HATCHERY FEEDS

Research and Development Plan
2000 - 2005

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FOREWORD

Nutrition during the early life stages is a major problem in intensive fish culture. Inadequate food sources, either in terms of quantity or quality, is a major cause of mortality. Live food such as zooplankton has been employed for culturing the early life stages of marine fish and is currently obligatory for successful culture past metamorphosis, when the fish are weaned onto dry formulated diets. Continuing research and development into production technology for a range of marine finfish species has consistently demonstrated the inadequacy of existing live prey organisms used for larviculture.

The Annual International Conference and Exposition of the World Aquaculture Society in Sydney in 1999 (WAS 99) provided the opportunity for representatives from all the research groups working with larval feeds and larviculture to meet. All concurred that there was a considerable Australian research commitment to the production of hatchery feeds and to the development of new feeds. However, with the expansion of aquaculture in Australia there was seen to be a need to improve coordination between the research organisations in the study of fish larvae feeds, and to identify opportunities and priorities for future research. Accordingly, FRDC subsequently requested us to prepare a strategic R&D plan to more appropriately match the needs of industry.

On 9–10 March 2000 we convened a Hatchery Feeds workshop in Cairns, with the following objectives:

1. To assess the status of hatchery feeds, including live and compounded feeds, and to identify research in progress.
2. To assess priorities for research and development needs in the area of hatchery feeds.
3. To identify constraints to the continued development of Australian aquaculture in the area of hatchery feeds.
4. To identify opportunities to enhance collaboration and information exchange amongst researchers and industry.
5. To develop a national R&D plan for hatchery feeds.

This document is the outcome of that meeting.

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EXECUTIVE SUMMARY

With the expansion of aquaculture in Australia there is a need to improve coordination between research organisations and industry in the study of fish larvae feeds, and to identify opportunities and priorities for future research. A world shortage of the brine shrimp *Artemia* has precipitated a crisis situation in aquaculture hatcheries. Accordingly, in late 1999 the FRDC commissioned a Hatchery Feeds workshop with the following objectives:

- To assess the status of hatchery feeds, including live and compounded feeds, and to identify research in progress.
- To assess priorities for research and development needs in the area of hatchery feeds.
- To identify constraints to the continued development of Australian aquaculture in the area of hatchery feeds.
- To identify opportunities to enhance collaboration and information exchange amongst researchers and industry.
- To develop a national R&D plan for hatchery feeds.

The aquaculture community was widely polled to establish industry priorities for future research. A questionnaire was sent to all stakeholders, together with an invitation to attend the workshop, which was held in Cairns on 9-10 March 2000. Researchers were invited to present the results of work in progress, and industry needs were canvassed in open forums.

For convenience, the subject was divided into 5 main areas of research: microalgae, rotifers, brine shrimp, copepods and artificial diets. Status reviews were commissioned in each of these areas, and priorities in each defined in the workshop. In all areas, the need to benchmark best practice and to more efficiently transfer research results to industry were highlighted. In addition to these common priority areas, the following specific areas were identified as worthy of further research:

- Microalgal production systems
- The role of microalgae in green-water systems
- Assessment and production of Australian rotifer strains and alternative feeds
- Production of brine shrimp in Australia rather than depending on imported product
- Early weaning of larvae on to artificial feeds
- Scaling up existing systems for copepod production
- Development of a knowledge-base for copepod production
- Improvement of diets for copepod production
- Identification of appropriate copepods as food for individual species
- Development of local microdiets.

SECTION ONE

Hatchery Feeds R&D Plan
2000 – 2005

INTRODUCTION

David McKinnon and Mike Rimmer[1]

In today's economic climate a successful aquaculture industry needs to be extremely cost-efficient. Hatcheries need a reliable supply of high quality feeds suitable for the rearing of early life stages of aquaculture species. At present larval culture in Australia and around the world is heavily dependant on two main food organisms: the rotifer *Brachionus plicatilis* and the brine shrimp *Artemia*. The use of these organisms can present problems such as:

1. Unsuitable size
2. Inadequate nutritional content
3. Inconsistent nutritional profile
4. Inadequate digestibility
5. Inconsistent supply
6. High production costs

In the short term the provision of alternative live prey items such as copepods may solve some of these problems. However, in the long term micro-particulate diets which can be produced cheaply and made available 'off the shelf' provide the best solution. Existing industry sectors have less need for new feeds than emerging sectors, which require the development of new or alternative hatchery feeds. Extensive production techniques provide an alternative to reliance on traditional live feeds, but seasonal limitations to the supply of live feed in extensive systems, especially in temperate areas, mean there is a need for further development.

Microalgae are the basis of all live feed production, but are often perceived as being difficult to grow reliably in quantity and therefore as expensive. Though some hatcheries find that microalgae are relatively easy to mass-culture most hatchery operators would prefer an off-the-shelf substitute. Mass production of micro-algae appears to be one area in which tested technology is available but where the extension and subsequent adoption of this technology is lacking. In addition, a larger suite of microalgal species and products (such as microalgal concentrates) would be available if import restrictions were less onerous. Existing quarantine procedures restrict progress in this regard and there is a need to involve AQIS in reassessing quarantine protocols.

Rotifers (*Brachionus* spp.) can be easily cultured at high density and have become the standard first feed organism for fish hatcheries. However, though rotifers can be easily enriched and are suitable for most fish currently used in aquaculture, developing industries are experiencing

[1] This introduction summarises the issues relating to hatchery feeds felt most pertinent in general discussion at the Hatchery Feeds Workshop, Cairns 9-10 March 2000.

problems with existing larval rearing techniques. Larvae of groupers (*Epinephelus* spp.) and tropical snappers (*Lutjanus* spp.) have a very small mouth gape, and are therefore only capable of taking small food items. The latter can ingest small rotifers, but are not always able to digest them.

The brine shrimp *Artemia* is the main organism used as 'live food' in the later stages of larviculture. However, dramatic decline in harvest from the Great Salt Lake, Utah, USA, (where 70% of the world's *Artemia* cysts originate) has caused the price to double during the last year, to $150/kg. The Australian *Artemia* market is currently around four tonnes, most of which is used in prawn hatcheries. In the short term, hatcheries need to improve the efficiency of use of *Artemia* to make the best of the existing supply. The worldwide *Artemia* shortage provides opportunities for Australian industry to produce brine shrimp, possibly from desalination projects, to take advantage of world demand and increasing prices. To date commercial production of brine shrimp in Australia relies on introduced strains of *Artemia* from San Fransisco bay (*Artemia fransiscana)*. Alternatively, native West Australian brine shrimp (*Parartemia spp.)* are expected to be commercially available in about one year

Progress in copepod cultivation in Australia has been substantial in the last few years and there is good reason to be confident that this work has commercial applicability. For instance, Darwin, Cairns and Adelaide scientists have all been developing culture of the same genus of copepod, *Acartia*. Perth scientists have developed an elegant automated system for culture of *Gladioferens imparipes*, supported by FRDC funding. Current interest in the aquaculture of high value marine finfish (e.g. coral reef fish, groupers, tuna) by both industry and research sectors may require the development of copepod culture to be successful. Copepods provide a useful augmentation to, if not replacement for, *Artemia*. However, there remains a need for a production system which can be easily adopted by industry and which is suitable for a wide range of species.

Dry diets for fish larvae present a viable alternative to live food. Advantages include 'off-the-shelf' availability, consistent nutritional profile, and the ability to adjust this to the specific nutritional requirements of individual fish species. Availability is especially important in remote locations and in commercial hatcheries limited by inadequate budgets, facilities and staff. To date, however, microdiets have not matched the growth and survival demonstrated by fish larvae fed live feeds such as rotifers and *Artemia* nauplii. During recent years intensive research has been conducted by a number of research groups around the world to develop microdiets that can partially or fully replace the use of live food, especially *Artemia*. Substantial advances have been developed especially in weaning diets and in shortening the live food period. The limited local market has hampered progress in diet development in Australia.

Australia supports a substantial scientific community engaged in aquaculture research. Government and university researchers compete for funding from various agencies such as FRDC and the CRC for Aquaculture. However, in the ongoing fight for this funding technology transfer to industry is sometimes overlooked. In particular, on-the-ground extension work, such as hands-on workshops,

is needed to efficiently transfer research results. It is also often the case that exchange of information is slow between aquaculture concerns, and there appears to be a need for more efficient avenues of communication such as meetings, workshops and electronic media. Whereas scientists often have the opportunity to visit overseas installations to learn of new developments, these opportunities are seldom available to hatchery managers or technicians. At present there is no clear way for hatcheries to gain access to overseas experience to increase the range of live food and to shortcut research. One means of achieving this would be to institute a mechanism by which exchange programs for industry technicians could occur.

Post-graduate degrees are a cheap way of funding research. Masters degrees are often more productive in that they provide more industry-relevant information in a shorter time frame than PhD degrees, which tend to take longer and be more academic. However, a gap in infrastructure remains in extending technology from research scale to commercial production scale.

More research is needed on the widespread problem of land salinity (inland-based marine aquaculture). This is being addressed through several Inland Saline Aquaculture projects, funded by FRDC, ACIAR, RIRDC and the CRC Aquaculture.

Our vision is for a strong Australian aquaculture industry, with a broad base. In order to achieve this goal we need to be better develop our resources, and we need ways to assess the economics of new systems and/or technologies. It is generally acknowledged that there is not enough research funding to go around, but if we can improve collaboration between researchers, industry and funding agencies we can improve the efficiency with which existing funds are used. Dedicated business units are one option by which the economics of new systems could be assessed. Indeed, a recurrent theme through the Hatchery Feed workshop was the need for the aquaculture community to promote best practice by benchmarking the most cost-effective hatchery feeds procedures.

A companion document, *Proceedings of a Hatchery Feeds Workshop, Cairns 9-10 March 2000* contains extended abstracts of presentations from the workshop and is available at http://www.aims.gov.au/hatchery-feeds. The contents of this document are in Appendix 3.

HATCHERY FEEDS R&D PLAN 2000-2005

This plan was developed at the request of FRDC. A questionnaire was circulated to all identified aquaculture stakeholders in January 2000, together with an invitation to attend a workshop held in Cairns on 9-10 March 2000. Forty eight people attended the workshop, including 17 from industry. The current status of R&D[2] was then used as a baseline for development of a 5-year plan for hatchery feeds research. For convenience, this research was divided into four key areas: microalgae, rotifers & brine shrimp, copepods, and artificial diets.

MISSION STATEMENT

To provide strategic research to improve the nutrition of early life history stages of aquaculture species, and to facilitate efficient technology transfer between research agencies and industry.

Status in 2000	Vision for 2005
▪ High production costs of traditional hatchery feeds	▪ Improved efficiency, reliability and quality
▪ Dependence on *Artemia*	▪ Short term: alternative diets ▪ Long term: artificial feeds
▪ Global *Artemia* shortage	▪ Brine shrimp (*Artemia* or *Parartemia*) in abundance
▪ Inadequate feeds for emerging sectors	▪ New and alternative feeds developed to suit each sector
▪ Inefficient technology transfer	▪ Improved communication between research agencies and industry
▪ Hatcheries seeking individual solutions	▪ Benchmarked code of best practice

[2] See Section 2 of this plan for Status Reviews, and the Proceedings of the Hatchery Feeds Workshop for other research in progress.

PRIORITY AREAS FOR RESEARCH AND DEVELOPMENT IN HATCHERY FEEDS IN AUSTRALIA

2000-2005

Key to Symbols

👍	High priority	➤	Links to
💰	High return	⏳	Longer term

GROUP A: MICROALGAE

👍 💰 ***A1. Production***

Transfer of existing technology: Cost-efficiency of algal production can be greatly improved with better transfer of existing technologies. Training-workshops such as the 'Microalgae for Mariculture' workshops operated by CSIRO but with more emphasis on current mass-culture production, specialised advice, or installation of commercial 'packages' for algal production are possible mechanisms.

On-site production versus remote production, including microalgal concentrates: There are economies of scale with algal production. Algal production constitutes the major hatchery cost: up to 30–50%. for small- to medium-sized hatcheries. It could be more cost-efficient if these hatcheries purchased their microalgae from a larger, centralised algal production facility (or larger hatcheries) able to produce cheaper biomass and to transport their product as concentrates or pastes. The potential for Australian industry to mass produce microalgae *per se*, not just as an adjunct to hatchery production, still needs R&D to identify the best mass cultivation technologies; for example, use of photo-bioreactors for mass production of the range of aquaculture strains.

Heterotrophic production: A limited number of microalgae (e.g. *Tetraselmis* strains) are capable of heterotrophic growth, which offers the potential for much cheaper biomass than conventional phototrophic production. The cost and specialised equipment required probably prevent this technology being used routinely by hatcheries, though perhaps this also might be a system to be adopted by a large, centralised producer of algal biomass for on-selling of concentrates to hatcheries. Thraustochytrids — algal-like micro-organisms — are also capable of heterotrophic growth and these have valuable nutritional profiles as they are rich in the essential fatty acid DHA. There are already commercial products derived from heteretrophically-grown thraustochytrids; that is, AlgaMac 2000 and Docosa Gold (dried preparations of *Schizochytrium* sp.) and these are finding popularity in hatcheries for *Artemia* enrichment.

A2. Ecological Systems

Microalgae for ecological systems, e.g. green-water: There is a need to identify microalgae that are 'good food' species, that can be seeded into natural ecosystems and that form sustained, stable blooms. Australian isolates should be the preferred for this application due to endemic species and ecosystem issues, and species selection would need to be targeted to the animal being cultured.

Managing pond and tank systems: A better understanding of pond dynamics will assist in the manipulation (i.e. management) of systems to promote the natural blooming of favourable microalgal species. Parameters that will influence the phytoplankton ecology will include fertilisation (i.e. nutrients), temperature, light, salinity and turbulence. This understanding may need to be developed for each specific site.

Substrate and structure (e.g. AquaMats): We need a better understanding of the influence of pond substrate and structure on the phytoplankton ecology.

A3. Reference collection and supply service

Supply of larger volumes: Some hatcheries have requested larger volumes of microalgae (e.g. 1–5 L), or even concentrates, to use as starter cultures.

Broader choice of species, including local strains and temperature-tolerant strains. Specific industry sectors or hatcheries desire more species than are currently available. However there is an issue regarding the use of new imported strains. Local strains, more suited for specific locations or application are needed — especially strains that are tolerant to the high temperatures encountered in tropical hatcheries and tolerant to wide temperature fluctuations.

Mixtures of algae: There may be some benefit in supplying mixtures of specific microalgae. Some research has been done in this area, but as yet the application of mixed cultures has not been successful. The best approach has been to mass culture individual species separately, and then apply them as mixtures at the stage of feeding to animals as a 'multi-species' diet. The composition of multi-species diets for specific target animals needs to be established.

Non-toxic strains: Use of non-toxic strains from algal groups often associated with toxic species needs more evaluation. In particular, more research is needed on strains for new live feeds; e.g. dinoflagellates for copepods (see Section 4).

Taxonomic guides: A taxonomic guide for good and problem algal species, perhaps including nutritional properties, temperature tolerances and mass culture suitability, would aid hatcheries undertaking mixed culture or green-water applications outside (e.g. prawn and oyster ponds).

Cost: Starter cultures are currently supplied to the industry by CSIRO on a cost-recovery basis. This can constitute a significant cost to hatcheries utilising many starter cultures; but the cost is generally accepted as reasonable by the industry.

Costs for starter cultures are equivalent to cultures supplied by other collections overseas.

Non-axenic isolates: The industry does not necessarily want axenic strains as is the case for many of the imported 'traditional' strains. This is reflected in both the desire by some hatcheries to use new imported strains and, conversely, the need to find equivalent endemic strains.

➢C3 ***A4. Assessment/Application***

Growth trials — ongoing assessment: Generally speaking, we have a good understanding of nutritional profiles of microalgae, from both Australian and overseas research. What is lacking is information on the nutritional needs of larvae, so we can match the microalgal diet to the target species. Growth trials, where microalgae of a defined composition are fed to larval food organisms, would assist in a better understanding of larval needs.

Transfer of microalgal nutrients through the food chain: Most research in this area has focussed on the transfer of polyunsaturated fatty acids from microalgae or other enrichment products to rotifers and *Artemia*. Microalgae are an important source of other key nutrients, for example vitamins, sterols and free amino acids. A better understanding of the transfer of these nutrients through different zooplankton to larvae is needed to provide more information on the requirements of larvae.

Assessment of off-the-shelf products: New commercial microalgal-like products such as dried thraustochytrids, AlgaMac 2000 and Docosa Gold are becoming more widely used by industry. Compositional analysis has shown that zooplankton enriched with these products have high concentrations of the essential fatty acid DHA. More commercial testing of these products as enrichments for zooplankton fed to target larvae is warranted.

A5. Production of zooplankton

Intensive production of rotifers, Artemia: Only several species of microalgae are used in the routine production and/or enrichment of zooplankton, including green-water applications. An examination of a broader range of species might reveal alternative microalgae that improve zooplankton production and/or nutritional characteristics.

Microalgal diet selection for copepods: Copepods are recognised as having superior nutritional qualities, yet difficulties in their intensive culture limits their utilisation in hatcheries. Alternative microalgae, especially dinoflagellates, might assist in improving their production.

GROUP B: ROTIFERS AND BRINE SHIMP

B1. Benchmarks for rotifer/brine shrimp production

Many different techniques are used to feed rotifers and brine shrimp in Australian hatcheries. We need to identify the best practices being used overseas for live food production and to translocate this technology to Australian hatcheries. In order to provide a benchmark for Australian production, we need information from individual hatcheries, such as the quantity of *Artemia* used and the level of fish production. Methods to improve feeding efficiency and reduce the use of *Artemia*, such as on-growing of nauplii, need to be introduced to hatcheries.

B2. Technology transfer

➢B8

There is a great deal of information on the production of live feeds throughout the world. The degree of technology used in Australian hatcheries is extremely variable. In order to raise the standard of live feed production across-the-board in Australian hatcheries, we must increase the level of technology transfer. This may be achieved by introducing regular workshops, producing manuals (printed and electronic) and reports, and initiating a website or mailing list through the internet.

B3. Assessment and production of Australian strains and alternative species

There is a need to establish a research program to identify new strains of endemic rotifer species that may be suitable for culture in Australian hatcheries. The program will require that we (a) initially isolate rotifers and (b) then develop techniques to mass culture them. If this is possible, the suitability of the rotifer as a live feed for marine fish will need to be evaluated in research and commercial hatcheries. New rotifers could be selected for size (very small for first feeding fish larvae; very large as a potential *Artemia* replacement) or productivity. Improvements in rotifer size and productivity may also be sought by initiating a selective breeding program for rotifer strains already cultured in Australian hatcheries.

B4. Australian production of Artemia *in ponds*

Australia has a large resource of saline ponds situated on the coast (usually for salt manufacture) and in inland Australia (natural ephemeral saline lakes; man-made saline evaporation basins as part of rising saline groundwater interception schemes). These lakes may be suitable for culture of either endemic brine shrimp such as *Parartemia* spp. or exotic *Artemia* spp. There is a need to evaluate the potential for commercial production of brine shrimp in Australian salt lakes.

B5. Weaning and co-feeds

There is a need to reduce the use of *Artemia* as a live food for marine fish. This may be achieved by developing new feeding strategies for fish larvae, such as an extension of the rotifer feeding phase and early weaning of larvae with artificial diets.

B6. Enrichment

Rotifers need to be enriched, particularly with (n-3)HUFA's prior to feeding to marine fish larvae. Several commercial enrichment diets are readily available to Australian hatcheries; however, the efficacy of the enrichment protocols used in Australian hatcheries is in doubt. Procedures need to be developed to regularly analyse HUFA content of enriched rotifers to ensure that target concentrations are being reached. Best practice methods currently in use in overseas hatcheries need to be determined and translocated to Australia. Flow-on effects of the enrichment of rotifers with vitamins etc. on the production of Australian marine fish larvae need to be quantified.

B7. Evaluation of alternative species from overseas

Possible alternative species to rotifers as live feeds may be cultured overseas. A program to identify new genera (e.g. the cladoceran *Moina*) that may be suitable for marine hatcheries would be useful. Once overseas genera are identified, similar species endemic to Australia may be isolated and evaluated.

B2

B8. Evaluation of new rotifer systems

Large-scale batch culture of microalgae is generally expensive and can account for 30–40% of total hatchery costs. Continuous microalgal production systems may reduce the cost of producing algae to feed rotifers. This technique should be investigated. Development and commercialisation of microalgae concentration in Australia may also provide an off-the-shelf feed for rotifer production. This may reduce the cost and increase the reliability of rotifer production. Significant mass-culture technology of rotifers has been developed overseas. For example, ultra-high-density production systems have been developed in Japan, which are based on feeding concentrated freshwater *Chlorella*. Final harvest densities in these systems can be 100 times greater, and production costs can be 65% less, than those of traditional culture methods. This technology needs to be transferred or adapted to Australian hatcheries.

B9. Evaluating ongoing technology

Large-scale production of juvenile rock lobsters requires large quantities of *Artemia*. To reduce costs and increase production we need to develop techniques for reliable production of advanced (on-grown) *Artemia*.

