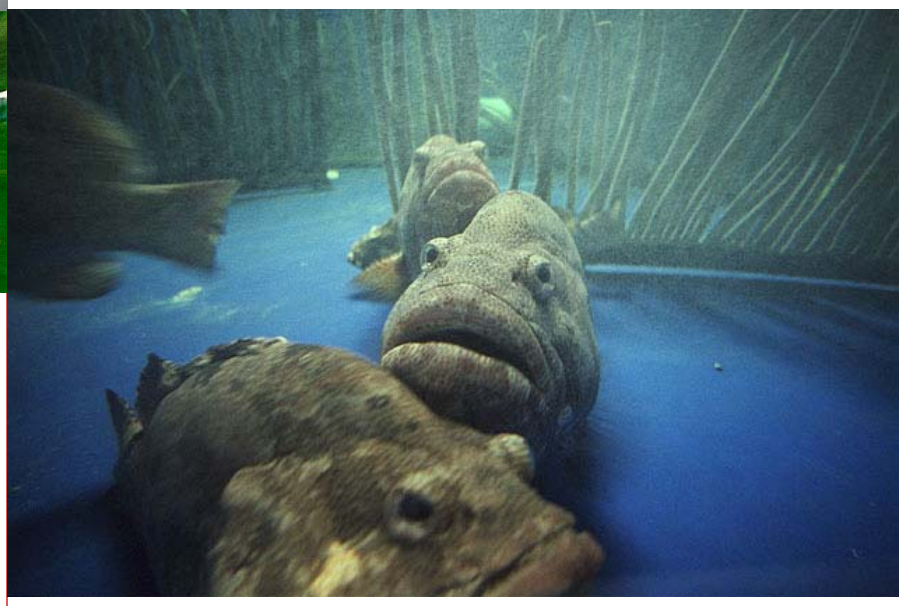
Reef Fish Aquaculture R&D Project

Annual Report 2003–2004



Queensland Government
Department of Primary Industries and Fisheries

Executive Summary

The Reef Fish Aquaculture R&D Project is based at Northern Fisheries Centre, Cairns, and provides a core platform for a suite of R&D projects addressing the development of aquaculture technologies for high-value marine finfish species. A Feasibility Study undertaken in 1996 indicated that the development of a reef fish aquaculture industry in Queensland would provide significant economic benefits to the state.

The project comprises three major core components, plus a number of associated projects:

Broodstock management and captive spawning

Three reef fish species are held at NFC – these species were selected to provide opportunities to interact closely with overseas laboratories that are working on the same species, and for their application in a reef fish aquaculture industry based in Queensland.

Flowery cod *Epinephelus fuscoguttatus*

Research undertaken up to and including the 2003–2004 season indicated that captive flowery cod have a short seasonal (summer) reproductive season. Fish held in ambient conditions do not spawn. Environmental control of reproduction (temperature and photoperiod) supports reproductive development and results in successful spawning events. The reliability and predictability of spawning events needs to be improved. Photoperiod control alone does not appear to impact reproductive development or spawning. Condensed photothermal regimes were unsuccessful in inducing a higher frequency of spawning events.

One problem with broodstock of this species is that males are aggressive and only one male can be held in each tank. This places heavy reliance on the reproductive performance of this single male.

Future research will focus on improving the predictability and reliability of spawning in flowery cod. Rapid changes in photoperiod ('spikes') will be trialed to induce spawning in reproductively mature fish. Given the limited response of this species to environmental control, more emphasis will be given to social interactions, including tank population changes to induce spawning and increasing the number of females per tank to extend spawning events.

Estuary cod *Epinephelus coioides*

Spawning in estuary cod does not appear to be strongly regulated by seasonal (temperature, photoperiod) cues. Spawning occurs over an extended period on both increasing and decreasing temperature and photoperiod phases of controlled photo-thermal regimes. There is significant variation in reproductive and spawning response of different populations to the same cues. However, constant temperature and photoperiod does not stimulate reproductive development.

Molecular tools were developed to assess levels of the insulin-like growth factors (IGFs) as measures of egg quality in marine finfish, including estuary cod.

Future research with estuary cod will attempt to further define environmental control regimes to provide reproductive maturity in captive broodstock populations.

Barramundi cod *Cromileptes altivelis*

Research on barramundi cod during 2003–2004 developed essential baseline data to assess the effectiveness of future hormonal manipulation by characterising their seasonal reproductive development. A number of hormonal treatments were developed to regulate and improve reproductive development, including 'slow release' hormonal treatments to successfully regulate

sex change in captive broodstock. The research also demonstrated differences in steroid regulation between the sexes.

In the short term, future research will focus on further clarification to refine hormonal treatment to control reproduction outputs.

