HATCHERY FEEDS

Proceedings of a workshop held in Cairns

9-10 March 2000

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About this Document

With the expansion of aquaculture in Australia, the Fisheries Research and Development Commission (FRDC) perceived 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. 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 contains extended abstracts from presentations delivered at that meeting by the research community. Due to time constraints, industry representatives did not give individual presentations, but their viewpoints were captured in an open forum (see Abstract 21). We did, however, invite industry representatives to submit written viewpoints, and these are included in the second half of this document. The R&D plan arising from the workshop is available in a companion document: *Hatchery Feeds Research and Development Plan 2000-2005*.

Both the *Hatchery Feeds Workshop Proceedings* and the *Hatchery Feeds Research and Development Plan 2000-2005* are available for download at the following site:

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

Readers should note that this document is unrefereed, and that the viewpoints expressed are those of the authors.

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Research in Progress

1. Priorities for live feed production and research in Tasmania

Stephen Battaglene, John Purser, Piers Hart and David Morehead[1]

The Tasmanian Aquaculture and Fisheries Institute (TAFI) conducts primary research into live feed production and mass produces feeds for a range of marine larvae. The research is undertaken at the School of Aquaculture in Launceston and the Marine Research Laboratories (MRL) in Hobart. Around 9 research scientists, 6 technicians and 16 post-graduate students study various aspects of live feed production or use live food in their research programs. Five groups of cultured live feeds are studied: microalgae, rotifers, brine shrimp, copepods and amphipods.

Live prey are used to cultivate a range of marine fish, crustaceans and molluscs including striped trumpeter (*Latris lineata*), sea horse (*Hippocampus abdominalis*), greenback flounder (*Rhombosolea tapirina*), damselfish (*Acanthochromis polyacanthus*), Tasmanian whitebait (*Lovettia sealii*), galaxids (*Galaxias maculatus*), blenny (*Parablennius tasmanianus*), black bream (*Acanthopagrus butcheri*), southern rock lobster (*Jasus edwardsii*), spotted handfish (*Brachionychthys hirsutus*) and Pacific oyster (*Crassostrea gigas*). Research has been conducted on optimal feed species, culture systems, densities and prey-selection at experimental and pilot-scale mass production levels.

Microalgae batch culture production is based on traditional algae species like *Chaetoceros muelleri, Isochrysis galbana, Pavlova pinguis, Tetraselmis suecia,* and *Nanochloropsis oculata*. Autoclaved 20-L carboys provide high quality axenic cultures and microfiltration (0.2 μm) is used in batch culture in tanks up to 1000 L, using metal halide lamps, CO_2 and aeration injection. Microalgae are used as a primary food source for oysters and other live feeds and in green-water culture of marine fish. Commercial oyster hatcheries also use continuous pasteurisation systems.

[1] University of Tasmania, Tasmanian Aquaculture and Fisheries Institute.
(See details in appendix.)

The large-strain rotifer *Brachionus plicatilis* is most commonly used to feed marine fish in Tasmania, although the easier-to-culture small strain *B. rotundiformis* has been trialled. Rotifers are batch-cultured at densities of 100 to 300 per millilitre on a diet of microlgae and bakers yeast, *Saccharomyces cerevisiae*. Experimental batches have also been cultured at densities 300 to 700 per millilitre using commercially available diets including Culture Selco and Rotimac. Rotifers are enriched with vitamins, algae and commercial products like DHA Selco, Protein Selco, and Algamac, depending on the nutritional needs of the target species. Research in collaboration with the CSIRO has been conducted on developing new methods for enrichment using vitamin C, bacteria and fungi. Rotifers are fed primarily to marine fish and are mass-cultured at the MRL for feeding to striped trumpeter in six 2000-L tanks. Striped trumpeter are fed rotifers twice a day at 5 per millilitre from first feeding for one month.

Brine shrimp are an important component of most larval diets, and especially for striped trumpeter, seahorses and southern rock lobster. The newly hatched *Artemia* nauplii are reared from decapsulated cysts and enriched using methods similar to those described for rotifers. Care is taken in selecting brands of *Artemia* with good nutritional profiles and in matching the appropriate sized *Artemia* to each developmental stage. There is a need for large-scale ongrowing of *Artemia* to around 12 mm in total length for feeding to southern rock lobster phyllosoma larvae and sea horses. It is believed that changing the tissue composition of the *Artemia* to a favourable biochemical profile probably requires an extended feeding regime of at least several days. Studies are being undertaken to better understand feeding regimes using ongrown *Artemia* and how to produce them cost-effectively. Problems with *Artemia* include protozoan diseases associated with the cysts, their high cost and a projected worldwide shortfall in supply.

Copepods are increasingly seen as a supplementary feed to rotifers and *Artemia* in the culture of 'difficult' marine fish. However, mass-culture of cold water copepods has been problematic. In Tasmania, copepod culture has been investigated through a research program within the Aquaculture CRC, although much of the field testing has been conducted using tropical species in Darwin. Three genera have been investigated experimentally: the harpacticoid *Tisbe,* cyclopoid *Apocyclops*, and calanoid *Acartia*. Most effort

has been expended on examining the life cycles, demographics, productivity, and environmental and dietary requirements of each species. *Tisbe* has been cultured in Launceston using batch, semi-continuous and recirculation systems on diets of microalgae, fortified yeast, pelleted feeds, vegetables, macroalgae and associated microflora. Much of the information is currently subject to confidentiality agreements. Striped trumpeter have been reared successfully using predominately harpacticoid copepods as a supplementary feed (Figure 1.1). They are collected from raceways at night using submersible pumps and floating net cages.

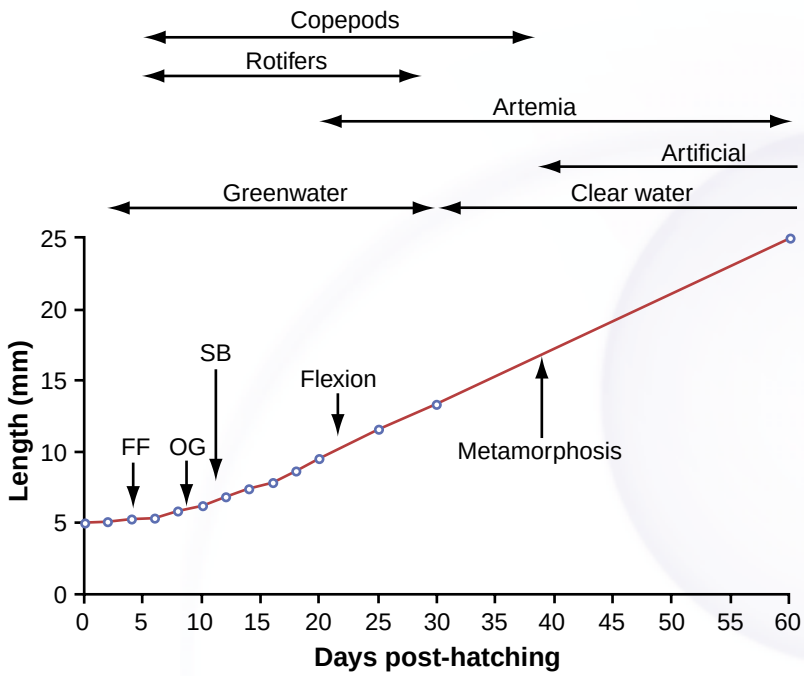

Figure 1.1. *Feeding regime and development of striped trumpeter larvae* (Latris lineata) *reared in green-water tanks using* Tetraselmis suecia.

Amphipods have been used to feed seahorses and the endangered handfish *Brachionychthys hirsutus*. The techniques for mass-culture of amphipods are still being developed through the work of post-graduate students and in collaboration with aquaculturists in the seahorse industry.

Priorities for live feeds research and development

Priorities for live feeds research and development in Tasmania fall into three categories:

1. Improvements are needed in the production and reliability of microalgae, rotifers and *Artemia* cultures. There is scope for adopting new, improved methods to increase the reliability and reduce the cost of these feeds. For example, microalgae could be cultured using continuous pasteurisation systems, and rotifers on commercially available algae pastes and dried products.

2. There needs to be development of live feeds that more closely match the nutritional profiles of the eggs and larvae of the target culture species. In the first instance this might involve better assessment of enrichment procedures and testing of enrichment diets. The longer-term aim should also be to develop cost-effective culture systems for selected alternative species like copepods and amphipods. The use of these species should also be linked to gaining a better understanding of green-water culture systems in order to optimise larvae survival and growth of cultured marine fish.

3. There needs to be a greater adoption and testing of early weaning practices using artificial feeds developed within Australia and overseas.

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2. CSIRO Microalgae Research Centre – microalgae for aquaculture, biotechnology and the environment

Susan Blackburn, Cathy Johnston and Dion Frampton[1]

The CSIRO Microalgae Research Centre

The CSIRO Microalgae Research Centre is an Australian centre for excellence in microalgae research. Located at CSIRO Marine Research in Hobart, Tasmania, it comprises the Collection of Living Microalgae, the Microalgae Supply Service, and research arms of aquaculture, biotechnology and environment.

The Collection of Living Microalgae is a culture collection of over 700 strains of microalgae, representing all marine microalgal classes and some freshwater classes. It is the largest in the southern hemisphere and one of only two extensive collections in the Asia-Pacific Region. The strains are unique in that the majority are from Australian waters spanning the tropics to Antarctica, representing some of Australia's microalgal biodiversity. The collection is housed in a world-class algal culture laboratory containing constant environment rooms and cabinets. This facility supports studies on microalgal growth, physiology, biochemistry and molecular genetics. While the collection is maintained in small volume cultures (40 mL to 250 mL) there is also a range of small- to medium-scale culture technologies including chemostats, laboratory columns and photobioreactors for experimental use. The microalgae culture facility has recently been listed by the Commonwealth Department of Science, Industry and Resources as a priority national facility.

As an adjunct to the collection, the centre operates a Microalgae Supply Service, a small business that supplies cultures to industry, researchers and teaching institutions. In particular it provides high quality 'starter cultures' as food for larvae and juvenile aquaculture animals. The Supply Service has clients as far afield as Europe, the Middle East and South Africa as well as the near Asia-Pacific Region.

[1] CSIRO Marine Research. (See details in appendix.)

Microalgae as live feeds

CSIRO has the only collection in the region holding all the 'traditional' aquaculture live food strains. The most commonly supplied strains are *Chaetoceros muelleri* CS-176, *Isochrysis* sp. (T.ISO) CS-177, *Pavlova lutheri* CS-182, *Chaetoceros calcitrans* CS-178 and *Tetraselmis suecica* CS-187. These mostly originate from the temperate northern hemisphere and are not necessarily appropriate or the best strains available for some environments, particularly in tropical Australia. In addition it is clear that some aquaculture animals (e.g. pearl oysters and abalone), as well as live feed animals (e.g. copepods) require strains that are different from the traditional strains. While these traditional strains have their use approved by AQIS, CSIRO also has concerns about extensive use of imported strains, and considers that any new strains used should preferably be endemic to Australia.

To examine all these issues, the Microalgae Research Centre has an active commitment to isolating new Australian strains and offering new strains for trial, free of charge to industry. Table 2.1 lists the commonly used Australian strains as well as new strains supplied to industry and researchers on a trial basis. Other less-commonly supplied strains such as the dinoflagellate *Heterocapsa niei* are clearly gaining prominence as alternative feeds for copepods.

Strain typing

Research in strain 'typing' being pursued by the Microalgae Research Centre in relation to its work in biotechnology is of potential significance to microalgal use in aquaculture. Our research comparing Australian and global populations from cultured strains has demonstrated great diversity within one species (Blackburn, 2000; Bolch *et al.,* 1999a, b). Strains of the one species can be very different physiologically, leading, for example, to very different growth characteristics and temperature tolerances. Knowing this can be critical to microalgal use and performance.

This diversity at the species level can be discriminated through various techniques. In our laboratory we have used several molecular techniques to demonstrate strain and population differences within one species. These methods include Randomly Amplified Polymorphic DNA using polymerase chain reaction (RAPD-PCR) as well as sequence analysis of non-conserved

regions of genes; for example the intergenic spacer of the B–A sub-unit of the phycocyanin gene (Bolch *et al.*, 1996, 1999a, b). These molecular (and some chemical) methods allow 'fingerprinting' of strains. Such strain typing may become inherent in quality control in industrial applications, including aquaculture, in the future.

Table 2.1. Australian Strains Supplied to the Australian Aquaculture Industry (June 1997 – June 1999).

Species	Strain No.	Source
Commonly used strains		
Navicula jeffreyi	CS-46	NSW
Nitzschia closterium	CS-5	NSW
Rhodomonas maculata	CS-85	NSW
Skeletonema sp.	CS-252	Qld
Nannochloropsis-like	CS-246	Qld
Pavlova pinguis	CS-375	Tas
Trial strains		
Amphora sp.	CS-307	Qld
Nitzschia sp.	CS-339/2	Qld
Nitzschia sp.	CS-339/3	Qld
Nitzschia sp.	CS-373	Tas
Nitzschia cf paleacea	CS-429	Tas
Nitzschia cf paleacea	CS-430	Tas
Nitzschia cf paleacea	CS-433	Tas
Chaetoceros sp.	CS-365/2	Tas

Remote high biomass production of microalgae

The potential of remote microalgal production, compared with on-site hatchery production, depends on high biomass production of high-quality microalgal food species, as well as methods to harvest and concentrate, preserve and transport. Some of the latter topics were investigated under the FRDC Projects 93/123 and 96/342 and the CRC for Aquaculture by Knuckey and Brown. There is still inadequate research being conducted into producing a high biomass of high-quality microalgae. As part of research in our laboratory we are collaborating with the University of Florence, Italy, on high biomass production of microalgae using several different photobioreactor technologies including alveolar panel reactors (Tredici, 1999). We have trialed novel microalgae in these systems including dinoflagellates, as well as a much used Australian strain, *Skeletonema* sp. CS-252, for live feeds. We have maintained a biomass of over 1 g L^{-1} (9 x 10^6 cells per millilitre) of the latter for over 40 days. Experiments to optimise growth and production are underway.

It is perhaps pertinent to the Australian industry that for two seasons, 14 finfish hatcheries in Italy have been buying *Nannochloropsis* slurries from a remote production facility developed by Xenia S.r.l. and the Centro di Biotechnologie Fotosintetiche (U Florence). These slurries are being used for both direct feed and green-water culture. The Italian industry is interested in pursuing new alternative species for remote production. However, internationally, there have been few trials of a wide range of microalgae in photobioreactor systems. The best technology for high biomass production of live food species of microalgae for aquaculture still requires research and development.

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3. Polyunsaturated fatty acid and ascorbic acid enrichment of zooplankton

Malcolm Brown[1], Graeme Dunstan[1], Piers Hart[2] and Arthur Ritar[2]

As part of Australia's Fisheries Research and Development Corporation project "The Development of Rock Lobster Propagation Techniques for Aquaculture in Australia", we assessed the biochemical composition of *Artemia* sp. fed different enrichment diets, including a comparison of different sized *Artemia*. The *Artemia* were then tested in feeding trials with lobster *Jasus edwardsii* phyllosoma. Here, we report on the content of polyunsaturated fatty acids (PUFA) and ascorbic acid (AsA) in the *Artemia*.

Experiment 1: Different enrichment diets. Artemia (1.5 mm) were enriched using a 6 h enrichment[3]. Products used for enrichment included Protein Selco, Super Selco, DHA Selco (all products from INVE), AlgaMac 2000 (Aquamarine Biofauna), *Isochrysis* sp. (T.ISO) and a "YM-20-like" manufactured diet. With respect to the PUFA docosahexaenoic acid (DHA), AlgaMac 2000 provided the best enrichment (18% of total fatty acids), followed by DHA Selco (10%) and the remaining products, 2 to 5%. AlgaMac 2000 also produced the highest DHA:EPA ratio in the *Artemia* of 4. Highest enrichment of AsA was provided by Protein Selco (2.2 mg AsA g^{-1} dry weight), and *Isochrysis* sp. (T.ISO) (1.2 mg g^{-1}) with other products producing 0.1 to 0.3 mg g^{-1}. When fed to lobster phyllosoma, there were some differences between the performance of the diets. In particular, the

[1] CSIRO Marine Research.

[2] Tasmanian Aquaculture and Fisheries Institute – TAFI.
(See details in appendix.)

[3] A 6 h enrichment is being used routinely for *Artemia* enrichment by TAFI. Producers of the commercial enrichment products advocate longer enrichment periods of 24 to 48 h. Therefore, what is being reported here is not necessarily implied as providing optimum *Artemia* enrichment, rather demonstrating the result of short-term enrichment, as adopted by TAFI.

"YM-20-like"-fed *Artemia*- which contained the least DHA and AsA - was the least effective for the phyllosoma.

Experiment 2: Different sized Artemia. *Artemia* of 3 different sizes (average: 0.8, 1.5 and 3.0 mm) were enriched with either DHA Selco, or *Isochrysis* sp. (T.ISO) using a 6 h enrichment. The fatty acid data from this experiment are still being finalised, but some initial trends were observed. The PUFA content was similar across *Artemia* size classes with DHA-Selco whereas with *Isochrysis* sp. (T.ISO) it was approximately twice as high in the smaller size *Artemia* compared to the 3.0 mm size. For AsA, the concentration in the *Artemia* increased with decreasing size of the Artemia and was higher with *Isochrysis* sp. (T.ISO) than with DHA-Selco (Fig. 3.1).

Lobster phyllosoma fed 0.8 mm *Artemia* had lower survival and growth than the 1.5 mm and 3.0 mm *Artemia*. This result was difficult to reconcile from the analytical data, and may be a function of differences in the ingestion and/or digestibility of the different sized *Artemia*.

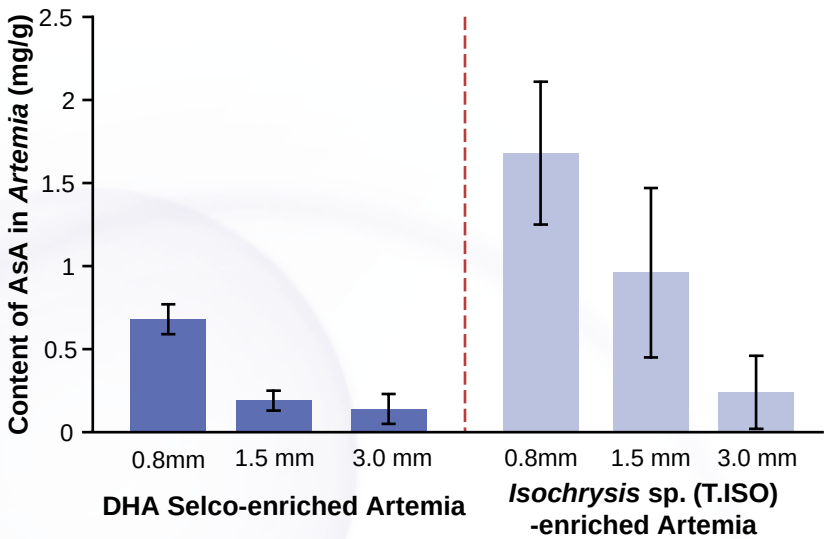

Figure 3.1. *Ascorbic acid in different sized* Artemia, *enriched with either DHA Selco or* Isochrysis sp. *(T.ISO)*

Summary and avenues for future research

Most data on the enrichment of *Artemia* are based on 24 or 48 h enrichment. We found that a 6 h enrichment was also effective, though our data (like other studies) did not discriminate between assimilated nutrients or bioencapsulated nutrients (i.e. contained in partly digested particles within the *Artemia* gut). Also, information on the ingestion and digestibility of *Artemia* fed different enrichments and of different sizes is required to provide a more complete assessment of nutritional value.

