparently increased to >250,000 t by 1997), including mullets, breams, groupers, tilapia, Asian sea bass, puffer fish and olive flounder.

Japan produces far more marine finfish than shrimp. Only a little more than 2000 t of kuruma prawn, *P. japonicus*, the species that began the industry, is still produced commercially in Japan. Japanese hatcheries, however, produce prodigious amounts of both finfish and shrimp for stock enhancement and sea ranching efforts. Oddly, yellowtail, *Seriola quinqueradiata*, the fish with the largest production in commercial aquaculture (nearly 140,000 t) is still dependent on wild-caught fry. Other major finfish produced commercially include red sea bream (>80,000 t), olive flounder (>8500 t), Tetraodontidae (nearly 6000 t) and jack mackerels, *Trachurus* spp. (>5700 t). These require hatchery production using the rotifer and *Artemia* techniques that the Japanese largely developed. Hatchery rearing with rotifers and *Artemia* is also necessary for the production of fry for the stock enhancement programmes. Major species with numbers of finfish fry released in 1995 are: *P. olivaceus* (23 million), *P. major* (19 million) and *A. schlegeli* (6 million) (Fushimi 1998). These impressive numbers are, however, surpassed by those for kuruma prawn, 305 million, which requires algae and *Artemia* in the hatcheries. Overall, Japan produced seed for stock enhancement of 80 species in a total of 284 facilities (such as national, prefectural and local hatcheries), with 11 species receiving more than 10 million seed and 33 species receiving at least 1 million seed (Fushimi 1998). This production included molluscs and echinoderms as well as fish and crustaceans, but clearly Japan is a major user of live feeds such as algae, rotifers and *Artemia* for marine finfish and crustacean culture in both commercial aquaculture and governmental stock enhancement efforts.

Finally, South Korea has been rapidly expanding its marine finfish culture, primarily *P. olivaceus* (>26,000 t), while maintaining production of a few hundred tonnes of red sea bream and yellowtail and >12,000 t of various other species. The flounder culture, as in Japan, is totally dependent on hatchery production of fingerlings, with both rotifers and *Artemia* required as live feed.

1.3.3 Europe

Although minimal production of penaeid shrimp species is reported in Albania, Cyprus, France, Greece, Italy and Spain (requiring use of algae and *Artemia*), the production of marine finfish far outweighs that of marine crustaceans in Europe. In the Mediterranean countries plus Portugal, the dominant species are sea bass, with over 24,000 t, and sea bream, with nearly 30,000 t, reported for 1997 (FAO 1999). Greece is by far the leader, with over 15,000 t of sea bass and over 18,000 t of sea bream. Cyprus, France, Greece, Italy, Portugal and Spain all produce minor to significant quantities of other finfish species as well, for an additional total of between 4500 and 5000 t. All of this production is based on hatchery-raised fry. Sea bass can feed on *Artemia* as a first feed, whereas sea bream also require rotifers prior to feeding on *Artemia*.

In northern Europe, the major species are cod and halibut and there is much greater usage of natural zooplankton in addition to, or in place of, rotifers and *Artemia*. Although research on culture of both species has been going on since the early 1970s, the actual commercial production is still rather small, but growing. Norway is the leader in cod production, with slightly more than 300 t of commercial production in 1997, but Norwegian scientists have also been engaged for a number of years in production of cod fingerlings for stock enhancement projects. Cod larvae are produced in tanks, ponds or blocked-off sections of fjords, and are fed natural zooplankton obtained from the same or similar enclosed bodies of water which have been fertilised to bring about phytoplankton blooms (Huse 1991). Halibut are produced in a variety of enclosed systems and can eat *Artemia* as first food, but Norwegian producers argue that natural zooplankton is also necessary during the larval stages for production of good-quality fry. Production of halibut has more recently been effected in Iceland, which has now become the leading producer of halibut juveniles, despite the fact that the halibut larvae in Iceland are raised without natural zooplankton (K. Pittman, personal communication).