Live prey production and research

Research in live prey has a focus to improve general efficiency of live prey production and to develop food for marine finfish larvae, in particular groupers[1]. Effort has been focused to:

- Improve nutritional value of rotifers
- Reduce scale-up time and increase production of rotifers
- Increase production of copepods (including AIDI)

Improve nutritional value of rotifers

Marine finfish larvae have specific dietary requirements, particularly during the early stages of development. Levels of specific highly unsaturated fatty acids (HUFAs) are critical, as are ratios of specific HUFAs. This research activity focussed on improving the nutritional value of rotifers (*Brachionus rotundiformis*) fed to early larval stages of marine finfish, with emphasis on fatty acid nutrition. A number of commercially available fatty acid enrichment products were trialed and analysed to assess their suitability for use with reef fish species. A modified 1 × 12 h enrichment protocol has been developed for use at NFC based on these results. This regime provides a consistently high level of the essential fatty acids DHA and EPA and a DHA:EPA ratio of about 4:1.

Reduce scale-up time and increase production of rotifers

Super-small (SS-strain) rotifers are cultured and fed to early larval stages of marine finfish. SS-strain rotifers have previously been isolated and cultured at NFC, and this research activity focussed on increasing production and decreasing production cost. The INVE medium-density culture system using Culture Selco 3000 was demonstrated to be effective in providing rotifer culture densities of 1500 – 1800/mL after 3–4 days of culture. The use of Culture Selco 3000 will reduce the reliance on algal cultures and the time required to scale-up rotifer cultures during spawning events. Supplementary use of oxygen for the rotifer cultures is required to support these densities.

Future research will evaluate other options for increasing the cost-effectiveness of rotifer culture by using commercially available microalgal concentrates instead of laboratory-cultured microalgae to support rotifer culture.

Increase production of copepods (including AIDI)

While rotifers provide an effective early diet for many marine finfish larvae, they are unsuitable as a sole diet for many tropical species. Larvae of groupers and snappers (Lutjanidae) have been shown to survive better and grow faster when fed copepods. A major component of the reef fish aquaculture project's live prey research has been focussed on developing culture techniques for copepods to support improved larval survival and growth.

Research during the 2003–2004 season evaluated microalgal species to replace *Rhodomonas*, which is an essential component in the diet of the tropical copepod *Acartia sinjiensis*. However,

[1] 'Grouper' is a term in common use outside Australia, which refers to members of the Subfamily Epinephelinae, Family Serranidae. There is no comparable term used in Australia, where individual species are known as 'cod': barramundi cod, estuary cod, flowery cod, etc. or coral trout.

Rhodomonas is difficult to culture and commercial hatcheries have indicated that it would be too unreliable for commercial use. An alternative Cryptomonad (CSIRO isolate CS-412) was evaluated and was found to be as nutritious for *A. sinjiensis* as *Rhodomonas* when fed on an equal ration (ash free dry weight basis).

Increasing copepod production by scaling up of existing methods used in 400 L tanks to 1200 L tanks resulted in 83% of the production per unit volume. With modifications to harvesting equipment, this percentage is likely to approach 100%. To achieve a production rate to supply the NFC experimental hatchery with 2 nauplii/mL (12 × 280 L larval rearing tanks), it is recommended to increase the copepod culture system to 2 × 5000 L tanks. This would require approximately 150 L of mature Cryptomonad culture per day as feed. The capacity to produce algal species required for the copepod diet, in particular Cryptomonad sp. CS-412 and *Isochrysis* sp. (T. ISO) exists within the current facility.

Another approach to providing large quantities of early stage copepod nauplii during the early stages of reef fish larval rearing is to collect and refrigerate copepod eggs over a time period of days or weeks, then hatch them simultaneously to provide large quantities of nauplii for feeding to fish larvae. Hatching of *A. sinjiensis* eggs was effectively (at least 90%) inhibited for 72 h at storage temperatures ≤ 10°C. Eggs and N1-stage nauplii could be cold-stored at 10°C for 72 h with no development or loss of viability. Storage at cooler temperatures (4°C) resulted in the rapid loss of viability of eggs. It is recommended to incorporate cold-storage techniques into the management of the upgraded copepod production (2 × 5000 L) facility. With an expected production of 5×10^6 nauplii/day/tank and the adoption of cold storage, up to 20×10^6 nauplii per 5000 L tank could be available for the crucial first feeding stage of finfish larvae.

Larval rearing

Larval rearing of reef fish remains problematic. Our research during the 2003–2004 season indicates that there is substantial variation between cohorts of larvae (i.e. separate spawns) which means that experimental work has to be replicated across cohorts in addition to the usual within-experiment replication. We found poor and inconsistent survival of both flowery cod and estuary cod to D4 / D5, and this impeded all larval rearing trials during the 2003–2004 season.