Nevertheless, the study provided useful information on the potential nutritional value of *Artemia* based on biochemical composition. The enrichment of different sized *Artemia* showed very interesting results for the transfer of AsA. It would be interesting to assess whether other vitamins or key nutrients are transferred at different rates according to the *Artemia* size, and also to examine retention rates after non-feeding periods.

4. Marine fingerling production at the Bribie Island Aquaculture Research Centre Intensive green-water culture: an historical perspective

Michael Burke[1]

Introduction

For the past 10 years, the Bribie Island Aquaculture Research Centre (BIARC) has been at the forefront of sub-tropical marine fingerling production for aquaculture research and restocking programs. Initially, the need for an intensive green-water culture (GWC) system was driven by the universal dissatisfaction with clear-water rotifer production. The clear-water system was too labour intensive, produced a lack of size ranges of prey items, unreliable rotifer production and daily disturbance of larval rearing tanks. The pond GWC method proved successful yet unreliable, particularly during monsoonal rains in Northern Queensland. The layer of fresh water on the top of the pond prevented swim bladder inflation by fish larvae at certain critical times. Out of necessity, the intensive GWC system emerged to service the need for a reliable larval rearing method that produced large numbers and excellent survival of fingerlings. Initially the system was developed for barramundi (*Lates calcarifer*) and was published in a QDPI Information Series *Development of a low-maintenance technique for rearing barramundi larvae.*

At the same time, the local hatchery industry was attempting to spawn and rear Australian Bass *(Macquaria novaemaculata*). Southern Queensland had recognised potential for aquaculture of this species because it is the northern end of the geographic distribution. Experience in NSW again highlighted the unreliability of clear-water rotifer systems, and the ensuing interaction of local industry with QDPI and the subsequent adoption of the GWC system kick-started the most successful stocking of Australian Bass in southern Queensland.

[1] QDPI Bribie Island Aquaculture Research Centre. (See details in appendix.)

In 1993, a Queensland State Government Inquiry into recreational fishing recommended that a pilot program to test the efficacy of an estuarine fish stocking program in south-east Queensland be undertaken. At the time, the program was the largest marine stocking exercise ever undertaken in Australia. Nearly two million sand whiting (*Sillago ciliata*) and 3/4 million dusky flathead (*Platycephalus fuscus*) fingerlings were produced through intensive GWC systems at BIARC. Again, the generic GWC was the enabling technology system that allowed the production of large numbers of fingerlings (Table 1) and a successful stocking program.

Since that time, BIARC has been involved in 'Mass Propagation of Snapper', a CRC-funded project with the NSW Fisheries (PI: Dr Geoff Allen and Mr Stuart Fielder). This also proved the success and adaptability of the GWC system (Table 1).

With the appointment at BIARC of Dr Abigail Elizur from the National Centre for Mariculture, Israel, the capacity to further extend the research and species list adaptable to this GWC technology has been greatly enhanced. Dr Elizur has 22 years research experience, 10 years in aquaculture research. She has devoted much of her time to the hormonal manipulation of gonadal development in finfish (puberty and spawning) and molecular studies on key reproductive hormones with emphasis on GNRH's (and receptors) and gonadotrophins.

With this experience, BIARC has also been able to produce 25 000 grey mullet *(Mugil cephalus*) fingerlings, a first for Australia. Together with the successful production of 80 000 rabbit fish (*Siganus fuscescens*) fingerlings, it will provide BIARC the capacity to further research the potential for biological remediation of eutrophic systems using finfish.

Table 4.1. Controlled green-water culture of finfish larvae.

Common name	Scientific name	Average survival%	Number produced (thousands)
Australian bass	*Macquaria novaemaculata*	80	250
Barramundi	*Lates calcarifer*	87	1,500
Summer whiting	*Sillago ciliata*	95	1,950
Dusky flathead	*Platycephalus fuscus*	95	750
Snapper	*Pagrus auratus*	60	194
Sea mullet	*Mugil cephalus*	20[2]	25

[2] As good as, if not better than survival rates elsewhere in the world, and a first for Australia.

5. Optimising penaeid larvae growth and nutrition: methods for *Artemia*, copepods and rotifers

Frances D'Souza[1]

The growth and nutrition of penaeid prawn larvae have been studied at the CSIRO Marine Laboratory for the last 20 years, initially from an ecological perspective but more recently to service the needs of aquaculture. The approaches that have been used are outlined in this paper; their application to other planktonic animals used as live prey for fish larviculture, particularly *Artemia*, copepods and rotifers, is discussed.

Optimising growth of prawn larvae

The Larvatron, funded by FRDC in 1985, was built as a fully automated apparatus for culturing larvae and zooplankton (Figure 5.1). With the ability to maintain up to 200 one-litre culture vessels in a small area, it reduces costs of labour, equipment and space. In each vessel, conditions such as salinity, temperature and algal cell density are monitored every 2 h and adjusted automatically to their pre-set levels through exchange of 10% of the culture volume. Continual cycling of the vessels around the track ensures uniform light and temperature conditions and avoids positional effects that can occur with bench culture systems.

The Larvatron is ideally suited to factorial experiments because of the large number of treatments and high replication afforded. The salinity and/or temperature tolerance of *Penaeus semisulcatus* larvae is one example of a factorial experiment performed with the Larvatron (Jackson and Burford, unpubl.) (Figure 5.2). Five salinities and five temperatures were assessed with four replicate cultures for each salinity–temperature combination. The hydrological tolerances of new species of copepods could be quickly determined with the Larvatron. Similarly, the factors affecting resting egg production in rotifers and copepods could be defined.

[1] CSIRO Marine Research. (See details in appendix.)

The Larvatron adapts easily as a screening tool, as demonstrated in a comparison of 20 algal diets fed to *P. monodon* larvae (D'Souza *et al.*, in press) (Figure 5.3). Five species of algae, fed as two forms (live or concentrated by centrifugation) and at two densities, were assessed with five replicates for each treatment. Finding the best algal diet for growth of new copepod, rotifer and *Artemia* strains or species could be simplified and made more rigorous with the Larvatron.

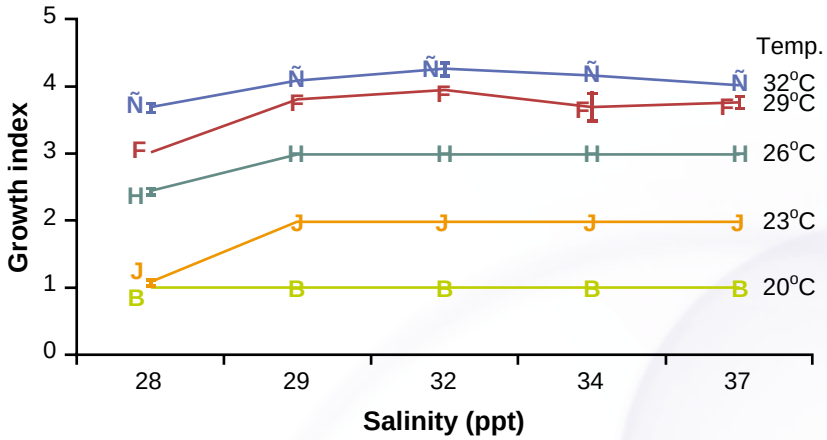

Figure 5.2. *Temperature and salinity tolerance of* Penaeus semisulcatus *larvae.*

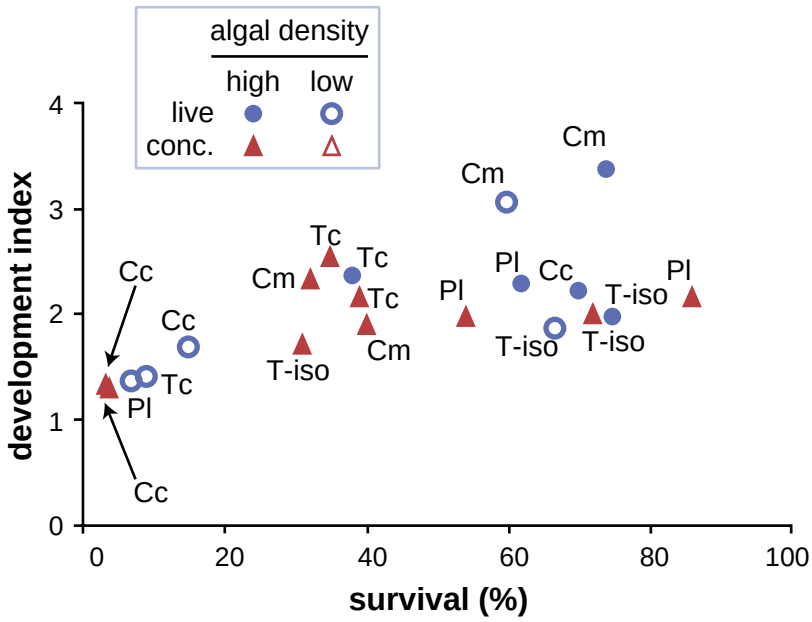

Figure 5.3. *Effect of algal diet (live or concentrate) fed at different densities (high = 100 000, low = 50 000 cells per millilitre) on growth and survival of* Penaeus monodon *larvae. Cm* = Chaetoceros muelleri; *Cc* = C. calcitrans*; Pl* = Pavlova lutheri*; T-ISO* = *Tahitian* Isochrysis *sp.; Tc* = Tetraselmis chuii.

Nutritional requirements of prawn larvae

A lack of defined formulated diets has meant that the nutritional requirements of prawn larvae have been studied largely by manipulating their natural diet of microalgae as well as by feeding microencapsulated nutrients. Live prey species also rely on microalgae. Thus the nutritional composition, enrichment and requirements of live prey organisms can also be examined using the following techniques.

1. **Mixed algal diets**

   Mixed algal diets promoted faster growth and higher survival of prawn larvae than did single algal diets (D'Souza and Loneragan, 1999). The nutritional compositions of the algae and the prawn larvae were determined to identify nutritional components essential or growth-promoting for the larvae. Arachidonic acid (20:4*n*-6) was identified as a possible growth promoter.

2. **Altering algal nutritional composition**

   By growing an alga in a nitrogen-deficient medium it is possible to increase its carbohydrate content three-fold and to reduce the *n*-3:*n*-6 fatty acid ratio by half (D'Souza and Kelly, 2000). The effect of feeding an alga grown in this manner on the growth and biochemical composition of prawn larvae has been studied. The high-carbohydrate, low-*n*3:*n*6 ratio alga did not affect survival but arrested development of the prawn larvae.

3. **Nutrient depletion studies**

   The changes in content of lipid, protein, carbohydrate and individual fatty acids of prawn larvae occurring during feeding and starvation have been used as a qualitative means of determining nutrient requirements (D'Souza, 1998). Nutrients that are conserved during starvation are considered more important to the animal than those that are metabolised. For example, when prawn larvae were starved over a 42-h period, eicosapentaenoic acid was conserved whereas linolenic acid was rapidly depleted within 12 h (Figure 5.4). In the absence of defined, formulated diets for fish larvae, the nutrient depletion technique would be a valuable tool for determining their nutritional requirements.

4. **Isotope-labelling of microalgae**

   The transfer of nutrients across trophic levels can be followed by feeding labelled microalgae to prawn larvae. Specific nutrients in the alga, such as fatty acids, can be enriched with natural or radioactive isotopes. In this way it is possible to determine the origin of individual fatty acids: whether they were synthesised or modified by the larvae, directly taken up from the algal diet or whether they originated from a maternal source through the egg yolk.

5. **Microencapsulation**

   Lipid has been encapsulated in gelatine–acacia microcapsules with the intention of examining the influence of encapsulated lipid on the growth of prawn larvae (D'Souza, Guest and Southgate, unpubl.). It is possible to encapsulate individual fatty acids to examine their role in larval metabolism.

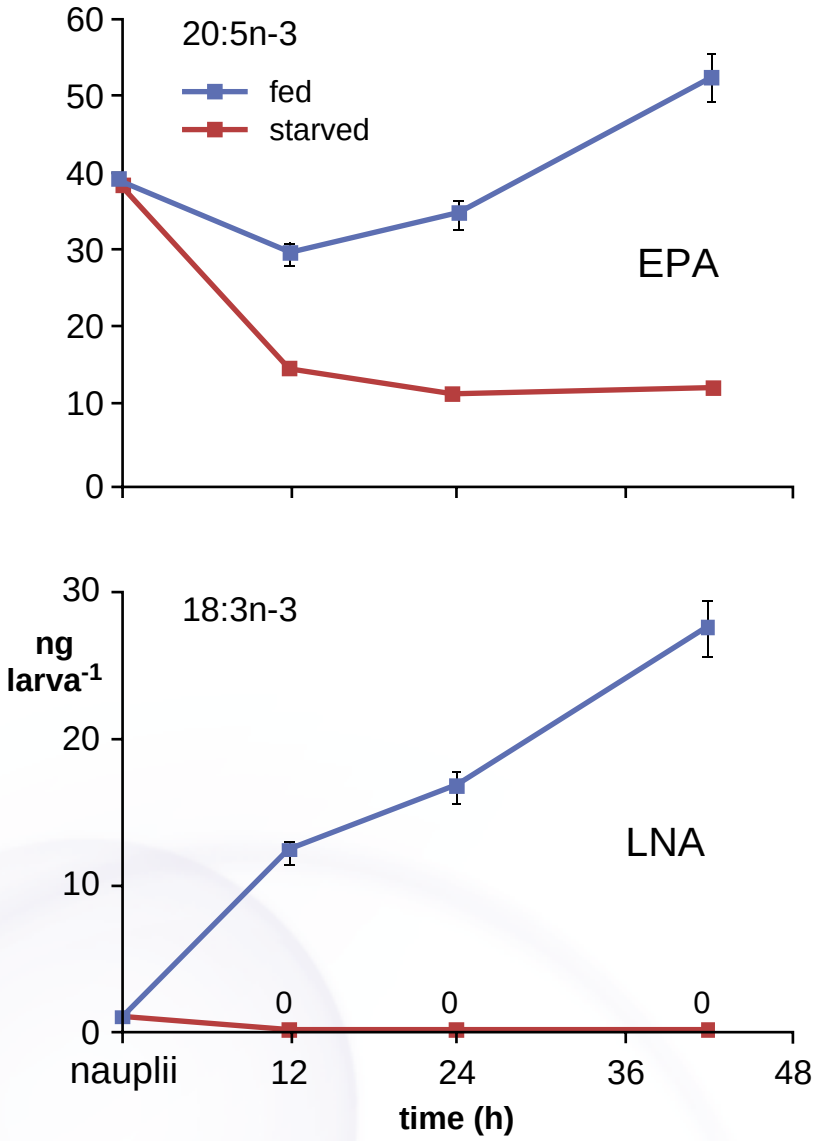

Figure 5.4. *Changes in fatty acid content in fed and starved* Penaeus *spp. larvae.*

Conclusion

Penaeid prawn larvae share aspects of their biology with live prey species for fish larvae. Thus, the techniques and equipment such as the Larvatron, developed for studying prawn larvae nutrition and growth, can easily be adapted for studies with *Artemia,* copepods and rotifers. The finfish aquaculture industry can greatly benefit from the knowledge and expertise gained from prawn larvae research.

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6. Live feed production in South Australian aquaculture

Wayne Hutchinson[1]

Over the past decade, the development of marine aquaculture has accelerated in South Australia (A$180 million in 1998–99) with a concomitant increase in production of live feed organisms. To date there has been little direct research focussed on live feed; however, a range of species are cultured in commercial facilities that have been established and which are now expanding their production capacity.

Conventional live feed organisms are cultured at abalone, bivalve and marine finfish hatcheries. Most abalone hatcheries settle larvae on plates conditioned to provide a film of seasonally occurring benthic diatoms, although some operators also introduce selected species (e.g. *Nitzchia* sp., *Naviculla* sp. or local isolates). Bivalve hatcheries utilise a range of microalgal species including small brown flagellates (*Isochryis* sp. 'Tahitian', *Pavlova lutheri*) and non-motile golden brown diatoms (*Chaetoceros calcitrans*, *Skeletonema costatum*, *Thalassiosira pseudonana*). The species composition fed depends on the stage of larval development. Marine finfish hatcheries use traditional live feed types with microalgae (*Nannochloropsis occulata*, *Tetraselmis* sp., *Isochrysis* sp. 'Tahitian', *Pavlova lutheri*) used as a component of feed and enrichment for rotifers, or for addition to larval rearing tanks in green- or brown-water larval culture methods. Large (*Brachionus plicatilis*) and small (*Brachionus rotundiformis*) strain rotifers are used as the first feed for marine finfish larvae followed by first stage (N1) or nutritionally enriched second stage (N2) *Artemia* nauplii hatched from cysts. In addition, Cognis Pty Ltd operate an extensive salt pond culture system on the northern approach to Whyalla to produce *Dunaliella salina*. Microalgae are harvested and processed to extract b-carotene.

[1] South Australian Research and Development Institute. (See details in appendix.)

With the growing maturity of industry groups that use live feeds, it is expected that research will be required to meet commercial requirements related to cost reduction, production efficiency, culture stability and product quality.

Copepod culture continues to be problematic but promising. We expect that marine finfish hatcheries will adopt production technology that can provide improved production levels and demonstrate measurable benefits when incorporated into existing hatchery feeding protocols. Since 1997, a PhD research project undertaken by Amanda Caughey has investigated the population dynamics and species composition of microalgae and copepods in a temperate saline pond at Port Augusta, SA. This project has been sponsored by the Playford Memorial Trust Aquaculture Scholarship facilitated by the University of Adelaide, the Port Augusta Council and SARDI. The ultimate objective of this research is to develop a mass culture system for production of King George whiting larvae for aquaculture and stock enhancement. The results of these trials will be presented in Ms Caughey's thesis.