B10. Culture systems

The development of extensive, fertilised-pond larval rearing techniques may overcome the need to conduct large-scale live-feed production in Australian hatcheries. Extensive larval rearing has been used successfully for a number of marine fish species. The suitability of this technique for larval rearing may be highly species specific, and this needs to be evaluated. There may be advantages in having an initial 10–14 day rearing phase in an intensive hatchery, followed by on-growing in extensive ponds. This would reduce the dependence on *Artemia*, and could significantly reduce the cost of fish production.

GROUP C: COPEPODS

C1. Scale-up of existing systems

➤C6

Considerable effort has been made in the development of copepod production systems around the country (e.g. *Tisbe* in Tasmania, *Gladioferens* in Perth, *Acartia* in Darwin and Cairns). These systems need to be scaled up and tested for effectiveness with other copepod species where necessary, and made available to industry. The effectiveness of these systems in suppling copepod food in commercial hatcheries needs to be assessed.

C2. Development of a knowledge base

There is a need to assemble available information on copepod culture into a central knowledge base to facilitate attempts by individual hatcheries to grow copepods. Though there is information available on the culture of specific copepods, at present this is difficult to identify and locate.

C3. Food type and feeding regimes

➤A4

Further research is required to identify better diets for copepods (e.g. dinoflagellates) as opposed to more conventional microalgal species. Improved feeding regimes need to be established, by determining the appropriate amounts and mixtures of various food items to maximise copepod production. Alternative diets, such as artificial feeds, also need to be investigated.

C4. Matching species

Fish species differ in their requirements for live feeds. Copepod species which are appropriate for each fish target and climatic zone (tropical, temperate, etc) need to be identified. For example, groupers and tropical snappers require sub-100µm food at first feeding, but this size requirement is soon passed by the nauplii of larger copepod species.

C5. Establishment of benchmarks for economical production

With the development of different production techniques for different copepod species, benchmark performance indices should be developed to identify the most cost-effective and efficient systems. Benchmarks should be established for production of nauplii for larviculture, for adults as Artemia replacements or supplements, and for matching the suitability of specific copepods to specific fish.

➢C1 ### *C6. The role of copepods in polyculture versus monocultures*

Green-water systems provide dietary diversity for larval fish. The effectiveness of presenting copepods as monocultures, as opposed to presenting them as part of a suite of potential food items, should be compared for each copepod species in cultivation. In addition, the ability of copepod species to persist in green-water situations needs to be assessed.

C7. Storage

There is benefit in being able to store live copepod material until needed. Spawning of broodstock can be fickle, and there would be considerable advantages in stockpiling copepod eggs and nauplii until the time which they are required. Usually, these copepod life stages are only required for a period of days, yet the production of sufficient numbers can take weeks to months. Promising short-term results have been obtained by refrigerating nauplii and adults but there is continued interest in being able to control the development of resting eggs. However, research to date has been opportunistic.

C8. Central zooplankton reference centre

A central facility should be established from which seed copepod cultures could be obtained by hatcheries. Without such a facility it is unlikely that individual hatcheries would develop the infrastructure to maintain copepods for their own intermittent use. Questions relating to the legality and appropriateness of relocating animals must be considered.

GROUP D: ARTIFICIAL DIETS

👍 💰 ➢B5 ### *D1. Test currently available diets*

A survey of the larvae and weaning diets commercially available in Australia is needed. The survey should focus on the main commercial species currently reared (i.e. barramundi and snapper).

The survey should use standard protocols and include the following topics:

- cost versus profit
- growth versus cost
- labour efficiency

D2. Develop standard testing systems

A standard system for testing microdiets needs to be developed. The performances of a given microdiet are greatly affected by the shape, size and volume of the larval tanks. The inert movements of the diet particles depend on hydrodynamics in the rearing tank. A standard testing system for both tropical and temperate areas will have the advantage of testing different diets with different fish species in the same conditions. Two standard systems, one for tropical fish species such as barramundi (at James Cook University, Townsville) and the other for temperate species such as snapper (at Fisheries Western Australia and/or Fremantle Maritime Centre, Perth) could be developed for this purpose.

The testing system should be on a commercial scale to allow immediate transfer of results to industry, without the need for up-scaling. A standardised system would also improve hatchery skills in general.

D3. Develop local diets

In the short term the development of local diets should focus on co-feeding, using both dry and live feeds. Research in this area should aim to shorten the weaning period and decrease the amount of *Artemia* being used. In the longer term research should aim at the complete replacement of *Artemia* with microdiets. Local microdiets will need to compete with overseas diets in terms of cost and performance.

The R&D of local microdiets will need to focus on improving:

- ingestion by using feed attractants
- digestion by using easy-to-digest proteins, binders and dietary enzymes etc.

Communication between hatcheries, research institutions and feed providers needs to be improved to aid development of local diets and to get feedback from the hatcheries that are using a particular diet.

TECHNOLOGY TRANSFER

Mike Rimmer

At the Hatchery Feeds Workshop, industry in particular felt that there is a gap between researchers and industry, and that many research results are not being provided to industry in a timely fashion. There is also inadequate exchange of information and experiences between hatcheries.

It was generally agreed that there was a need for more industry and research meetings to provide forums for the exchange of information, and for researchers to gain better access to industry needs. It is clear that industry prefers workshops with a 'hands-on' style, where the technology is demonstrated in a practical fashion. In addition, there was an identified need for on-the-ground extension work to transfer research results to industry.

Another issue discussed was the adoption of overseas technology, rather than 're-inventing the wheel' in Australia. There is a wealth of overseas experience in aquaculture, including hatchery feeds development, and much of this could be rapidly transferred to Australian hatcheries. Industry participants felt that technology transfer from overseas could be more usefully undertaken by having hatchery technicians (rather than researchers) visit overseas aquaculture operations.

In short, workshop participants recognised the need to improve technology transfer not only amongst Australian researchers and industry, but also with regard to the adoption of overseas technology. Some methodologies for improving technology transfer, as well as communication generally, include:

- an e-mail discussion list
- a dedicated web site, with links to other relevant web sites
- regular meetings of workshop participants and other interested parties[3]. These could be undertaken in conjunction with existing national meetings, such as the Australian Society for Fish Biology, or the Australian Marine Science Association.

These improved communication strategies will be implemented as an outcome of the Hatchery Feeds Workshop (see 'Communication Strategies').

There are opportunities to access funding for the development of networks designed to enhance technology transfer and generally promote better linkages between researchers and industry; for example, through the Department of Industry, Science and Resources Technology Diffusion Program. These will be further investigated by the workshop organisers with a view to developing

[3] It was suggested that the next meeting of a group interested in hatchery feeds and related issues should take place during AquaFest 2000, to be held in Hobart, Tasmania, in October 2000.

mechanisms to support regular meetings of researchers and industry personnel with an interest in the field of hatchery feeds.

COMMUNICATION STRATEGIES

David McKinnon

Participants at the Hatchery Feeds Workshop resolved to improve communication between stakeholders. An E-mail mailing list has been established as:
hatchery-feeds@aims.gov.au

To subscribe to the list, send a message to
majordomo@aims.gov.au

with the following in the body of the message:

subscribe hatchery-feeds
end

To unsubscribe to the list send a message to
majordomo@aims.gov.au

with the following in the body of the message:

unsubscribe hatchery-feeds
end

A web page includes all messages sent to this mailing list, as well as related documents such as this R&D plan and the Proceedings of the Hatchery Feeds Workshop, in Adobe Acrobat format. The address of the page is:

http://www/aims.gov.au/hatchery-feeds

REVIEW PROCESS

Mike Rimmer

Regular review of industry needs and research outcomes is a useful mechanism for providing a focussed research effort. This could be a relatively informal process (similar to that undertaken during the Cairns workshop) or it could be formalised through the adoption of a program and/or project model. With the latter model, individual *projects* make up an overall *program* of activities that addresses the identified needs of industry. Projects can be institutionally funded, or funded by external agencies. The overall approach is to identify the work that is currently being undertaken, and to develop projects to fill gaps in the priority research topics. Such an approach reduces overlap between research projects, and provides an integrated approach to addressing industry needs.

The capacity to review progress regularly, either formally or informally, will depend on the ability of workshop participants and other interested parties to attend meetings and other workshops. As noted above, the workshop organisers will investigate opportunities to develop a regular series of such gatherings.

SECTION TWO

Scientific Background to the Development of the Plan

This section comprises invited status reviews of each of the main areas of hatchery feeds research. Each review is intended to encapsulate the present state of knowledge, and to highlight areas where further research is needed. These reviews, plus presentations by individual researchers at the Hatchery Feeds Workshop were used as the scientific basis for development of the R&D plan.

The views expressed are those of the authors.

STATUS REVIEW 1: Microalgal feeds

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SCOPE OF REVIEW

This review provides a background on the role of microalgae in Australian aquaculture, especially in food chains leading to the production of fish. The current status of knowledge is summarised and potential areas of research and industry development are identified. The review is divided into six sections: (a) attributes of microalgae and species used, (b) nutritional properties, (c) production systems, (d) alternatives to fresh algae, (e) application of algae for larval fish culture and (f) avenues for future research.

ATTRIBUTES OF MICROALGAE AND SPECIES USED

Microalgae are used in aquaculture as live feeds for all growth stages of molluscs, for the larval stages of crustaceans and some fish species, and for zooplankton used in aquaculture food chains. In the Australian context, microalgae therefore have a key role in the larval production of Pacific and Pearl oysters, prawns, barramundi and juvenile abalone, as well as other emerging species. Over the years, several hundred microalgae have been tested as food, but probably less than twenty have been successful and have widespread use. Microalgae must possess a number of key attributes to be useful aquaculture species. They must be of an appropriate size for ingestion (e.g. from 1 to 15 microns for filter feeders) and readily digested. They must have rapid growth rates, be amenable to mass culture, and also be stable in culture under any fluctuations in temperature, light and nutrients that may occur in hatchery systems. Finally, they must have a good nutrient composition, including an absence of toxins that might be transferred up the food chain.

Popular strains identified for bivalve culture by Persoone and Claus (1980) included *Isochrysis galbana, Pavlova lutheri, Tetraselmis suecica, Phaeodactylum tricornutum, Pseudoisochrysis paradoxa, Chaetoceros calcitrans, Skeletonema costatu*m and *Isochrysis* sp. (T.ISO). It is noteworthy that now, over 20 years later, hatcheries are still using essentially the same strains for their production. This is reflected by data from the CSIRO Microalgae Supply Service (Fig. 1). The oyster industry has most requests, the most popular species being *Isochrysis* sp. (T.ISO), *P. lutheri, C. calcitrans* and *C. muelleri.* For the prawn industry, *C. muelleri* is the most popular. Relatively few requests are received from fish hatcheries (for enriching zooplankton); species used are *Isochrysis* sp. (T.ISO), *P. lutheri, T. suecica* and *Nannochloropsis oculata.*

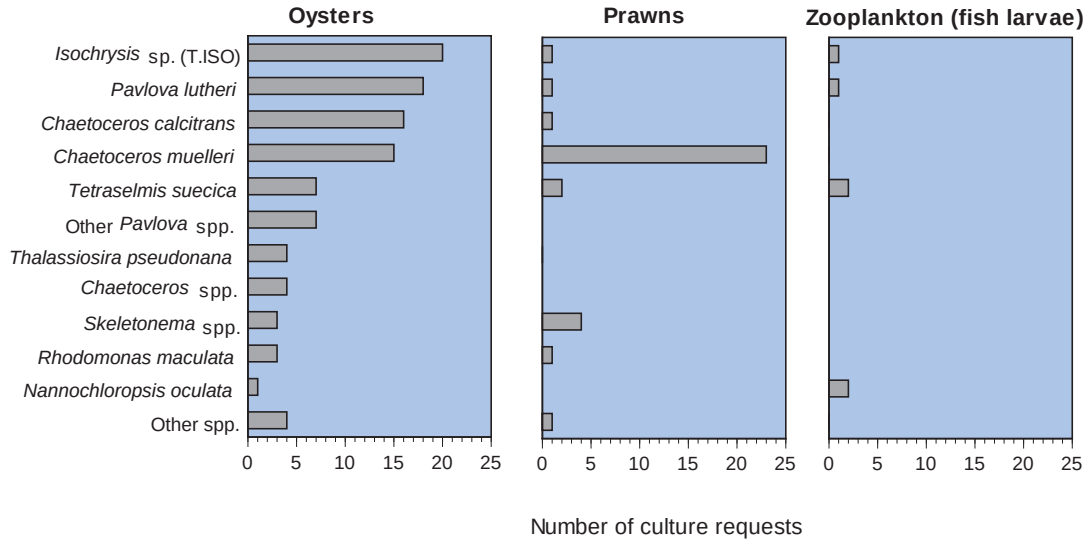

Fig. 1 Microalgae requested for aquaculture from CSIRO in Australia during 1997-98

Approximately 10–15% of microalgae requested by Australian hatcheries are local isolates. As the development of Australian strains has been a priority for industry, it has also been a key focus of CSIRO microalgal research over the last decade. Other research groups (e.g. NTU, NSW Fisheries, JCU) have also made valuable contributions. Compared to 'conventional' overseas strains, some Australian strains may be more suited to specific environmental conditions (e.g. light, temperature, seawater chemistry). Also, in some instances Australian strains could better match the nutritional needs of local animals. Finally, there may be less concern from government regulatory agencies about any potential, inadvertent introduction or release of Australian strains into natural waterways. Examples of Australian strains being used by industry include *Pavlova pinguis*, *Skeletonema* sp. CS-252, *Nannochloropsis* sp. CS-246, *Rhodomonas salina* CS-24 and *Navicula jeffreyi*.

NUTRITIONAL PROPERTIES OF MICROALGAE

Microalgal species can vary significantly in their nutritional value, and this may change under different culture conditions (Brown *et al.,* 1997). Nevertheless, a carefully selected mixture of microalgae can offer an excellent nutritional package for larval animals, either directly or indirectly (through enrichment of zooplankton). Microalgae that have been found to have good nutritional properties — either as monospecies or within a mixed diet — include *C. calcitrans*, *C. muelleri, P. lutheri. Isochrysis* sp. (T.ISO), *T. suecica, S. costatum* and *Thalassiosira pseudonana* (Enright *et al.,* 1986a; Thompson *et al.,* 1993; Brown *et al.,* 1997). Biochemical and nutritional assessment of microalgae used, or of potential use, in Australian have been assessed through several FRDC Projects (e.g. projects 86/81, 90/63 and 94/83 to CSIRO; 95/131 to NTU), so we have a good understanding of their profiles.

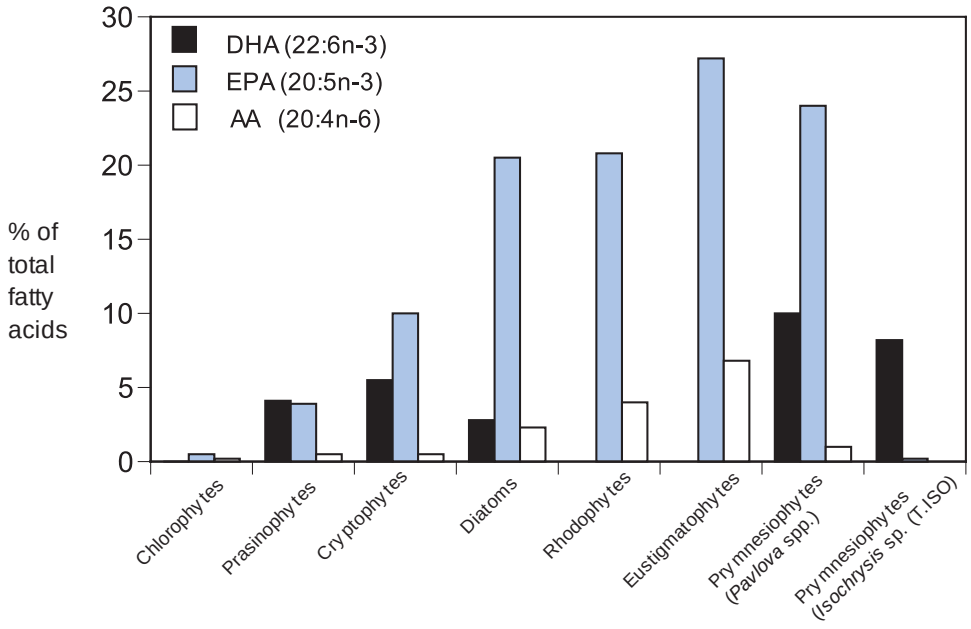

Fig 2. Content of PUFAs in different classes of microalgae.
Compiled by data published by Dunstan, Volkman et al. (see FRDC Projects 86/81 and 90/63)

In general, microalgae provide a rich source of carbohydrate, and have a well-balanced amino acid composition (Brown, 1991). While the gross composition of microalgae can influence nutritional value (Enright *et al*., 1986b), it is the balance of other key nutrients that possibly have most influence. Polyunsaturated fatty acids (PUFAs), especially docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) and arachidonic acid (AA) — which are known to be essential for various larvae (Langdon and Waldock, 1981; Sargent *et al*., 1997) — vary significantly between algal classes and algal species (Fig. 2). While most species have moderate to high concentrations of EPA, relatively few are rich in DHA. *Isochrysis* sp. (T.ISO), *Pavlova lutheri, Micromonas pusilla* and *Rhodomonas salina* are examples of DHA-rich microalgae.

Microalgae vary in their vitamin content. Ascorbic acid shows the greatest variation, 16–fold (1–16 mg g^{-1} dry weight; Brown and Miller, 1992). Concentrations in other vitamins typically show a 2- to 4-fold difference between species (Seguineau *et al*., 1996; Brown *et al.,* 1999) (Table 1.1). To put the vitamin content of the microalgae into context, data should be compared with the nutritional requirements of the consuming animal. Unfortunately, nutritional requirements of larval or juvenile animals that feed directly on microalgae are, at best, poorly understood. However, the requirements of the adult are far better known (e.g. for marine fish and prawns; Tacon, 1991; Conklin, 1997) and, in the absence of information to the contrary, will have to serve as a guide for the larval animal. These data suggest that a carefully selected, mixed-algal diet should provide adequate concentrations of the vitamins for aquaculture food chains (Table 1.1).

Sterols (Knauer *et al*., 1999), minerals (Fabregas and Herrero, 1986) and pigments (see discussion in later section of this review) may also contribute to nutritional differences in microalgae.

Table 1.1 Range of vitamin content of microalgae (μg g^{-1} dry weight) used in aquaculture. Combined data from Brown *et al.* (1999), Seguineau *et al.*, (1996) and Brown and Miller, (1992). Requirements of prawn from Conklin,(1997) and marine fish from Tacon (1991) for yellowtail/seabass/seabream/grouper. Retinol requirements of fish can be met through pro-vitamin A metabolites such as β–carotene.

VITAMIN	CONCENTRATION RANGE (μg g^{-1})	REQUIREMENTS OF PRAWN	REQUIREMENTS OF MARINE FISH
ascorbic acid (C)	1,000 – 16,000	200	200
β–carotene	500 – 1,200		
niacin	110 – 470	40	150
α–tocopherol (E)	70 – 350	100	200
thiamine (B_1)	30 – 110	60	20
riboflavin (B_2)	25 – 50	25	20
pantothenic acid	14 – 38	75	50
folates	7 – 24	10	5
pyridoxine (B_6)	4 – 17	50	20
cobalamin (B_{12})	1.7 – 7.4	0.2	0.02
biotin	0.7 – 1.9	1	1
retinol (A)	<0.25 – 2.2	1.6	1.9
ergocalciferol plus cholecalciferol (D_2, D_3)	<0.9	0.1	0.025

PRODUCTION SYSTEMS

Typical systems used indoors for microalgal mass culture include carboys (10–20 L), polythene bags (100–500 L) and tubs (1000–5000 L). These are usually operated in batch or continuous mode. For larger volumes, out-door tanks or ponds are used, operated semi-continuously. Depending on their scale, hatcheries may produce between several hundred to tens of thousands of litres of algae daily. Cell densities range from 10^5–10^7 cells per millilitre with these standard systems, and production costs can range from US$50–200, or 20–50% of hatcheries' operating costs (Coutteau and Sorgeloos, 1992). There are clear economies of scale with algal production, so that production costs become especially significant to smaller hatcheries. Consequently, there has been much effort directed at examining alternatives to the production of fresh algae, and also more cost-efficient production systems.

Large-scale photobioreactors, either for indoor or outdoor production, have been assessed (Tredici and Materassi, 1992; Chrismadha and Borowitzka, 1994). Essentially these can be considered as variations of the 'standard' culture systems, but with a much higher surface area to volume ratio (SA:VOL). Consequently, light is less likely to become limiting and systems are characterised by higher productivity and greater cell biomass at harvest — potentially effecting a low production cost. However, these systems do have major disadvantages. Oxygen concentrations (resulting from photosynthesis) can build up because of the high biomass, and therefore give rise to 'photoinhibition' — thus restricting productivity. Because of the high SA:VOL, cultures can overheat in outdoor systems. Also, because of the high biomass, the systems need turbulent flow to ensure nutrient exchange and to avoid light-limitation, thereby making them unsuitable for fragile species. In fact, most aquaculture strains have not been effectively cultured in such systems. Exceptions include *Nannochloropsis* spp. (Tredici and Materassi, 1992) and *Skeletonoma* spp. (S. Blackburn *et al*., unpublished data).