However, limited results indicate that survival did improve with decreasing light intensity for both flowery cod and estuary cod. Larvae have poor feeding ability at first feeding with low ingestion rates of rotifers. With the improvements in rotifer and copepod production outlined above, future work will incorporate feeding of both rotifers and copepods to assess their impacts on growth and survival of larvae.

Grow-out

In response to industry-expressed concerns regarding the likely availability of grow-out sites for reef fish aquaculture, a workshop was held to evaluate the priorities for development of reef fish grow-out systems. The workshop concluded that there was greater likelihood of some production systems being less contentious than others, and that these production systems should be the focus of ongoing research and industry development activities. In particular, sea cages within the GBRMP are likely to be highly contentious issues and are likely to face widespread community opposition, particularly from environmental lobbying groups. Based on this perspective, it is appropriate to focus reef fish aquaculture development on production systems and areas that are likely to have a greater probability of acceptance.

Associated projects

Improved hatchery and grow-out technology for grouper aquaculture in the Asia-Pacific region (ACIAR project FIS/97/73)

This project (completed December 2003) has successfully improved hatchery survival of reef fish from around 3% to 30% for estuary cod (Philippines) and around 5% to 40–50% for barramundi cod (Indonesia). Research results from this project have been incorporated into the Reef Fish Aquaculture R&D Project.

In addition, the research outcomes from this project have supported the development of commercial grow-out diets in Indonesia and the Philippines. The development of a collaborative network of researchers and industry in the Asia-Pacific region has been successful in supporting information dissemination and improving collaboration and research coordination in the region.
Outcomes from the project have provided direct benefits to Queensland through training of Queensland hatchery staff in rearing techniques for reef fish.

Improved hatchery and grow-out technology for marine finfish aquaculture in the Asia-Pacific region (ACIAR project FIS/2002/077)
This is the follow-on project to FIS/97/73, and commenced in July 2004. This project will again focus on developing hatchery and grow-out technologies for high-value marine finfish species, including coral trout (*Plectropomus leopardus*).

Economic and Market Analysis of the Live Reef Fish Food Trade in the Asia-Pacific (ACIAR project ADP/2003/022)
This project, which commenced in July 2004, will evaluate and model supply and demand in the live reef food fish trade, including the likely impacts of aquaculture production on market demand and price. The project will evaluate the impact on consumer perspectives of using pellet diets for grow-out of reef fish.

Minimizing environmental effects of finfish grow-out cages in Indonesia and Australia (ACIAR project FIS/2003/027)
This project, which commences in January 2005, will evaluate the environmental impacts of marine finfish cage aquaculture, particularly nutrient outputs. A component will support coastal planning for aquaculture in Indonesia based on the carrying capacity of coastal environments.

Table of Contents

Authors

Compiled by
Mike Rimmer

Broodstock management and captive spawning
Elizabeth Cox, Julian O'Brien, Robin DeVries, Joseph Sariman, Adam Reynolds

Live prey production and research
Richard Knuckey, Gale Semmens, Provan Crump, Damien McKeering

Larval rearing
Elizabeth Cox, Peter Fry, Anjanette Johnson

Grow-out, associated projects
Mike Rimmer

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Background

The Queensland Government is investing heavily in Reef Fish Aquaculture research and development. The project originated when it became apparent that the trade in export of live coral reef fish from Queensland to Hong Kong was highly lucrative; for example, very high value species such as barramundi cod (*Cromileptes altivelis*) wholesale in Hong Kong for US$70 /kg (whole live product) (McGilvray and Chan 2001).

In 1995–96 DPI&F undertook a feasibility study to assess opportunities to develop an aquaculture sector to take advantage of this market. The Reef Fish Aquaculture Feasibility Study comprised the following separate studies:

1. Market Analysis
2. Domestic Reef Fishing Industry Assessment
3. R&D Case Studies
4. Infrastructure Requirements
5. Grow-out Site Identification and Evaluation
6. R&D Requirements and Costing
7. Benefit-Cost Analysis
8. Financial Evaluation

The study concluded that:

- There is a small, well established, rapidly expanding market in Hong Kong, and southern China.
- The existing live reef fishery is unlikely to expand significantly, leaving aquaculture as an option for supplying the predicted increased demand[2].
- Development of a reef fish aquaculture industry would require a long-term government research program, which could be expected to last at least 10 years and cost in the order of $14 m.
- Risk would be reduced by investigating several species simultaneously, and by dividing the program into 3 phases.
- A cost-benefit study indicated that the resultant industry would provide a benefit:cost ratio of the order of 16.6:1.
- Financial studies showed that the resultant industry would be profitable, and that every $1 invested would realise $2.30 – $4.00 benefits.