The use of saline groundwater for aquaculture has not been fully evaluated. Since 1997, a research project aligned to the Aquaculture CRC has been undertaken by the Coorong District Council and SARDI to develop a closed production system at the Bedford Groundwater Interception Scheme at Cookes Plains, SA. The objective of this research is to develop aquaculture production systems to assist reclamation of land affected by dryland salinity. At this site, snapper (*Pagrus auratus*), *Artemia* and *Dunaliella salina* have been cultured in tanks and lined ponds housed within polytunnels. All used water has been directed to evaporation ponds for salt production. In future, the use of *Artemia* within this system will be expanded to take advantage of their uses for nutrient harvesting, partial feed replacement for fish, and biomass and cyst production.

7. The *Parartemia* Working Group

Brenton Knott[1] and Colin Adams[2]

Brine shrimp nauplii are widely used in aquaculture for feeding early-stage fish and crustacean larvae. The nauplii exhibit two essential qualities for this strategy: they are of an appropriate size to be ingestible, and they move actively in the water column, establishing themselves as targets for young carnivores. However, as many of the other papers presented at this workshop have emphasised, the brine shrimp widely used in aquaculture, *Artemia*, is not entirely satisfactory. *Artemia* can produce cysts under prescribed conditions and these, since they float, are easily harvestable. Consequently, there is world-wide interest in *Artemia* production. However, except for limited, small-scale production, the bulk of the cysts used today emanate from a wild-capture harvest susceptible to over-fishing and reduced production due to uncontrollable climatic influences. The net result is an unreliable supply of a product which is not entirely satisfactory anyway, with concomitant fluctuations in price. This is not a satisfactory scenario for driving new industries.

Australian sources

The members of the *Parartemia* Working Group take an entirely different approach. Australian saline lakes harbour a rich fauna of endemic species which have been blithely ignored in the haste to pursue overseas non-solutions to the supply of aquaculture feeds. In particular, Australian saline lakes harbour a rich diversity of endemic brine shrimps, genus *Parartemia*, which, like *Artemia*, can produce cysts from which nauplii hatch, but under quite different limnological conditions. This raises the possibility that the local brine shrimps can be exploited for cyst production, either:

[1] The University of Western Australia.

[2] Wheatbelt Development Commission. (See details in appendix.)

1. as successfully as the overseas production of *Artemia*, thereby resulting in a more reliable supply of a local product with lower amplitudes of price fluctuation for local users of brine shrimp cysts, or
2. even more successfully, generating an export market as well.

The current preoccupation with *Artemia* as the only brine shrimp to be considered is generating a major problem for Australian biodiversity, and possible economic exploitation of the saline lake resources. There is a dangerous mind-set operating by individuals and within government aquaculture organisations which at best sees no problem in introducing *Artemia* into local saline lakes and is even encouraging their spread. The argument typically applied is that *Artemia* have been in Australia for a number of decades now and they have not been a problem. *Artemia* were introduced into a number of strictly coastal salt-works and for a number of years no spread has been observed into the hinterland saline lakes, the area of natural *Parartemia* distribution. However, this situation is now changing: *Artemia* are beginning to move into the *Parartemia* areas, a spread facilitated both by increasing degradation of saline lakes — reflecting the sins of poor catchment use since the advent of European agriculture - and also by human encouragement. It is crucial that we acknowledge that Australian salt lakes are in no way comparable to the massive American lakes from which 1200 tonnes of *Artemia* cysts are harvested each year; these lakes are large, deep (more than 90 m) and permanent. Inland Australian lakes are shallow (under 1 m), do not fill regularly, and have short inundation cycles; because of this they will not produce commercial yields of exotic grazing animals.

The biological and economic values of our natural saline lake resources have never been evaluated; consequently, they cannot be compared with production based on exotic species. It is quite consistent with Australian practice that we destroy one endemic resource which *may* be of far higher value in favour of a lesser, but known, exotic resource. The net result could be the replacement of many local species with a cosmopolitan weed species — a modern-day version of the rabbit.

8. Research in progress at the Live Prey Research Unit, QDPI Northern Fisheries Centre, Cairns

Richard Knuckey, Gale Semmens and Bernard Della-Rodolfa[1]

The Northern Fisheries Centre (NFC) undertakes research, development and extension activities in the field of live prey to support the aquaculture industry in Queensland. Our Live Prey Unit comprises three main areas:

- microalgae
- rotifers
- copepods.

These are integrated to deliver a continuous supply of live prey organisms to support larviculture research at NFC.

Microalgae

We maintain a small collection of microalgae that are acclimatised to tropical conditions (Table 8.1). These are grown primarily to feed rotifers and copepods.

Recently we ran experiments to evaluate the potential for boosting productivity of mass (500-L) algal cultures through the addition of CO_2 via feedback of the culture pH. Typically, algal productivity declines with increasing volume, largely due to poor nutrient dynamics, gas exchange and light attenuation. The addition of CO_2 increased the growth rate and productivity of several species. For *Rhodomonas*, culture density could be doubled by the addition of CO_2 (Figure 8.1A). However, no benefit was demonstrated for *Nannochloropsis oculata* (Figure 8.1B)

[1] QDPI Northern Fisheries Centre, Cairns. (See detail in appendix.)

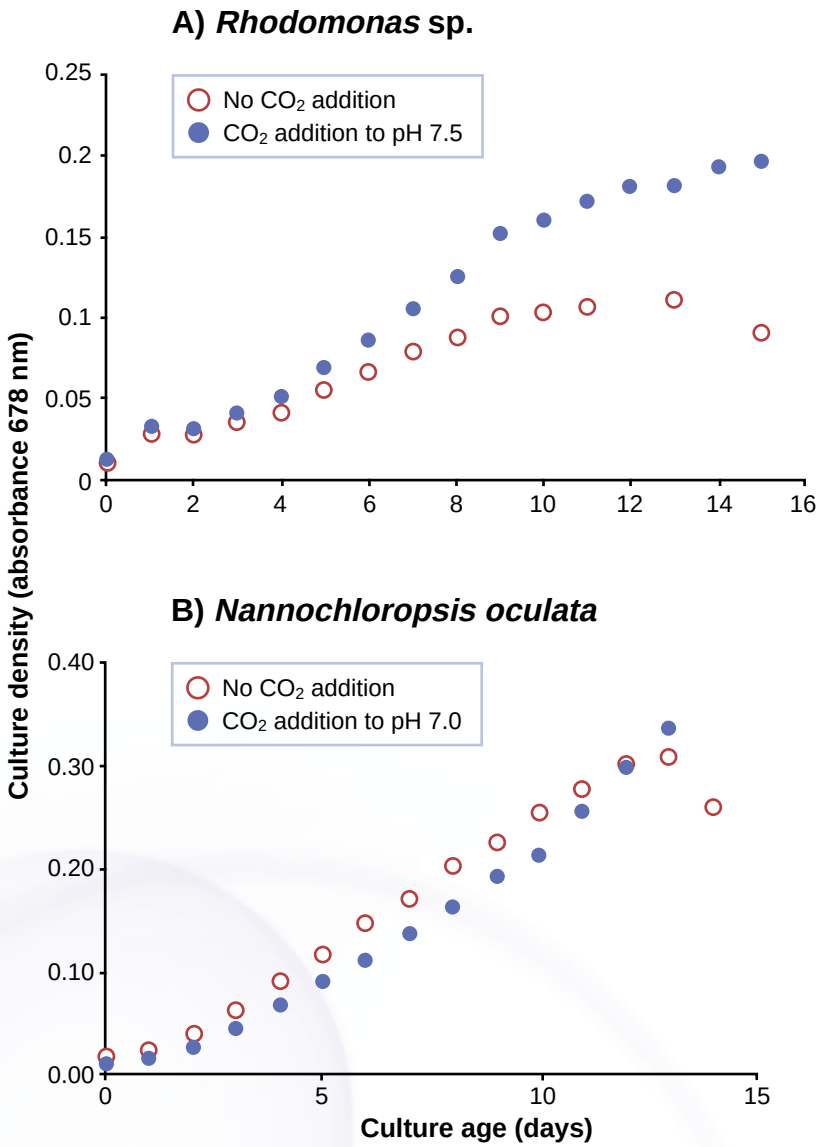

Figure 8.1. *Growth curves (absorbance versus time) for aerated cultures of (a)* Rhodomonas *sp. and (b)* Nannochloropsis oculata *with and without CO2 addition. CO2 dosed to control pH to 7.5 for* Rhodomonas *sp. and pH 7.0 for* N. oculata. *Average values, n = 2.*

Table 8.1. Microalgae maintained and supplied to industry by NFC.

Class	Species	Primary use
Eustigmatophyceae	*Nannochloropsis oculata*	Rotifer production
Chlorophyceae	*Dunaliella tertiolecta*	Stimulate mixis in rotifers
Cryptophyceae	*Rhodomonas* sp.	Major component of copepod diet
Dinophyceae	*Heterocapsa niei*	Boosts copepod productivity
Prasinophyceae	*Tetraselmis chuii, suecica*, NT sp.	Minor component of copepod diet
Prymnesiophyceae	*Isochrysis* sp. (T.ISO), NT sp.	Minor component of copepod diet

Rotifers

Previously, *Brachionus plicatilis* (L-type) rotifer was cultured at NFC for barramundi larviculture research. However, this species is too large (about 240 µm lorica length) for many marine finfish larvae. For our current research on grouper species we have isolated a strain of *B. rotundiformis* from a local lake. This isolate is within the size range classified as SS-type (Table 8.2).

Table 8.2. Lorica size (mean ± SD; range in square brackets; *n* = 50) of L-, S- and SS-type *Brachionus* (Su *et al*., 1994) compared to the NFC SS-type isolate (*n* = 97).

Rotifer type	Length (µm)	Width (µm)
L	219 ± 13 [193-243]	186 ± 13 [161-208]
S	176 ± 16 [158-205]	133 ± 10 [124-151]
SS	147 ± 11 [111-163]	123 ± 8 [93-134]
SS-NFC	151 ± 15 [96-173]	113 ± 8 [64-137]

The size distribution of the isolate showed it to have an average lorica length of 151 µm with the distribution skewed toward the smaller sized rotifers (Figure 8.2).

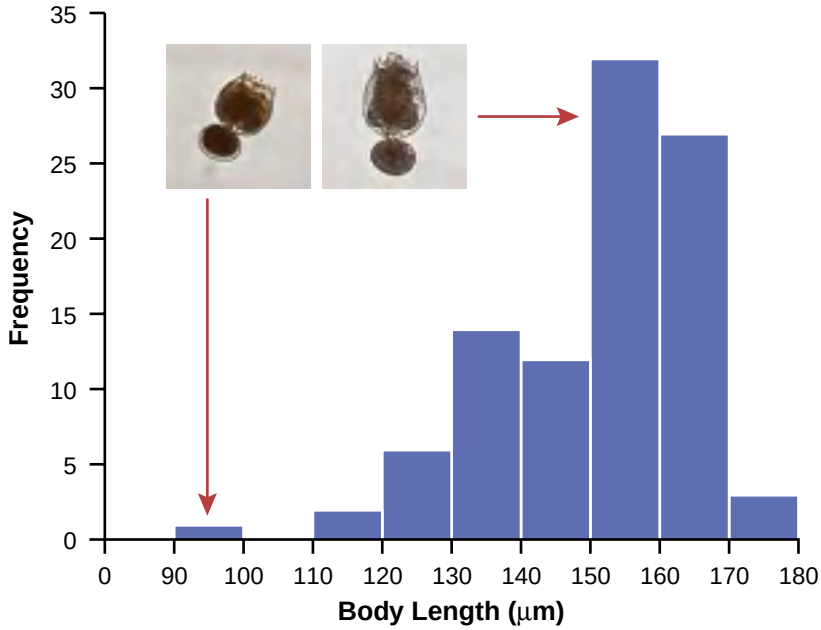

Figure 8.2. *Histogram of the size distribution of lorica length of egg bearing female NFC rotifers (n=97).*

For early-stage grouper larvae, even SS-type rotifers need to be screened (under 90 μm) before feeding. To achieve a stable population of such small rotifers, we need to select for the smallest rotifers in the population. Initially, we will select for the largest and smallest 0.01% of the population and assess size in relation to heritability, rate of reversion and reduction in productivity associated with a decrease in size.

Copepods

Copepod nauplii have been shown to be essential prey items during the first 4 days of feeding for snapper larvae (Lutjanidae) and to improve survival of grouper larvae. They are beneficial because of their small size, digestibility, fatty acid profile and their swimming motion.

Copepods

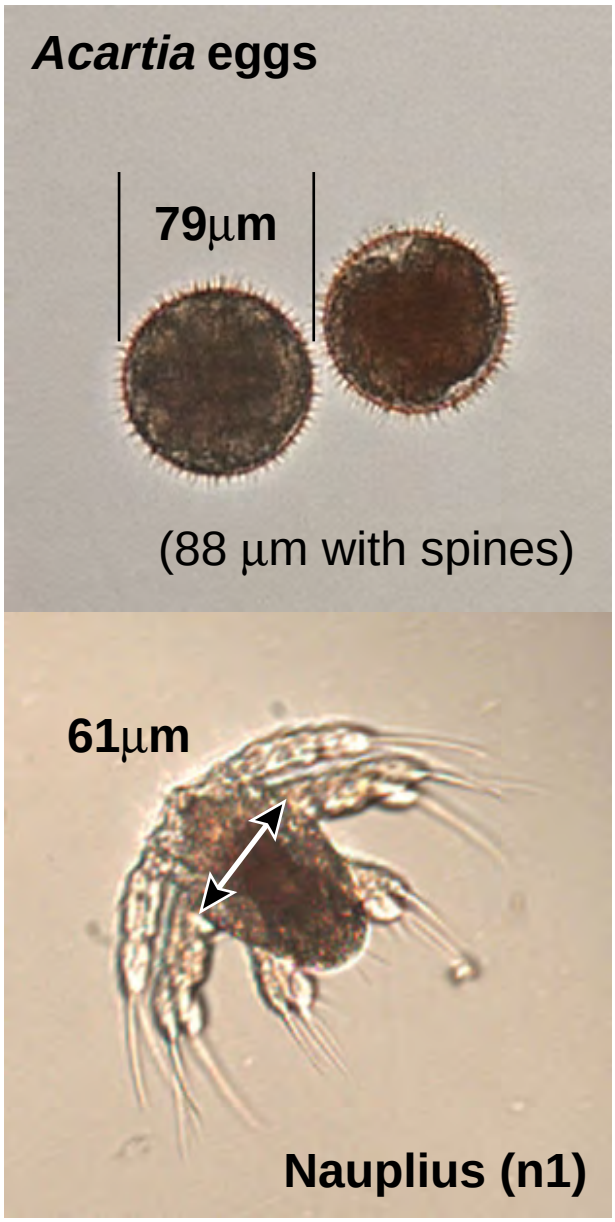

Figure 8.3. *Eggs and nauplius of* Acartia *sp.*

At NFC we have focused on developing production technology for the calanoid copepod *Acartia (Acanthacartia)* sp. These are cultured in 400-L conical bottom tanks with a central airlift that screens out adult copepods but not eggs and nauplii (Figure 8.3), which are collected in a fine screen tray at the top of the airlift. Collected eggs and nauplii are taken and counted each morning to measure productivity. Initially, tanks were operated as a batch system, operating for 7–10 days until productivity fell because of loss (death) of most of the adult population (Figure 8.4A). More recently, the algal feed rate was increased by 25% and the adult population was supplemented daily with copepodids that had been grown on from some of the harvested eggs (Figure 8.4B). This improved the stability of the adult population, extended the production cycle and doubled the number of eggs and/or nauplii harvested from a tank over a batch cycle.

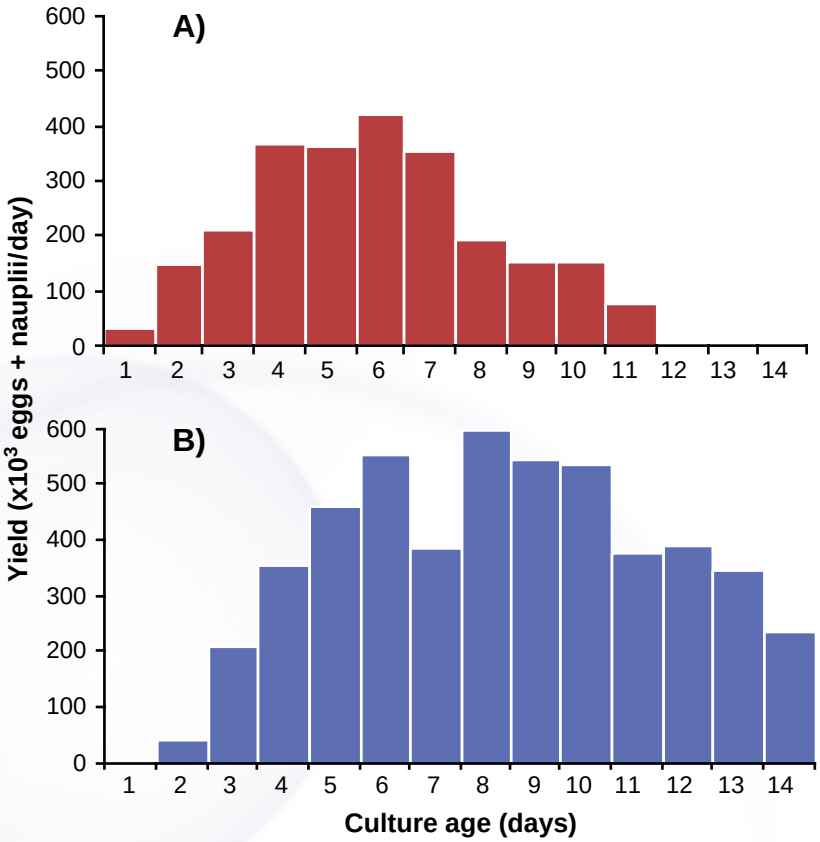

Figure 8.4. *Average daily production of* Acartia *eggs and/or nauplii from 400-L culture tanks operated with a single addition of adults (A) and with the initial adult population supplemented with daily addition of copepodids (B).*

Copepod production per tank cycle increased from 2.5 million to 5 million and the production period was extended from 4 days to 10 days. With four 400-L tanks operating, about 2 million nauplii per day could be supplied as larval feed. In the future we shall continue to refine the culture methods for *Acartia* and also for the second copepod species that we maintain, *Bestiolina similis*. We are currently assessing the potential of cold storage of eggs and nauplii to boost numbers available to fish larvae during the critical early days of development.

Future research

In the future, we shall investigate interactions of live prey on fish larval development. This work will include investigating the effect of diet on the nutritional value of live prey and the effect of prey type on larval gut development in relation to gut morphology and digestive enzyme activity.