Fermentation technology should also be considered. This technology is well established for low-cost production of bacteria and yeast, and there are some microalgae capable of heterotrophic growth. The advantages include a high-density and high biomass production, and elimination of light — a major cost for phototrophic production. Because lower volumes are required for producing the same biomass (compared to conventional algal systems) this provides a greater degree of control. Production costs of between US$2–25 per kilogram dry weight have been projected by using this technology (Gladue, 1991). Unfortunately, few aquaculture species have been identified that can grow heterotrophically. *Tetraselmis* spp. are exceptions, though these are generally recognised as having moderate food value, unless forming part of a mixed diet. There is a high capital cost also associated with fermentation; 2-L units can cost US$5000–10,000, whereas 10,000-L units may exceed US$1 million.

ALTERNATIVES TO FRESH ALGAE

There have been a few algal products produced by fermentation and available commercially as dried powders. One of the first was Algal 161 from CellSys. This was produced from *Tetraselmis suecica* and cost US$180/kg. This product had moderate value as a diet component for molluscs (Laing, Child and Janke, 1990), though it did not have a high-market penetration and is now unavailable. More recently, several products based on thraustochytrids (microorganisms whose taxonomy may be related to certain algal classes) from the genus *Schizochytrium* have been marketed through Aquafauna Biomarine Inc. (e.g. AlgaMac 2000) and Sanders Brine Shrimp Co. (e.g. Docosa Gold). These products are characterised by high concentration of DHA (Barclay and Zeller, 1996), and so are being applied as alternatives to commercial oil enrichments (e.g. Selco) for zooplankton fed to larvae. These products are further discussed in the section 'Application of algae for larval fish culture'.

Algal pastes or concentrates have also been examined as alternatives to live algae. The advantage of such products is that they can be used 'off-the-shelf', thus providing potential cost efficiencies to hatcheries. Concentrates are prepared by flocculation (final product about 1:100 concentration) or by centrifugation (about 1:500 concentration). Two Australian research projects have recently assessed these two methods. A new flocculated process was developed by Richard Knuckey as part of a CRC for Aquaculture project. Centrifugation and associated post-harvest storage techniques were assessed by Mike Heasman's group at NSW Fisheries (FRDC Projects 93/123 and 96/342). Both studies found that microalgal species were variable in their suitability, with diatoms the most promising. Concentrates were prepared with shelf lives between 2 and 8 weeks when stored at ≤4°C. These have been assessed in feeding trials with the larvae and spat of Sydney rock oyster (Heasman *et al*., in press) and Pacific oyster (McCausland *et al.* 1999; M. Brown, R. Knuckey and R. Robert, unpublished data) and prawn larvae (D'Souza *et al*., unpublished data). Concentrates were particularly effective as partial diets (e.g. up to 80%) with growth rates similar to or marginally inferior to complete live diets. This technology is at a stage for transferring to Australian hatcheries. This could be effected either through direct transfer of information so hatcheries can produce their own concentrates on-site, or the establishment of a central facility preparing concentrates for distribution to hatcheries. More R&D on post-harvest preservation methods is required to extend shelf-lives beyond 4 to 8 weeks, and also for the preparation of concentrates from flagellates.

APPLICATION OF ALGAE FOR LARVAL FISH CULTURE

Microalgae have an important role in enriching zooplankton for on-feeding to fish and other larvae. In addition to providing protein (essential amino acids) and energy, they provide other key nutrients such as vitamins, essential PUFAs, pigments and sterols, which are transferred through the food chain.

For example, rotifers fed microalgae become rapidly enriched with ascorbic acid (AsA). After 24 h, rotifers fed on *Isochrysis* sp. (T.ISO) and *Nannochloropsis oculata* contained 2.5 and 1.7 mg g^{-1} DW, respectively, whereas rotifers fed on baker's yeast (itself deficient in AsA) contained only 0.6 mg g^{-1} DW (Brown *et al*., 1998). After an ensuing 16 h of non-feeding, rotifers lost <10% of their AsA, retaining ≈ 50% of total ingested AsA. Similarly, concentration of AsA in *Artemia* may be enriched by feeding with microalgae (Merchie *et al.,* 1995). Little information is available on the transfer of other vitamins from microalgae through the food chain to fish larvae.

PUFA-rich microalgae such as *Pavlova* spp. and *Isochrysis* sp. (T.ISO) can be fed to zooplankton to enrich them in DHA (Nichols *et al.,* 1989). However, often these do not provide the level of enrichment often sought for zooplankton, and commercial oil-emulsions (e.g. DHA Selco from INVE) are often used. Recently, 'algal-like' products such as AlgaMac 2000 and Docosa Gold (dried preparations of the thraustochytrid *Schizochytrium* sp.) — which contain 5–15% of their DW as DHA — have been utilised. These have produced similar levels of enrichment of DHA within the zooplankton compared to the commercial oils (Gara *et al*., 1998; G. Dunstan, P. Mansour, M. Brown, unpublished data), and also produce DHA to EPA ratios between 1 and 2, which is considered favourable for fish larval nutrition (Rodríguez *et al.,* 1998). Research in progress by the University of

Tasmania (T. Lewis *et al.*) and the CSIRO (M. Brown, S. Blackburn *et al.*) is also assessing live and dried Australian thraustochytrids as dietary constituents and for enrichment.

A study by Rønnestad *et al.* (1998) has highlighted that microalgal pigments transferred through to zooplankton may contribute to nutritional value. They found that the dominant pigments in the copepod *Temora* sp. were lutein and astaxanthin, whereas in *Artemia* it was canthaxanthin. When these prey items were fed to halibut larvae, adequate amounts of vitamin A were found in halibut fed on copepods, but not with halibut fed on *Artemia*. The authors ascribed this to the ability of the larvae to convert lutein and/or astaxanthin, but not canthaxanthin, into vitamin A. They recommended that *Artemia* should routinely be enriched with astaxanthin and lutein (the latter pigment common in 'green' microalgae, e.g. *Tetraselmis* spp.) to improve their nutritional value.

A common procedure during the culture of both larval fish and prawns is to add microalgae (i.e. 'green-water') to intensive culture systems together with the zooplankton prey (Tamaru *et al.,* 1994). Addition of the microalgae can improve the production of larvae in such systems, though the exact mechanism of action is unclear. Theories advanced include (a) light attenuation (i.e. shading effects) have a beneficial effect on larvae, (b) maintenance of the nutritional quality of the zooplankton, (c) an excretion of vitamins or other growth-promoting substances by algae and (d) a probiotic effect of the algae. Most likely, the mechanism may be a combination of several of these possibilities. A maintenance of NH_3 and O_2 balance has also been proposed, though this has not been supported by experimental evidence (Tamaru *et al.,* 1994). The most popular algal species used for green-water applications are *Nannochlorospsis oculata* and *Tetraselmis suecica*. More research is needed on the application of other microalgae — especially species rich in DHA — to green-water systems. Green-water may also be applied to extensive outdoor production systems, through fertilisation of ponds to stimulate microalgal growth, and correspondingly zooplankton production as food for larvae introduced into the ponds.

AVENUES FOR FUTURE RESEARCH

The high production costs of microalgae remains a constraint to many hatcheries. Despite efforts over several decades to develop cost-effective artificial diets to replace microalgae as hatchery feeds, on-site microalgal production remains a critical element of most marine hatcheries. Improvements in alternative diets may continue, but production costs of microalgae may also decrease due to the uptake of new technology by hatcheries (e.g. continuous bag system, from Seasalter Shellfish (Whitstable) Ltd.[4]). Therefore, it is unlikely that microalgae will be totally replaced, at least in the medium term.

We now have a good selection of microalgal strains to support the aquaculture industry. However for some particular applications or industry sectors, new Australian strains with improved nutritional quality or growth characteristics could improve hatchery efficiencies. For example, copepods are

[4] see Proceedings of the Hatchery Feeds Workshop

recognised as excellent feeds for fish larvae, but they have proven difficult to produce in intensive systems. The use of alternative microalgal species could improve their production rates. Also, the prawn and pearl oyster industries still have a demand for new tropical microalgal strains with broad temperature tolerances.

Apart from improvements in the cost-efficiencies of on-site algal production, an alternative is the centralisation of algal production at specialised mass-culture facilities, using heterotrophic methods or photobioreactors to produce cheap algal biomass. These technologies could be married with post-harvest processing such as spray-drying, or algal concentration (centrifugation or spray-drying) to develop off-the-shelf algal biomass for distribution to hatcheries. More research is required to enhance the shelf lives of concentrates and for the development of concentrates of popular flagellates such as *Isochrysis* sp. (T.ISO) and *Pavlova lutheri*.

The use of microalgae either as a full or partial (i.e. in conjunction with products like Selco and AlgaMac 2000) enrichment should be considered for improving the nutritional quality of zooplankton. It is now well recognised that microalgae contain an array of essential nutrients that may be transferred through food chains, especially PUFAs. Microalgae (e.g. *Isochrysis* sp. (T.ISO) and *Pavlova lutheri*) can provide a moderate enrichment of DHA, though not as effective as commercial oil emulsions like DHA Selco. The new 'algal-like' thraustochytrid products are extremely efficient in DHA enrichment of zooplankton with good DHA:EPA ratios. New thraustochytrids are being investigated with other nutritional characteristics, for example high concentrations of AA (Lewis *et al.,* 1999). Some work has been documented on the transfer of ascorbic acid from microalgae through to zooplankton and fish larvae, but much less is known about other vitamins. Though microalgae have generally been proposed here as good sources of vitamins, they can vary significantly in their composition. Therefore, zooplankton could be deficient in one or more vitamins when enriched using certain dietary regimens. Future research should focus on this issue, and the transfer of other essential nutrients (pigments, sterols) to zooplankton fed different diets and grown under different culture conditions.

Finally, a better understanding of the mechanism of green-water systems — both in intensive and extensive culture — will aid in optimising it's application to larval culture. A broader range of microalgal species, especially mixtures and including species rich in DHA, should be assessed in green-water systems.

REFERENCES

Barclay W. and Zeller S. (1996). Nutritional enhancement of *n*-3 and *n*-6 fatty acids in rotifers and *Artemia* by feeding spray-dried *Schizochytrium* sp. *Journal of the World Aquaculture Society, 27*, 314–322.

Brown, M. R. (1991). The amino acid and sugar composition of 16 species of microalgae used in mariculture. *J. Exp. Mar. Biol. Ecol.*, 145: 79–99.

Brown, M. R. and Miller, K. A. (1992). The ascorbic acid content of eleven species of microalgae used in mariculture. *J. Appl. Phycol.*, *4*, 205–215.

Brown, M. R., Jeffrey, S. W., Volkman, J. K. and Dunstan, G. A. (1997). Nutritional properties of microalgae for mariculture. *Aquaculture*, *15*, 315–331.

Brown, M. R., Mular, M., Miller, I., Trenerry, C. and Farmer, C. (1999). The vitamin content of microalgae used in aquaculture. *J. Applied Phycology, 11*, 247–255.

Brown, M. R., Skabo, S. and Wilkinson, B. (1998). The enrichment and retention of ascorbic acid in rotifers fed microalgal diets. *Aquac. Nutrition, 4*, 151–156.

Chrismadha, T. and Borowitzka, M. A. (1994). Effect of cell density and irradiance on growth, proximate composition and eicosapentaenoic acid production of *Phaeodactylum tricornutum* grown in a tubular photobioreator. *J. Applied Phycology, 6*, 67–74.

Conklin, D. E. (1997). Vitamins. In L. R. D'Abramo, D. E. Conkin and D. M. Akiyama (Eds.), *Crustacean nutrition: Advances in world aquaculture, Vol. 6, World Aquaculture Society*, (pp. 123–149).

Coutteau, P. and Sorgeloos, P., (1992). The use of algal substitutes and the requirement for live algae in the hatchery and nursery rearing of bivalve molluscs: An international survey. *J. Shellfish Res., 11*, 467–476.

Enright, C. T., Newkirk, G. F. Craigie, J. S. and Castell, J. D. (1986a). Evaluation of phytoplankton as diets for juvenile *Ostrea edulis* L. J. *Exp. Mar. Biol. Ecol., 96*, 1–13.

Enright, C. T., Newkirk, G. F., Craigie, J. S. and Castell, J. D. (1986b). Growth of juvenile Ostrea edulis L. fed *Chaetoceros calcitrans* Schütt of varied chemical composition. *J. Exp. Mar. Biol. Ecol., 96*, 15–26.

Fabregas, J. and Herrero, C. (1986). Marine microalgae as a potential source of minerals in fish diets. *Aquaculture*, *51,* 237–243.

Gara, B., Shields, R. J. and McEvoy, L. (1998). Feeding strategies to achieve correct metamorphosis of Atlantic halibut, *Hippoglossus hippoglossus* L., using enriched *Artemia*. *Aquaculture Research, 29*, 935–948.

Gladue, R. (1991). Heterotrophic microalgae production: Potential for application to aquaculture feeds. In *Rotifer and Microalgae Culture Systems. Proceedings of a U.S. – Asia Workshop, HI* (pp. 275–286).

Heasman, M., Diemar, J., O'Connor, W., Sushames, T. and Foulkes, L. (2000). Development of extended micro-algae concentrates harvested by centrifugation for bivalve molluscs – A summary. *Aquaculture Research*, in press.

Knauer, J., Barrett, S. M., Volkman, J. K. and Southgate, P. C. (1999). Assimilation of dietary phytosterols by Pacific oyster *Crassostrea gigas* spat. *Aquaculture Nutrition, 5*, 257–266.

Laing, I. Child, A. R. and Janke, A. (1990). Nutritional value of dried algae diets for larvae of manila clam (*Tapes philippinarum). J. Mar. Biol. Ass. U.K., 70*, 1–12.

Langdon, C. J. and Waldock, M. J. (1981). The effect of algal and artificial diets on the growth and fatty acid composition of *Crassostrea gigas* spat. *J. Mar. Biol. Ass. U.K., 61,* 431–448.

Lewis, T. E., Nichols, P. D. and McMeekin T. A. (1999). The biotechnological potential of thraustochytrids. *Marine Biotechnology 1*, 580-587.

McCausland M. A., Brown M. R., Barrett S .M., Diemar J. A. and Heasman, M. P. (1999). Evaluation of live and pasted microalgae as supplementary food for juvenile Pacific oysters *(Crassostrea gigas). Aquaculture, 174*, 323–342.

Merchie, G., Lavens, P., Dhert, Ph., Dehasque, M., Nelis, H., De Leenheer, A. and Sorgeloos, P. (1995) Variation of ascorbic acid content in different live food organisms. *Aquaculture, 134*, 325–337.

Nichols, P. D., Holdsworth, D. G., Volkman, J. K., Daintith, M. and Allanson, S. (1989). High incorporation of essential fatty acids by the rotifer *Brachionus plicatilis* fed on the prymnesiophyte alga *Pavlova lutheri. Aust. J. Mar. Freshwater Res., 40*, 645–655.

Rodríguez C., Pérez J. A., Badía P., Izquierdo M. S., Fernández-Palacios, H. and Lorenzo Hernández A. (1998) The *n*-3 highly unsaturated fatty acid requirements of gilthead seabream (*Sparus aurata* L.) larvae when using an appropriate DHA/EPA ratio in the diet. *Aquaculture, 169*, 9–23.

Rønnestad, I, Helland, S. and Lie., Ø. (1998). Feeding *Artemia* to larvae of Atlantic halibut (*Hippoglossus hippoglossus* L.) results in lower larval vitamin A content compared with feeding copepods. *Aquaculture, 165*, 159–164.

Sargent, J. R., McEvoy, L. A. and Bell., J. G. (1997). Requirements, presentation and sources of polyunsaturated fatty acids in marine fish larval feeds. *Aquaculture*, *155,* 117–127.

Seguineau, C., Laschi-Loquerie, A., Moal, J. and Samain, J. F. (1996). Vitamin requirements in great scallop larvae. *Aquacult. Int.*, *4*, 315–324.

Tacon, A. G. J. (1991). Vitamin nutrition in shrimp and fish. In D. M. Akiyama and R. K. H. Tan (Eds.), *Proc. of the Aquaculture Feed Processing and Nutrition Workshop, Thailand and Indonesia, September 1991* (pp. 10–41). Singapore: American Soybean Association.

Tamaru, C. S., Murashige, R. and Lee, C.-S. (1994). The paradox of using background phytoplankton during the larval culture of striped mullet, *Mugil cephalus* L. *Aquaculture, 119*, 167–174.

Tredici, M. R. and Materassi, R. (1992). From open ponds to vertical alveolar panels: The Italian experience in the development of reactors for the mass cultivation of phototrophic microorganisms. *Journal of Applied Phycology, 4*, 221–231.

Thompson, P. A., Guo, M.-X. and Harrison, P. J., 1993. The influence of irradiance on the biochemical composition of three phytoplankton species and their nutritional value for larvae of the Pacific oyster (*Crassostrea gigas*). *Mar. Biol., 117,* 259–268.

STATUS REVIEW 2: Rotifer culture

A REVIEW OF THE STATUS OF PRODUCTION IN AUSTRALIA

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PREAMBLE

The intention of this paper is to provide an overview of the status of rotifer (*Brachionus* spp.) culture in Australian marine finfish hatcheries and to identify areas of future research and development that may improve the production of cultured marine fish in Australia. I do not intend to provide an extensive review of the use of rotifers in aquaculture as this has been more than adequately covered elsewhere (see Lubzens, 1987; Lubzens *et al*., 1989). However, I will discuss culture methods commonly used or under development overseas to allow benchmarking of the status of Australian rotifer culture.

INTRODUCTION

The technology for rearing marine fish larvae throughout the world has been increasing for the last 30 years (Yoshimura *et al*., 1996). This has been due largely to the availability of marine rotifers (*Brachionus* spp.), as live feeds for first-feeding fish larvae. More than 40 species of fish have been reared successfully (greater than 1000 larvae) by feeding rotifers (Yoshimura *et al*., 1996) and many more fish species have been reared experimentally. In Australia, rotifers are used extensively in intensive hatcheries to rear barramundi, (*Lates calcarifer*), and pink snapper (*Pagrus auratus*), which dominate the marine fish farming industry. Technology is also developing for the production of new fish species, and rotifers have been used to rear larvae of fish such as Australian bass (*Macquaria novemaculeata*), mulloway (*Argyrosomus japonicus*), striped trumpeter (*Latris lineata*), bream (*Acanthopagrus* spp.), groupers (*Epinephelus* spp.), whiting (*Sillago* spp.), flathead (*Platycephalus fuscus*), mullet (*Mugil cephalis*), and West Australian dhufish (*Glaucasoma hebraicum*).

Rotifers have become essential for rearing many marine fish because they satisfy most feed requirements of early-stage marine fish larvae. First-feeding fish larvae are usually small in size (2–7 mm, total length), have poorly developed eyes, do not swim well but are mostly present in the water column, and require easily digested diets with a high caloric value. Rotifers are commensurately small (90–320 μm), swim slowly and stay suspended in the water column, thus being available for capture and consumption by fish larvae. Of significant importance is the fact that rotifers can be cultured at relatively high densities and large numbers can be produced daily to supply the demand from hatcheries producing large numbers of fish (Kafukue and Ikenoue, 1983). It has been estimated that between 40,000 and 100,000 rotifers are required to feed one fish larvae until it can consume

another food source (Kafuku and Ikenoue, 1983). The nutritional profile of rotifers, such as content of highly unsaturated fatty acids (HUFA's) and vitamins, can also be manipulated before the rotifers are fed to fish larvae (Lubzens, 1987).

The major challenge for operators of fish hatcheries is to provide adequate numbers of rotifers for fish larvae during the critical first-feeding life stages. The production of high quality rotifers must be reliable and the system economically viable. Most hatcheries propagate rotifers using mass cultivation techniques. The following parameters, as outlined by Lubzens (1987), must be considered before commencing mass cultivation:

1. A rotifer strain must be selected on the basis that it is an appropriate size (usually small enough) for the larvae and that it is suitable for the culture conditions.
2. The food quality and quantity for the rotifers must be adequate.
3. Water quality parameters in the culture tanks (e.g. salinity, pH, temperature) must be controlled and waste products must be removed.

ROTIFER PRODUCTION

Three important aspects of rotifer production are the rotifer strain, rotifer culture methods and rotifer nutrition.

Rotifer strain

The body size of the rotifer selected for culture in the hatchery is extremely important as prey selection by fish larvae is size-dependent, and the preferred size of the prey increases as the larvae grow (Hunter, 1980). Consequently, two species of rotifer, a small sized *Brachionus rotundiformis* (formerly known as S-type, Segers, 1995) and a large sized *B. plicatilis* (formerly known as L-type, Segers, 1995) are typically cultured in marine fish hatcheries. Many strains of *B. rotundiformis* and *B. plicatilis* have been isolated (see Fu and Hirayama, 1991) and cultured throughout the world. The size of the rotifer strains is determined genetically and only minor changes in body size (up to 15%) have been manipulated by modifying feed type or salinity (Fukusho and Iwamoto, 1980).

Productivity of rotifers can vary significantly with strain and is strongly influenced by culture conditions. The optimum temperature and salinity for rotifers are highly strain specific; however in general, *B. rotundiformis* and *B. plicatilis* are most productive at high (30–35°C) and low (15–25°C) temperatures, respectively (Snell and Carillo, 1984; Fukusho and Iwamoto, 1980). Both species are in general euryhaline and are productive between 4 and 35 ppt (Hirayama and Ogawa, 1972).