Based on the feasibility study, the Queensland Government provided funding under several Aquaculture New Initiatives to fund the development of technologies for reef fish aquaculture. This research and development (R&D) program has focussed on the development of production technology for reef fish fingerlings, because this is the main constraint to the development of this sector in Queensland.

The project is based at Northern Fisheries Centre (NFC), Cairns. The construction and commissioning of the new Aquaculture and Stock Enhancement Facility at NFC has provided substantially increased infrastructure capacity for aquaculture R&D, particularly for the Reef Fish Aquaculture R&D Project and associated projects.

[2] Since this report was published, supply from wild fishery in Queensland has further contracted due to reductions in effort and catch in the Queensland Coral Reef Line Fishery.

Introduction

The Reef Fish Aquaculture Research and Development (R&D) Project provides a core platform for the development of aquaculture technologies for high-value marine finfish. The project is organised into three major area of R&D which reflect the major areas of production technology for marine finfish:

1. Broodstock – provision of fertilised eggs from captive broodfish.
2. Larval rearing – production of fingerlings from fertilised eggs.
3. Live prey – production of live prey organisms to provide food for the developing larvae.

These core areas of research address the major bottleneck to the development of sustainable aquaculture of reef fish: the production of fingerlings for grow-out.

Using this core platform, the project has also attracted significant external funding to support related areas of R&D:

- Improved hatchery and grow-out technology for grouper aquaculture in the Asia-Pacific region (ACIAR project FIS/97/73), and its successor:
- Improved hatchery and grow-out technology for marine finfish aquaculture in the Asia-Pacific region (ACIAR project FIS/2002/077).

These projects focus on improving production technology for high-value marine finfish through R&D on hatchery and grow-out technologies.

The Reef Fish Aquaculture R&D Project is also strongly linked with other related projects, including:

- Environmental impacts of marine cage aquaculture in Australia and Indonesia (ACIAR project FIS/2003/027).
- Economic and market analysis of the live reef fish food trade in the Asia-Pacific (ACIAR project ADP/2003/022).

The interaction of these projects provides a basis for a coordinated R&D effort towards sustainable reef fish aquaculture in Queensland, covering most of the essential aspects of industry development and support:

- Fingerling production technology
- Grow-out technologies, particularly diet development
- Environmental impacts of cage aquaculture
- Assessment of product quality and market impacts
- Evaluation of supply and demand and potential market responses

This report focuses on the outcomes of the core (Queensland Government funded) project, the Reef Fish Aquaculture R&D Project for the financial year 2003–2004. Summary reports of related projects are also included (from p. 46) to provide an overview of the entire R&D effort.

Species

The Reef Fish Aquaculture R&D Project is working with three reef fish (grouper) species:

Barramundi cod *Cromileptes altivelis*

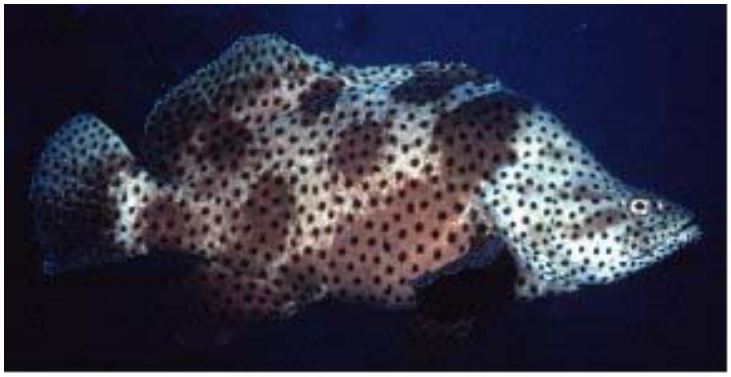


Photo: GBRMPA

Cromileptes altivelis is found through the Western Pacific from southern Japan to Palau, Guam, New Caledonia, and southern Queensland (Australia); and in the eastern Indian Ocean from the Nicobars to Broome, Western Australia. It is a high-value species in the live reef fish trade, bringing up to US$70 per kg wholesale. Juvenile fish are in demand as aquarium inhabitants. Commonly known as humpback grouper or polkadot grouper, it is known as barramundi cod in Australia, kerapu tikus or kerapu bebek in Indonesia, and señorita in the Philippines.

Because barramundi cod does not adapt well to environments subject to physicochemical perturbations it is not well suited to inshore coastal aquaculture. In Australia, this species is likely to support relatively small-scale grow-out in recirculating aquaculture systems. This species is relatively slow growing, taking 1.5 – 2 years to reach market size.