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9. Production of polyunsaturated fatty acids by Australian thraustochytrids: Aquaculture applications

Tom Lewis[1], Peter Nichols[2] and Tom McMeekin[1]

Interest in the nutritional importance of polyunsaturated fatty acids (PUFAs) has increased markedly during the past decade. As PUFAs are necessary constituents of cell membranes and of many cell-signalling systems, deficiencies in dietary PUFAs may lead to poor growth, low vitality and/or disease. The essentiality of PUFAs as dietary components for marine finfish and crustacean larvae has been amply demonstrated (e.g. Sorgeloos and Leger, 1992).

PUFAs are generally classified into two main groups: the omega-6 (ω6 or *n*-6) and omega-3 (ω3 or *n*-3) series. Of the *n*-6 PUFAs, arachidonic acid [AA; 20:4 (*n*-6)] is of particular importance, as it is a major precursor of many prostaglandins and eicosanoids. Eicosapentaenoic acid [EPA; 20:5 (*n*-3)] and docosahexaenoic acid [DHA; 22:6 (*n*-3)] are two *n*-3 PUFAs which are currently receiving much attention and have been termed 'essential' fatty acids.

At present, selected fish oils and microalgal species are the main industrial sources of PUFAs. However, supplies of fish oil may be unreliable due to the failure or variability of various fisheries. There is concern that insufficient fish oil will be available in the future to meet the expected growth in world demand for *n*-3 oils (Tacon, 1995).

Phototrophic microalgae are also used to provide PUFAs for aquaculture operations (e.g. Volkman *et al.,* 1989). However, the *de novo* synthesis of *n*-3 and *n*-6 PUFAs by heterotrophic microorganisms may provide a cheaper and easier means of producing PUFA-rich biomass and oils. Microheterotrophs do not require all of the elements necessary for the culture of autotrophs (e.g. light, carbon dioxide), and are seen by some as a

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potential alternative to traditional commercial sources of PUFAs. Certain bacteria have been shown to produce EPA and DHA (Nichols *et al*., 1993). These PUFA-producing bacteria also have been successfully used as a means to enrich rotifers (*Brachionus plicatilis*) with these fatty acids (e.g. Lewis *et al*., 1998b).

Another potential source of *n*-3 oils is the little-studied group of microheterotrophs called thraustochytrids. Thraustochytrids are common marine microheterotrophs that feed as saprobes or occasionally as parasites. Thraustochytrids have a wide geographic distribution, with strains isolated from Antarctica, the North Sea, India, Micronesia, Japan and Australia (reviewed by Lewis *et al*., 1999).

Several recent studies have catalogued the ability of some thraustochytrid strains to produce:

- a high biomass in culture,
- a high proportion of lipid as part of this biomass,
- a high proportion of PUFAs in the lipid.

Most reports concerning the production of PUFAs by thraustochytrids have dealt almost exclusively with DHA production, as this compound is the most abundant PUFA produced by many of the strains of thraustochytrids reported to date.

Data available in the scientific literature demonstrate the large variation in biomass, lipid and maximum DHA yields obtained for different thraustochytrid strains. For example, *Schizochytrium aggregatum* produced a biomass of 0.9 g L^{-1} after 10 days (Vazhappilly and Chen, 1998), while a biomass of 48 g L^{-1} after 4 days was achieved using *Schizochytrium* sp. SR21 (Yaguchi *et al*., 1997). Perhaps more importantly, PUFA production by a single strain (*T. roseum* ATCC 28210) cultured under different conditions also showed marked differences. For this strain, a batch-fed flask culture yielded 2100 mg L^{-1} of DHA (Singh and Ward, 1996), as compared to an unsupplemented flask culture, which yielded 650 mg L^{-1} of DHA (Li and Ward, 1994).

Although production of DHA has been the main focus of recent attention, it is evident that some thraustochytrid strains also produce other PUFAs. Lewis

et al. (1998a) isolated a number of thraustochytrid strains with a range of different PUFA profiles, including one strain (ACEM E) which produced AA to 30% of the total fatty acids (TFA), with no other PUFA exceeding 10% TFA (Figure 9.1).

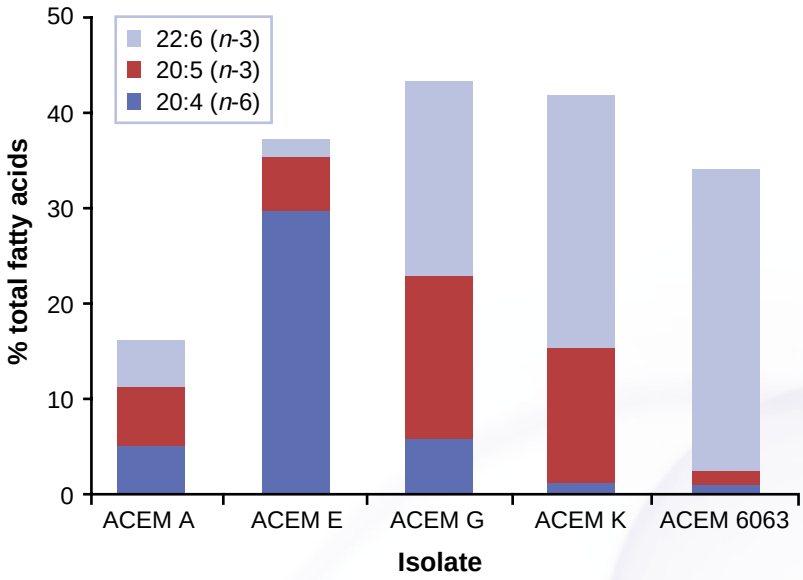

Figure 9.1. *PUFAs in new Australian thraustochytrids (expressed as percentage of total fatty acids; after Lewis et al. 1998a).*

Given the diversity of PUFA profiles seen for thraustochytrids examined to date, we feel there is great potential to use these organisms (singly or in combination) to produce live or manufactured feeds containing PUFA profiles tailored to the specific PUFA requirements of aquaculture species.

We have fed two strains of our thraustochytrids (ACEM A and ACEM 6063), both separately and mixed with each other or with microalgae, to rotifers. These experiments revealed two noteworthy results:

- Our strains of Australian thraustochytrids can be used to enrich rotifers with PUFAs to levels that are reported to be nutritionally significant for many aquaculture species (i.e. 1–2% w/w dry weight).

- Marked variations in the final PUFA profile of enriched rotifers can be achieved by changing the strain(s) with which rotifers are enriched (Figure 9.2).

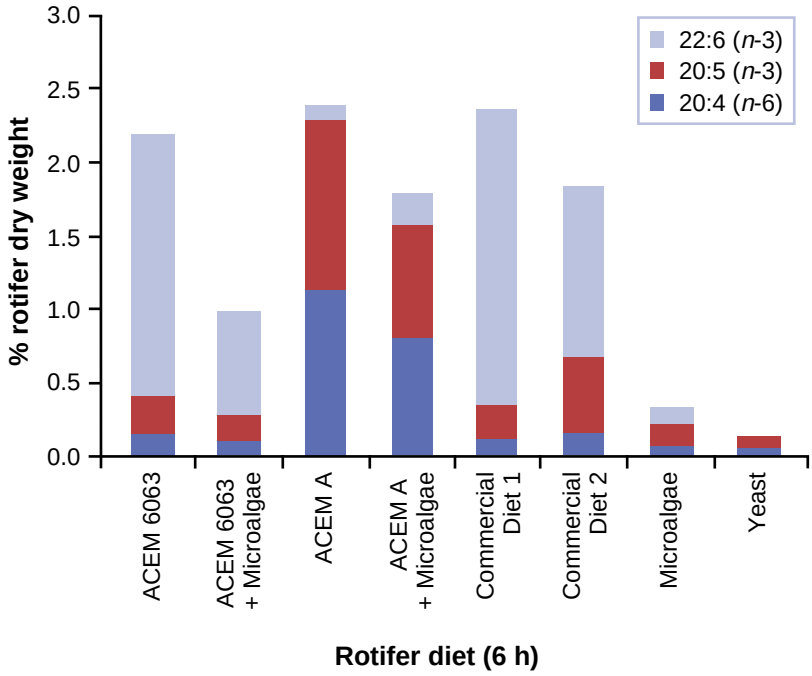

Figure 9.2. *PUFA enrichment of rotifers using Australian thraustochytrids, compared with two commercial PUFA enrichment diets and the microalgae Isochrysis sp. (clone T.ISO) and bakers yeast.*

Thraustochytrids are already being used in the USA for commercial production of PUFA-rich products. A *Schizochytrium* strain is the basis for two products marketed for enriching rotifers (*Brachionus* spp.) and brine shrimp (*Artemia* spp.) with PUFAs, prior to feeding these organisms to cultured finfish larvae (Barclay and Zeller, 1996; www.aquafauna.com; www.sandersbshrimp.com). These products have entered the market in direct competition with microalgal and fish-oil-based products. It is possible, however, that thraustochytrids may offer some advantage over other oils as

sources of PUFAs for aquaculture. Many aquaculture species require proportionally more DHA than EPA in their diets (Narciso *et al*., 1999). The PUFA profiles of many thraustochytrids fit this criterion, while most oils derived from the fish meal industry contain more EPA than DHA.

Thraustochytrids are clearly a new and potentially competitive player in the PUFA market. Considerable work is required before the production of oil from these organisms significantly increases its share of the market for PUFA-rich products. To achieve this aim, the following key stages need to be negotiated:

1. Further isolation, screening and maintenance of PUFA-producing strains: Several strains with potential for the commercial production of DHA-rich oils have been already isolated. However, if thraustochytrids that produce higher PUFA yields and/or more attractive PUFA-profiles can be isolated and optimised, demand for these isolates and compounds may well increase.
2. Optimisation of efficiency of PUFA production: The types and amounts of PUFAs produced by individual strains of thraustochytrids are susceptible to manipulation by varying culture conditions. Enhancement of PUFA profiles using molecular techniques may also be considered. Different markets will provide demand for strains that produce high levels of PUFAs measured either in terms of biomass (i.e. PUFA production w/w cell mass) or volume (i.e. PUFA production w/v fermentation medium).
3. Determination of appropriate conditions for long-term storage of microbial cells and/or their products: The form and stability of thraustochytrid biomass and/or oils will be major factors in determining the suitability of these products for use as food additives.
4. Development of production technologies to meet market demands for cost-effective and safe trophic transfer of PUFAs to the target consumer(s). The bottom line for the biotechnological future of thraustochytrid-oils will be their competitiveness against other PUFA-rich oils. Examples given above indicate that large-scale culture of thraustochytrids for commercial purposes already is, or soon will be, economically feasible.

Acknowledgments

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10. Hatchery feeds for the mud crab *Scylla serrata*: towards a nutritionally complete diet

David Mann, Tom Asakawa, Morris Pizzutto, Clive Keenan and Ian Brock[1]

There has been increasing interest in the aquaculture of mud crabs (*Scylla* spp.) both in Australia and throughout Asia. In Australia, the lack of technology for the hatchery production of crablets has been one of the main constraints to developing a mud crab grow-out industry. Recently, the Bribie Island Aquaculture Research Centre (BIARC) conducted an Australian Centre for International Agricultural Research (ACIAR) funded project aimed to develop techniques for the aquaculture production of the crab. The hatchery component of this project identified larval nutrition as a critical factor in the mass production of crablets. The culture of mud crab larvae is currently based on a diet of the brine shrimp, *Artemia* spp., particularly in the late larval instars. Declining survival near the end of the larval cycle and a high incidence of failure to complete the first metamorphosis indicate a possible nutritional deficiency.

Commercially exploited sources of *Artemia* occur throughout the world and it is well documented that the nutritional content of newly hatched nauplii varies considerably among the types sourced from different geographic locations. When newly hatched nauplii are being used, therefore, the selection of *Artemia* type for feeding cultured marine larvae is critical to supplying an appropriate nutritional profile. Additionally, the natural harvest of *Artemia* cysts has recently been declining and has resulted in rising cyst prices and some cyst types becoming unavailable. A series of experiments was conducted at BIARC to investigate nutritional factors influencing the value of *Artemia* nauplii as feed for mud crab, *S. serrata*, larvae.

The experiments compared the survival and growth of mud crab larvae fed nine types of *Artemia* nauplii hatched from cysts. The cysts were sourced

[1] QDPI, Bribie Island Aquaculture Research Centre

from San Francisco Bay, Great Salt Lakes, China, Vietnam and other locations unspecified by the supplying companies. The influence of nutritionally enhancing *Artemia* nauplii of different types with a commercial lipid emulsion (Super Selco, INVE Aquaculture) and selected microalgae species (*Nannochloropsis oculata* and Tahitian *Isochrysis* sp.) was also investigated. All diets were biochemically analysed for protein, lipid, ash and gross energy, as well as for fatty acid and amino acid profiles and vitamins A, E and C.

Only three of the nine *Artemia* types assayed in this experiment were able to support development of a significant proportion (>40%) of larvae through to the megalopa stage: one from China, one from Vietnam and the other a graded type (AF grade, INVE Aquaculture) sourced from an unspecified location. Similar survival performance of all *Artemia* treatments was observed initially with a point of divergence between 'good' and 'poor' treatments occurring around day 12 (Figure 10.1). Following the point of divergence, larvae from the poorer performing treatments suffered a high incidence of moult death syndrome (MDS), an inability to complete the moulting process at the first metamorphosis.

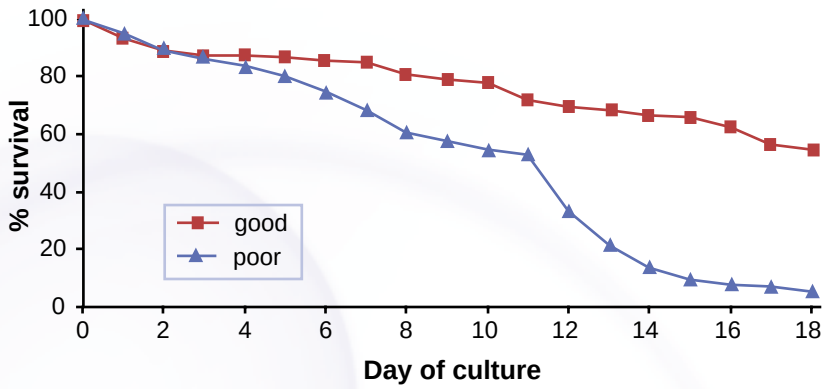


Figure 10.1. *Survival of mud crab larvae fed 'good' and 'poor'* Artemia *types.*

Neither larval survival nor growth were significantly affected by enrichment of nauplii with a prepared lipid emulsion (Figure 10.2) or selected microalgae species used alone and in combination in the larval culture medium.

Biochemical analyses confirmed that the lipid emulsion enrichment technique increased levels of the fatty acids DHA and EPA and others in the different *Artemia* types. However, the experimental results indicated that a simple elevation of these particular fatty acids in the diet does not correct the apparent nutritional deficiency of the nauplii.

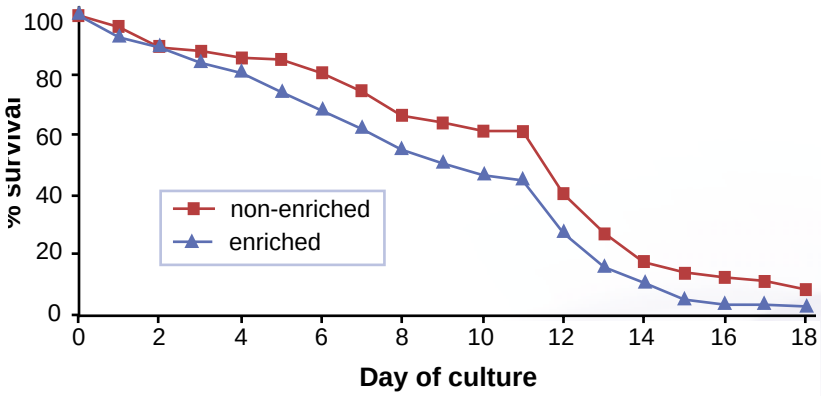


Figure 10.2. *Survival of mud crab larvae fed enriched and non-enriched* Artemia *nauplii.*

All biochemical data have now been collected and are being statistically analysed with the aim of identifying the critical limiting components, or class of components, in the larval diet. A preliminary statistical treatment of the incomplete data-set indicated that phospholipid content of the *Artemia* is positively correlated with mud crab larval survival and is also strongly represented in the multiple parameter model for mortality rate.

The simplest solution for a mud crab hatchery is to secure a source of high-nutrition *Artemia* cysts. In the short term this seems to be a poor option due to low availability and high prices. The three best-performing cyst types identified in this study are not commercially available. The longer-term solution is to use information on the nutritional requirements generated from this study to customise an enrichment formulation for crab larvae. Alternatively, a particulate dietary supplement — one that specifically corrects the deficiency in the standard *Artemia* diet — could be formulated

to feed direct to the larvae. There is already information indicating that crab larvae ingest inert micro-particulate diets and that some improvement in larval performance can be achieved. One further option is to explore the use of other live food sources, such as copepods, that are potentially a nutritionally complete prey item for crab larvae.

The production of crabs is set to undergo rapid development, and investors in Australia are already involved. Overseas, mud crab aquaculture continues to grow in popularity leading to a strong demand for crablets and therefore hatchery production. Determining the critical nutritional requirements of the larvae is an important step towards developing reliable hatchery techniques.

11. Victorian hatchery feed production and development

Lachlan McKinnon and Brett Ingram[1]

The Victorian aquaculture industry is relatively small by Australian standards, with the majority of production occurring in the well established freshwater salmonid sector. Almost 800 tonnes of salmonid fingerlings worth an estimated $5.8M were produced in Victoria in 1998 (Fisheries Victoria Aquaculture Information Bulletin, 2000). A small ornamental (predominantly *Carassius auratus*) aquaculture industry and small scale yabby (*Cherax destructor*) farming have also been in operation for many years. More recent developments in industry and at the research level, have been occurring in the hatchery production and growout of native fish, such as Murray cod (*Maccullochella peelii chpeelii*) and silver perch (*Bidyanus bidyanus*). Two tonnes of native fish fingerlings worth $0.6M were produced in hatcheries in 1998 (Fisheries Victoria Aquaculture Information Bulletin, 2000). The intensive culture of shortfinned eel (*Anguilla australis*) is also beginning to develop in Victoria, but relies on the supply of wild caught glass eels as seedstock.