In Australia, very few strains of rotifer are cultured in marine fish hatcheries. Until the mid-1990s, all marine fish hatcheries cultured a single strain of *B. plicatilis* (160–260 μm lorica length) which was imported from Hawaii to the NSW Fisheries, Brackishwater Fish Culture Research Centre (now Port Stephens Research Centre, PSRC) in the late 1970s. Starter cultures of rotifers were distributed from this centre to hatcheries around Australia. This rotifer became the mainstay for production of

barramundi, Australian bass and snapper; however, rearing success was limited for fish larvae with very small mouths at first-feed, such as whiting (*S. ciliata*; *S. maculeata*) and groupers. As a result, in 1995 a project was initiated by NSW Fisheries and the Cooperative Research Centre for Aquaculture to introduce from Japan the smallest strain of rotifer *(B. rotundiformis*; 90–190 μm lorica length) used in fish hatcheries at the time. This new rotifer was evaluated for its suitability as a first-feed for several Australian fish species. No improvement in growth or survival was found when snapper larvae were fed the *B. rotundiformis* (S. Fielder, unpublished data[); however, many barramundi hatcheries experienced improved hatchery production when the small rotifer was fed initially to larvae.

Two research and production hatcheries have also recently isolated and developed methods to mass cultivate local isolates of *B. rotundiformis*. The Queensland Department of Primary Industries' Northern Fisheries Centre at Cairns and The Northern Territory Department of Primary Industries and Fisheries at Darwin are now culturing a strain of *B. rotundiformis*, collected from their own local estuaries. Size characteristics of the local rotifers are similar to those of the imported *B. rotundiformis*.

Clearly, the diversity of rotifer strains available currently to Australian marine fish hatcheries is extremely limited. Increasing the number of rotifer strains may be advantageous by allowing selection of rotifers with different size profiles and high production rates. The importance of providing very small rotifers at first-feeding has already been mentioned; however, there is also an opportunity to identify a very large strain of rotifer and develop suitable mass cultivation techniques. Currently, there is a severe world-wide shortage of *Artemia* cysts available for marine hatchery operations due to poor harvest of cysts from the Great Salt Lakes in 1997–99 (Lavens and Sorgeloos, 2000). Hatchery operators will be forced to develop new larval feeding strategies to maintain or increase the current level of fish production. Production of a large rotifer (>400 μm) may therefore be a satisfactory replacement for *Artemia*.

New strains of rotifer for Australian hatcheries could be accessed by (a) importing rotifers already isolated and cultured in hatcheries outside of Australia, and (b) identifying, isolating and developing culture techniques for new rotifer strains endemic to Australia. The rotifers would then need to be evaluated for their suitability as live-feeds for Australian marine fish larvae.

Rotifer culture methods

Most rotifers are indiscriminate filter feeders and will feed on algae, yeast, bacteria and microparticles up to approximately 25 μm in size (Komis, 1992). There are many different methods and adaptations used to mass culture rotifers. In general, rotifer production is based on either (a) a diet of marine microalgae and bakers yeast, or (b) a single diet of commercially produced fortified yeasts. Within each of these feed types, rotifers are cultured using either batch, semicontinuous or continuous methods (see Lubzens, 1987 for description).

In Australia, most marine fish hatcheries use batch or semicontinuous methods and feed fresh microalgae species such as *Nannochloropsis*, *Tetraselmis*, *Isochrysis* and *Pavlova* in conjunction with

baker's yeast. Rotifer production tanks range in size from 1,000–50,000 litres. The density of rotifers at harvest is generally low (100–300 rotifers per millilitre). A significant problem experienced with this method of culture is that rotifer cultures can crash quickly and unpredictably. This can be due to deteriorating water quality following a build-up of metabolites or poor food quality such as low-density microalgae. Other problems with this method are associated with the production of microalgae. The volume of microalgae required is large and is often two to three times the volume of the rotifer cultures. Production of fresh microalgae is expensive in terms of labour, seawater use and tanks and can account for 30–40% of the total hatchery costs (Borowitzka, 1999). The quality of fresh microalgae can also be variable due to environmental factors and seasonal changes. Also, a great deal of hatchery space is needed to produce microalgae. If alternative methods of feeding rotifers were used, this space could become available for fish rearing.

Alternative feeds to fresh algae such as commercial fortified yeasts (e.g. culture-Selco - Inve, Microfeast products) have been used at Australian hatcheries with varied success. Several hatchery managers have stated that on occasion, rotifer production was good with culture Selco but sustainable production of rotifers was often not possible. Techniques for sustainable production of rotifers using products like culture- Selco have been developed and assessed, and are widely used overseas (see Komis, 1992), thus suggesting that there is a need for improved technology transfer from the commercial producers of the feeds and production systems to Australian hatchery operators. Feeding regimes that incorporate fortified yeast and microalgae diets may also increase the number of rotifers and improve the reliability of rotifer production in Australian fish hatcheries.

There is also significant opportunity to replace fresh, hatchery produced algae with off-the-shelf concentrated, viable algae products. For example, research conducted by Heasman *et al.* (in press) evaluated several techniques for concentrating microalgae and determined the optimum storage methods and times for a range of microalgal species. Comparable growth and survival of juvenile bivalves were obtained when they were fed concentrated (up to 8 weeks old) or fresh microalgae. Similarly, Lubzens *et al.* (1995) showed that concentrated and frozen *Nannochloropsis* sp. was suitable for large-scale rotifer production. Commercialisation of this technology in Australia should be encouraged to provide consistently high-quality, off-the-shelf microalgal products for use in marine fish hatcheries.

There is also scope to investigate the transfer of technology developed by Japanese researchers, who were able to culture rotifers at extremely high densities (Yoshimura *et al.*, 1996). The system, ultra-high-density culture, is based on the use of concentrated freshwater *Chlorella* as food for rotifers. Rotifers are mass-cultured at 10,000–30,000 individuals per millilitre. Cultures are supplied with constant pure oxygen and the pH of the water is adjusted to 7 by addition of HCl to avoid the presence of free-ammonia in the system. Organic detritus is removed from the water column using suspended filter mats. This technique has been used by commercial hatcheries in Japan and the total cost of production of rotifers was reduced by almost 65% compared with conventional methods (Yoshimura *et al.*, 1996).

Nutrition of rotifers

The nutritional quality of food is critical for normal development and survival of marine fish larvae. It is well known that perhaps the most important dietary factors to influence the growth and survival of marine fish larvae are the highly unsaturated fatty acids (HUFA) of the *n*-3 series (Lubzens *et al*., 1989), in particular eicosapentaenoic acid (EPA, 20:5*n*-3) and docosahexaenoic acid (DHA, 22:6*n*-3) (Watanabe, 1979; Watanabe *et al*., 1983). The total requirement of HUFAs and ratio of DHA:EPA in the food also varies widely with fish species (e.g. Koven, *et al*., 1990; Mourente *et al*., 1993; Rodriguez *et al*., 1994).

The fatty acid profile of rotifers is largely determined by the diet (Watanabe *et al*., 1983) as rotifers have limited capacity to synthesize long chain fatty acids (Lubzens *et al*., 1985). Also, cultured rotifers, particularly those fed with baker's yeast, tend to be deficient in HUFAs. As a consequence, procedures have been developed to enrich the HUFA content of rotifers before feeding them to fish larvae. The enrichment techniques involve feeding rotifers for 8–24 h with (a) microalgae high in (*n*-3)HUFAs such as *Isochrysis* and *Pavlova* spp. (b) yeast enriched with (*n*-3)HUFA (c) emulsions which are based on (*n*-3)HUFA-rich fish or cuttlefish oils or (d) microparticulate diets high in (*n*-3)HUFAs.

Most Australian marine fish hatcheries practice enrichment of rotifers by either feeding fresh microalgae or commercial emulsions alone or in combination. In the case of feeding commercial emulsions, the enrichment procedures provided by the suppliers are usually followed; however, very little, if any, confirmation of the final HUFA content of rotifers is conducted, especially in commercial hatcheries. Therefore, rotifers are being fed to fish larvae on the assumption that the HUFA content is adequate. Clearly, there is a need to develop protocols in hatcheries, which regularly assess the efficiency of enrichment. Also, as attempts are made to rear new fish species, research to determine the total HUFA and DHA:EPA requirement of target fish larvae is essential. A further necessary challenge will be then to develop enrichment procedures for rotifers to match the HUFA requirement of the fish larvae. Rotifers have also been enriched with vitamins and antibiotics (Gatesoupe, 1982) and opportunities therefore exist to investigate the efficacy of different enrichment techniques that may increase marine fish production.

OTHER CURRENT OR PLANNED OVERSEAS RESEARCH

A great deal of other research on rotifer production for aquaculture is currently being undertaken overseas and there are opportunities to potentially transfer or adapt new technology developed elsewhere to Australia. Briefly, examples of new research include:

1. Production and preservation of resting eggs (Hagiwara *et al*., 1997). Mass production of resting eggs similar to *Artemia* cysts has been achieved for *B. rotundiformis* and *B. plicatilis*. Techniques have been developed to store rotifer eggs in cans, thus potentially providing access to off-the-shelf product.
2. Selection of rotifers with different genetically controlled sizes and growth rates, tolerant of low dissolved oxygen and high ammonia concentration (Yoshimura *et al*., 1996).
3. Cryo-preservation of amictic eggs for maintenance of useful genotypes.

POSSIBLE FUTURE AREAS FOR ROTIFER RESEARCH AND DEVELOPMENT

Production of rotifers in Australian marine fish hatcheries is based on the use of a small number of genetically different strains and standard, low-technology, production systems. Productivity of rotifer culture systems is generally low, the reliability of production is variable and significant hatchery resources are needed to produce the necessary number of rotifers.

Improvement in performance of Australian marine fish production — through increased survival and growth of fish larvae, reduced costs of production of fingerlings, and increased numbers of fish species successfully reared in Australia — may be achieved if the following aspects of rotifer production technology are addressed:

1. Importation and evaluation of new rotifer strains from overseas. Rotifers selected for size and growth rate. Quarantine issues may be problematic in importing an exotic species to Australia.

2. Isolation and evaluation of new local strains of rotifer. Rotifers may be more suited to local culture conditions. Particular attention could be paid to selection of a very large rotifer to potentially replace *Artemia* as a live food.

3. Genetic selection. Instigate a genetic selection program of rotifers currently in use in Australian hatcheries. Traits to select for could include size (small or large), reproductive capacity, tolerance of poor water quality.

4. Reliability and cost of rotifer production. Increase the reliability and decrease the costs of rotifer production by:
   (a) development and commercialisation of concentrated microalgae production
   (b) transfer of technology from Japan for ultra-high-density rotifer production.

5. Hatchery protocols. Develop hatchery protocols to validate that target HUFA content of enriched rotifers is being met.

6. HUFA requirements. Identify essential HUFA requirement of new fish species and develop enrichment techniques to meet these levels in rotifers.

7. Live feed and production systems. Identify new live feeds and production systems for fish species which cannot be cultured with rotifers e.g. copepod culture.

8. Artificial diets. Develop artificial diets or feeding strategies to totally or partially replace rotifers as live feed for marine fish larvae.

REFERENCES

Borowitzka, M. A. (1999). Production of microalgal concentrates for aquaculture (Part 1: Algae Culture). Final Report to the Fisheries Research and Development Corporation (Australia). Project No 93/123. 70 pp.

Fu, Y. and Hirayama, K. (1991). Morphological differences between two types of the rotifer *Brachionus plicatilis*. *Journal of Experimental Biology and Ecology 151*(1), 29–41.

Fukusho, K. (1983). Present status and problems in culture of the rotifer *Brachionus plicatilis* for fry production of marine fishes in Japan. In H. R. Fuentes, J. G. Castillo and L. H. Disalvo (Eds.), *Advances and Perspectives in Aquaculture. Proceedings of a Symposium*, (pp. 361–374). Coquimbo, Chile: Universidad del Norte.

Fukusho, K., Iwamoto, H. (1980). Cyclomorphosis in size of the cultured rotifer, *Brachionus plicatilis*. *Bull. Natl. Res. Inst. Aquacult., 1*, 29–37.

Gatesoupe, F. J. (1982). Nutrition and antibacterial treatments of live food organisms: The influence on survival, growth rate and weaning success of turbot (*Scophthalmus maximus*). *Ann. Zootech, 31*, 353–368.

Hagiwara, A., Balompapueng, M. D., Munuswamy, N. and Hirayama, K. (1997). Mass production and preservation of the resting eggs of the euryhaline rotifer *Brachionus plicatilis* and *B. rotundiformis*. *Aquaculture, 155*, 223–230.

Heasman, M., Diemar, J., O'Connor, W., Sushames, T. and Foulkes, L. (in press). Development of extended shelf-life micro-algae concentrate diets harvested by centrifugation for bivalve molluscs – A summary. *Aquaculture Research*.

Hirayama, K. and Ogawa, S. (1972). Fundamental studies on physiology of rotifers for its mass culture. I. Filter feeding of rotifer. *Bull. Japan. Soc. Sci. Fish., 38*, 1207–1214.

Hunter, J. R. (1980). The feeding behaviour and ecology of marine fish larvae. In J. E. Bardach, J. J. Magnuson, R. C. May and J. M. , Reinhart (Eds.), *Fish behaviour and its use in the capture and culture of fishes. ICLARM Conf. Proc. 5*, (pp. 287–330) Manila, Philippines: ICLARM.

Kafuku, T. and Ikenoue, H. (1983). *Modern methods of aquaculture in Japan: Developments of aquaculture and fisheries science, 11*. Kodansha Ltd. Tokyo: Elsevier.

Komis, A. (1992). Improved production and utilization of the rotifer *Brachionus plicatilis* Müller, in European sea bream (*Sparus aurata* Linnaeus) and sea bass (*Dicentrarchus labrax* Linnaeus) larviculture. Doctor in Agricultural Sciences Thesis, University of Gent.

Korunuma, K. and Fukusho, K. (1987). *Rearing of marine fish larvae in Japan*. Ottawa: IDRC.

Koven, W. M., Tandler, A., Kissil, G. W., Sklan, D., Friezlander, O. and Harel, M. (1990). The effect of dietary (*n*-3) polyunsaturated fatty acids on growth, survival and swim bladder development in *Sparus aurata* larvae. *Aquaculture, 91*, 131–141.

Lavens, P. and Sorgeloos, P. (2000). The history, present status and prospects of the availability of *Artemia* cysts for aquaculture. *Aquaculture, 181*, 397–403.

Lubzens, E. (1987). Raising rotifers for use in aquaculture. *Hydrobiologia, 147*, 245–255.

Lubzens, E., Tandler, A. and Minkoff, G. (1989). Rotifers as food in aquaculture. *Hydrobiologia, 186/187*, 387–400.

Lubzens, E., Gibson, O., Zmora, O. and Sukenik, A. (1995). Potential advantages of frozen algae (*Nannochloropsis* sp.) for rotifer (*Brachionus plicatilis*) culture. *Aquaculture, 133*, 295–309.

Mourente, G., Rodriguez, A., Tocher, D. R. and Sargent, J. R. (1993). Effects of dietary docosahexaenoic acid (DHA; 22:6n-3) on lipid and fatty acid compositions and growth in gilthead sea bream (*Sparus aurata* L.) larvae during first feeding. *Aquaculture, 112*, 79–98.

Rodriguez, C., Perez, J. A., Lorenzo, A., Izquierdo, M. S. and Cejas, J. R. (1994). *n*-3 HUFA requirement of larval gilthead seabream *Sparus aurata* when using high levels of eicosapentaenoic acid. *Comp. Biochem. Physiol. 107A*, 693–698.

Snell, T. W. and Carillo, K. (1984). Body size variation among strains of the rotifer *Brachionus plicatilis. Aquaculture, 37*, 359–367.

Segers, H. (1995). Nomenclatural consequences of some recent studies on *Brachionus plicatilis* (Rotifera, Brachionidae). *Hydrobiologia, 313*, 121–122.

Watanabe, T. (1979). Nutritional quality of living feeds used in seed production of fish. *Proc. 7th Japan-Soviet Joint Symp. Aquaculture, Sept. 1978*, Tokyo, 49–66.

Watanabe, T., Kitajima, C. and Fujita, S. (1983). Nutritional values of live organisms used in Japan for mass propagation of fish: A review. *Aquaculture, 34*, 115–143.

Yoshimura, K., Hagiwara, A., Yoshimatsu, T. and Kitajima, C. (1996). Culture technology of marine rotifers and the implications for intensive culture of marine fish in Japan. *Mar. Freshwater Res., 47*, 217–222.

STATUS REVIEW 3: *Artemia*

PAST, PRESENT AND FUTURE STATUS

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INTRODUCTION

The larval culture of fish (and most marine organisms) is generally carried out under intensive controlled environment regimes. These hatchery conditions require specific culture techniques and a continuous supply of suitable feeds for the larvae. These feeds need to satisfy the nutritional requirements of the larvae, as well as being amenable to culture and being available in a ready-to-use form.

Marine fish larvae are usually very small, extremely fragile and are generally not physiologically well developed. The mouth size of first feeding larvae usually restricts the size of the food particles that can be ingested. The developmental status of the larval digestive system also dictates the larvae ability to digest and assimilate food. For example, first feeding Gilthead seabream (*Sparus aurata*, a close relative to the pink snapper, *Pagrus auratus*) larvae do not have a functional stomach, but only a short digestive tube with a low level of enzyme activity. The digestive system develops during the first weeks after hatching and, only after the 'metamorphosis' stage (e.g. from larval stage to fingerling), does the digestive system become fully functional (Kolkovski *et al*., 1993; Kolkovski, 2000).

It follows, therefore, that many fish larvae will have to rely on a food source that has the following characteristics: (a) suitable size for ingestion, (b) easily digestible, (c) contains enzyme systems which allow autolysis, and (d) supply all the essential nutrients for the larvae (Bengston *et al*., 1991; Kolkovski *et al*., 1998).

From the practical viewpoint of the culturist, a good larval diet should be readily available, cost-effective, relatively simple to culture and be consistently available in sufficient quantities.

Only a few live feeds are typically used in larval culture with the choice being dependent on the mouth gape and life stage of the larvae. A standard feeding routine for sparid species (snapper, seabream etc.) and many other marine species includes rotifer *Brachionus plicatilis* (50–200 μm) for the first two weeks after hatching, followed by *Artemia* nauplii (300–500 μm).

The brine shrimp, *Artemia,* has been intensively used as a live food for fish culture since the 1960s. Live *Artemia* nauplii and/or adults are currently used in virtually all commercial shrimp and fish hatcheries. Over 85% of all marine animals now cultured utilise *Artemia* as a partial or sole diet during their larval phase (Sorgeloos *et al*., 1993). The widespread use of *Artemia* in aquaculture has improved

both cyst availability and quality through more appropriate harvesting and processing methods. The availability of the *Artemia* cysts combined with long shelf life and easy procedures for hatching makes them a convenient live food for marine fish larvae. However, there are disadvantages to the use of *Artemia*. In most cases, *Artemia* nauplii are not the optimal live food organism in terms of nutritional requirements for fish larvae. The biggest disadvantages, however, are reliability of supply, product quality variation between sources and variable cost.

LIFE CYCLE

Artemia life cycles begin by hatching from dormant cysts which are metabolically inactive encysted embryos. Dormancy can persist for several years as long as the cysts are kept dry. When the cysts are placed in salt water, they re-hydrate and the embryos resume development.

At low salinities (<85 ppt) and optimal food levels, fertilised females usually produce free swimming nauplii (ovoviviparous reproduction) at a rate of up to 75 nauplii per day. They may produce 10–11 broods over an average life cycle of 50 days. Under ideal conditions adult *Artemia* survive for several months and produce up to 300 nauplii every 4 days. Cyst production (oviparous reproduction) is considered to be induced by high salinity, under conditions of high eutrophication (large O_2 fluctuations between day and night) and chronic food shortages. At high salinities (>150 ppt) and low oxygen concentrations, the embryos develop to the gastrula stage. They then become surrounded by a thick shell and enter dormancy (diapause). Females can release up to 75 cysts per day which float in the highly saline water. The floating cysts are eventually blown ashore where they accumulate in large masses and dry. Development is resumed when the cysts are re-hydrated and the life cycle is begun again (Lavens and Sorgeloos, 1996).

NUTRITIONAL VALUE

Nutritional value is adversely affected by utilisation of metabolic reserves during the non-feeding nauplius (Instar I) stage. However, the nutritional value of feeding *Artemia* nauplii (Instar II) can be improved by enrichment with highly unsaturated fatty acids (HUFA) especially *n*-3 and *n*-6 HUFA such as docosahexaenoic acid (DHA 22:6*n*-3), eicosapentaenoic acid (EPA 2:5*n*-3) and, arachidonic acid (ARA 20:4*n*-6) (Czesny *et al*., 1999; Sorgeloos *et al*., 1993). The ratio between these fatty acids is considered extremely important. The enrichment procedure can improve the nutritional value of the *Artemia* nauplii to match most of the copepod species, as well as other wild zooplankton that are considered to have high nutritional value in terms of fatty acid composition (Table 3.1; Dhert, 1999).

Vitamin C (ascorbic acid) is also considered to have a positive effect on marine fish larvae. Low doses (25–50 ppm) are reported to have a positive effect on growth and to reduce skeletal deformities. With 'giga' doses (500–2000 ppm), improvements to the immune response, disease and stress resistance have been observed (Merchie *et al*., 1997; Kolkovski *et al*., 1998). Techniques for enrichment of *Artemia* with therapeutic compounds like antimicrobial drugs have been also published (Dhert, 1999; Lavens and Sorgeloos, 1993; Sorgeloos, 1999).