Flowery cod *Epinephelus fuscoguttatus*

Photo: GBRMPA

Epinephelus fuscoguttatus is widely distributed in the Indo-Pacific region, including the Red Sea, and occurs at most (probably all) of the tropical islands of the Indian and west-central Pacific oceans (east to Samoa and the Phoenix Islands) along the east coast of Africa to Mozambique, and it has also been reported from Madagascar, India, Thailand, Indonesia, the tropical coast of Australia, Japan, Philippines, New Guinea, and New Caledonia. It is a medium-priced species in the live reef fish trade and juveniles are in demand by farmers in Southeast Asia because this

species survives well and grows rapidly in culture. Widely known as tiger grouper, this species is called flowery cod in Australia, kerapu macan in Indonesia, and lapu-lapu in the Philippines.

Flowery cod is a fast-growing species that will reach market size (around 500 g) in 9–12 months. It is relatively hardy and a good candidate for large-scale cage aquaculture, or pond aquaculture where adequate salinities can be maintained throughout the year.

Estuary cod *Epinephelus coioides*

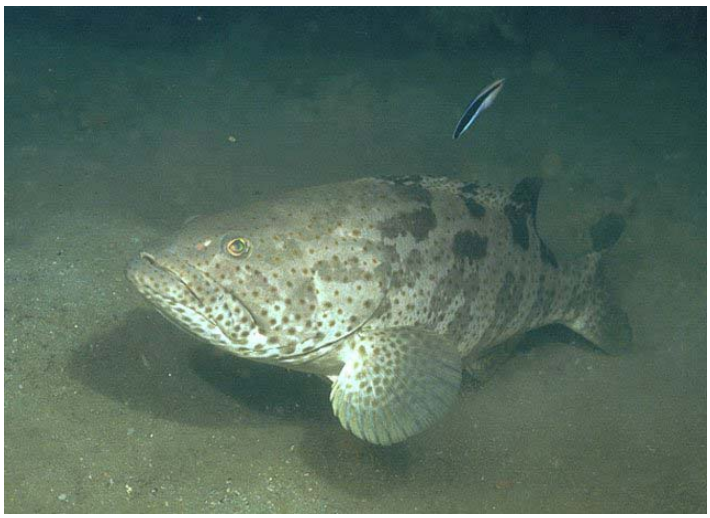


Photo: David Cook

Epinephelus coioides is a mainstay of the live reef food fish trade and is now widely cultured throughout Southeast Asia. It is found from the Red Sea south to at least Durban and east to the western Pacific, where it ranges from the Ryukyu Islands to Australia and eastwards to Palau and Fiji. Other localities include the Persian Gulf, India, Reunion, Mauritius, Andaman Islands, Singapore, Hong Kong, Taiwan and the Philippines and it has been reported from the Mediterranean coast of Israel. This species is frequently misidentified in the aquaculture literature as *E. tauvina* or *E. malabaricus* and is sometimes incorrectly named *E. suillis* (a synonym). *E. coioides* is widely known as green grouper, estuary cod in Australia, kerapu lumpur in Indonesia, and lapu-lapu in the Philippines.

Estuary cod grows rapidly in culture and reaches market size (around 500 g) in 9–12 months. It is a hardy species and can tolerate substantial variations in water quality, reflecting its estuarine habitat in the wild. Consequently, it is an ideal candidate for coastal pond aquaculture in tropical Queensland.

Benefits to Queensland

The Reef Fish Aquaculture R&D Project aligns directly with the DPI&F vision of *profitable primary industries for Queensland*, and makes a tangible contribution to the department's mission to *maximise the economic potential of Queensland primary industries on a sustainable basis*. The development of reef fish aquaculture will provide an important new primary industry sector that will provide direct and indirect economic benefits to rural Queensland. As noted above, the Reef Fish Aquaculture Feasibility Study indicated that the development of a reef fish aquaculture industry in Queensland was likely to be highly profitable.

The Reef Fish Aquaculture R&D Project directly contributes to the realisation of DPI&F Key Strategies (as outlined in the Strategic Plan 2004–09):

Increase productivity
Invest in R&D that will result in innovative products, technologies and practices that will maximise growth, value-adding and productivity.

- ✓ The Reef Fish Aquaculture R&D Project is utilising world-class research, as well as developing new and innovative techniques, to develop sustainable production technologies for reef fish aquaculture:
  - control of broodfish reproduction through environmental and hormonal manipulation;
  - production of new live prey organisms to support hatchery production of high-value marine finfish species;
  - assessment of the nutritional requirements of reef fish larvae, and development of methods to improve larval nutrition;
  - development of high-sensitivity enzyme analysis techniques to evaluate the capacity of marine fish larvae to digest different prey types;
  - development of grow-out diets for reef fish aquaculture.