More recently, marine finfish such as black bream (*Acanthopagrus butcheri*), snapper (*Pagrus auratus*) and greenback flounder (*Rhombosolea tapirina*) have been produced on a small commercial scale and at research level. In terms of shellfish, commercial abalone (*Haliotis rubra* and *H. laevigata*) production has been developing over the last 3-4 years, and some commercial interest and government R&D investment is currently being made into the aquaculture of scallops (*Pecten fumatus*). The aquaculture of blue mussels (*Mytilus edulis planulatus*) is well established in Victoria. Algal requirements for both benthic and planktonic species is an area of interest, with some R&D currently underway, including FRDC funded research into the seeding of benthic diatoms onto settlement plates used in abalone aquaculture.
Until the recent entry into marine finfish culture in Victoria, hatchery feed

[1] Marine and Freshwater Resources Institute. (See details in appendix.)

requirements were limited largely to formulated diets for salmonids, and *Artemia* for native fish. Freshwater native fish too small to accept *Artemia* at first feed, and juvenile yabbies, have been catered for largely in fertilised plankton ponds, into which juveniles are stocked to graze on naturally produced live feed. These ponds, which have been specifically designed, are fertilised with organic and inorganic nutrients to encourage the development of phytoplankton blooms and, in turn, zooplankton blooms (rotifers, cladocerans, copepods) upon which the stocked fry feed. Management of these ponds is being developed and pond production is becoming more routine and reliable. Weaning diets for Murray cod and shortfinned eels are presently being developed and some commercial success has been seen with "crashing" Murray cod larvae and glass eels on to commercially available starter diets. Hatchery feeds for goldfish are generally limited to *Artemia* or larvae are placed directly into plankton ponds.

The development of microalgae and rotifer culture systems, and *Artemia* for marine production, at both private and government hatcheries has been more recent in Victoria. In some cases booster feeds and alternatives to microalgae for rotifer culture are being used. Limited application of commercial microdiets has also been undertaken in marine finfish hatchery production. The present level of hatchery production in Victoria is low however, but is expected to increase over the next few years with the anticipated development in marine finfish aquaculture.

The primary areas for R&D in hatchery feeds identified for Victorian aquaculture include the development of semi-continuous culture methods for freshwater rotifers (eg *Brachionus* spp., *Keratella* spp.), copepods (eg *Calamoecia, Boeckella*) and cladocerans (eg. *Daphnia, Moina*), and the development of compounded starter diets for Murray cod, eels, and marine finfish.

12. Cultured copepods as live food for fish

Mick Payne[1]

Intensive fish cultivation requires the large-scale production of live prey. The following are summaries of experiments aimed at maximising production of *G. imparipes* nauplii.

Copepod diets

The effect of five diets on copepod growth and fecundity were tested: *Isochrysis galbana, Chaetoceros muelleri, Dunaliella tertiolecta, Nannochoropsis oculata* and bakers yeast. Copepods did not survive to maturity on a diet of bakers yeast. Highest nauplius production was recorded on a diet of *I. galbana* and lowest on a diet of *N. oculata* (Figure 12.1).

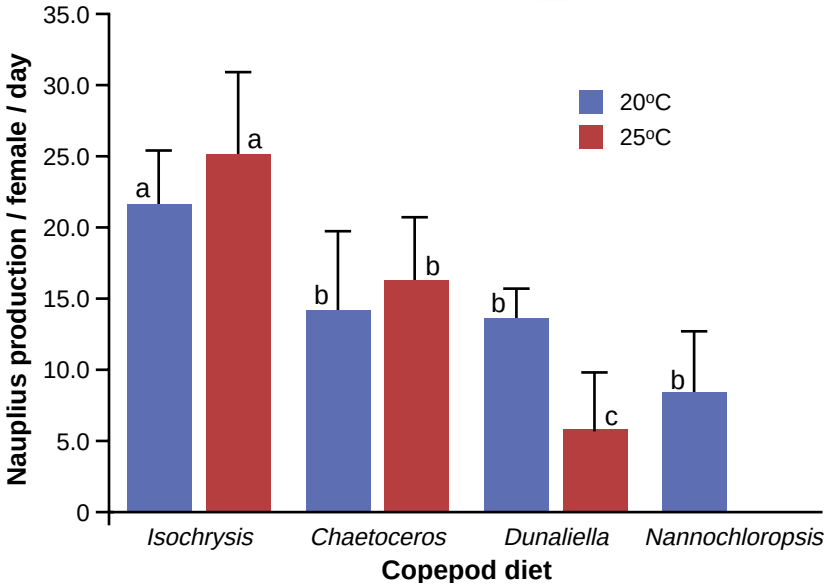


Figure 12.1. *Effect of diet on nauplius production by* Gladioferens imparipes.

[1] Fremantle Maritime Centre. (See details in appendix.)

Salinity

Fecundity was recorded for copepods grown at salinities of 25%, 50%, 75% and 100% seawater. Nauplius production was not reduced at any of the test salinities.

Storage in refrigerator

Nauplii and adult copepods were stored at both 4 and 8°C. Survival was poor at 4°C. At 8°C, nauplii survival was greater than 95% after 12 days and survival of adults was approximately 70% after 42 days (Figure 12.2).

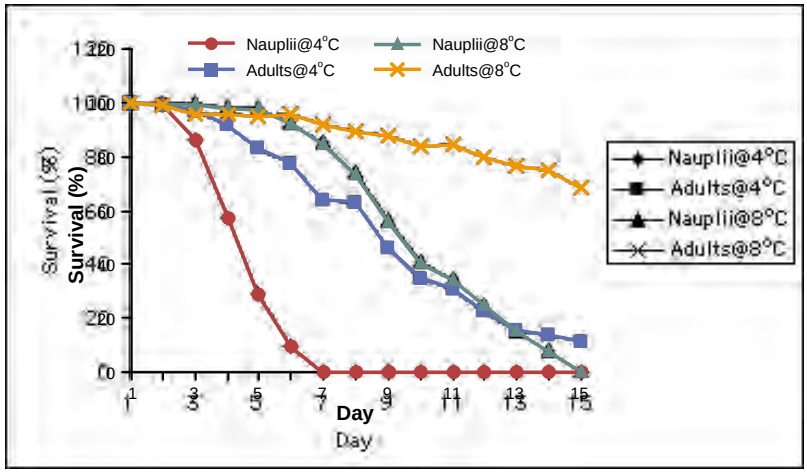


Figure 12.2. *Survival of adult and naupliar* Gladioferens imparipes *after refrigeration.*

Enrichment

The HUFA content of copepod nauplii fed *I. galbana* was determined. DHA content was greatest after 6 h of enrichment. EPA content did not change. When *I. galbana* was combined with *N. oculata* during enrichment, the EPA and arachidonic acid content of nauplii increased (see Table 12.1).

Table 12.1. HUFA Content of Copepod Nauplii (-; not deleted).

Time (h)	Arachidonic	EPA	DHA	DHA:EPA
0	-	1.4 ± 0.4	6.9 ± 0.2	4.9
0.5	-	1.4 ± 0.3	7.8 ± 0.1	5.6
2	-	1.4 ± 0.2	7.9 ± 1.2	5.6
4	-	1.3 ± 0.1	8.6 ± 0.6	6.6
6	-	1.3 ± 0.1	9.1 ± 0.2	7.0
6+Nanno	0.9 ± 0.4	2.8 ± 0.2	10.1 ± 0.9	3.6

Diet for pink snapper larvae

Snapper *(Pagrus auratus)* larvae were fed diets consisting of rotifers only and rotifers supplemented with copepod nauplii. Growth, survival and swim-bladder inflation were higher in those larvae fed the supplemented diet, but not by a significant margin (Figure 12.3).

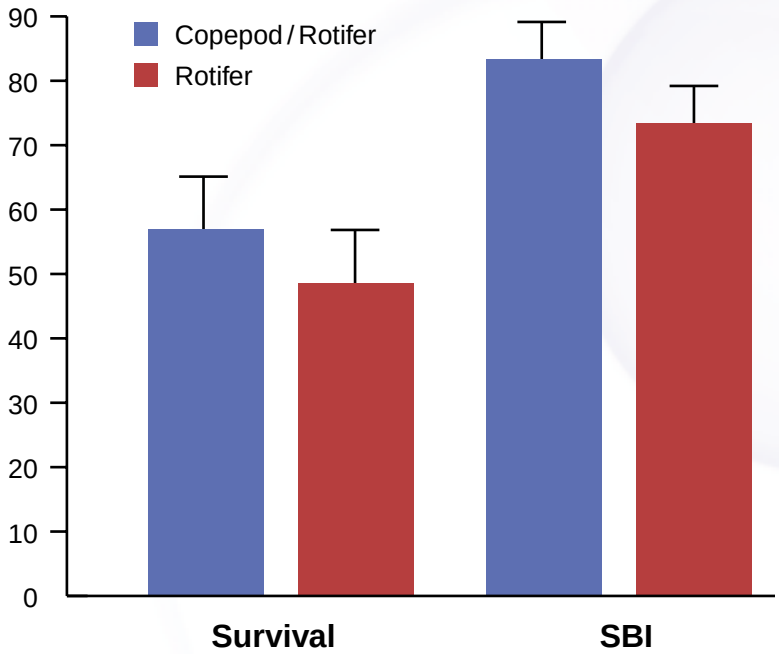


Figure 12.3. *Percentage survival and swim bladder inflation (SBI) of snapper larvae fed diets of rotifers, or rotifers supplemented with copepods.*

Diet for WA seahorses

Growth and survival of juvenile WA seahorses *(Hippocampus subelongatus)* were substantially greater on a diet of copepod nauplii than on a diet of enriched *Artemia* nauplii (Figure 12.4).

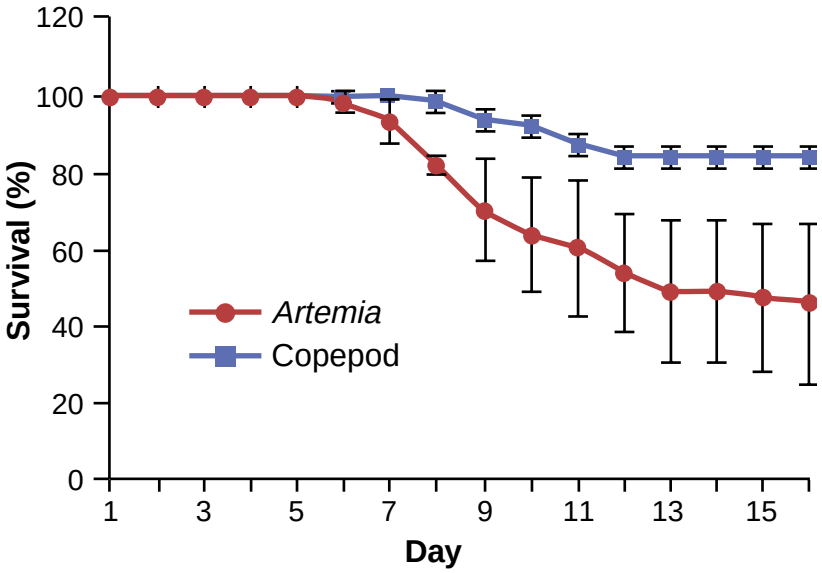


Figure 12.4. *Percentage survival of juvenile WA seahorses fed diets of enriched* Artemia *nauplii or copepod nauplii.*

Diet for Westralian dhufish larvae

Dhufish *(Glaucosoma hebraicum)* larvae were fed diets of rotifers only and an equal combination of rotifers and copepod nauplii. Growth and survival were substantially greater on the combined diet (Figure 12.5).

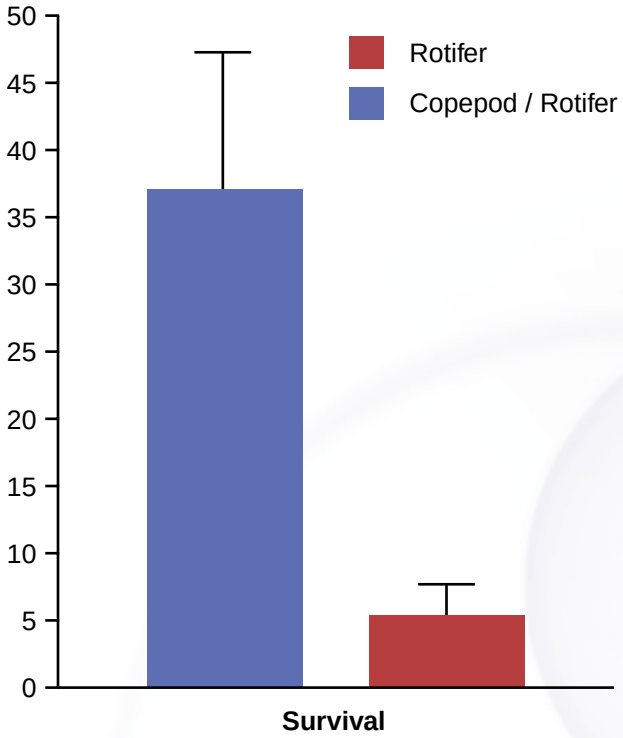


Figure 12.5. *Percentage survival of dhufish larvae fed diets of rotifers, or rotifers supplemented with copepods.*

Current status

Much background work has been completed on *G. imparipes*, and two practical, large-scale culture systems are being developed at the Fremantle Maritime Centre. The first consists of two 1000-L tanks based on the automated system described previously but without the automation. This system produces nauplii for feeding direct to fish larvae. The second system consists of a 5000-L tank. This tank is maintained as a semi-continuous system, requiring only occasional draining and restocking. The main purpose of this system is to provide adult copepods to stock into green-water larviculture systems.

13. Live food and feeding ecology of larval snapper *(Pagrus Auratus)*

Jian G. Qin and Troy Hillier[1]

The relationships between mouth morphology and live food selection were studied in snapper from first feeding larvae to metamorphosis. The mouth width was a limiting factor for snapper larvae to ingest food particles before the body length reached 5.6 mm (20-day old); then the size of the gape opening became a limiting factor for food ingestion. The change of the limiting factor from mouth width to gape size coincided with the onset of larval metamorphosis. The relationship between mouth size and food particles predicted that it would not be physically possible for larval snapper to consume a rotifer (0.13 mm) until the fish reached a notochord length of 2.4 mm; *Artemia* nauplii (0.45 mm) until 4.8 mm; and copepods (0.83 mm) until 6.8 mm. The food selectivity trial for larval fish confirmed that these physical limitations were the major factor determining the prey size that could be ingested by a fish of given size. Consumption rate analyses showed that 6-day-old larvae were capable of consuming 17 rotifers when subjected to a 12:12 lighting regime. The daily consumption rate increased 3-fold to 49 rotifers for 12-day-old larvae. These results suggest that the size of live food offered to fish larvae should be determined by mouth morphology at different developmental stages.

Mortality in snapper larvae has been associated with the onset of first feeding and the transition between live feeds. Exogenous feeding of snapper larvae (as for many other planktivorous fish) is limited by the size of mouth. Although larval snappers' foraging behaviour has been studied, knowledge on gape size limitation and food selectivity in snapper larvae is lacking. The gape limitation for live food greatly affects the survivorship of the larval snapper. Snapper larvae have a limited 'window' of opportunity during which they can start their first feeding. If the live food provided is too large for the mouth size then the snapper will inevitably be unable to feed and

[1] School of Biological Sciences, Fliners University. (See details in appendix.)

subsequently die from starvation. Understanding the ontogenetic diet change will assist in providing the appropriate food for fish larvae at each developmental stage.

As the larval snapper grow and develop, their visual acuity also refines. This implies that not only will the snapper be able to consume more live food, but also will they become more efficient predators, able to capture more prey items. The ability to capture and ingest more prey items means that the consumption rate and daily food requirements will increase dramatically, so the amount of live food provided may also need to be increased to achieve better fish growth and survival.

The objectives of this study were to determine (a) the morphological limitations that inhibit larval snapper from capturing a particular prey item, (b) how this relates to the actual fish size at which a particular prey item is ingested, and (c) the changes in selectivity of prey with increasing fish size. This study also aimed to determine the food consumption rate for larval snapper at different sizes.

The experiments were conducted in 30-L aquaria with one side blackened out to provide contrast for the live food. Snapper were initially stocked at a density of 50 larvae per litre. All aquaria were equipped with air-stones to maintain the oxygen level above 3.5 mg L–1. The temperature was controlled at 20.8 ı 0.4°C. Fish larvae were first fed on day 3 with small strain rotifers (0.14 mm) at a density of 15 rotifers per millilitre, moving to the large strain rotifers (0.33 mm) by day 9. At day 15, *Artemia* nauplii (0.45 mm) were introduced to the aquaria at a density of 5 nauplii per millilitre. All *Artemia* were removed from the system every second day and replaced with newly hatched nauplii, since juvenile *Artemia* are too large for the larval snapper.

Five fish were randomly selected from each aquarium every day starting on day 3 and continuing until day 24 to measure the morphological change of fish mouth. Five fish were randomly selected from each aquarium every 2 days starting on day 4 for the food selectivity trial. All measurements were obtained using a dissecting microscope and eyepiece micrometer. The upper and lower jaw lengths were measured to determine the gape dimension (Figure 13.1), as well as the notochord length of each fish. However, the mouth

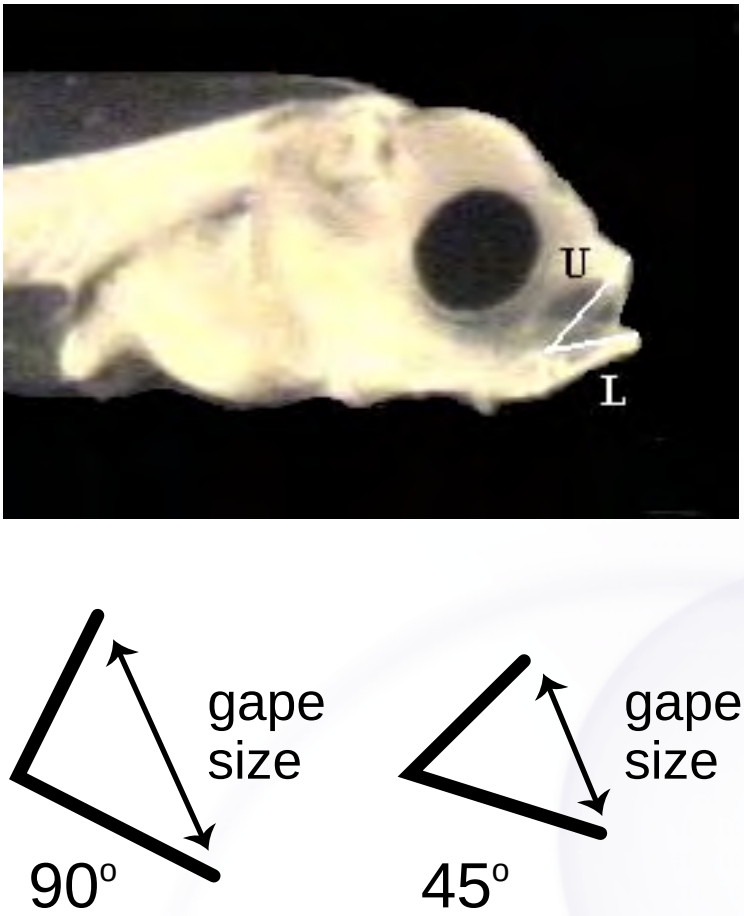


Figure 13.1. *Fish mouth and gape size.*

width was taken only in the feeding morphology trial, while the stomach contents and numbers were recorded only for the food selectivity trial.