AVAILABILITY

Over 90% of the world production of *Artemia* cysts is harvested in the Great Salt Lake (GSL), Utah, USA. In 1997, some 6000 hatcheries required over 1500 metric tons of dry cysts annually, or 7500 tonnes of raw, wet cysts. The majority of the cyst consumption is by the prawn industry (80–85%), while other mariculture industries use only 15–20% of total world consumption. However, the mariculture industry is, at the moment, almost totally dependent on *Artemia* cysts as a secondary food organism after the rotifer.

The Great Salt Lake in Utah, USA is divided into two arms by an east-west rock-filled causeway that carries trains. For years, the connections in the causeway between the north and south arm have not been maintained and are partially blocked, leading to almost complete separation between the two arms of the lake. The 1997 El Nino phenomenon dropped the salinity levels in the south arm to 70–80 ppt while the north arm is at saturation.

On 27 October 1997, the Utah Division of Wildlife Resources ordered an emergency closure of the GSL brine shrimp cysts harvest, due to the threat of over-harvesting and the poor quality of *Artemia* cysts. The harvest from the GSL was even worse the following season (1998–1999) and the current season was closed after only 800 metric tonnes were harvested.

Currently, the low salinity in the south arm allows the possible entrance of predators (e.g. fish, Corixid beetles etc.), changing the primary producers from *Dunaliella viridis* to diatoms that are not considered optimal for *Artemia* nauplii (Lavens and Sorgeloos, 2000). The salinity is too low for cyst production and reduces the buoyancy of those that are produced. The salinity in the north arm is near saturation (240 ppt), resulting in very low cyst production, coupled with poor quality (Leger, 1999).

It is believed that the total of *Artemia* cysts harvested in this season (1999–2000) will not even satisfy 20% of the global demand (Table 3.2; Lavens and Sorgeloos, 2000).

This situation led to a dramatic increase of cyst prices. The current price for brine shrimp cysts is $80–150 per can (425 g), if it is available at all. Other sources of *Artemia* cysts are available, but the quality and the hatching percentage are variable.

The history and current situation of the GSL is described in detail in Lavens and Sorgeloos (2000).

POSSIBLE SOLUTIONS

Possible solutions include the diversification of *Artemia* sources, the more-efficient use of *Artemia* and the development of *Artemia* replacements.

I. Diversification of *Artemia* sources. New sources are currently under intensive investigation as potential *Artemia* cyst sources. Some of these sites are already producing cysts, including:

1. Natural sites such as:
   (a) China — Aibi Lake
   (b) Siberia — Bolshoye Yarovoye
   (c) Kazakstan
   (d) Turkmenistan — Kara Bogaz Gol
   (e) Argentina
   (f) Iran — Lake Urima

2. Semi-natural or managed sites such as:
   (a) San Francisco Bay
   (b) Vietnam
   (c) Colombia
   (d) Brazil
   (e) Australia — Cargill, Dampier and Shark Bay salt fields

II. Efficient use of *Artemia*

1. Improvement of zootechniques such as:
   (a) Decapsulation
   (b) Enrichments — lipids, vitamins, probiotics
   (c) Standard hatching-enrichment protocols (temperature, pH, density, oxygen etc.)

III. Development of *Artemia* replacements:

1. Other live feeds such as:
   (a) Copepods
   (b) *Parartemia*
   (c) 'Mega' rotifers
   (d) Other zooplankton

   These live food organisms have the potential for partial replacement of *Artemia* cysts. However, the use of any other live food organisms will be limited in the near future, to species such as groupers (*Epinephelus* spp.) and *Lutjanus* spp. that are considered difficult to rear.

2. Dry microdiets
   Today, a full replacement of *Artemia* is possible in a few marine fish species, but always at the cost of culture time, low growth and survival. It is predicted that in the near future, more-efficient and higher quality microdiets will be available as a partial replacement for *Artemia* using co-feeding methods to shorten the weaning period. A complete *Artemia* replacement for most marine fish larvae is still a long-term goal.

Table 3.1. DHA/EPA in wild zooplankton/copepod and enriched *Artemia* nauplii (data from Dehert, P., 1999)

COPEPOD SPECIES	DHA mg/g dry weight	EPA mg/g dry weight	DHA:EPA
Pseudocalanus acuspes	24.3	21.5	1.1
Pseudocalanus acuspes	25.8	31.6	0.8
Acartia longiremis	20.6	17.5	1.2
Calanus glacialis	24.4	20.0	1.2
Calanus finmarchicus	30.9	23.1	1.3
Pseudocalanus sp.	31.8	22.1	1.4
Temora longicornis	31.9	18.4	1.7
Wild zooplankton	32.9	21.1	1.6
Centropages hamatis	37.7	17.2	2.2
Tropical wild zooplankton	32.0	13.0	2.5
AVERAGE	29	21	1.5
Enriched *Artemia* nauplii	16–28	10–28	0.5–1.4

Table 3.2. Harvests of *Artemia* cysts (metric tonnes of raw wet weight cysts) from Great Salt Lake, Utah USA (Lavens and Sorgeloos, 2000)

SEASON	FIRMS	LICENSES	HARVEST (T)
1988–1989	7	–	2170
1989–1990	12	–	5020
1990–1991	19	24	4860
1991–1992	11	26	5870
1992–1993	12	20	4900
1993–1994	12	18	4030
1994–1995	14	29	2680
1995–1996	21	69	6640
1996–1997	32	79	6600
1997–1998	32	79	2020
1998–1998	39	79	<2000
1999–2000*	**<20**		**<800**

* 1999–2000 season data based on Leger, 1999 and pers. comm.

REFERENCES

Bengston, D. A., Leger, P. and Sorgeloos, P. (1991). Use of *Artemia* as a food source for aquaculture. In R. A. Browne, P. Sorgeloos and C. N. A. Trotman (Eds.), Artemia *Biology* (pp. 255–285). Boca Raton, FL: CRC Press.

Czesny, S., Kolkovski, S., Dabrowski, K. and Culver, D. (1999). Growth, survival, and quality of juvenile walleye *Stizostediom vitreum* as influenced by *n*-3 HUFA enriched *Artemia* nauplii. *Aquaculture, 178*, 103–115.

Dhert, P. (1999). Culture and manipulation techniques in live food production. *Advanced Bio-technology in hatchery production workshop proceedings* (abstract). Honolulu, HI: Oceanic Institute.

Kolkovski, S., Czesny, S., Yackey, C., Moreau, R. and Dabrowski, K. (1998). The effect of vitamins C and E in *n*-3 HUFA enriched *Artemia* nauplii on growth, survival and stress resistance of walleye *Stizostedion vitreum* fry. *World Aquaculture'98* (pp. 303–304). Las Vegas NV.

Kolkovski, S., Tandler, A., Kissil, G. Wm. and Gertler, A. (1993). The effect of dietary exogenous digestive enzymes on ingestion, assimilation, growth and survival of gilthead seabream *Sparus aurata* larvae. *Fish Physiol. and Biochem., 12*(3), 203–209.

Kolkovski, S. (2000). Digestive enzymes in fish larvae - Application and implication: A review. *Aquaculture* (in press).

Lavens, P. and Sorgeloos, P. (1996). *Manual on the production and use of live food for aquaculture*. FAO Fisheries Technical Paper, Rome: FAO.

Lavens, P. and Sorgeloos, P. (2000). The history, present status and prospects of the availability of *Artemia* cysts for aquaculture. *Aquaculture, 181,* 397–403.

Leger, P. (1999). Great Salt Lake out of shape. *Fish Farming International, 26,* 28–29.

Merchie, G., Levans, P. and Sorgeloos, P. (1997). Optimization of dietary vitamin C in fish and crustacean larvae: A review. *Aquaculture, 155*, 165–181.

Sorgeloos, P., Levans, P., Leger, P. and Tackaert, W. (1993). The use of *Artemia* in marine fish larviculture. *TML Conference Proceedings, 3,* 73–86.

Sorgeloos, P. (1999). Use of brine shrimp *Artemia* spp. in finfish larviculture. *Advanced Bio-technology in hatchery production workshop* (abstract). Honolulu, HI: Oceanic Institute.

STATUS REVIEW 4: Copepod culture

COPEPODS AS HATCHERY FEEDS IN AUSTRALIA

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Copepods are small crustaceans that occur naturally in all aquatic habitats. At present there are over 10,000 species known, with this likely to double in the next 50 years (Huys and Boxshall, 1991). They are the most numerous metazoan animals in the world (Hardy, 1965), based on a calculation of the abundance of planktonic copepods and the volume of the ocean. This does not take into account the many species occupying benthic, interstitial and commensal habitats. Copepods constitute the principal trophic link between microbial systems and higher trophic levels, and are the major food items of larval fish in the sea (Hunter, 1981).

Free-living copepods range in size from 0.2 mm to 28 mm, but most planktonic forms are between 0.5 mm and 2.5 mm. Most copepods have 6 naupliar and 6 copepodite stages, the last of which is the adult. Copepods carry their eggs in either a single egg sac or a pair of sacs, but many pelagic species release their eggs directly into the water column ('broadcasting'), since the carrying of egg sacs renders the adult more liable to visual predation.

From an aquaculture perspective, the distinction between broadcasting species and brooding species is potentially an important one. Eggs and early nauplii of copepods are important components of first-feeding fish larvae. Because broadcast eggs suffer a very high rate of mortality (Peterson and Kimmerer, 1994), the rate of egg production of broadcasters is higher than that of brooders (Kiørboe and Sabatini, 1994). However, by coincidence rather than science, the difference in egg production rate per female may be offset by the ability to culture brooders in higher density than broadcasters (Støttrup, pers. comm., 1999).

THE USE OF COPEPODS IN AQUACULTURE OVERSEAS

The initial interest in culturing copepods in the laboratory was prompted by research on many aspects of their biology. Most early work concentrated on *Calanus finmarchicus*, a large and abundant planktonic species in the North Atlantic, which proved important in understanding the herring fishery. This work was summarised by Marshall and Orr (1972), and later developments in copepod culture reviewed by Kinne (1977) and Paffenhöfer and Harris (1979).

The importance of copepods as prey for wild fish made it inevitable that their use in aquaculture would be investigated, and this development has been reviewed by Nellen (1986). Research in Europe and Japan has focussed on the harpacticoid copepods *Tisbe* spp. and *Tigriopus*, and the calanoids

Eurytemora and *Acartia*. Increased survival of turbot (*Scophthalmus maximus*) and seabream (*Pagrus major*) was obtained when small copepod nauplii were supplied. However, copepod culture in the northern hemisphere still presents considerable problems. By nature they do not reach the densities obtained by rotifers or microalgae, and are more sensitive to water quality and handling. Planktonic filter-feeding copepods such as *Acartia tonsa* require large volumes of water and yield about 530 eggs per litre, whereas 100,000 nauplii per litre have been obtained for *Tisbe holothuriae* with a small-volume method that uses 3-litre trays (Støttrup and Norsker, 1997; Støttrup *et al.*, 1998).

In Hawaii, *Euterpina acutifrons* (Harpacticoida: Tachidiidae) has been successfully cultured and used as food for larval mahimahi (*Coryphaena hippurus*; Kraul, 1990). Despite fish larvae selecting nauplii over rotifers of similar width, survivorship was low during the first week, possibly because of toxicity associated with either the media or microorganism associated with it. Older larvae were able to resist this effect and grew well on a copepod diet till day 21.

In Thailand, green-water culture techniques are employed to rear larvae of *Lutjanus argentimaculatus* (red snapper, mangrove jack; Singhagraiwan and Doi, 1993). Early attempts to rear this fish species using rotifers alone as larval diets resulted in 100% mortality. In subsequent trials, naturally occurring copepods (*Acartia*, *Pseudodiaptomus*, *Oithona*, *Longipedia*) were added to green-water tanks for 1–2 days. This water was introduced into the larval rearing tanks when the larvae were between 3 and 8 days old. When larvae were 6 days old, rotifers were introduced; when 10 days old, *Artemia* were introduced. Copepod nauplii comprised 88% of gut contents of larvae up to 15 days old. In later work, Doi *et al.* (1994) developed cultures of *Acartia sinjiensis* in outdoor tanks, and demonstrated that early nauplii of this species constituted an ideal diet for larvae of *L argentimaculatus* to about day 4 (Doi *et al.*, 1997).

In Taiwan, grouper larvae are raised in two ways: the 'indoor method' and the 'outdoor method' (Rimmer, 1998). In the outdoor method, oyster trochophores are added from day 4 for 2 days, then wild zooplankton, cultured in small ponds using decomposing trash fish to build up numbers. Fine nets are used to collect (mostly) rotifers for early larvae, and coarser nets are used to collect copepods for older larvae. Survival of the larvae is usually less than 7%, and high mortality at first feed is regarded as the major problem associated with larval rearing.

WHY COPEPODS?

In the wild, copepods are the natural prey of virtually all fish larvae. Surrogates such as rotifers and *Artemia* nauplii are widely used as larval prey for finfish species in aquaculture, but poor survival of some species has prompted aquaculturalists to investigate the use and development of the natural trophic relationship between copepod nauplii and first feeding larvae. The provision of copepod food can result in:

1. improved larval survival (Shields *et al.*, 1999)
2. higher growth rates (Støttrup and Norsker, 1997)
3. better pigmentation (Spenelli, 1979; Støttrup *et al.*, 1998)

4. improved gut development (Luizi *et al*., 1999)
5. a source of exogenous enzymes (Munilla-Moran *et al*., 1990).

Nutritional composition

Diets deficient in essential nutrients are thought to be the main reason for the high mortality of young fish (D'Abramo and Lovell, 1991). Copepods are particularly rich in 16:0, 16:1, 20:5 ω-3 and 22:6 ω-3 fatty acids (Moreno *et al*., 1979, and references therein). The ability to synthesise these lipids, especially 22:6 ω-3, makes them very attractive larval feeds (Nanton and Castell, 1999). In addition, other compounds such as enzymes and carotenoids are important, depending to some extent on the target fish species Toledo *et al.* (1999) found ω-3 HUFA content of copepods to be twice that of rotifers, and that *Acartia* had the best profile of those copepods tested (*Acartia*, *Pseudodiaptomus* and *Oithona*).

Size and digestibility

Some high-value tropical finfish, especially the groupers, have a very small mouth gape at first feeding. This has led to the development of small-strain rotifer cultures for use as larval feeds. Best survival of larvae has been achieved by the provision of copepod nauplii, usually within green-water culture systems (e.g. Singhagraiwan and Doi, 1993; Toledo *et al*., 1999). Digestibility of rotifers can be a problem with other fish species, such as striped trumpeter (*Latrus lineata*).

STATUS OF RESEARCH INTO COPEPOD CULTURE IN AUSTRALIA

Increasingly, finfish hatcheries have been experimenting with copepod culture to solve practical problems associated with poor survival and growth of certain fish species. This has prompted funding agencies to invest in research on the subject, and there have been two recent research projects:

1. Andria Marshall was funded by the CRC for Aquaculture in 1994 to develop copepod culture for finfish aquaculture, and a thesis entitled 'The culture and assessment of three copepod species as live feeds for marine fish larvae' submitted to the University of Tasmania in 2000. The project was split between studies in Tasmania (*Tisbe*) and in the Northern Territory (*Apocyclops* and *Acartia*).
2. In 1996, the FRDC funded a project entitled 'Intensive cultivation of a calanoid copepod for live food in fish culture' by Dr. R. Rippingale, Curtin University. This work is now finalised and a report has been submitted.

These projects, and other research into culture of copepods for use in aquaculture, are listed in Table 4.1, and a summary of the status of each is given in the following paragraphs. Table 4.2 summarises the strengths, weaknesses, opportunities and threats (SWOT analysis) of each.

Table 4.1. Recent research conducted in Australia on the culture of copepods for aquaculture, and the target fish species.

INSTITUTION	COPEPOD SPECIES	COPEPOD CULTURE METHOD	FISH TARGET SPECIES
NTDPIF	*Acartia* sp.	Intensive	*Lutjanus johnii*
QDPI NFC	*Acartia* sp.	Intensive	Groupers [*Epinephelus fuscoguttatus* (flowery cod), Barramundi cod (*Cromileptes altivelis*)]; Mangrove jack (*Lutjanus argentimaculatus*)
Curtin University / Fremantle Maritime Centre	*Gladioferens imparipes*	Intensive, in conjunction with green-water	WA dhufish (*Glaucosoma hebraicum*) Snapper (*Pagrus auratus*) Seahorse (*Hippocampus angustus*) ornamentals (e.g. clownfish, pipefish)
Bluewater Barramundi	*Acartia*, mixed	green-water	Groupers (Goldspot cod *Epinephelus coioides*), mangrove jack
TAFI	*Tisbe?*	green-water	Striped trumpeter *Latris lineata* Flounder *Rhombosolea tapirina*

Table 4.2: Copepod species under consideration for development as aquaculture feeds in Australia: A SWOT analysis.

SPECIES	STRENGTHS	WEAKNESSES	OPPORTUNITIES	THREATS
Gladioferens imparipes	Long term culture Technology developed High naupliar prodn. achieved	Endemic to WA Demersal habit Brooder Large nauplii	Extend technology to other species: *G. pectinatus, Pseudodiaptomus*, etc?	Fish species have low market value; technology may not be implemented because of marginal benefit
Acartia spp	Widespread Broadcaster Easily caught (light) Easily cultured Technology developed	Taxonomic confusion Predator avoider Cannibalism an impediment to high density culture Only early nauplii are small enough for small mouth-gape larvae	Best candidate for development of canned product (resting eggs)	Subject to periodic crashes ; may require greater dietary diversity?
Bestiolina similis	Easily cultured Small nauplii Good nutritional profile? Occurs naturally in high density	Restricted to tropics	Good prospects for development	Poorly studied
Euterpina acutifrons	Widespread Easily cultured Established technology (Hawaii) small nauplii	Brooder	Demonstrated suitable as food for mahi mahi; adaptable to tuna	Toxicity problem? (Kraul, 1990)
Tisbe spp	Widespread Easily cultured (weed) European technology small nauplii	Taxonomic confusion Brooder Hyperbenthic	Extend European (temperate) findings to tropics?	Little studied in Australia

Acartia spp.

Acartia spp. (Calanoida: Acartiidae) are common components of coastal marine waters worldwide and have become a favourite of marine scientists and aquaculturalists worldwide. Because it is positively phototactic *Acartia* can be attracted by light at night in high densities, and used to seed green-water cultures. *Acartia* responds well to culture conditions because it feeds on a wide range of prey items and is robust. However, it is highly cannibalistic of its own nauplii (e.g. Ohno and Okamura, 1988), which prevents the achievement of high-density cultures.

Andria Marshall (CRC Aquaculture: UTAS) investigated the use of a tropical species of the subgenus *Acanthacartia* at NTDPIF, and obtained culture densities of 1200 per litre on a mixed algal diet as above, but with *Heterocapsa* and *Rhodomonas* added. Survival rates of golden snapper (*Lutjanus johnii*) were as high as 40% when larvae were fed *Acartia*, as opposed to <0.1% obtained with rotifers.

Northern Territory Department of Primary Industries and Fisheries (NTDPIF) further developed culturing systems for *Acartia* (Schipp *et al.*, 1999). This research sought to improve on the Thai method of green-water culture, which relies on wild plankton, by establishing cultures of *Acartia*. They were able to produce an average of 750 juveniles and 319 adults per litre over an 8-day culture cycle. Contamination of the cultures with rotifers was a problem, which was solved by the development of a 'zooplankton washer' which removed all animals (including copepod nauplii) smaller than 190 μm. Adult copepods were then taken from the cultures and used to seed green-water cultures containing larval *Lutjanus johnii*. This approach severs the dependence on wild plankton for supply of copepod material, but stops short of developing an intensive system for producing larval prey items (copepod nauplii).

Queensland Department of Primary Industries Northern Fisheries Centre (NFC) developed *Acartia* (*Acanthacartia*) cultures in parallel with the work at NTDPIF (Semmens *et al.*, 1999). Maximum egg production rates were achieved when copepods were fed the dinoflagellate *Heterocapsa*. However, *Rhodomonas* was necessary for continuous culture, and is probably more suitable for juvenile copepod stages. Research into copepod cultivation at NFC has been driven by the apparent requirement of groupers for copepod nauplii at first feeding.

Bluewater Barramundi Pty Ltd has diversified from production of barramundi into gold-spot estuary cod *Epinephelus coioides*. Plankton were attracted by light in Mourilyan Inlet and *Acartia* sp. adults isolated from the collections. These cultures were scaled up by about a factor of 10 each week, until they reached densities of about 100 adults per litre, when sufficient nauplii were obtained to use as food for estuary cod. Survivorship, normally less (about 1%) when conventional larval feeds were used, improved to about 40% when copepods were used as prey items.

Acartia *and resting eggs*

Acartia is one of several genera known to have resting eggs (Kasahara *et al.*, 1974; Grice and Marcus, 1981; Uye, 1985). This feature is attractive for aquaculture, and is sometimes raised as having potential to become the copepod equivalent of the brine shrimp cyst. The practicalities of achieving this goal, however attractive, are considerable. Two observations from aquaculture in Australia are worth noting:

1. In one South Australian enterprise producing King George whiting (*Sillaginodes punctata*) the management system included periodic draining of the ponds (**Amanda Caughey, University of Adelaide**, pers. comm.). Subsequent to refilling, populations of *Acartia* (*Acartiura*) sp. developed rapidly. It is probable that these populations arose from subitaneous eggs in the sediment.