  All of these technologies are essential for developing a profitable and sustainable aquaculture industry sector.

- ✓ The Reef Fish Aquaculture R&D Project is facilitating the development of strategic partnerships with major research funding agencies and leading research institutes:
  - Postgraduate students at James Cook University are developing improved mechanisms to control reproduction in reef fish.
  - Collaborative research between DPI&F and the Australian Institute for Marine Science is developing improved live prey organisms to support reef fish aquaculture.
  - The Australian Centre for International Agricultural Research (ACIAR) has funded three major research projects to support the development of sustainable aquaculture of high-value marine finfish in the Asia-Pacific region. The Reef Fish Aquaculture R&D Project provides the core platform for the ACIAR research program.
  - The Reef Fish Aquaculture R&D Project provides a core platform for the Asia-Pacific Marine Finfish Aquaculture Network, facilitated by the Network of Aquaculture Centres in Asia-Pacific (NACA).

Facilitate enterprise access to sources of skill development, knowledge and recognised training across the sector

- ✓ Technologies and skills developed under the Reef Fish Aquaculture R&D Project are transferred to industry via work placements, information workshops and formal training courses. As the technology for reef fish aquaculture production improves, training and skills development opportunities will expand.
- ✓ Aquaculture is largely undertaken in rural and regional areas of Queensland. The development of reef fish aquaculture will provide development and employment opportunities in regional Queensland, including Cape York and Torres Strait areas.

Develop future growth industries

- ✓ Reef fish aquaculture is recognised as a future growth industry. Aquaculture of high-value marine finfish is a sector growing rapidly in the Asia-Pacific region and Queensland is well placed to take advantage of this growth sector.
- ✓ The Reef Fish Aquaculture Feasibility Study indicated that the development of a reef fish aquaculture industry sector in Queensland would make a major economic contribution to the state.

Develop markets

Invest in R&D and management arrangements to develop solutions tailored to identified market needs and trade opportunities.

- ✓ Reef fish aquaculture will provide significant export market development for Queensland, because the primary markets are the high-value live fish markets of Hong Kong and southern China.
- ✓ The development of marine finfish aquaculture will provide additional employment opportunities in regional Queensland. Currently, the Queensland finfish aquaculture sector employs one full-time position per 15 tonnes production (not including casual labour).
- ✓ The development of reef fish aquaculture will strengthen the economy by providing opportunities for support services, such as equipment suppliers, marketing specialists, etc. to expand.

Results 2003–2004

Broodstock management and captive spawning

Objectives

The broodstock component objectives for 2003–2004 were:

1. Develop environmental control regimes to provide reliable spawning of reef fish (flowery cod and estuary cod).
2. Resolve difficulties in barramundi cod male reproductive behaviour including failure to fertilise and sex reversion of males back to females (AIDI).

Flowery cod

Introduction

Previous research at NFC has shown that flowery cod have a short reproductive season under natural, ambient conditions. Artificially controlled, condensed photo-thermal regimes have been used since 1999 to control reproduction and spawning of captive brood fish. Using these artificially controlled temperature and photoperiod regimes, it has been possible to both extend and phase shift the reproductive season of this species. However, whilst environmental control has been shown to consistently induce reproductive development, spawning (or release and fertilisation of eggs) does not always follow. Further work is needed to identify additional cues that will induce predictable spawning. This is an important aspect in developing reproductive technologies that can be transferred to developing industry proponents.

This trial examined whether temperature is a key factor in the control of reproduction and spawning or whether photoperiod or the combination of photoperiod and temperature is required.

Methodology

Environmental control regimes

Two 60m³ tanks and one 30m³ tank were each stocked with one male and three to five females. Both 60m³ tanks were exposed to the same 120-day condensed photoperiod cycle. Tank 1 was also exposed to a controlled temperature cycle and tank 2 was exposed to natural ambient temperatures. Therefore, temperature in the second tank did not follow increasing and decreasing photoperiod as in Tank 1. It was anticipated that simultaneous spawning in both tanks would indicate that temperature was not a key, controlling factor.

The 30m³ (control) tank was exposed to natural ambient photoperiod and temperature. These fish were monitored for seasonal reproductive development under ambient captive conditions between May 2003 and March 2004. When spawning had not occurred by March, all fish were relocated to tanks with environmental control.