The mouth width was taken by a direct measurement. A comparison of the food size and mouth dimensions was made to determine the physical limitations that may stop a larval snapper from feeding on a given sized prey. In the food selectivity trial three types of live food were used including rotifer *(Brachionus plicatilis)*, *Artemia* nauplii and cyclopoid copepods to determine the food selectivity of snapper of a particular size.

The abundance of prey items in the stomach and the environment was used to determine the electivity index (ε) for each prey item. The electivity index was used to determine whether or not a particular prey item was selected for or against in respect to the other food items available in the environment. The electivity index was calculated with the following equation:

$$\varepsilon_i = \frac{m\alpha_i - 1}{(m-2)\alpha_i + 1}, i = 1,\ldots, m$$

Where ε_i is the electivity index for prey item i, αi is the preference index for prey item *i*, and *m* is the number of prey species in the environment. The electivity values range from –1 to 1, where 1 indicates a positive selection for that prey item, –1 indicates an avoidance of that prey item and zero implies a random selection. The αi for each prey item was calculated using the following equation:

$$\alpha_i = \frac{r_i/n_i}{\sum_{i=1}^{m} r_i/n_i}, i = 1,\ldots m$$

Where r_i and n_i are the proportions of prey type i in the stomach and the environment.

Snapper larvae in the food consumption trials were fed rotifers at a density of 15 rotifers per millilitre. The rotifers used for the consumption experiment were dyed using methyl red for 30 minutes. Rotifers at a density of 15 per millilitre were added to the aquarium 2 hours after lights had been switched on. Five fish were randomly selected every hour, commencing one hour after the dyed rotifers were introduced into the system, and continuing for six consecutive hourly samples. The consumption rate was determined by the following equation:

$$F = \frac{\left(S_t - S_0 e^{-Rt}\right)R}{1 - e^{-Rt}}$$

Where F is the consumption rate (prey number per hour), S_t is the number of prey in the gut after time t, S_0 is the number of prey in the gut at the beginning of the time period, R is the evacuation time, and t is the time between samples. The evacuation time was determined by the passage of food items in the digestive tract.

Food Electivity

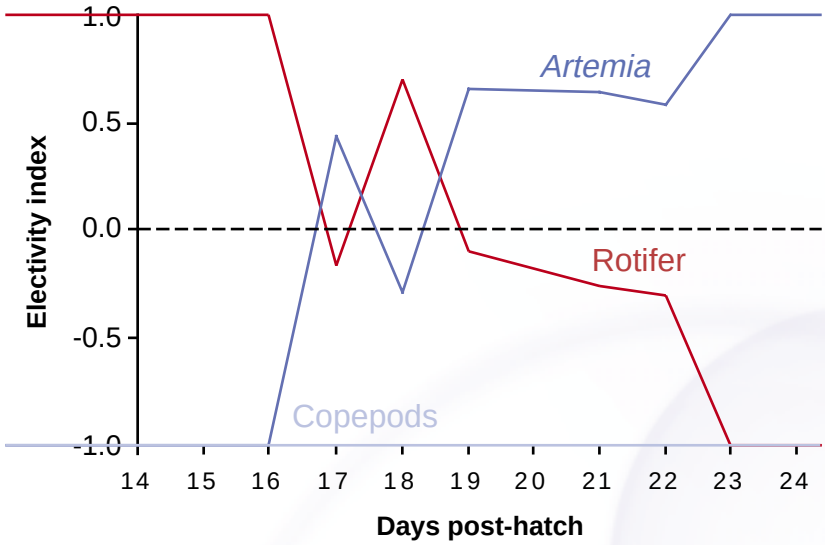


Figure 13.2. *Food electivity over time.*

The gape and mouth width dimensions were not linearly related in pre-metamorphic snapper but followed a natural logarithmic relationship. The gape opening and mouth width began in a linear relationship; however, once the gape reached a particular point (gape of 90°, 0.36 mm; and 45°, 0.27 mm) the mouth width increased dramatically. This point corresponded to a notochord length of 3.6 mm (10 days old). This could be due to the larval organism undergoing preliminary morphological changes in preparation for metamorphosis.

When comparing the width and gape dimensions against those of the live food, the gape was always the limiting factor when assuming that the mouth could open only to a 45° angle. However, when considering a 90° mouth opening, the width became the limiting factor until the fish reached a body length of 5.6 mm (20 days old), at which point gape became limiting.

Observations on feeding and sampled larvae showed that a 90° gape opening was not only feasible but also common, thus all results would be based on this measurement. The pre-metamorphic snapper (less than 20 days old) were limited in their food selection not by the mouth gape but by the width of the mouth.

The snapper larvae that were 4.2–4.9 mm long (i.e., 14–17 days old) selected for rotifers. After 19 days old, snapper reached a length of 5.3 mm and selected for *Artemia* nauplii. This was supported by the results of the electivity index, where before day 17 (length <4.7 mm) there was a positive selection for rotifers and a negative selection for both A. salina and copepods (Figure 13.2). By day 19 there was an increasing selection for *Artemia* nauplii and an increasing selection against rotifers. The electivity index for copepods was –1 for the entire experimental period. By day 24, fish larvae (6.4 mm long) were attempting to consume copepods. However, copepods were too large for the snapper larvae to capture.

Consumption rates for 6- and 12-day post-hatch larval snapper were measured at 20.8 ± 0.4°C. An evacuation time was 2 hours for 6-day-old larvae (2.8 mm long) decreasing to 1 hour for 12-day-old larvae (3.4 mm long). Two peaks of consumption rate were observed in a 6-hour feeding period. The total daily food consumption was estimated at 17 rotifers for 6-day-old larvae and 49 rotifers for 12-day-old larvae over a 12:12-h lighting cycle, assuming that fish did not feed in the dark.

In summary, it is theoretically possible for larval snapper (assuming a gape of 90° opening) to consume small-strain rotifers by day 3 (mouth first open), *Artemia* nauplii by day 16 (4.8 mm long), and copepods by day 27 (6.8 mm long). Once the larvae were physically capable of consuming *Artemia*, it took only 3 days for the snapper to positively select for the prey (i.e., electivity index of –1 on day 16 to +0.75 on day 19). This suggests that once larvae are physically able to consume a larger prey item, they will quickly select for the larger, more energy-rewarding prey.

14. Intensive cultivation of a calanoid copepod

Rob Rippingale[1]

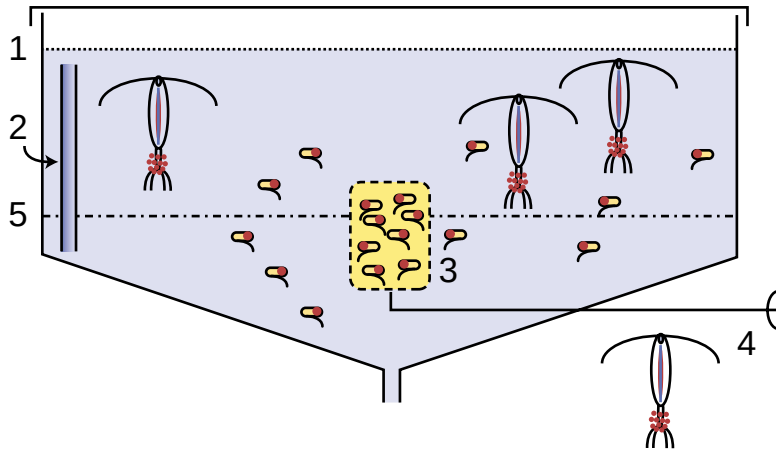
A calanoid copepod, *Gladioferens imparipes*, from estuaries of south-west Western Australia, has been kept in intensive cultivation and used experimentally in the diet of larval fish. An intensive cultivation was developed as a part of FRDC Project 96/398. This system is described.

Nauplii of the same age are introduced to one of two 500-L culture vessels with an initial density of one nauplius per litre. Unicellular algae, usually *Isochrysis galbana*, is added daily to provide food. When mature females are present, usually after 14–21 days at 23°C, a daily regime of nauplius collection is initiated. One cohort of nauplii is used to inoculate the second tank. After about three weeks of daily nauplius collections from the broodstock tank it is cleaned and stocked with a new cohort of nauplii. Mature animals in the second tank now become the broodstock.

The culture system is closed (Figure 14.1). Provision for the management of water quality is linked with procedures for nauplius collection under PLC control. Nauplius collection involves daily removal of about 200 L of water from the broodstock tank (Figure 14.2). Nauplii are concentrated into a few litres and most of the 200 L is treated with biological filtration and protein skimming for 24 hours before being returned to the broodstock tank during the next cycle of nauplius collection.

The culture system is designed around particular aspects of the biology of *G. imparipes*. In at least some natural habitats, *G. imparipes* is a pioneer grazer in the ecological succession of seasonal estuaries. After the cessation of winter flow, this copepod exploits the new growth of microalgae. Adults feed only on small particles and various species of algae provide adequate food. Nauplii are not predated and can co-exist safely with the adults. In common

[1] Curtin University, Western Australia. (See details in appendix.)



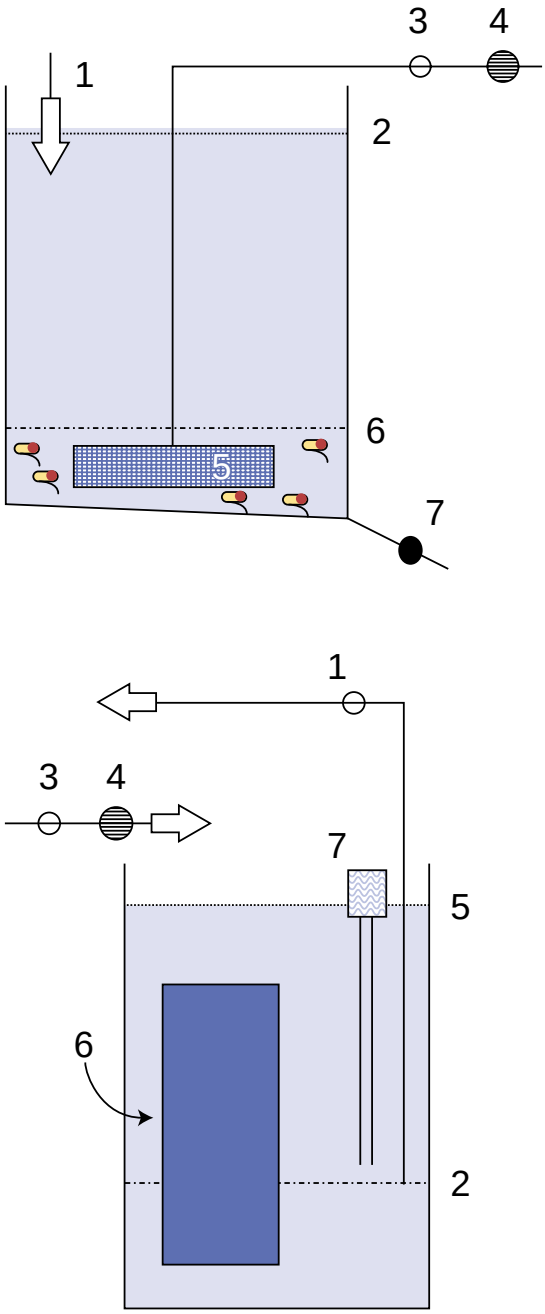
The main culture vessel, 500L capacity, filled to the line 1, covered to exclude light.

- Six stand pipes (2), with air injected, provide oxygenation and water movement. Air is turned off prior to nauplius collection allowing debris to sink.
- A light within a 140µm mesh cage (3) is activated by PLC when water movement has stopped. Nauplii are attracted to the light and accumulate within the mesh cage.
- When nauplii have accumulated in the cage a tap (4) opens to drain water containing nauplii into the nauplius holding tank. Water drains to the level 5.
- Reconditioned water is then pumped to the culture vessel, restoring the level to 1.

Figure 14.1. Gladioferens imparipes *culture system.*

with some other genera of estuarine copepods, *Gladioferens* adult females hold clutches of embryos until the nauplii hatch and swim freely. Healthy nauplii have a strong photopositive response to directional light but late copepodids and adults do not.

The late-stage copepodid and adult copepods attach to firm underwater surfaces for extended periods of time using fine hair sensillae on the dorsal prosome. Surfaces of firm, 3-mm mesh are provided in the culture tanks. Animals attach to these and to the internal surfaces of the tank. Movement of water by continual airlift results in gentle currents moving past the stationary animals. The broodstock culture is kept in darkness until a PLC switches off the aeration, allowing the water to become still. The PLC then activates a light within a 150-µm screen, attracting nauplii to a point from which adults are excluded. Nauplii are then flushed from the culture vessel



14.2 (a)

- At each nauplius collection, 180L of water containing nauplii siphons (1) from the culture vessel to fill this nauplius holding tank. A float switchstops the flow at level 2.
- When water from the reconditioning tank has been returned to the culture vessel, water from this holding tank is pumped (3) through a 50µm mesh (5), which retains the nauplii, and a 5µm cartridge filter (4),which removes debris, to the reconditioning tank. A float switch at level 6 inactivates the pump.
- Nauplii, now concentrated into 10L of water to level 6, are drained manually (7) from this holding tank.
- Nauplius numbers in replicate 1mL samples are counted to estimate the daily nauplius collection from the culture.

14.2 (b)

- Water that has been reconditioned in this unit is pumped(1) to refill the culture vessel after the daily collection of nauplii. This reduces the level to 2.
- Water from the nauplius holding tank is then pumped (3) through a 5µm cartridge filter (4) into this reconditioning tank, stopped by a float switch at level 5.
- During the 24 hours between nauplius collections, this water is circulated through a biological,filter (5) and treated with a protein skimmer (7).

Figure 14.2. *Sequence of operation of the culture system.*

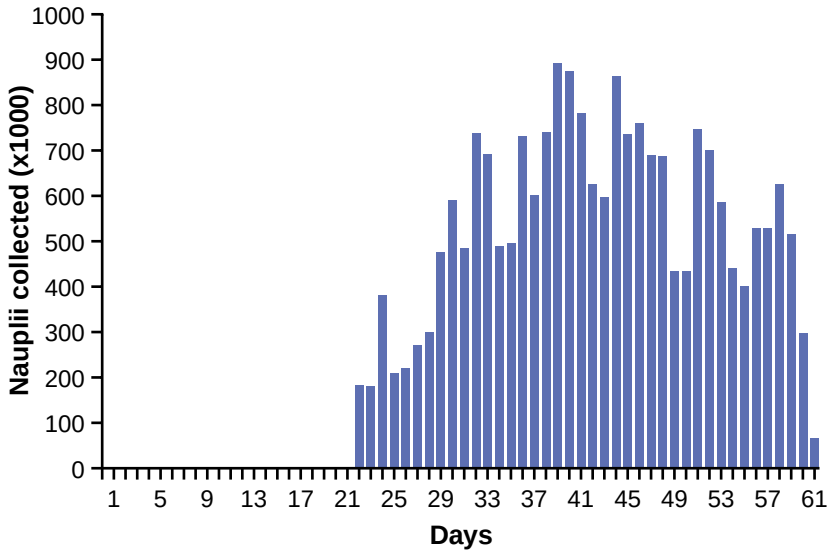


Figure 14.3. *Daily nauplius production from a 500L copepod culture; mean production of 540,000 nauplii per day for 40 consecutive days.*

and concentrated in approximately 10 L of water. A sub-sample of the nauplii is counted to assess production. The removal of water associated with nauplius collection involves about 40% of the volume of the broodstock tank. This water is replaced by water which was previously cleaned by mechanical filtration, biological filtration and protein skimming.

Daily collection of about 500 000 nauplii can be sustained from the system (Figure 14.3).

The closed system with recycled water allows for copepod production in a locality without a ready supply of clean seawater. Where seawater is readily available the system can be simplified and scaled up to larger tanks.

The reliability of cultures of *G. imparipes,* and the readiness with which all life history stages are taken as food by some juvenile fish, suggest that with further development of culture systems they may become important as a live food in particular aquaculture niches.

Development of the copepod culture and evaluation of *G. imparipes* as food for larval fish is described in the report for FRDC Project 96/398 titled *Intensive Cultivation of a Calanoid Copepod for Live Food in Fish Culture*. Procedures for maintaining cultures of the copepod are described in a manual produced by FRDC titled *Intensive cultivation of a calanoid copepod* Gladioferens imparipes. *A guide to procedures*.

15. Development of artificial diets for fish larvae

Paul Southgate[1] and Sagiv Kolkovski[2]

Introduction

A generalised feeding protocol for marine finfish larvae begins with the provision of rotifers at first feeding followed by *Artemia* nauplii and larger Artemia as larvae increase in size, to a point where artificial diets are introduced and larvae are weaned from live feed organisms. In addition to rotifers and *Artemia*, microalgae are usually cultured in mariculture hatcheries to feed them. Therefore, most mariculture hatcheries culture three different live foods (microalgae, rotifers and *Artemia*) to provide food for the larvae of a single target species.

Artificial diets

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 outlined in Table 15.1. Although cost is the major impetus for research into development of artificial diets, from a nutritional standpoint, live foods (rotifers and/or *Artemia*) are far from ideal. Artificial diets offer the opportunity to develop diets with better nutritional compositions.

[1] School of Marine Biology and Aquaculture, James Cook University, Townsville.
[2] Mariculture Research and Advisory Group, Fisheries Western Australia. (See details in appendix.)

Table 15.1. Problems Associated with the Use of Live Foods.

- **Expense**
  Live food organisms may contribute up to 50% of hatchery operating costs. Most of this cost is associated with labour.
- **Facilities**
  Live food production requires substantial commitment of space and infrastructure.
- **Nutritional inconsistency and/or deficiency**
  Live foods vary in their nutritional composition according to source, age and culture techniques. *Artemia* and rotifers lack some essential nutrients and must be enriched prior to use.
- **Availability**
  Australian hatcheries rely on a continuing supply of adequate quantities of imported *Artemia* cysts. There are also quarantine issues.
- **Disease and/or crashes**
  The introduction of disease, and live food culture 'crashes' can be major problems for mariculture hatcheries.

Artificial diets for finfish larvae must be attractive and readily ingested. They must also be stable in aqueous suspension and maintain integrity. They should be digestible, their nutrients readily assimilated by fish larvae, and of appropriate nutritional composition. One of the major potential advantages of artificial diets is the ability to adjust their nutritional composition to suit the exact requirements of target larvae. This is not possible with live foods. Two types of micro-particle have been used to present artificial diets to fish larvae; these are (a) micro-encapsulated diets (MED) and (b) micro-bound 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 may restrict leaching of water soluble dietary components and therefore reduce the attractiveness of the food particles. The capsule wall is also thought to impair digestion of the food particle and, as such, MED are considered to be of limited use for marine fish larvae. MBD, on the other hand, do not have a capsule wall; this facilitates greater digestibility and increased attractability through greater nutrient leaching. Both MED and MBD are generally dried prior to use and this may hinder their digestion.