2. **Schipp** (pers. comm.) observed that some of the *Acartia* eggs from stocks cultured at NTDPIF have a thick chorion, suggestive of resting eggs. If confirmed, this will be the first record of tropical copepods producing resting eggs.

Gladioferens imparipes

Gladioferens (Calanoida: Centropagidae) is a genus unique to Australia, New Zealand and Antarctica. It is typically found in estuaries, and has representatives that occur across the full range of salinities from freshwater to fully marine. The most common representatives are *G. imparipes*, which is endemic to Western Australia, and *G. pectinatus*, which occurs on the east coast from Victoria to North Queensland. Both these species have been cultured in the laboratory, (Takano, 1971; Arnott *et al.*, 1986). Both are strongly euryhaline and can be cultured successfully at the salinity of seawater. The genus broods its eggs in a single sac, and has some interesting adaptations to estuarine life, including a strongly demersal habit in the adult. Copepodite stages have dorsal arrays of hairs on the prosome which appear to enable them to adhere to substrate (Rippingale, 1994), either to avoid predation or to maintain their distribution against currents.

Rippingale (FRDC: Curtin University) developed an automated culture system which produced about 450,000 nauplii per day for over a year; the nauplii produced were used in experiments with seahorses, snapper (*Pagrus auratus*) and dhufish (*Glaucosoma hebraicum*). Though these fish species do not require copepod food (i.e. can be raised on rotifers and *Artemia*), higher survival and faster growth were obtained when copepods were offered. Fish had no difficulty in preying on copepodites adhering to aquarium walls.

This project was completed in 1999, and a manual of procedures for intensive cultivation by commercial hatcheries was produced. Consequently, it is the most fully developed application of copepods in Australian aquaculture to date.

Tisbe spp.

Tisbe (Harpacticoida: Tisbidae) is a genus characterised by high rates of speciation and the occurrence of many cryptic species. This has resulted in its becoming the marine *Drosophila* for geneticists. It is a ubiquitous inhabitant of aquarium systems, surviving on bacteria and fouling growth on the inside of pipes etc.

Andria Marshall (CRC Aquaculture: UTAS) obtained culture densities of over 1000 *Tisbe* per litre when fed mixed algal diets of *Tetraselmis* and *Isochrysis*. Flounder (*Rhombosolea tapirina*) larvae ingested *Tisbe* nauplii in preference to rotifers.

TAFI report that small-scale cultures of harpacticoid copepods, probably *Tisbe*, have been held to decrease reliance on naturally occurring stocks. These copepods have been used to ameliorate nutritional problems in larviculture of striped trumpeter (see below). With the TAFI system, naupliar concentrations of up to 35 per millilitre have been obtained (J. Purser, pers. comm. 2000).

Other species

Andria Marshall (CRC Aquaculture: UTAS) As is the case for *Tisbe*, *Apocyclops dengizicus* (Cyclopoida: Cyclopidae) occurs as a contaminant in aquaria, but in the wild occurs in tropical salt lakes. Cyclopoid copepods are usually small, but *A. dengizicus* is large for the group, at about 1.5 mm. Marshall was able to obtain culture densities of 4500 per litre on the same mixed diet as for *Tisbe*. Barramundi (*Lates calcarifer*) larvae consumed the copepod when healthy, but the converse was the case when the fish were unhealthy!

At **AIMS**, we have held *Bestiolina similis* (Calanoida: Paracalanidae) in continuous culture for 6 months on a mixed algal diet of *Isochrysis*, *Tetraselmis*, *Rhodomonas* and *Heterocapsa*. The ability of paracalanid copepods to store lipids in dorsal sacs (Moreno *et al.*, 1979), as well as their vulnerability to fish predation (Kimmerer and McKinnon, 1989) and high fecundity would seem to make them ideal candidates for aquaculture.

NEEDS, BY SECTOR

Most fish species in Australian aquaculture can be raised through the larval stages with rotifers and *Artemia* nauplii before weaning on to particulate diets. Generally, fish which produce big eggs (and consequently, big larvae), present less of a problem for larviculture than highly fecund species which produce small eggs. In the case of the latter, both research and industry have become indifferent to the very low survival rates obtained with conventional larviculture techniques. A larval survival rate of 5% is accepted as satisfactory. Although this may not be a problem for highly fecund fish in terms of supply of larvae for grow-out, it does introduce the possibility of inadvertent selection of deleterious genes. At the very least, there is plenty of room for improvement.

The success of larviculture of finfish currently cultured in Australia falls into three groups:

GROUP 1: Species for which current technology is apparently satisfactory. This is the case for the salmonids. Commercial feed companies, such as Ridley Aqua Feed and Pivot Aquaculture, provide salmonid feeds appropriate for fish of all ages. The technology is well established and benefits from overseas research support. There are overseas data to suggest that pigmentation is improved when carotene-rich zooplankton are provided as feeds (Sargent *et al*., 1979; Spenelli, 1979). However, these components can usually be enhanced in the diet artificially.

GROUP 2: Species which can be raised with conventional technology but which could benefit from live food developments. Enriched rotifers are the most common larval diets for WA dhufish, yellowtail kingfish (*Seriola lalandi*), pink snapper (*P. auratus*) and barramundi. Low survivorship of larvae is characteristic of aquaculture practice for these species, but because adult fecundity is high, sufficient fry survive to make the industry viable. Though more appropriate larval diets may improve growth and survival of these species, it may not be worth the added expense. The degree of benefit derived from the provision of live feeds varies between these fish species. For instance conventional feeds are presently regarded as adequate for snapper and kingfish, whereas improvements in growth and survival of dhufish make the provision of copepod food desirable, especially since the technology for supply of *Gladioferens imparipes* has been developed and successfully applied.

GROUP 3: Species for which conventional larval rearing technology is severely lacking. These include striped trumpeter, groupers and, arguably, tuna. Coincidentally, these species also attract the highest market prices (Table 4.3), making the investment into R&D feasible. Each of these problem areas is discussed below.

Table 4.3: Fish species for which there are problems in larviculture, ranked by market price.

FISH SPECIES	WHOLESALE MARKET VALUE $AUS KG^{-1}	MARKET	COPEPOD SPECIES MOST LIKELY TO ACHIEVE SUCCESSFUL LARVICULTURE
Southern Bluefin Tuna *Thunnus maccoyi*	$260	Japan	Small species?
Giant grouper *Epinephelus lanceolatus*	$163 (small) $64 (large)	Hong Kong	*Acartia* Other small species?
Maori wrasse *Cheilinus undulatus*	$150 (small) $83 (large)	Hong Kong	*Acartia* Other small species?
Barramundi cod *Cromileptes altivelis*	$140	Hong Kong	*Acartia* Other small species?
Red grouper *Epinephelus akaara*	$121	Hong Kong	*Acartia* Other small species?
Leopard coral trout *Plectropomus leopardus*	$80	Hong Kong	*Acartia* Other small species?
Flowery cod *Epinephelus fuscoguttatus*	$70 (small) $44 (large)	Hong Kong	*Acartia* Other small species?
Spotted coral trout *Plectropomus areolatus*	$61	Hong Kong	*Acartia* Other small species?
Striped trumpeter *Latris lineata*	$18	Local good prospect for Japanese sashimi market.	*Tisbe*
Dhufish *Glaucosoma hebraicum*	$30	Local good prospect for Japanese sashimi market.	*Gladioferens imparipes*

Striped trumpeter

Striped trumpeter are found in deep water off Tasmania, and are a valued table fish in that State, second only to blue-eye trevalla (*Hyperaglyphe antarctica*). The Atlantic salmon industry is keen to diversify its target species, and has closed the life cycle. The potential domestic market and suitability of striped trumpeter for the Japanese sashimi market make this fish a desirable candidate for aquaculture. At TAFI, research into the rearing of striped trumpeter has encountered problems with high larval mortality, developmental irregularities (mouth deformities) and behaviour (spinning behaviour, walling syndrome). These seem to be related to nutritional problems, and especially poor digestibility of rotifer food in early larval development. To solve this problem, harpacticoid copepods, probably *Tisbe*, were isolated from aquarium raceways and used to seed green-water

cultures containing fish larvae. The success of this approach is uncertain at present, however, because copepod nauplii were so rapidly digested by the larvae that it was difficult to determine feeding rates.

Groupers and other high value tropical finfish

Groupers (Serranidae) and tropical snappers (Lutjanidae) are in great demand in the live fish trade in East Asia, and attract very high market prices (Table 4.3). QDPI commissioned a Reef Fish Aquaculture Feasibility Study in 1995–96, to assess the potential of the aquaculture industry to supply Chinese markets, and the implications of this study for grouper aquaculture are more fully discussed by Rimmer *et al.* (1997). Though R&D costs are expected to be high, this industry is potentially highly profitable. The main constraint to industry development is the supply of large numbers of fingerlings for grow-out. QDPI have a well developed R&D plan for development of reef fish aquaculture in Queensland, Phase 1 of which is to span 4 years and concentrate on spawning and larval rearing technology. However, the level of funding is below that identified in the Feasibility Study as being necessary for development of a viable industry.

Groupers and tropical snappers are characterised by having larvae with a very small mouth gape, which prevents ingestion of all but the smallest rotifer strains. Moreover, they do not digest rotifers well, which are often excreted whole, or even live (Schipp *et al.*, 1999). Ali *et al.* (1998) report that *Epinephelus fuscoguttatus* ingests both *Acartia* and *Paracalanus* nauplii in preference to rotifers. Generally, the best success to date in rearing groupers and snappers has been obtained when copepod nauplii have been provided, usually employing some variant of the green-water method described above.

Southern bluefin tuna

The supply of southern bluefin tuna for the Japanese sashimi market is currently the second biggest fishing industry in Australia ($60.9 million in 1997–98, second only to pearl oysters, $229.4 million; 1999–2000 Austasia Trade Directory). At present it comprises sea ranching of young fish and depends on wild harvest of appropriately-sized fishes. There is a lot of interest in closing the life-cycle of this species from the tuna industry itself. Lee (1998) summarised the feasibility of aquaculture of southern bluefin tuna (SBT) in Australia. There is little or no information available on SBT themselves, but methods available for other tuna species, especially the northern bluefin tuna (NBT), ought to be applicable. NBT have been reared in Japan on a diet of rotifers and *Artemia,* but the problem of high juvenile mortality at first feeding and at around metamorphosis is well recognised and may be related to the interaction of early gut development and diet (Kaji *et al.*, 1996). In Panama, copepods were used in conjunction with rotifers and *Artemia* as larval feeds (Lee, 1998), but there is no information on the efficacy of the copepod component of the diet.

In the wild, the larval diet of SBT and other tuna species comprises different life stages of copepods, as well as Cladocera and Appendicularia (Uotani *et al.*, 1981). Interestingly, these authors report that tuna larvae select for 'cyclopoid' copepods (actually the poecilostome family Corycaeidae) and the cladoceran *Evadne*, and against calanoid copepods. However, this may be the result of these

organisms leaving identifiable remains in the gut contents, and there is certainly evidence of ingestion of calanoid copepod nauplii and copepodites (their Fig. 3).

Lee (1998) concludes that considerable research is still required in larval rearing and larval nutrition, and that current technology is inadequate for the mass production of juveniles. As is the case for groupers, the provision of more appropriate larval feeds, ideally copepods, is necessary for successful industry development. An FRDC-funded strategic plan for the propagation of SBT is presently being developed by Agriculture, Fisheries and Forestry – Australia (AFFA).

MISSION: TO PROVIDE COPEPODS AS FEEDS IN AQUACULTURE

The problem

The greatest problem facing aquaculturalists both in Australia and overseas in the use of copepods as live feed organisms is the failure to produce cultures of sufficient density. For instance, rotifers such as *Brachionus plicatilis* can be easily cultured to densities as high as 500 per millilitre (Nellen, 1986), corresponding to 75 mg L^{-1} dry weight. Schipp *et al.* (1999) claim a 'mean peak density' of *Acartia* nauplii of 2 per millilitre, exceeding that of most temperate *Acartia* species. This corresponds to a biomass of 0.1 mg L^{-1}, figuring the average weight of an *Acartia* nauplius to be about 0.05 μg (Landry, 1978). Higher densities were obtained by (Støttrup and Norsker, 1997); over 100 *Tisbe* nauplii per millilitre in small-scale cultures. Nevertheless, copepod cultures are unlikely to achieve the return possible with rotifers, at equivalent levels of effort. On the other hand, nauplii need to be provided for only a short period.

Removal of two of the major obstacles could certainly improve current methods:

1. The problem of cannibalism (Uye and Liang, 1998), which is well recognised by aquaculture scientists especially with *Acartia* (Støttrup *et al.*, 1986; Ohno *et al.*, 1990). All copepods, even those traditionally regarded as suspension feeders, ingest microzooplankton, including nauplii of their own or other species. The problem is exacerbated with *Acartia* because of its ability to feed both as a suspension feeder and as a raptor (Saiz and Kiørboe, 1995, and references therein).
2. The attainment of maximal egg production rates through dietary diversity. In the field, copepods maintain a diverse diet, comprising phytoplankton, microzooplankton and detritus (Kleppel, 1993). Maximum egg production rates are achieved when the combination of food items achieves the best nutritional mix (Kleppel *et al.*, 1998). The provision of a small suite of microalgal species in copepod culture may be insufficient to achieve maximal rates.

Other perceived problems, such as high maintenance costs and the requirements for high water quality, are more tractable and could be resolved by technological solutions such as flow-through systems and turbidostat technology. However, copepod nauplii need to be provided for only a short period of time, spanning first feeding, and older larvae can be raised on conventional diets.

The goal

The goal is to develop sustainable, easily maintained culture systems for copepods. These must be capable of producing high densities of appropriately sized nauplii on demand, for at least the period of first feeding of larvae. It is unlikely that copepods will provide a replacement for *Artemia*, but copepod nauplii are the best candidates for rotifer substitutes in the aquaculture of high-value finfish species unable to prey on or digest rotifers. Copepods are also valuable supplements to conventional live feeds.

Broodstock can be fickle in the timing of spawning. Copepod culture systems must be sufficiently flexible to accommodate the timing of first-feeding of fish larvae.

The challenge

For Group 2 Fish Species: Good progress in R&D has already been made in southern Australia for the provision of copepod food for this group of fish species. The challenge now is to:

1. Extend the technology developed for *Gladioferens imparipes* to other copepod species. Because *G. imparipes* is endemic to W.A., responsible aquaculture development should guard against its introduction to the eastern states. However, there are obvious candidates in the east for which the same technology may be applicable, such as *G. pectinatus* and *Pseudodiaptomus* spp. This technology depends upon strong positive phototaxis of nauplii, and it is unclear how general this behaviour is.
2. Apply and further extend the culture of *Tisbe* spp. using methods developed at TAFI and European research as a basis for development.

The future for the application of these technologies will be decided on economic rather than scientific grounds. Are the advantages conferred by provision of copepod food sufficient to justify the added expense of providing them?

For Group 3 Fish Species: Development of copepod culture for these fish has concentrated on *Acartia*, for reasons of convenience and tradition outlined above. However, though *Acartia* nauplii have a proven track record as prey items for groupers and jacks, the culture techniques developed to date do little more than break the dependence on wild stocks. This is in itself a worthwhile achievement, as the continual introduction of wild stocks into hatcheries brings with it the risk of disease.

All the fish species in this group are tropical, and characterised by small mouth gape at first feeding. The need then, is for the provision of large amounts of sub-100-μm food. This could be achieved in either of two ways:

1. Extend the methods already developed for *Acartia* to increase egg production rates and culture densities. *Acartia* nauplii exceed 100 μm about N3 – N4, developmental stages they are likely to reach about 24 hours after hatching from the egg. To provide adequate amounts of suitable prey items from *Acartia* will mean optimising egg production rates by dietary diversity and circumventing cannibalism.

2. The small size and occasional high density of tropical copepods such as the paracalanids *Bestiolina similis* and *Parvocalanus crassirostris* make them good candidates for aquaculture. Both of these species are obligate suspension feeders, and would have less of a problem with cannibalism. In addition, *B. similis* in particular is naturally more fecund than *Acartia*. Alternatively, it may be possible to extend the technology developed for *Tisbe* spp. in temperate regions to the tropics.

In view of the high market prices attracted by Group 3 fish species, further investment into R&D into alternative diets for larviculture is warranted.

REFERENCES

Ali, A., Mohn Salleh, M. T. and Siti Noraziah, A. Z. (1998). Food preference in early larvae of brown-marbled grouper. *Aquaculture Asia III*, 39–43.

Arnott, G. H., Brand, G. W. and Kos, L. C. (1986). Effects of food quality and quantity on the survival, development, and egg production of *Gladioferens pectinatus* (Brady) (Copepoda: Calanoida). *Aust. J. Mar. Freshwat. Res., 37*, 467–473.

D'Abramo, L. R. and Lovell, R. T. (1991. Aquaculture research needs for the year 2000: Fish and crustacean nutrition. *World Aquacult., 22*, 57–82.

Doi, M., Ohno, A., Taki, Y., Singhagraiwan, T. and Kohno, H. (1997). Nauplii of the calanoid copepod, *Acartia sinjiensis* as an initial food organism for larval red snapper *Lutjanus argentimaculatus*. *Suisanzoshoku, 45*, 31–40.

Doi, M., Singhagraiwan, T., Singhagraiwan, M., Kitade, M. and Ohno, A. (1994). Culture of the calanoid copepod *Acartia sinjiensis* , in outdoor tanks in the tropics. *Thai Mar. Fish. Res. Bull., 5*, 27–36.

Grice, G. D. and Marcus, N. H. (1981). Dormant eggs of marine copepods. *Oceanogr. Mar. Biol. Ann. Rev., 19*, 125–140.

Hardy, A. (1965). *The open sea: Its natural history*. Cambridge: Houghton Mifflin.

Hunter, J. R. (1981). Feeding ecology and predation of marine fish larvae. In R. Lasker (Ed.), *Marine fish larvae: Morphology, ecology and relation to fisheries*, (pp: 33–77). Seattle: University of Washington Press.

Huys, R. and Boxshall, G. A. (1991). *Copepod Evolution*. London: The Ray Society.

Kaji, T., Tanaka, M., Takahashi, Y., Oka, M. and Ishibashi, N. (1996). Preliminary observations on development of Pacific bluefin tuna *Thunnus thynnus* (Scombridae) larvae reared in the laboratory, with special reference to the digestive system. *Marine and Freshwater Research, 47*, 261–269.

Kasahara, S., Uye, S. and Onbe, T. (1974). Calanoid copepod eggs in sea-bottom muds. *Mar. Biol., 26*, 167–171.

Kimmerer, W. J. and McKinnon, A. D. (1989). Zooplankton in a marine bay. III. Evidence for influence of vertebrate predation on distributions of two common copepods. *Mar. Ecol. Progr. Ser., 53,* 21–35.

Kinne, O. (1977). *Marine ecology: A comprehensive integrated treatise on life in oceans and coastal waters. Vol III Cultivation part 2*. John Wiley.

Kiørboe, T. and Sabatini, M. (1994). Reproductive and life cycle strategies in egg-carrying cyclopoid and free-spawning calanoid copepods. *Journal of Plankton Research, 16*, 1353–1366.

Kleppel, G. S. (1993). On the diets of calanoid copepods. *Mar. Ecol. Progr. Ser., 99*, 183–195.

Kleppel, G. S., Burkart, C. A. and Houchin, L. (1998). Nutrition and the regulation of egg production in the calanoid copepod *Acartia tonsa*. Limnol. *Oceanogr., 43*, 1000–1007.

Kraul, S. (1990). Production of live prey for marine fish larvae. Advances in tropical aquaculture, Tahiti. Feb 20 – March 4 1989. Aquacop. IFREMER. *Actes de Colloque, 9*, 595–607.

Landry, M. R. (1978. Population dynamics and production of a planktonic marine copepod, *Acartia clausii*, in a small temperate lagoon on San Juan Island, Washington. *Int. Rev. Gesamten Hydrobiol., 63*, 77–119.

Lee, C. L. (1998). *A study on the feasibility of the aquaculture of the Southern Bluefin Tuna. Final Report — December 1998*. Agriculture Fisheries and Forestry - Australia, Canberra ACT and Fisheries WA 1–92.

Luizi, F. S., Gara, B., Shields, R. J. and Bromage, N. R. (1999). Further description of the development of the digestive organs in Atlantic halibut (*Hippoglossus hippoglossus*) larvae, with notes on differential absorption of copepod and *Artemia* prey. *Aquaculture, 176*, 101–116.

Marshall, S. M. and Orr, A. P. (1972). *The biology of a marine copepod*. Berlin: Springer.

Moreno, V. J., Moreno, J. E. A. and Brenner, R. R. (1979). Lipid composition of *Paracalanus parvus*. *Oceanol. –Acta, 2*, 373–378.

Munilla-Moran, R., Stark, J. R. and Barbour, A. (1990). The role of exogenous enzymes in digestion in cultured larvae (*Scophthalmus maximus* L.). *Aquaculture, 88*, 337–350.

Nanton, D. A. and Castell, J.D. (1999). The effects of temperature and dietary fatty acids on the fatty acid composition of harpacticoid copepods, for use as a live food for marine fish larvae. *Aquaculture, 175*, 167–181.