Assessment of reproductive development

The behaviour and spawning response of both populations was monitored over a 12-month period during which the condensed regime was run over three consecutive cycles. Flowery cod do not appear to develop an external opening to the oviduct until spawning making assessment of reproductive development difficult. Nassau grouper (*Epinephelus striatus*) are also reported as not having an external opening to the oviduct until just prior to spawning, making it very difficult to take ovarian biopsies without causing injury and infection (Tucker *et al.* 1991). This study used regular sampling periods to assess both fish weight and body depth, which changes as females

approach spawning. Males were checked for the presence of milt. These measurements in conjunction with fish behaviour are used to assess reproductive status and predict spawning.

Potential spawning cues

During this trial there were two events that indicated additional potential spawning cues. Firstly, at the commencement of the photoperiod regime in May 2003, there was an accidental overnight increase in photoperiod. Secondly, in March 2004, there was a relocation of reproductively developed females that had not yet spawned, into the only tank fitted with both temperature and photoperiod control.

Results and Discussion

Response to environmental control

As seen in previous years of study with this species at NFC, reproductive development did occur over summer in the brood fish held under ambient conditions, however spawning did not follow. This appears to be a typical response for this species held under ambient captive conditions where reproductive maturation occurs once a year with resorption of oocytes occurring at the end of the summer period.

Variable responses were observed between the two populations exposed to artificial environmental control. Those fish exposed only to photoperiod control did not spawn and not all females developed reproductively. Whilst this may be due to the non-synchronised temperatures, these fish were newer acquisitions that had not been held in captivity for long and had not previously spawned. Assessment of the male in this tank at the end of the trial indicated that it was not in good physical condition, which may have impacted the performance of the females.

The fish exposed to both temperature and photoperiod control developed reproductively on the increasing temperature and photoperiod phase of each cycle (Fig. 1). However, spawning response of this population did vary and appeared to be responsive to several cues. Firstly, at the commencement of the trial, an accidental overnight increase in photoperiod resulted in spawning two weeks later in mid June 2003. Spawning did not then occur as expected on the increasing temperature and photoperiod phase of the first cycle but did occur on the second and third increasing cycle phase (Fig. 1). Additionally, a relocation of females between the second and third cycle (increasing the tank population from three to eight females) resulted in five spawning sequences, each of 5–6 nights duration, over a period of two months.

This was also the first instance of spawning occurring during a decreasing temperature and photoperiod phase suggesting that the population change and/or the increase in total number of fish was a strong enough cue to override the decreasing environmental regime.

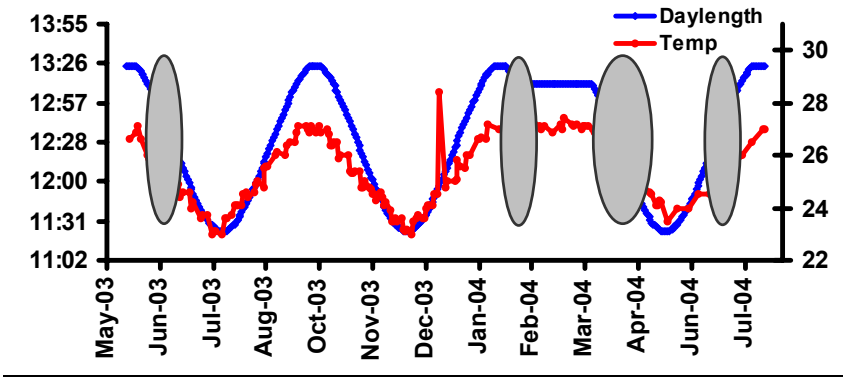


Figure 1 Spawning incidence of flowery cod in response to a repeated 120-day condensed photothermal regime. Filled circles indicate spawning periods.

Lunar spawning pattern
Collation of all spawning data from January to June 2004 indicated that most spawning occurs during the full moon phase (from the first to last quarter) with the least number of spawning events occurring during the new moon phase (Fig. 2).

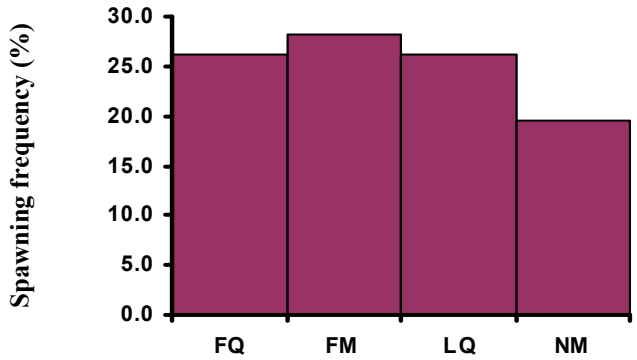


Figure 2. Spawning frequency of flowery cod during 2004 relative to lunar phase. NM = new moon, FQ = first quarter, FM = full moon, LQ = last quarter.

Sex change
To date, sex change has been infrequent and has not impacted on the maintenance of spawning populations. Population sex ratios have remained fairly stable and neither female nor male fish are difficult to source from the wild.