Many studies have been conducted to assess the nutritional value of micro-particulate artificial diets for marine finfish larvae. Generally, they result in lower survival and poorer growth of larvae compared to those fed live foods. The major problems in developing artificial diets for marine finfish larvae are (a) diet attractiveness and (b) poor development of the digestive system in young fish larvae. Artificial diets need to be attractive to marine fish larvae and to be ingested at a similar rate to live prey. One of the major problems in this regard is that carnivorous fish larvae rely heavily on the visual stimulus of moving prey to initiate a capture response. The lack of movement of artificial diets in aqueous suspension is a major factor influencing their low ingestion rate. Most marine finfish larvae are poorly developed at hatching; 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. The digestive tract is fully developed only after 'metamorphosis', when the stomach with gastric glands and pyloric caeca are developed. 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 that of adult fish. Each enzyme develops independently during ontogenesis, with variations 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.

Live food organisms consumed by the larvae are thought to assist digestion by 'donating' their digestive enzymes, either by autolysis or as zymogens that activate larval endogenous digestive enzymes. 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 that enhance digestion. 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 contain 60–90% dry matter while zooplankton has only 10%).

Inclusion of digestive enzymes, especially proteases, in the diets for fish larvae has been reported to significantly improve nutrient utilisation and performance of larvae, but still not as much as larvae fed on live food. The

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 including digestive system neuropeptides to formulated diets has also been investigated in recent years. The results suggest that including bombesin may increase assimilation of diets and larval growth. However, other trials with juvenile fish have shown no effect of these additions of the neuropeptide.

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, the profitability of larval production may also be increased 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. For example, weaning of European seabass (*D. labrax*) larvae 15 days earlier has enabled savings in *Artemia* production of up to 18%. 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, odour 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 incorporating digestive enzymes into artificial food particles and by developing 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 needed to establish more specific requirements. This knowledge may be acquired by developing more attractive and digestible artificial food particles whose nutritional composition can be manipulated in nutritional studies.

(4) **Culture system design**

The settling of artificial food particles can reduce water quality and 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.

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

Industry Perspectives

16. Latest developments in low cost, low impact phytoplankton production for feeding molluscs and zooplankton for the shrimp and fish farming industry, or: How to produce it?

John Bayes[1]

Heterotrophic or Autotrophic?

Heterotrophic production, bioreactors and the like we can dismiss right away. No farmer in his right mind would start up food production for humans or his cattle in that way; so why wish it on the aquaculture industry?

Autotrophic

Just grow it! Most of the requirements are free. The light, water, CO_2, even nutrients are not expensive. The main ones are N and P, available at A$300/tonne.

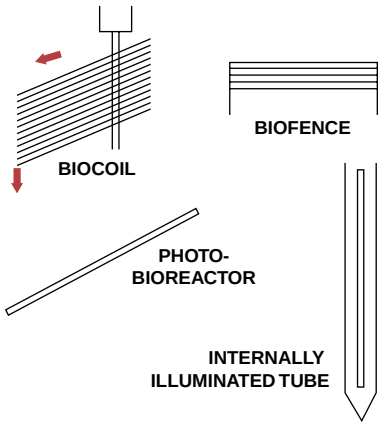


Figure 16.1 *Illustrations of a biocoil, biofence, photo-bioreactor and internally illuminated tube.*

[1] SeaSalter Shellfish (Whitstable) Ltd. (See details in appendix.)

There are four main strategies to culturing algae — what I call Contraptions, Batch system, Continuous, and Managed Ponds. The Contraptions (Figure 16.1), for want of a better word, embrace highly intensive cultures in small bodies of water. They all suffer the same shortcomings (Table 16.1). Outdoors, or in a greenhouse, they are too unstable and therefore need to be kept in an insulated and artificially illuminated room. This alone means they price themselves out of the market and should be relegated to the laboratory bench. The contest, if there is one, is between batch systems and continuous flow systems.

Table 16.1. Disadvantages of systems competing with the Seasalter Bag Systems.

Disadvantages of systems "competing" with the "Seasalter" Bag Systems .
1. Low thermal capacity and so extremely unstable.
2. Low total volume so that they can not be run continuously unless they are illuminated during the night.
3. Total No. of cells produced is a fraction of what our units produce.
4. None has a foolproof water treatment process .
5. The algae does not have long enough residence time in the system to build up optimum lipid levels
6. To produce more than one species you have to have a separate unit for each one
7. All make heavy demands on power.
8. All of them stand up, so they cost a lot of money.
9. They are conspicuous and so may require planning permission.
10. Difficult to clean.
OUTDOOR LAY FLAT SYSTEMS have zero environmental impact, and are very cheap to install.

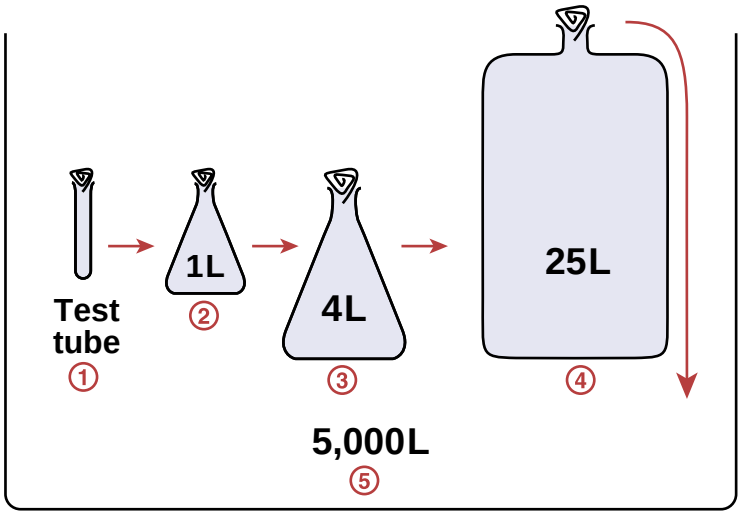


Figure 16.2. *Sketch showing volumes ranging from test tube, through 1-litre, 4-litre and 25-litre flasks, up to a 5000-litre tank.*

In Figure 16.2, set aside Stages 1–4, and look first at Stage 5. Hands up those of you who can get your batch tanks from inoculation to a useable cell count in less than 5 days. Your tank is out of action for the first 4 and when it is ready you have to use it all. The fact that batch cultures frequently crash means your algae capacity has to be 5 to 10 times that of a continuous flow system. That alone should wipe it out.

But look at this: Items 1–4 are hard work, and cost a lot to set up and run. You need test tubes, lights, shelving, air-conditioning, an autoclave and personnel — at least one person full-time to operate the system, possibly 7 days a week.

Continuous cultures can run for months, even years in some cases. The water supply is so clean that we can start a bag from a test tube if we wish, but we don't, because it would take weeks. The important thing is that the culture in the flow-through system is as good as the starter, so what do we use as a starter? An existing bag! You can do away with flasks and autoclaves completely.

We use polybags because they are cheap and disposable. No need for bin scrubbers. Throwing away a couple of kilos of polythene every few days is

probably more environmentally friendly than driving to work; and using my system will see to it that fewer people do that! The object of the exercise is to utilise, as much as possible, the available natural light — so your algae system could lie flat like a pillow (Figure 16.3). If the CO_2 is added with the incoming water and not mixed with the air, aeration is required only to stir the culture. There must be many ways to do that.

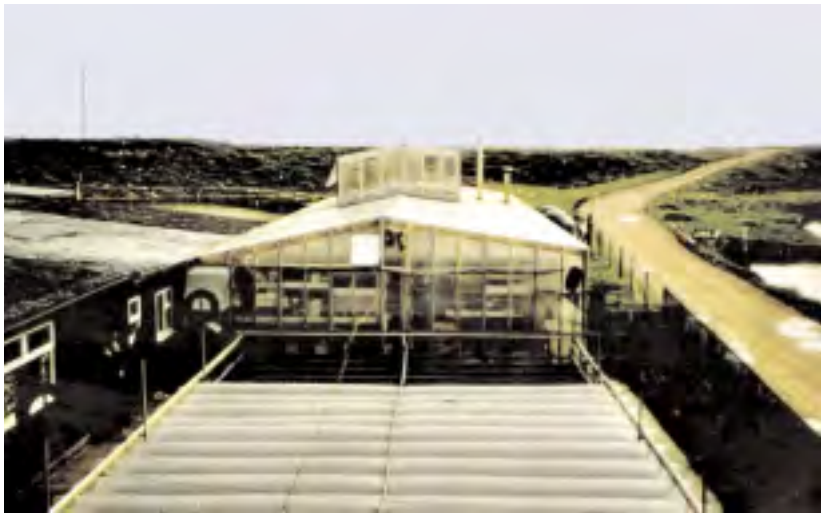


Figure 16.3. *Lay flat algal production system.*

How to set about growing microalgae

1. Overcome the low thermal capacity by having most of the water external to the culture. Float the bags in a pond if you like, and perhaps have the pond hyper-saline so that in the event of loss of air, the bag does not sink. Such an arrangement might enable the bag to be agitated, and perhaps circulated other than by aeration. This will facilitate the production of long chains of diatoms, which are easier for copepods to grasp. In summer it could be the cooling medium. Cylindrical bags floating in this way would take on a pillow shape, and it might be possible to gently rotate them, to prevent sedimentation.
2. Retain the high volume by having a greatly increased area while depth is kept to 10–20 cm. It is obvious, from the efforts around to date, that circulation has proved a bit of a problem. First we need to establish the degree, if any, to which this is necessary. If it is, find a means of doing it, perhaps by moving the bag rather than the water. Aeration itself is certainly not necessary, but we have on occasion found that the culture can be less dense at the inlet end that at the harvest end, due to lack of mixing.
3. Use natural light. Assuming that nutrients and CO2 are in plentiful supply, all cultures are light limited. Using artificial light is a waste of money. It follows that natural light is the obvious solution and yield will be a function of how much surface you can cover and the extent to which you can minimise reflection.
4. Maintain a 4–5 day retention time in the shallow bags. This should not be a problem.
5. Using the above, flexibility of the bag system remains.
6. There are no power requirements, other than for water treatment.
7. The sun shines for an average of only 4 hours a day in east Kent, and even less in Walney. Most of the time algae are supported by daylight, which is diffuse and comes as much from the north as from the south; so pointing the panels toward the sun is a waste of time and, even more importantly, of money.

Lay-flat has all the answers. Just reducing the depth will increase the cell density.

We have algae systems running in many parts of the world. Obviously, in places like Newfoundland Island and northern Norway, the only option is an insulated building; but coming a little further south to Nova Scotia, we are putting up a system in a greenhouse with a special snow-shifting polythene cover. In the UK we can grow algae outdoors most of the time, but it pays to cover the bags during the winter.

In the tropics, air temperature tends to be even. For example, the system in the Philippines is around 32^0C, so all that is required is to avoid direct sunlight. South Australian summer temperatures can be higher than 35^0C and active cooling is necessary here with shading and mist spray. Rod Grove-Jones, who runs that system, has said that he would be happy to explain it to visitors.

Zooplankton production

Turn now to zooplankton production. I have approached the subject strictly from a layman's point of view. I have no fish and so I have no requirement for zooplankton. Increasingly, our systems are used by fish farmers.

First, copepods and shrimp. I know very little about these and suspect I am not alone. Copepods crop up from time to time in my ponds, sometimes at high density. Efforts to cultivate them on our algae have been largely unsuccessful. To my eye it seems that the feeding mechanism of both shrimp and copepods is adapted to larger cells, or chains of cells, than we are currently offering, This is the field I wish to explore and a subject perhaps for further research by yourselves. *Skeletonema* and *Thalassiosira* can form long chains but not in the vigorously agitated cultures we are producing. The 'un-aerated' bags referred to earlier might produce a much better food for these crustaceans.

Rotifers are an entirely different story. Early on, I encountered some aquaculture folklore. For example, it is claimed that rotifers:

1. Die after 40 generations.
2. Should not be handled with screens.
3. Are difficult to separate from their faeces.
4. Cannot be stored in the refrigerator.
5. Very quickly metabolise and use up algae they have consumed.
6. Easily succumb to viral infestations.

Claims 1 to 4 have proved to be complete nonsense and it is beginning to look as though, suitably stored, their last algae diet may remain intact for days at least.

So far as 6 is concerned. Is there any need to expose them to virus?

My interest in zooplankton production stems from two things. One is that mollusc hatching is coming to the end of the road; we can produce far more than we can sell and, for various reasons, sales are declining. The other is that our algae production facilities are now so cheap to run that we believe we could supply significant quantities of zooplankton to the fish farming industry.

We have already crossed some bridges: Back to my 'folklore' again.

1. Rotifers reproduce about once per day. After 200 days I saw no signs whatever of deterioration in the stock, so gave up.
2. Using exactly the same procedures as we do for oyster larvae proved quite straightforward and at the same time assured against point 3 (about faeces).
3. Drained and stored in the fridge for 5 days left them all alive and ready to swim again within minutes of re-introduction to algae. The gut appeared to be full but that needs to be tested analytically before we can conclude anything about point 5.

There does seem to be a very real prospect of shipping rotifers to fish farms at any point of the globe if we can get the price right.

The rotifers can grow directly in algae from the flow-through system, which I would remind you contains only water that has been pasteurised. This means we can guarantee that it is free from any form of zooplankton or competing organisms. I would need to know more about the nature of the virus before commenting on that but there is a real possibility that it would not pass through the pasteuriser. If it did, there are other very simple pre-treatments that would eliminate it from the water before it reaches the algae culture.

There is a drawback. For the guarantees to stay in place the practice, much beloved of fish farmers for reasons of economy, of feeding rotifers on yeast, would have to go.

It is a fact that yeast is much cheaper than phytoplankton (Table 16.2). I hope in the discussion that follows to try to balance that equation.

Table 16.2. Yeast Cells Required to Produce 1.5 X 10^9 Rotifers/Day.

Yeast		Algae
Cost of yeast	$0	all inclusive $100.00
+ Water treatment	$50	
+ Labour	$25	
+ Amortisation tanks	$?	
+ Algae enrichment facility	$?	
Crash/total Loss?		0 risk.
Contingency purchase from another farm can be $10,000	0 risk.	
Viral infection?	0 risk??	

Notes:

1. Quality off-the-shelf algae substitutes are not available. If they were to come available they might cost as much as the real thing.
2. If you are going to spend $1,000,000 on a fish farm, why not include 10% for a really reliable algae unit?

Research Priorities

1. The need to establish the optimum diet for zooplankton used in aquaculture. But what species? (including types more readily captured and ingested by zooplankton); how grown? light limited? nutrient limited? which commensal bacteria?
2. We need chemical profiles that enhance the zooplankton to effectively reduce problems such as albinism in flat fish and susceptibility to disease.
3. Food storage. There is a perceived need to store algae. Vast amounts of money have already been spent on this and research continues. Why is this? It's easier to reach down a tin of beans from the shelf than to make a fresh salad — we never deep-freeze lettuces.

There simply may be no cheap substitute for the real thing. Would it not be better to consolidate research on maximising production of top quality algae from naturally available resources? My company has already gone a long way down that road.

17. *Artemia:* The turning point – Industry research priorities in a world short of Artemia

Liz Evans[1]

Artemia facts

- Less than 20% of world demand is available due to continued poor harvest from the Great Salt Lake which has come about mostly from non-conducive environmental conditions for cyst production. New sources such as Turkmenistan cannot fill the GSL gap and meet the growing demand.
- There is a rapid escalation in price which will affect all aquaculture businesses — research and commercial alike.
- There is a shift towards higher value product; shrimp and marine finfish are dominating.
- The usage period needs to be *absolutely minimised*; in all species there is a need to reduce the use of *Artemia* by feeding rotifers for longer and weaning to artificial diets sooner.
- Maximum benefit has to be derived from each hatched nauplii. *Artemia* can no longer be looked on as an inexhaustible 'bank'. Care must be taken in *all aspects* of *Artemia* use.
- Effective diets for co-feeding are available. The introduction of a dry diet of appropriate size, even at the onset of *Artemia* feeding, assists to reduce *Artemia* usage.

Deriving maximum benefit...rotifers

- Maximum benefit *must be* derived from the live feeds available.
- Use rotifers for longer periods — for up to three days longer before introducing *Artemia.*
- Efficient batch culture of rotifers is required. Over 800 million active rotifers per millilitre of water can easily be harvested every 5 days in a well-run batch rotifer culture system.

[1] Primo Aquaculture Pty Ltd. (See details in appendix.)

- Enrich rotifers — tissue enrichment to maximise benefit to larvae using either a very good quality *Nannochloropsis* and *Isochrysis* mix for 12 hours (plus), or DHA Protein Selco for 6 hours. If also using Culture Selco for the culture, the final enrichment levels are the highest possible for tissue enrichment, which is preferable to gut-only enrichment.
- Possible use of different rotifer strains like the Super Small strain rotifers from Japan which are here in Australia. There may be Australian strains that have potential?
- My experience is that there are varying levels of success in commercial hatcheries with batch rotifer culture. Help is therefore required to assist hatcheries to develop and adopt effective batch culture procedures and techniques. There are nothing like 'hands on' demonstrations by people who have practical skills in commercial-scale rotifer production to help iron out the 'glitches' in a commercial hatchery.

Deriving maximum benefit... *Artemia*

- To derive the maximum benefit from *Artemia*, hatcheries need to follow standard operating procedures — recommended guidelines for hatching and storage of *Artemia* nauplii result in a *much more efficient* use of the *Artemia* cysts.
- Recommended instructions for temperature, pH, cyst density, etc. are geared towards the premium production and storage of energy-rich Instar I *Artemia* with a large yolk reserve. Points to remember are:

    1. Hatch the required amount only, and hatch according to the instructions.
    2. Temperature is to be as constant as possible.
    3. Keep the pH constant — above 8.
    4. Aerate sufficiently to keep all cysts in suspension.
    5. Use low hatch density, e.g. 1 g/L.

- Fatten the *Artemia* nauplii through enrichment. Commercial products that can increase the biomass of *Artemia* by up to 50% and deliver nutritional value *are available*. Enrichment of *Artemia*

with HUFAs and other elements not only provides for a living capsule to transfer essential nutrients to the cultured animals, but also results in a larger and more energy-rich *Artemia*.