Nellen, W. (1986). Live animal food for larval rearing in aquaculture: Non-*Artemia* organisms. In M. Bilio, H. Rosenthal and C. J. Sindermann (Eds.), *Realism in Aquaculture: Achievements, constraints, perspectives* (pp. 215–250). Bredene, Belgium: European Aquaculture Society.

Ohno, A. and Okamura, Y. (1988). Propagation of the calanoid copepod, *Acartia tsuensis*, in outdoor tanks. *Aquaculture, 70,* 39–51.

Ohno, A., Takahashi, T. and Taki, Y. (1990). Dynamics of exploited populations of the calanoid copepod, *Acartia tsuensis*. *Aquaculture, 84*, 27–39.

Paffenhöfer, G.-A. and Harris, R. P. (1979). Laboratory culture of marine holozooplankton and its contribution to studies of marine planktonic food webs. *Adv. Marine Biol., 16*, 211–308.

Peterson, W. T. and Kimmerer, W. J. (1994). Processes controlling recruitment of the marine calanoid copepod *Temora longicornis* in Long Island Sound: Egg production, egg mortality, and cohort survival rates. *Limnol. Oceanogr., 39*, 1594–1605.

Rimmer, M. (1998). Grouper and snapper aquaculture in Taiwan. *Austasia Aquaculture, 12*, 3–7.

Rimmer, M., O'Sullivan, M., Gillespie, J., Young, C., Hinton, A. and Rhodes, J. (1997). Grouper aquaculture in Australia. *SPC Live Reef Fish Information Bulletin, 3*, 15–23.

Rippingale, R. J. (1994). A calanoid copepod *Gladioferens imparipes*, holding on to surfaces. *Hydrobiologia, 292/293*, 351–360.

Saiz, E. and Kiørboe, T. (1995). Predatory and suspension feeding of the copepod *Acartia tonsa* in turbulent environments. *Marine Ecology Progress, Series 122*, 147–158.

Schipp, G. R., Bosmans, J. M. P. and Marshall, A. J. (1999). A method for hatchery culture of tropical calanoid copepods, *Acartia* spp. *Aquaculture, 174*, 81–88.

Semmens, G. L., Stewart, M. D., Fry, P. A., Rimmer, M. A. and McKinnon, D. (1999). Comparison of yield produced by the copepod *Acartia* fed on different microalgae. WAS 99 Abstract.

Shields, R. J., Bell, J. G., Luizi, F. S., Gara, B., Bromage, N. R. and Sargent, J. R. (1999). Natural copepods are superior to enriched *Artemia* nauplii as feed for halibut larvae (*Hippoglossus hippoglossus*) in terms of survival, pigmentation and retinal morphology: Relation to dietary essential fatty acids. *J. Nutr., 129*, 1186–1194.

Singhagraiwan, T. and Doi, M. (1993). Induced spawning and larval rearing of the red snapper *Lutjanus argentimaculatus* at the Eastern marine Fisheries Development Center. *Thai Mar. Fish. Res. Bull. 4*, 45–57.

Spenelli, J. (1979). Preparation of salmonid diets containing zooplankton and their effect on organoleptic properties of pen-reared salmonids. In: *Proceedings of the world symposium on finfish nutrition and fishfeed technology*, Hamburg 20–23 June 1978. Berlin. *2*, 383–392.

Støttrup, J. G. and Norsker, N. H. (1997). Production and use of copepods in marine fish larviculture. *Aquaculture, 155*, 231–247.

Støttrup, J.G., Richardson, K., Kirkegaard, E. and Pihl, N. J. (1986). The culture of *Acartia tonsa* Dana for use as a live food source for marine fish larvae. *Aquaculture, 52,* 86–96.

Støttrup, J. G., Shields, R., Gillespie, M., Gara, M. B., Sargent, J. R., Bell, J. G., Henderson, R. J., Tocher, D. R., Sutherland, R., Næss, T., Mangor Jensen, A. Naas, K., van der Meeren, T., Harboe, T., Sanchez, F. J., Sorgeloos, P., Dhert, P. and Fitzgerald, R. (1998). The production and use of copepods in larval rearing of halibut, turbot and cod. *Bull Aquacul. Assoc. Canada 98-4*, 41–45.

Takano, H. (1971). Notes on the raising of an estuarine copepod, *Gladioferens imparipes* Thomson. *Bull. Tokai Reg. Fish. Res. Lab., 64*, 81–87.

Toledo, J. D., Golez, Ma. S., Doi, M. and Ohno, A. (1999). Use of copepod nauplii during early feeding stage of grouper *Epinephelus coioides*. *Fisheries Science, 65*, 390–397.

Uotani, I., Matsuzaki, K., Makino, Y., Noda, K., Inamura, O. and Horikawa, M. (1981). Food habits of larvae of tunas and their related species in the area northwest of Australia. *Bulletin of the Japanese Society of Scientific Fisheries, 47*, 1165–1172.

Uye, S. (1985). Resting egg production as a life history strategy of marine planktonic copepods. *Bull. Mar. Sci., 37*, 440–449.

Uye, S.-I. and Liang, D. (1998). Copepods attain high abundance, biomass and production in the absence of large predators but suffer cannibalistic loss. *Journal of Marine Systems, 15*, 495–501.

STATUS REVIEW 5: Artificial feeds

DEVELOPMENT OF ARTIFICIAL DIETS FOR FISH LARVAE

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INTRODUCTION

A generalised feeding protocol for marine finfish larvae begins with rotifers at first feeding, followed by *Artemia* nauplii and larger *Artemia* as larvae increase in size (Dhert *et al*., 1990). Artificial (formulated) diets are then introduced and larvae are weaned from live feed organisms. In addition to rotifers and *Artemia,* microalgae are usually cultured in mariculture hatcheries to provide food for rotifers and *Artemia*. Most mariculture hatcheries culture three different live foods (microalgae, rotifers and *Artemia*) to provide food for the larvae of a single target species.

Not surprisingly, efforts have been made to develop artificial diets to replace live foods for marine fish larvae. The major factors influencing this development are expense of live food production, infrastructure requirements for live food production, nutritional inconsistency and deficiencies of live foods, their availability and potential as vectors for disease introduction.

- **Expense**
  Live food organisms may contribute up to 50% of hatchery operating costs with most of this cost associated with labour. Lavens *et al.* (1995) estimated labour to contribute up to 68% of rotifer production costs and *Artemia* production has been estimated to represent 79% of production costs of European seabass (Person-Le Ruyet *et al*., 1993).

- **Infrastructure/Facilities**
  Live food production requires substantial commitment of space and infrastructure.
  Marine finfish hatcheries usually culture microalgae, rotifers and *Artemia* each requiring specific culture conditions and dedicated facilities (Southgate and Partridge, 1998).

- **Nutritional inconsistency/deficiency**
  Live foods vary in their nutritional composition according to source, age and culture techniques (Sorgeloos *et al*., 1986; Leger *et al*., 1986; Ben-Amotz *et al*., 1987). *Artemia* and rotifers lack some essential nutrients and must be enriched prior to use (Sorgeloos *et al*., 1986; Leger *et al*., 1986). This process further adds to the cost of live food production. Although cost is the major impetus for research into development of artificial diets, from a nutritional standpoint, live foods (rotifers/*Artemia*) are far from ideal.

- **Availability**
  Australian hatcheries are reliant on a continuing supply of adequate quantities of imported *Artemia* cysts. It is likely that this supply may become a major bottleneck for Australian hatcheries as a result of recent declines in harvests of *Artemia* cysts (Leger, 1999) coupled with increasing demand for *Artemia* cysts from a rapidly growing world aquaculture industry. There are also quarantine issues associated with importing *Artemia* cysts and rotifer cultures into Australia.

- **Disease/crash**
  Other potential problems associated with live foods include "crashes" (rapid, large-scale mortality) which can leave hatcheries short of food, and the potential for disease introduction to larval cultures (Southgate and Partridge, 1998).

ARTIFICIAL DIET DEVELOPMENT

The high cost of live food production in marine fish hatcheries could be reduced by cheaper production of live foods and earlier weaning onto formulated feeds. However, complete or significant replacement of live foods with artificial diets is the ultimate goal of research in this field. Perhaps the most significant advantage of artificial 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. This is not possible with live foods. Artificial diets offer the advantages of nutritional consistency and off-the shelf convenience. Successful artificial diets must support similar growth and survival to live foods and, for finfish larvae, must satisfy a number of criteria (Table 5.1).

Table 5.1 Desired characteristics of artificial diets for finfish larvae (Southgate and Partridge, 1998)

CHARACTERISTIC	COMMENTS
ACCEPTABILITY	Artificial diets must be attractive and readily ingested. Diet particles must be of a suitable size for ingestion and must illicit a feeding response from the larvae. Diet particles must remain available in the water column.
STABILITY	Artificial diet particles must maintain integrity in aqueous suspension and nutrient leaching should be minimal. Some nutrient leaching may be beneficial in enhancing diet attractability
DIGESTIBILITY	Artificial diets should be digestible and their nutrients readily assimilated
NUTRIENT COMPOSITION	Artificial diets should have an appropriate nutritional composition. Materials added to the diet as binders or the components of microcapsule walls should have some nutritional value.
STORAGE	Artificial diets must be suitable for long term (6-12 months) storage with nutrient composition and particle integrity remaining stable.

Two types of microparticulate particles have been used to present artificial diets to fish larvae; these are (1) micro-encapsulated diets (MED) and (2) microbound diets (MBD). Both have been used extensively in nutritional studies with finfish larvae. The major difference between the two is that MED have a membrane or capsule wall which separates dietary materials from the surrounding medium. The capsule wall helps maintain integrity of the food particle and until eaten and helps maintain water quality (Meyers, 1979); however, it may restrict leaching of water soluble dietary components and therefore reduce the attractability of the food particles. The capsule wall is also thought to impair digestion of the food particle (Southgate and Lee, 1993) and a number of studies have reported poor growth and survival of fish larvae fed MED (Teshima *et al*., 1982; Walford *et al*., 1991). Although effective for presenting artificial diets to bivalves and crustaceans (Jones *et al*., 1993), MED may have limited use for marine fish larvae.

MBD consist of dietary components held within a gelled matrix or binder (Lopez-Alvarado *et al*., 1994). They do not have a capsule and this has been suggested to facilitate greater digestibility and increased attractability through greater nutrient leaching (Southgate and Lee, 1993). Many different binders have been used in MBD including polysaccharides from seaweed such as agar, carrageenan and alginate and proteins such as zein and gelatin (Meyers *et al*., 1972; Adron *et al*., 1974; Hashim and Mat Saat, 1992; Person-Le Ruyet *et al*., 1993; Lee *et al*., 1996). They vary considerably in their properties and nutritional value and choice of binder can significantly influence the rate of ingestion of artificial food particles and nutrient assimilation (Partridge and Southgate, 1999). Water stability of MBD is also influenced by the binder employed. Heinen (1981) assessed water stability of artificial diets made from 11 different binders; MDB made from agar and alginate were amongst the most stable in terms of integrity, while carrageenan was amongst the poorest. Both MED and MBD are generally dried prior to use and this may hinder their digestion.

STATUS OF ARTIFICIAL DIET DEVELOPMENT

Many studies have been conducted to assess the nutritional value of microparticulate artificial diets for marine finfish larvae (Table 5.2). Generally, they have resulted in lower survival and poorer growth of larvae compared to those fed live foods and often lead to higher incidence of deformities (Person-Le Ruyet *et al*., 1993). The data indicates that total replacement of live prey with artificial diets is still not possible for the larvae of most marine fish.

Table 5.2. Summary of studies on replacement of live foods for first feeding marine fish larvae (from Southgate and Partridge, 1998).

SPECIES	DIET TYPE	RESULT	AUTHOR
Pleurnoectes platessa (plaice)	- gelatin-bound MBD.	- survival 50% of live fed controls.	- Adron *et al.* (1974).
Solea solea (sole)	- zein bound MBD.[1] - MED.[2]	- lower survival and growth than live fed controls.[1,2]	- Gatesoupe *et al.* (1977).[1] - Appelbaum (1985).[2]
Dicentrachus labrax.(European seabass)	- zein bound MBD.	- lower survival and growth than live fed controls.	- Gatesoupe *et al.* (1977).
Sparus aurata. (gilthead sea bream)	- MBD with/without exogenous enzymes.	- best survival and growth with enzymes; still less than live feeds.	- Kolkovski *et al.* (1991).
Lates calcarifer. (barramundi, Asian seabass)	- protein walled MED.[1] - gelatin-bound and carrageenan-bound MBD.[2]	- no survival after 10 days.[1] - no survival after 8 days.[2]	- Walford and Lam (1991).[1] - Southgate and Lee (1993).[2]
Gadus morhua.(Atlantic cod)	- MED.	- poor survival and growth.	- Garatun-Tjeldsoto *et al.* (1989).
Clupea harrengus. (Atlantic herring)	- MED.	- poor survival and growth.	- Fox (1990).
Pagrus major.(red sea bream)	- nylon-protein MED.[1]	- little survival and growth.[1]	- Kanazawa *et al.* (1982).[1]
Paralichthys olivaceus.(starry flounder; flatfish)	- nylon-protein MED.[1] - zein-bound and carrageenan-bound MBD.[2]	- poor survival and growth.[1] - good survival and growth; less than live feeds. Better results with zein-bound MBD.[2]	- Teshima *et al.*, 1982 - Kanazawa and Teshima (1988).[2]
Oplegnathus fasciatus. (knife jaw)	- nylon-protein MED.	- low survival and very little growth.	- Teshima *et al.* (1982).

Despite this, partial replacement of live foods with artificial feeds can result in considerable cost savings in live feed production (Jones *et al.*, 1993). For example, Kanazawa and Teshima (1988) reported that newly hatched red seabream (*Pagrus major*) larvae fed a 1:1 combination of rotifers and MBD, grew as well as larvae receiving live food alone. Similarly, growth of day 8 *Sparus aurata* larvae fed a diet where 80% of rotifers were replaced with MBD was similar to the treatment receiving 100% live food (Tandler and Kolkovski, 1991).

Weaning fish larvae onto artificial feeds at the earliest possible age is another effective means of reducing the cost of live food (Lavens *et al.,* 1995). For example, *D. labrax* larvae weaned 15 days earlier has enabled savings in *Artemia* production of up to 80% (Person-Le Ruyet *et al.*, 1993). Promising results from early weaning have also been reported for *Pagrus major* (Teshima *et al.*, 1982) and *Lates calcarifer* (Juvario *et al.*, 1991).

CONSTRAINTS TO DEVELOPING ARTIFICIAL DIETS FOR MARINE FINFISH LARVAE

The relatively poor performance of artificial diets in studies with marine fish larvae is thought to result primarily from their reduced rates of ingestion and poor digestion.

a. Ingestion

Successful artificial diets must be ingested at a rate similar to live food. This is a particular problem with carnivorous fish larvae, which rely on the visual stimulus of moving prey to initiate a capture response (Dabrowski, 1984; Kamler, 1992) and is likely to be a factor in explaining why formulated diets are less effective than live foods in nutritional studies (Weinhart and Rösch, 1991; Fuchs and Nedelec, 1989). Efforts to over come this problem have included inclusion of various chemicals (using light refraction) to impart a sense of motion to artificial food particles (Meyers, 1979) and incorporation of food dyes into MBD to simulate the colour of *Artemia* nauplii (Adron *et al.,* 1974). Amino acids, which naturally emanate from live prey organisms, have been shown to enhance larval feeding response and can be incorporated into artificial diets to improve attractability (Rottiers and Lemm, 1985; Doving and Knutsen, 1991; Kolkovski *et al*., 1993).

b. Digestion

Most marine finfish larvae are poorly developed at hatch (Lavens *et al*., 1995); the digestive tract in the larvae of most species is a straight tube that, with time, becomes segmented into the different sections of the gut (Jones *et al*., 1993). The digestive tract is fully developed only after 'metamorphosis', when the stomach with gastric glands and pyloric caeca are developed (Walford and Lam, 1993). At first feeding, the digestive tract, in most fish species, contains the enzymes related to metabolism (digestion, absorption and assimilation) of molecules such as proteins, lipids and glycogen. Enzyme activity has been observed to be relatively low compared with adult fish. Each enzyme develops independently during ontogenesis, with variation related to fish species and temperature. Secretion of acid and pepsin to aid digestion occurs only after 'metamorphosis' is completed and a functional stomach is present. Improving ability to digest artificial food particles with age has also been shown for *Lates calcarifer* larvae. Southgate and Lee (1993) reported that first feeding *L. calcarifer* readily ingested MBD but were unable to digest the food particles; larvae reared on MBD alone suffered complete mortality by day 10. However, the same diet supported good rates of growth and survival when presented to older *L. calcarifer* larvae as a weaning diet (Lee *et al*., 1996).

Live food organisms consumed by the larvae are thought to assists digestion (Dabrowski, 1984; Hjelmeland *et al.,* 1988, Kolkovski *et al*., 1993) by 'donating' their digestive enzymes, either by autolysis or as zymogens that activate larval endogenous digestive enzymes. The contribution of prey enzymes to digestion in 3 day old turbot (*Scophthalmus maximus*) larvae approaches 60% for protease activity, 27% for amylase activity, 88% for exonuclease activity and 94% for esterase activity (Munila-Moran *et al.,* 1990). Likewise, Lauff and Hofer (1984) estimated that exogenous proteases contribute up to 80% of the total proteolytic activity in first feeding whitefish (*Coregonus sp.*).

However, other evidence has led to contradictory views regarding the role of the live food contribution in the digestion process of fish larvae. Live food organisms contain a "package" of enzymes, gut neuropeptides and nutritional 'growth' factors which enhance digestion (Table 5.3). These substances are frequently omitted in formulated diets. Moreover, particulate diets for larvae contain proteins and other ingredients that are difficult to digest, (especially since formulated diets are 60-90% dry matter while zooplankton is only 10%).

Inclusion of digestive enzymes, especially proteases, in the diets for fish larvae has been reported to significantly improved nutrient utilisation and performance of larvae. Kolkovski *et al.* (1991) reported that the inclusion of commercially available pancreatic enzymes into MBD at a level of 0.05% increased assimilation by up to 30% when fed to *Sparus aurata* larvae. In a subsequent study with *Sparus aurata* larvae, it was shown that although MBD containing pancreatin at 0.05% supported significantly greater larval growth than diets containing no supplemental enzyme, there was no significant improvement if the level of enzyme was increased to 0.1% (Kolkovski *et al*., 1993, Table 5.4). Inclusion of pre-hydrolysed proteins in artificial diets has given mixed results depending on percentage of hydrolysate and larval age.It remains unclear whether a unique combination of hydrolysates coupled with nutrient absorption transporters can be comparable to live zooplankton. The effect of inclusion of digestive system neuropeptides to formulated diets has also been investigated in recent years (Kolkovski *et al*., 1999, Kolkovski 2000). The results suggest that inclusion of bombesin may increase assimilation of diets and larval growth. However, other trials with juvenile fish have shown no effect of these addition of the neuropeptide.

Table 5.3. Digestive enzyme contribution by live food organisms (from Kolkovski 2000).

SPECIES	LIVE FOOD ORGANISM	FINDINGS	AUTHORS
Carp *Cyprinus carpio*, Grass carp *Ctenophayngodon idella*, Salmon *Salmo gairdneri*, whitefish *Coregonus lavaretus*	Copepods, Cladocera, rotifer, Artemia	10%-98% of proteolytic activity is due to the food organisms	Dabrowski and Glogowski (1977a)
Turbot *Scophtalmus maximus*	Artemia, rotifers, copepods	Exogenous digestive enzymes contribution: proteases 43-60% esterase 89-94% exonuclease 79-88% amylase 15-27%	Munila-Moran *et al.* (1990)
Herring *Clupea herrengus*	copepods	0.5% of total trypsin content in intestine is derived from the live food	Pedersen *et al.* (1987), Pedersen and Hjelmeland (1988)
Whitefish *Coregonus* sp.	Monia sp.	70% of the trypsin activity in intestine derived from the live food	Lauff and Hoffer (1984)
Japanese sardine *Sardinops melanotictus*	Rotifer protease	0.6% of total protease activity in larvae	Kurokawa *et al.* (1998)

Table 5.4. Dietary digestive enzyme supplementation in microdiets (from Kolkovski 2000)

SPECIES	ENZYME SUPPLEMENTATION	FINDINGS	AUTHORS
Carp *Cyprinus carpio*	bovine trypsin	increased proteolytic activity	Dabrowski and Glogowski (1977b) and Dabrowska *et al.* (1979)
Salmon *Salmo salar*	Dietary amylase	No effect on growth or protein utilization	Carter *et al.* (1992)
Salmon *Salmo salar*	Dietary mixture of pancreatic enzymes	Positive effect on growth and protein utilization in soybean based diet	Carter *et al.* (1994)
Carp *Cyprinus carpio*	polyzyme mixture	increased weight gain	Bogut *et al.* (1995)
Gilthead seabream *Sparus aurata*	pancreatin (porcine pancreatic extract)	30% increase in MD assimilation, double growth rates	Kolkovski *et al.* (1993)
SeaBass *Dicentrarchus labrax*	pancreatin	no effect	Kolkovski *et al.* (1997b)
Gilthead seabream *Sparus aurata*	lipase	300% increase in glycerol trioleate absorption in 45 day old juvenile. No effect on younger larvae	Koven *et al.* (1993)
Yellow perch *Perca flavescens*	pancreatin	no effect	Kolkovski *et al.* (1999a)

FUTURE RESEARCH REQUIREMENTS

There is little doubt that the development of suitable artificial diets for marine finfish larvae would increase the profitability of larval production by reducing or eliminating the requirement for live feed organisms. However, research into artificial diets for finfish has not received as much attention as that for crustacean larvae for which successful artificial diets have been developed and are now commercially available. Total replacement of live prey with artificial food particles is still not possible for most marine fish larvae and more research is required in this field. However, increased profitability of larval production may also be achieved by reducing the requirement for live feed organisms through partial replacement of live foods. While not totally replacing live foods, this option can result in considerable cost savings. More research is required to establish the degree to which live feeds can be replaced with existing artificial diets and the degree to which weaning onto artificial diets can be advanced. Development of more suitable artificial diets for marine finfish larvae will require research into the following key areas:

(1) Improved ingestion

Artificial diets are ingested at a lower rate than life foods and are negatively buoyant. This may lead to overfeeding and water quality problems. Better ingestion of artificial diets requires more "attractive" diets and important factors to be addressed include buoyancy, colour, "smell" and movement. Many feed attractants have been identified, but we still don't understand completely their mode of action on feeding behaviour and the digestive tract.