1.3.4 North America

Relatively little culture of marine finfish and crustaceans is reported from North America. Culture of cold-water finfish in Canada, using rotifers and *Artemia* as live feed for larvae, is still in the trial phases. In the USA, commercial production is reported for red drum (*Sciaenops ocellata*) and summer flounder (*Paralichthys dentatus*), both of which require rotifers and *Artemia* as prey in the hatchery. Oddly, FAO includes hybrid striped bass (*Morone saxatilis* × *Morone chrysops*) as a marine fish species in its statistics, even though the fish are reared in fresh water. The larvae of those bass are mostly raised in earthen ponds, which are fertilised in spring to induce blooms of phytoplankton and zooplankton before the introduction of the fish larvae (Harrell 1997). Since the early 1980s, hatchery production of a few species has been necessary for enhancement, restoration of stocks or mitigation of environmental impacts; striped bass (*M. saxatilis*), red drum (*S. ocellata*) and spotted sea trout (*Cynoscion nebulosus*) are the most noteworthy of these. In addition, the USA reported production of 1200 t of *Litopenaeus vannamei* in 1997, originating from intensive hatcheries with heavy use of algae and *Artemia*.

Mexico has become a large producer of shrimp (*L. vannamei*), with over 17,000 t of production reported in 1997.

1.3.5 Oceania

The majority of production here is penaeid shrimp. Australia reported nearly 1600 t of *P. monodon* production in 1997 and New Caledonia over 1100 t of *Penaeus* spp. All are from hatchery origin, requiring algae and *Artemia*. Other island nations (Fiji Islands, French Polynesia, Guam and the Solomon Islands) all report minor production (<50 t each) of various penaeids. Australia also reported over 500 t of Asian sea bass production.

1.3.6 South America, including Central America and the Caribbean

With the exception of Chile, the culture of marine finfish and crustaceans in this region is overwhelmingly dominated by penaeid shrimp. Ecuador is the clear leader, with 1997 production of over 120,000 t. Other countries producing between 2000 and 10,000 t include Brazil, Colombia, Costa Rica, Guatemala, Honduras, Nicaragua, Panama, Peru and Venezuela. Although wild seed is still used in some places, the trend is for increased reliance on hatchery production of postlarvae. The hatchery techniques are by now quite standard throughout the region, with algae and *Artemia* as the live feeds of choice, just as they are elsewhere in the world. Chile has been rapidly increasing its finfish aquaculture industry and is poised to become the world leader in salmon production, but it also is producing turbot in significant quantities for export to Europe. Hatchery rearing of these turbot depends on both rotifers and *Artemia* in the same way that they are used by the European turbot industry.

To summarise this geographical review, hatchery production of penaeid shrimp postlarvae around the world depends on the use of live algae for the early stages and *Artemia* for the later stages. Usage of formulated diets to supplement and eventually replace *Artemia* is apparently increasing (see below), but live feed is still dominant at this point. For marine finfish, hatchery production of juveniles globally is normally accomplished just with *Artemia*, if the mouth gape is large enough at first feeding, or with rotifers and *Artemia*, if a smaller initial feed is required. It should be pointed out that algae is routinely used in marine fish culture of the so-called ‘green-water’ method, but it is still not clear to what degree the algae may be contributing directly to the nutrition of the larvae (Reitan *et al*. 1997). The use of natural zooplankton, or the use of cultured foods other than algae, rotifers or *Artemia*, is limited to a few places in the world, but it can be very important in those particular places.

1.4 Why is Live Feed Necessary?

Fish biologists categorise larvae of two types: precocial and altricial. Precocial larvae are those that, when the yolk sac is exhausted, appear as mini-adults, exhibiting fully developed fins and a mature digestive system including a functional stomach. Such fish can ingest and digest formulated diets as a first food and are best exemplified by the salmon and trout raised extensively in hatcheries around the world without the benefit of live food. Altricial larvae are those that, when the yolk sac is exhausted, remain in a relatively undeveloped state. The digestive system is still rudimentary, lacking a stomach, and much of the protein digestion takes place in hindgut epithelial cells (Govoni *et al*. 1986). Such a digestive system seems (at this point) to be incapable of processing formulated diets in a manner that allows survival and growth of the larvae comparable to those fed on live feed. Altricial larvae therefore appear to require live feed, but there may be other reasons besides the digestibility question. Live feeds are able to swim in the water column and are thus constantly available to the