- Standard enrichment procedures (48 hours) on the average result in a 30% increase in *Artemia* biomass. In practice, this means that one can still feed the same amount of *Artemia* meat while hatching 30% fewer *Artemia* cysts. *Artemia* biomass can be increased by 50% when one goes beyond the present standard procedures and enriches *Artemia* for 72 hours.
- Once again, I know from experience that 'hands on' demonstrations by people who have knowledge in commercial-scale *Artemia* hatching and enrichment procedures can greatly assist hatchery operators to help iron out the bottlenecks in a commercial hatchery and improve their economic performance.

Maximum benefit also includes adopting a procedure of co-feeding

- Use co-feeding diets — Introduce a dry diet of appropriate size at the onset of *Artemia* feeding. Diets such as Lansy R1 or NRD 1/2 are especially designed for the co-feeding phase where a dry diet is introduced early. The diet should be presented in a solution and hand-fed by beaker, several times a day.
- Wean as early as possible — The use of high-quality weaning diets also assists in early weaning due to their physical and nutritional character. Weaning must be undertaken using the correct strategy which takes into consideration tank hydrology, feed demand, larval conditions and patience!
- Increase the survival of post-weaned fish through the use of appropriate diets. The degree of effective *Artemia* substitution is a function of the nutritional and physicochemical characteristics — the 'quality' — of the diets, plus zootechnical aspects in the applied culture techniques. Today, hatcheries and researchers alike should focus on making the most out of these diets in order to effectively substitute *Artemia* nauplii.
- It is important to note that the quality differences between different feed products will obviously become more significant when substituting *Artemia* at higher levels. The selection of any

nutrient product today should therefore be dominated by diet quality over any other parameter.
- Assistance is needed to develop practical feeding guides for commercially cultured species in the various regions of Australia in order to improve the fundamentals.

Examples of improvements required in two Australian commercial species dependent on *Artemia*

- Snapper
- Barramundi

The following figures are based on a survey, by Primo Aquaculture P/L, of commercial hatcheries (February 2000).

Snapper fry production per kilogram of Artemia

20 mm fry post-weaned
Best production run in Australia = 9250 fry
Average in Australia = 7900 fry
Average in Japan 1999 = 22 000
Best practice needed to achieve in 2000 in Australia = 20 000 fry

Barramundi fry produced per kilogram of Artemia

20 mm fry post-weaned in intensive system
Best run in Australia 2000 = 36 570 fry
Average run in Australia 1999 = 17 200 fry
Best practice needed to achieve in 2000 in Australia = 25 000 fry

Conclusion...

- This *is not* the time to top tune.
- This *is* the time to ensure that all commercial systems are running smoothly and achieve maximum efficiency to survive.
- Commercial products available *are a result of research* that is ongoing.
- Therefore, the immediate scientific priority for industry should be the transfer of existing applied technologies to assist the development of the industry.

Reference

Artemia Crisis… and Solutions, *INVE Aquaculture N.V.*

18. Production of Live Microalgal Feed

Rodney Grove-Jones[1]

We have been using continuous algal production systems of original UK design in our oyster hatchery for the past seven years. The system has evolved over that period and continues to do so. Our experiences with producing high quality micro algae at a production scale are quite at odds with those expressed at the workshop. We find our continuous microalgal system both reliable and relatively cheap to run. It would appear that fish and prawn hatcheries could benefit from adopting algal culture techniques developed in the mollusc hatchery industry.

Our experiences culturing microalgae as live feed for oyster larvae are quite different to those reported by the majority of speakers at the workshop. We have found algal production using the continuous pasteurisation method to be both reliable and relatively inexpensive.

Our cultures are maintained in the log phase at stable cell densities of about 3-4 million cells per ml (Iso eq) for several weeks by continuously adding small quantities of treated seawater and harvesting continuously by overflow at the same rate. Consequently, cell densities do not become so high as to be self limiting and crashes due to exceeding sustainable densities do not occur.

The use of heat instead of filtration to limit the entry of undesirable bacteria to production scale cultures is another factor that reduces the likelihood of contamination and consequent loss of cultures. The system uses the basic Pasteurian principles of killing bacteria by heating seawater and then excluding or limiting opportunities for problematic bacteria to reinfect.

[1] The South Australian Oyster Hatchery Ltd. (See details in appendix.)

Pasteurisation is extremely effective in killing the marine bacteria of concern and is also very reliable. There are no filters to fail and pass unwanted pathogens, or to monitor and clean or replace. In the event of an extended power loss or the failure of the heating element (rare), the system automatically shuts down so that unpasteurised water cannot reach the cultures.

The pipework post pasteuriser is borosilicate glass and sealed as far as the culture vessel. Glass has the advantages of being relatively inert chemically, transparent and can take steam. Live steam is used to clean the pipes regularly, and steam, unlike chlorine, insures all micro organisms are killed. Furthermore it is non toxic and leaves no residues. The culture vessels are plastic bags and are used only once (for several weeks) then discarded. New bags are effectively sterile as far as the chance of contamination with marine bacteria is concerned and no bags are cleaned or reused. This saves labour and removes another opportunity for infection. The chance of infection is further reduced by an automatic harvest and distribution (to larvae) system that eliminates the need for staff to handle the algae or culture vessels.

Once it is set up, the main operating costs to run the system are labour and power. Typically, the labour input to run a system producing up to 10,000 litres per day is less than 15 hours per week. Power requirements are 3-4 kilowatts assuming you have a water and air supply (generally present in a hatchery situation). These are a fraction of the costs commonly associated with production scale algal cultures yet these figures are reality in several mollusc hatcheries around the world.

It is clear that the finfish, prawn and other hatchery industries using fresh micro algae as feed for larvae or live feed species can improve their success with algal production by adopting existing technologies from the mollusc hatchery industry.

19. A synopsis of aquaculture in Western Australia

Adam Maskew[1]

Resources ideally suited for the development of aquaculture distinguish the State of Western Australia. It is the largest State of the Commonwealth of Australia with a coastline extending for some 13 000 km. This long coastline offers enormous potential for the development of marine and brackish water aquaculture of a great variety of commercial species. Commercial species of interest range form cold-temperate species, through warm-water and sub-tropical species to tropical species. In addition to its long coastline, WA has the largest artificial tropical freshwater lake in the Southern Hemisphere, Lake Argyle, which has a surface area of 980 km^2. These, combined with the growing need to utilise unproductive saline-affected areas, indicate that WA has the potential to develop a large and diverse aquaculture industry.

Considerable commercial interest has been expressed for the development of a marine, brackish and freshwater aquaculture industry in Western Australia. Currently, there are licenses for the culture and sale of barramundi (*Lates calcarifer*), snapper (*Pagrus auratus*), black bream (*Acanthopagrus butcheri*), WA dhufish (*Glaucosoma hebraicum*), King George whiting (*Sillaginodes punctata*), barramundi cod (*Cromileptes altivelis*), estuary cod (*Epindephelus coioides*), silver perch (*Bidyanus bidyanus*) and sooty grunter (*Hephaestus jenkinsi*). In addition to these finfish species there are licenses for the culture and sale of prawn, abalone, sea-cucumber, trochus, mussels, oysters and ornamental fish. Companies interested in all these culture species are mostly involved in research and development activities and have not reached the stage of successful commercialisation.

With most sectors of the industry being in their infancy, there are only a handful of species which are being reliably produced from hatcheries. Some sectors of the industry are developing hatchery techniques through

[1] Oceanwest Fisheries. (See details in appendix.)

technology transfer and adaptation to local conditions. The remaining sectors of the industry are transferring technology and adapting to suit their culture species, but there is a gap in available knowledge for the reliable commercial production of seed stock. The Western Australian aquaculture industry has identified a need to develop and refine hatchery production of target species.

An integral component of the hatchery production of target species is the utilisation of algae, live feeds and weaning diets. The needs of the Western Australian industry can be summarised as follows.

Algae

- The development of 'low-tech' reliable algae production methods;
- The availability of concentrate or freeze-dried algae cells; and
- Availability of larger volumes of starter algae culture.

Live food

- Development of organisms suitable as an *Artemia* replacement;
- Development of commercial-scale culture of smaller live food organisms such as copepods;
- Identification of methods for extensive larvae culture of target species; and
- Determination of nutritional value of live foods when cultured on different algae or enriched with various products.

Weaning diets

- Development of suitable diets for co-feeding of larvae; and
- Assessment and development of suitable diets for weaning of juveniles.

In addition to these needs of the industry, bench-marking for juvenile production of target species is required. This benchmark would be used to set a 'best practice' hatchery regime for the production of target species.

Aquaculture in Western Australia has the potential to become a large and diverse industry. The industry will develop only when hatchery production of target species is commercially viable. To assist the industry to develop commercially viable hatchery production of juveniles, research is required. Research is particularly required to develop and refine algae, live food and weaning diets for use in hatcheries.

20. Ocean Wave Seafoods

Antonio Mozqueira[1]

One of the newest aquaculture facilities (entering its second year of operation), Ocean Wave Seafoods is the largest multi-species marine hatchery in Victoria. Covering an area of 14.5 ha, the farm is located at Lara in the vicinity of Avalon airport, 20 km east of Geelong and only 40 minutes from the centre of Melbourne.

Initial production objectives are for 100 tonnes of abalone and 50 tonnes of finfish per annum. Although abalone is our main production species, extensive facilities have been set aside for the production of finfish and associated R&D. Current species farmed include:

- Ocean trout — Fingerling (100 g) to commercial size in 8 months (2.5 kg).
- Greenback flounder — Experimental quantities. Produced in-house. Commercial size (about 30 cm) in 2_ years.
- Black bream — Experimental quantities. Produced by MAFRI. Commercial size estimated between 16 and 18 months (350 g).

Additional species under consideration are yellowtail kingfish and snapper, as well as other species of shellfish.

Research projects have been set up under a joint venture partnership with Fisheries Victoria, Marine and Freshwater Resources Institute (MAFRI), the Zoology Department of Melbourne University and the Department of Applied Biology of the Royal Melbourne Institute of Technology (RMIT). These projects include acclimatisation and growth trials of ocean trout, abalone broodstock conditioning and black bream grow out

[1] Ocean Wave Seafoods. (See details in appendix.)

Fish production facilities include insulated, purpose-built microalgae, rotifer and *Artemia* production rooms. Initial work has followed the simplest approach where microalgae production was not used; instead, we have opted for clear-water production using the INVE model of enriching diets.

Over the coming months, microalgae production will be undertaken in anticipation of next season's finfish run.

Current subjects requiring our attention are:

- Limited availability of rotifers;
- Mal-pigmentation of flounder due to inadequate early diet;
- Faltering *Artemia* supplies; and
- Increasing efficient use of available *Artemia*.

Ocean Wave Seafoods is interested in collaborative R&D and in assisting new entrants to the industry.

21. Issues raised in general discussion at the Hatchery Feeds Workshop

Mike Rimmer[1]

The aim of this session was to capture industry needs and concerns with respect to hatchery feeds issues raised in general discussion on 10 March 2000. The following list was raised by participants.

General

- What does the aquaculture industry need with regard to live feed?
- Efficiency, reliability, quality.
- Reduce cost; maintain quality.
- Quality diets formulated for specific species.
- Need short- and long-term solutions:
- Short term: copepods, alternative live prey items;
- Long term, micro-particulate diets.
- We should aim to promote best practice by benchmarking the more cost-effective procedures for using hatchery feeds. These benchmark values should be periodically reviewed to take into account improvements.

Microalgae

- Microalgae tend to get 'bad press' because they are perceived as being difficult to grow reliably in quantity, and thus expensive. Some participants indicated that microalgae were in fact relatively easy to mass-culture, but most hatchery operators would prefer an off-the-shelf substitute.
- Australia should adopt tested technology for mass production of algae.

[1] QDPI Northern Fisheries Centre. (See details in appendix.)

- A larger suite of microalgae, and microalgal products (such as concentrated Chlorella), would be available if import restrictions were less onerous. Existing quarantine procedures restrict progress in this regard — need to involve AQIS in modifying quarantine protocols.

Rotifers

- Hatcheries would like a large rotifer, to reduce brine shrimp usage.
- Australia has the world's largest rotifer (freshwater).

Brine shrimp

- Highest priority is to overcome the brine shrimp shortage.
- Hatcheries need to improve efficiency of use of *Artemia*, to make the best of the existing supply.
- A good way to improve hatchery efficiency is to benchmark best practice
- Australian *Artemia* market is currently around four tonnes.
- Major *Artemia* users are prawn hatcheries, but they are not particularly efficient.
- The worldwide *Artemia* shortage provides opportunities for Australian industry to produce brine shrimp, possibly utilising saline water sources, to take advantage of world demand and increasing prices.
- There may be opportunities to combine desalination projects with *Artemia* production.
- *Parartemia* is expected to be commercially available in about one year.

Copepods

- Copepods may provide a replacement for brine shrimp.
- Need a production system that can be used by farmers. Current methods are too labour-intensive or still experimental.
- Need culture technique that can be used for a wide range of copepods.

Artificial feeds

- Need weaning diets to co-feed or replace *Artemia*.
- Existing artificial feeds are not as good as live feeds but economics will change this.
- Research needs to focus on diets for a limited number of species.
- Most hatchery diet work has taken place overseas, although some work has been done locally.
- Lack of local diet development reflects the limited local market for these products.
- Combination of artificial substrates and liquid diets was given as an example of an innovative approach to improving the efficiency of live feeds in prawn and finfish hatcheries.

Extension and technology transfer

- Industry feels that there is a lack of transfer of information. Technology transfer tends to be overlooked in the ongoing fight for research funding.
- There is a gap between research and technology — not enough transfer between researcher and aquaculture operators, and between operators themselves.
- Need more industry and research meetings to transfer technology.
- Hands-on workshops preferred by industry.
- On-the-ground extension work is needed to transfer research results.
- Use overseas experience to increase the range of live food and to shortcut research (for example, look at Chinese experience).
- Exchange programs targeted at industry technicians are most useful.
- Most benefit is from hands-on overseas visits by industry personnel.
- Who scales up the technology from research scale to commercial production scale?
- ost-graduate degrees are a cheap way of funding research. Industry feels that Masters degrees are 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.

Industry development and economics

- Industry would be strengthened by broadening its base.
- Existing industry sectors are seen as having less need for new feed species; whereas emerging sectors have more need for development of new or alternative hatchery feeds.
- Methods to assess the economics of new systems and/or technologies would be valuable: dedicated special business units are one option.
- Underdevelopment of our own resources (not enough research funding to go around).
- Collaborative approach, involving other funding bodies, would be more efficient.
- More research needed on 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.

Extensive production

- Provides an alternative method to reliance on traditional live feeds, such as *Artemia*.
- However, there are seasonal limitations to supply of live feed in extensive systems.
- Need to develop extensive techniques for different climatic zones.

22. Clean Seas Aquaculture Pty Ltd

Brendan Spillman[1]

Clean Seas Aquaculture is a land-based marine finfish hatchery. The company began construction of the hatchery in January 1997 and had its first production run of snapper in August 1997. Production is based on the standard rotifer, *Artemia* and particulate diet system.

Species cultured

Snapper (*Pagrus auratus*) black bream (*Acanthopagrus butcheri*)
Future projects: yellowtail kingfish (*Seriola lalandii*) mulloway (*Argyrosomus hololepidotus*).

Algae

Nanocloropsis oculata is the main species cultured due to its suitability to bulk tank culture. Other species that have been cultured in the past include Tetraselmis and Isochrysis although these species do not seem to last very long in bulk culture. Algae are used for feeding to rotifer cultures as well as larval rearing in green-water.

The recent addition of an algal laboratory, were stock cultures are maintained, has increased species availability. Cultures are up-scaled to 20-L carboys which are then used as inoculums for 2.5-t fibreglass tanks housed indoors with skylights. Water is filtered to 1 µm, chlorinated and de-chlorinated prior to addition. Fertilisation of culture water is achieved using Aquasol at a rate of 40 g/t.

[1] Clean Seas Aquaculture Pty Ltd. (See details in appendix.)

Rotifers

Rotifer culture is undertaken in two 10-t fibreglass tanks and eight 1-t poly tanks. Feed consists of algae (daily) and fresh bakers yeast (twice daily). This is supplemented with twice-daily additions of a homemade boost product consisting of fish oil, egg yolk and water. Both tank types are run on a batch system, 1-t tanks running for about 6 days and 10-t running for about 12 days before harvest and re-inoculation.

Feed rates: Algae 100–200 L/d for 1-t tanks; 200–600 L/d for 10-t tanks.
Yeast 0.2–0.4 g per million rotifers twice per day.

This system is quite reliable with cultures crashing very rarely.
Small strain rotifers cultured at 300–500 per millilitre.

Enrichment is carried out in four 200-L poly tanks with Super Selco at 10–15 g per tank, twice daily (6–12 h)

This system allows harvest and feedout of 600–700 million rotifers per day without decline in overall population.

Artemia

Currently using CIS cysts, non-decapsulated. Hatching and culture in nine 500-L fibreglass tanks. Enrichment with DC DHA Selco 12 h and/or 24 h depending on number of enrichment tanks required.

This system allows a maximum of 2.4 kg of cysts to be hatched daily. Enrichment in five tanks allows five feedouts per day. *Artemia* are fed until they are approximately 50–60 days old.

Particulate diets

NRD diets (INVE Aquaculture) are used until approximately 60–70 days old, when it is replaced by Pivot and Gulf Feeds diets. Sales of fingerlings are made in another 10–20 days.

Problems

As we have all seen in the past, wild harvests of *Artemia* cysts are unpredictable and therefore unreliable. To continue to develop various aquaculture ventures which heavily rely on this unpredictable harvest is obviously extremely risky. Commercial production of *Artemia* cysts in Australia's salt production areas or salt lakes could be a way of increasing reliability of this organism.

The development of a reliable alternative live food organism such as copepods is also an option. This would have more wide-reaching benefits, for example enabling the commercial culture of those species difficult to rear on *Artemia*. Pond culture of zooplankton, which would then be fed to fish in the hatchery, has possibilities. However, if out-of-season production is targeted, this strategy may not be viable.

However, I believe that both of these potential solutions to the *Artemia* crisis are only a short- to medium-term replacement. Ideally, we would be far better off to replace as much of the live food chain as we can. Development of an artificial replacement for *Artemia* would provide a more reliable alternative and would be likely to decrease costs due to reduced labour, floor space and equipment.

Particulate diets

We don't seem to have any problems with snapper in the early stages of culture. Our main problem remaining occurs beyond *Artemia*, when fingerlings are also weaned from imported to locally manufactured diets. At this time we experience problems with fish becoming thin and dying. Fingerlings transferred to sea cages do very well. However, fed the same diet in the hatchery they waste away. Is this due to weaning too late or a problem with diet composition, digestibility etc.? Are they getting something from wild prey in cages which helps in the digestion of artificial food?

What is the minimum size for weaning off *Artemia*?

Improvement in availability of quality, locally manufactured, particulate diets specifically formulated for target species will lower weaning size.

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