(2) Improved digestion

Once ingested, artificial diets need to be efficiently digested. Improved digestibility may be possible through more selective use of binders, by incorporation of digestive enzymes into artificial food particles and through the development of "soft" food particles. We now know more about pancreatic hydrolazes in larval fish but understand less about mechanisms responsible for their developmental patterns.

(3) Nutritional requirements

Little is known about the nutritional requirements of marine finfish larvae. Research is required to determine specific nutritional requirements. This knowledge may be acquired by the development of more attractive and digestible artificial food particles whose nutritional composition can be manipulated in nutritional studies.

(4) Culture system design

Settling of artificial food particles can cause water quality problems and reduce the availability of food particles to fish larvae. Appropriate system design (e.g. tank shape and aeration) can be used to maximise the availability of artificial food particles within the water column and to reduce settling and resulting water quality problems (Backhurst and Harker, 1988).

Development of successful artificial diets for fish larvae will require a multidisciplinary approach addressing all of these factors.

REFERENCES

Adron, J.W., Blair, A., and Cowey, C. B., 1974. Rearing of plaice (*Pleuronectes platessa*) larvae to metamorphosis using an artificial diet. *Fishery Bulletin 72(2),* 353-357.

Appelbaum, S., 1985. Rearing of the Dover sole, *Solea solea* (L), through its larval stages using artificial diets. *Aquaculture 49*, 209-221.

Backhurst, J. R., and Harker, J. H., 1988. The suspension of feeds in aerated rearing tanks: the effect of tank geometry and aerator design. *Aquacultural Engineering 7*, 379-395.

Ben-Amotz, A., Fishler, R., and Schneller, A., 1987. Chemical composition of dietary species of marine unicellular algae and rotifers with emphasis on fatty acids. *Marine Biolog, 95,* 31-36.

Bogut, I., Opacak, A. and Stevic, I. 1995. The influence of polyzymes added to the food on the growth of carp fingerlings *Cyprinus carpio*. *Aquaculture* 129, 252.

Carter, C. G., Houlihan, D. F. and McCarthy, I. A. 1992. Feed utilization efficiencies of Atlantic salmon *Salmo salar* L. parr: effect of a single supplementary enzyme. *Comp. Biochem. Physiol.* 101, 369-374.

Carter, C. G., Houlihan, D. F. Buchanan, B. and McCarthy, I. A. 1994. Growth and feed utilization efficiencies of seawater Atlantic salmon *Salmo salar* L. fed a diet containing supplementary enzymes. *Aquacult. Fish. Manag*. 25: 37-46.

Dabrowska, H., Grudniewski, C. and Dabrowski, K. 1979. Artificial diets for common carp, effect of the addition of enzyme extracts. *Prog. Fish Cul*, 41, 196-200.

Dabrowski, K. and Glogowski J. 1977a. Studies on the proteolytic enzymes of invertebrates constituting fish food. *Hydrobiologia* 52, 171-174.

Dabrowski, K. and Glogowski, J. 1977b. Studies on the role of exogenous proteolytic enzymes in digestion processes in fish. *Hydrobiologia* 54, 129-134.

Dabrowski, K., 1984. The feeding of fish larvae: present state of the art and perspectives. *Reproduction Nutrition Development 24(6),* 807-833.

Dabrowski, K. R., 1986. Ontogenetical aspects of nutritional requirements in fish. *Comparative Biochemistry and Physiology 85A(4),* 639-655.

Dhert, P., Lavens., P., Duray., and Sorgeloos, P., 1990. Improved larval survival at metamorphosis of Asian seabass (*Lates calcarifer*). *Aquaculture 90*, 63-75

Doving, K. J., and Knutsen, J. A., 1991. Feeding responses and chemotaxis in marine fish larvae. *In:* Kaushik, S., J., and Luquet, P., (editors), *Fish Nutrition in Practice*, Ed. INRA, Paris 1993 (Les Colloques, No 61) pp. 579-587.

Fox, C., 1990. Studies on polyunsaturated fatty acid nutrition in the larvae of marine fish - herring *Clupea harengus* L. Doctoral thesis. University of Stirling, United Kingdom.

Fuchs, J., and Nedelec, G., 1989. Larval rearing and weaning of seabass, *Lates calcarifer* (Block), on experimental compounded diets. *In:* Advances in Tropical Aquaculture. *IFREMER Actes de Colloque 9*. pp. 677-697.

Garatun-Tjeldsoto, O., Opstad, I. Hansen, T., and Huse, I., 1989. Fish roe as a major component in start-feed for marine fish larvae. *Aquaculture 79*, 353-363.

Gatesoupe, F. J., Girin, M., and Luquet, P., 1977. Recherche d'une alimentation artificielle adaptée à l'élevage des starades larvaires des poissons. II. Application à l'élevage larvaire du baret de la sole. Proceedings of Third Meeting of the ICE Working Group on Mariculture, Best, France, 1977. Actes Colloq. CNEX.0.4, pp. 59-66.

Hashim, R., and Mat Sat, N. A., 1992. The utilisation of seaweed meals as binding agents in pelleted feeds for snakehead (*Channa striatus*) fry and their effects on growth. *Aquaculture 108,* 299-308.

Heinen, J., M., 1981. Evaluation of some binding agents for crustacean diets. *Progressive Fish Culturist 43(3),* 142-145.

Hjelmeland, K., Pedersen, B. H., and Nilssen, E. M., 1988. Trypsin content in intestines of herring larvae, *Clupea harengus*, ingesting inert polystyrene spheres or live crustacea prey. *Marine Biology 98*, 331-335.

Jones, D. A., Kamarudin, M. S., and Le Vay, L., 1993. The potential for replacement of live feeds in larval culture. *Journal of the World Aquaculture Society 24(2),* 199-210.

Juvario, J. V., Durary, M. N., and Fuchs, J., 1991. Weaning of seabass, *Lates calcarifer* B., larvae to artificial diet. *In:* Lavens, P., Sorgeloos, P., Jaspers, E., and Ollevier, F., (editors), Larvi '91 - Fish and Crustacean Larviculture Symposium, European Aquaculture Society, Special Publication No. 15, Gent, Belgium, pp. 183.

Kamler, E., 1992. *Early Life History of Fish: An Energetics Approach*. Chapman and Hall, London.

Kanazawa, A., Koshio, S., and Teshima, S., 1989. Growth and survival of larval red sea bream *Pagrus major* and Japanese flounder *Paralichthys olivaceus* fed microbound diets. *Journal of the World Aquaculture Society 20(2),* 31-37.

Kanazawa, A., Teshima, S., Inamori, S., Sumida, S., and Iwashita, T., 1982. Rearing of larval red sea bream and ayu with artificial diets. *Memoirs of the Faculty of Fisheries, Kagoshima University*, *31*, 185-192.

Kanazawa, A., and Teshima, S., 1988. Microparticulate diets for fish larvae. *In*: Sparks, A., K., (editor), New and Innovative Advances in Biology/Engineering with Potential for Use in Aquaculture, Proceedings of the Fourteenth U.S.-Japan Meeting on Aquaculture, Massachusetts, 1985. (NOAA Technical Report, NMFS 70), pp.57-62.

Kolkovski, S., Tandler, A. and Kissil, G. Wm., 1991. The effect of dietary enzymes with age on protein and lipid assimilation and deposition in *Sparus aurata* larvae. *In:* Kaushik, S., J., and Luquet, P., (editors), *Fish Nutrition in Practice*, Ed. INRA, Paris 1993 (Les Colloques, No 61) pp. 569-578.

Kolkovski, S., Tandler, A., Kissil, G. Wm., and Gertler, A., 1993. The effect of dietary exogenous enzymes on ingestion, assimilation, growth and survival of gilthead seabream (*Sparus aurata*, Sparidae, Linnaeus) larvae. *Fish Physiology and Biochemistry 12(3),* 203-209.

Kolkovski, S., Arieli, A. and Tandler, A. 1997a. Visual and chemical cues stimulate microdiet ingestion in gilthead seabream, *Sparus aurata*, larvae. *Aquacult. Int.* 5, 527-536.

Kolkovski, S., Tandler, A. and Izquierdo, M. S. 1997b. Effects of live food and dietary digestive enzymes on the efficiency of microdiets for seabass *Dicantrarchus labrax* larvae. *Aquaculture* 148, 313-322.

Kolkovski, S., Yackey, C., Czeny, S. and Dabrowski, D. 1999. The effect of microdiet supplementation of dietary digestive enzymes and hormones on growth and enzyme activity in yellow perch juveniles. *Prog. Fish Cult.* (In press).

Kolkovski, S. 2000. Digestive enzymes in fish larvae and juveniles - implications and applications to formulated diets. *Aquaculture* (In press)

Koven, W. M., Kolkovski, S., Tandler, A., Kissil, Wm. G. and Sklan, D. 1993. The effect of dietary lecithin and lipase, as a function of age, on n-9 fatty acid incorporation in the tissue lipids of *Sparus aurata* larvae. *Fish Physiol. and Biol.* 10, 357-364.

Kurokawa, T., Shiraishi, M. and Suzuki, T. 1998. Qualification of exogenous protease derived from zooplankton in the intestine of Japanese sardine *Sardinops melanoticus* larvae. *Aquaculture* 161, 491-499.

Lauff, M., and Hofer, R., 1984. Proteolytic enzymes in fish development and the importance of dietary enzymes. *Aquaculture 37*, 335-346.

Lavens, P., Sorgeloos, P., Dhert, P., and Devresse, B., 1995. Larval foods. *In:* Bromage, N., and Roberts, R., J., (editors), *Broodstock Management and Egg and Larval Quality*. Blackwell Science, Oxford, pp. 373 - 397.

Lee, P. S., Southgate, P. C., and Fielder, D. S., 1996. Assessment of two microbound artificial diets for weaning Asian sea bass (*Lates calcarifer*, Bloch). *Asian Fisheries Science*.

Leger, P. 1999. Great Salt Lake out of shape. *Fish Farming International*. December, 1999. 28-29.

Leger, P., Bengston, D. A., Simpson, K. L., and Sorgeloos, P., 1986. The use and nutritional value of *Artemia* as a food source. *Oceanography and Marine Biology: An Annual Review 24,* 521-623.

López-Alvarado, J., Langdon, C. J., Teshima, S., and Kanazawa, A., 1994. Effects of coating and encapsulation of crystalline amino acids on leaching in larval feeds. *Aquaculture 122*, 335-346.

Meyers, S. P., Butlers, D. P., and Hastings, W. H., 1972. Alginates as binders for crustacean rations. *Progressive Fish Culturist 34(1),* 9-12.

Meyers, S. P., 1979. Formulation of water-stable diets for larval fishes. *In:* Proceedings from the World Symposium on Finfish Nutrition and Fishfeed Technology Vol II, Hamburg, Berlin, pp. 13-21.

Munilla-Moran, R., Stark, J. R., and Barbour, A., 1990. The role of exogenous enzymes in digestion in early turbot larvae, Scophthalmus maximus L. *Aquaculture 88*, 337-350.

Partridge, G.J. and Southgate, P.C. 1999. The effect of binder composition on ingestion and assimilation of microbound diets (MBD) by barramundi *Lates calcarifer* Bloch larvae. *Aquaculture Research 30*, 879-886.

Pedersen, B.H. and Hjelmeland, K., 1988. Fate of trypsin and assimilation efficiency in larval herring *Clupea harengus* following digestion of copepods. *Mar. Biol*. 97, 467-476.

Pedersen, B.H., Nilssen, E.M. and Hjelmeland, K. 1987. Variation in the content of trypsin and trypsinogen in larval herring *Clupea harengus* digesting copepod nauplii. *Mar. Biol*. 94, 171-181.

Person-Le Ruyet, J., Alexandre, J. C., Thébaud, L., and Mugnier, C., 1993. Marine fish larvae feeding: Formulated diets or live prey? *Journal of the World Aquaculture Society 24(2),* 211-223.

Rottiers, D. V., and Lemm, C. A., 1985. Movement of underyearling walleyes in response to odour and visual cues. *Progressive Fish Culturist 47(1*), 34-41.

Southgate, P. C. and Lee, P., 1993. Notes on the use of microbound artificial diets for larval rearing of seabass (*Lates calcarifer*). *Asian Fisheries Science 6,* 245-247.

Southgate, P.C. and Partridge, G.J. 1998. Development of artificial diets for marine finfish larvae: problems and prospects. In: De Silva (Ed.). *Tropical Mariculture*, Academic Press, London. pp. 151-169.

Sorgeloos, P., Lavens, P., Leger, P., Tackaert, W. and Versichelel 1986. *Manual for the Culture and Use of Brine Shrimp Artemia in Aquaculture*. FAO Ghent, Belgium.

Tandler, A., and Kolkovski, S., 1991. Rates of ingestion and digestibility as limiting factors in the successful use of microdiets in *Sparus aurata* larval rearing. *In:* Lavens, P., Sorgeloos, P., Jaspers, E. and Ollevier, F., (eds), Larvi '91 - Fish and Crustacean Larviculture Symposium, European Aquaculture Society, Special Publication No. 15, Gent, Belgium, pp. 169-171.

Teshima, S., Kanazawa, A. and Sakamoto, M., 1982. Microparticulate diets for the larvae of aquatic animals. *Mini Review and Data File of Fisheries Research, Kagoshima University 2,* 67-86.

Walford, J., Lim, T. M. and Lam., T. J., 1991. Replacing live foods with microencapsulated diets in the rearing of seabass (Lates calcarifer) larvae: do the larvae ingest and digest protein-membrane microcapsules. *Aquaculture 92*, 225-235.

Walford, J. and Lam, T. J., 1993. Development of digestive tract and proteolytic enzyme activity in seabass (*Lates calcarifer*) larvae and juveniles. *Aquaculture 109*, 187-205.

Weinhart, G. and Rösch, R., 1991. Food intake of larvae of *Coregonus lavaretus* L. Do they really ingest less dry diet than *Artemia* nauplii? *In:* Lavens, P., Sorgeloos, P., Jaspers, E., and Ollevier, F., (editors), Larvi '91 - Fish and Crustacean Larviculture Symposium, European Aquaculture Society, Special Publication No. 15, Gent, Belgium, pp. 144.

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Appendix 1

QUARANTINE ISSUES

Samantha Duggan

Information regarding the import and export of food ingredients/live organisms etc. between Australia and overseas can be found on the following web site:

http://www.aqis.gov.au

However, AQIS is not involved with polices and issues concerning the movement of plant and animal species between states within Australia. A National Translocation Policy has been established from which the states develop their own policy regarding the translocation of aquatic organisms, but this is mainly concerned with fish and other aquatic vertebrates. This policy can be seen on the following web site:

http://www.brs.gov.au/fish/translocation.html

Each state appears to have its own regulations regarding the import and export of new organisms, based on the species that are being moved and where they are from. This enables governing bodies the chance to ensure that they won't pose any threat to the marine species and environments that are already present. The Aquaculture Act is presently being reviewed, and the issues behind the movement of other marine organisms will be looked at.

To find out the translocation regulations that may be in place for a particular state, the following contact names and numbers may be of some assistance.

NORTHERN TERRITORY

Contact: Steve Wilmore
Senior Licensing Officer
Department of Primary Industry and Fisheries
GPO Box 990
Darwin 0801
Ph: (08) 8999 2370
steve.wilmore@dpif.nt.gov.au

It is necessary to obtain a Section 16 permit if you want to import any fish or aquatic life into the Northern Territory. Depending on the organism you may also need to obtain a health/disease free certificate. These requirements are both dependent on the species being moved.

TASMANIA

Contact: David Tollard
Quarantine Centre
Department of Primary Industry, Water and Environment
1 Macquarie Wharf
Hobart 7000
Ph: (03) 6233 3352
Mobile: 0418 347161
david.tollard@aqis.gov.au

SOUTH AUSTRALIA

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Primary Industries and Resources
Compliance Section
16th Floor PO Box 282
25 Greenfell Street Port Adelaide 5015
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pendle.max@saugov.sa.gov.au
http://www.pir.sa.gov.au

There is no set legislation in place at the moment, though the Aquaculture Act is currently under review, and some of the issues regarding the translocation of marine organisms other than fish may be looked at. The main concern is where the organisms entering South Australia were coming from, what they are and whether they are disease free.

NEW SOUTH WALES

Contact: Steve Boyd
NSW Fisheries
Port Stephens Office
Taylors Beach Road Private Bag 1
Taylors Beach 2316 Nelson Bay NSW 2315
Ph: (02) 4916 3821
boyds@fisheries.nsw.gov.au

Under the Fisheries Management Act, a permit is required if an organism being translocated into NSW is not native to the state. However, this again will depend on what exact species is being imported.

VICTORIA

Contact: Richard McLoughlin
Director of Fisheries
Department of Natural Resources and Environment
Fisheries Victoria
8 Nicholson Street
East Melbourne 3002
Ph: (03) 9637 8512
richard.mcloughlin@nre.vic.gov.au
http://www.nre.vic.gov.au

QUEENSLAND

Contact: Rob Swindlehurst
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Department of Primary Industry
GPO Box 46
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Ph: (07) 3224 2257
swindlr@dpi.qld.gov.au
http://www.dpi.qld.gov.au

At the moment there are no restrictions over the movement of algae and copepods into Queensland though this may depend on the specific organism.

WESTERN AUSTRALIA

Contact: Jackie Chappell
Translocation Officer
WA Fisheries
168-170 St. George Terrace
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Ph: (08) 9482 7385
jchappell@fish.wa.gov.au

While there are a lot of policies in place for moving fish into Western Australia, at the moment there is no policy in place for the importation of live feeds into the state. Fisheries WA are starting to look at the implications of introducing these organisms into WA, but if the organisms are being introduced into a closed system there is little concern at present.

Appendix 2

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Appendix 3

CONTENTS OF THE *PROCEEDINGS OF A HATCHERY FEEDS WORKSHOP, CAIRNS 9-10 MARCH 2000*

available at http://www.aims.gov.au/hatchery-feeds

RESEARCH IN PROGRESS

Stephen Battaglene, John Purser, Piers Hart and David Morehead. Priorities for live feed production and research in Tasmania.

Susan Blackburn, Cathy Johnston and Dion Frampton CSIRO Microalgae Research Centre – microalgae for aquaculture, biotechnology and the environment

Malcolm Brown, Graeme Dunstan, Piers Hart and Arthur Ritar Polyunsaturated Fatty Acid and Ascorbic Acid Enrichment of Zooplankton

Michael Burke Marine fingerling production at the Bribie Island Aquaculture Research Centre. Intensive green water culture – an historical perspective.

Frances D'Souza Optimising penaeid larvae growth and nutrition: Methods for *Artemia*, copepods and rotifers.

Wayne Hutchinson Live feed production in South Australian aquaculture

Brenton Knott and Colin Adams The *Parartemia* Working Group

Richard Knuckey, Gale Semmens and Bernard Della-Rodolfa Live Prey Research Unit, QDPI Northern Fisheries Centre, Cairns.

Tom Lewis, Peter Nichols and Tom McMeekin Production of polyunsaturated fatty acids by Australian thraustochytrids: aquaculture applications.

David L. Mann, Tom Asakawa, Morris Pizzutto, Clive P. Keenan and Ian J. Brock Hatchery feeds for the mud crab *Scylla serrata*: Towards a nutritionally complete diet.

M. F. Payne Cultured copepods as live food for fish

Jian G. Qin and Troy Hillier Live Food and Feeding Ecology of Larval Snapper (*Pagrus auratus*)

R.J. Rippingale Intensive cultivation of a calanoid copepod

Paul Southgate and Sagiv Kolkovski Development of artificial diets for fish larvae

INDUSTRY PERSPECTIVES

John Bayes The Seasalter Shellfish (Whitstable) Pty. Ltd. algal culture system.

Liz Evans Artemia, The Turning Point: Industry research priorities in a world short of *Artemia*.

Rodney Grove Jones. Production of live microalgal feed

Adam Maskew A synopsis of aquaculture in WA.

Antonio Mozqueira Ocean Wave Seafoods

Mike Rimmer Issues raised in general discussion at the Hatchery Feeds Workshop, Cairns 9-10 March 2000.

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