Behaviour
Flowery cod display highly distinctive pre-spawning behaviour for a period of several weeks prior to spawning. Competition between females to pair with the male can lead to sporadic aggression and it is common to see some scale loss and teeth marks on the flanks and fins during spawning periods. The removal of smaller, less dominant females is not usually necessary.

Captive breeding populations
Only one male per breeding population can be held in a 30 to 60m^3 capacity tank. The introduction of additional males leads to physical aggression that results in the death of the less dominant male if it is not removed from the tank.

Summary – Flowery cod

Research to date indicates that:
- Only one male can be held per breeding population due to aggression between multiple males.
- Captive flowery cod have a short seasonal (summer) reproductive season.
- Reproductive development of flowery cod held under ambient captive conditions does not result in spawning.
- Fish exposed to only photoperiod control have not spawned.
- Exposure to condensed temperature and photoperiod regimes does control reproductive development and induce spawning.
- Spawning patterns indicate a lunar rhythm with most spawnings occurring around the full moon.

This suggests that until other cues are identified, photo-thermal control is essential in order to both control and extend seasonal reproduction for this species. Condensed photo-thermal cycles have been shown to consistently induce reproductive development in flowery cod even when phase

shifted into the winter months. Whilst this technique has significant potential in providing controlled and extended spawning periods for industry production, the identification of additional cues to deliver reliable spawning requires further assessment. Currently, cues identified for assessment to be used in conjunction with condensed photo-thermal cycles include:

- photoperiod spikes,
- population changes to stimulate spawning,
- increased population size to extend spawning.

Estuary cod

Introduction

Research into reproduction and spawning of estuary cod began in late 2002. The first objective was to establish whether reproductive development and spawning of this species was seasonally cued as observed for the flowery cod. This would then allow an environmental regime to be developed to increase frequency of, and control over spawning periodicity.

Secondly, the effect of constant summer temperature and photoperiod on reproductive development could be tested. The potential of short duration stimulus such as temperature to induce spawning could then be examined.

Methodology

In order to assess spawning seasonality of captive estuary cod exposed to a controlled environment, one male and five to six female estuary cod were held in each of two 30m³ tanks that were concurrently exposed to a 360-day simulated photo-thermal regime. At the end of this trial, fish in both tanks were then held on a constant summer temperature and photoperiod regime for six months. Regular sampling (ovarian biopsy, weight, total length), behavioural observations and spawning frequency were recorded during these periods.

Results and Discussion

Spawning seasonality

The performance of estuary cod exposed to a 360-day photo-thermal regime indicated that spawning was not cued to a short seasonal response. However, spawning frequency did increase during summer months. In addition, the spawning response from each population varied greatly. Fish in the first tank spawned for a short period and displayed more seasonal reproductive development, from October to December 2003. In the second tank, spawning occurred over an extended period from June through to January (Fig. 3).

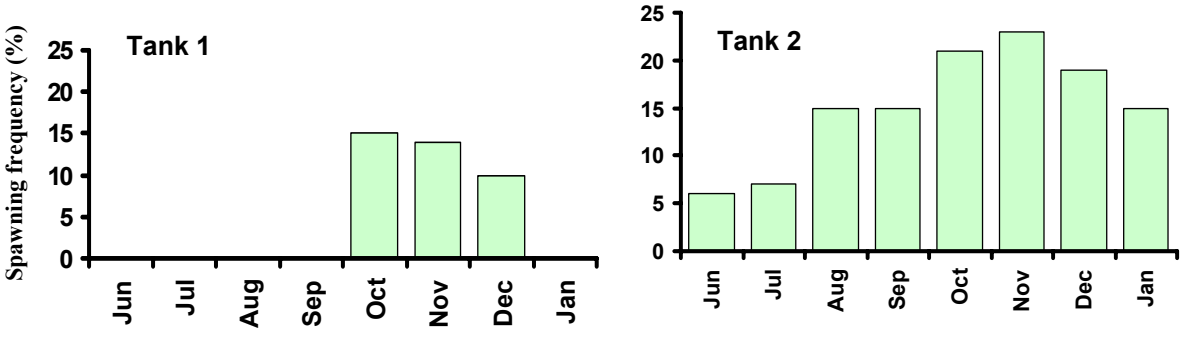


Figure 3 Spawning frequency of estuary cod exposed to a 360-day photo-thermal regime.

Spawning events did not appear to be strongly linked to lunar periodicity. From 167 nights of spawning during 2003, 24% of spawning events occurred during the last quarter to new moon and new moon to first quarter phase, 25% during the first quarter phase to full moon phase and 27% during the full moon to last quarter phase (Fig. 4).

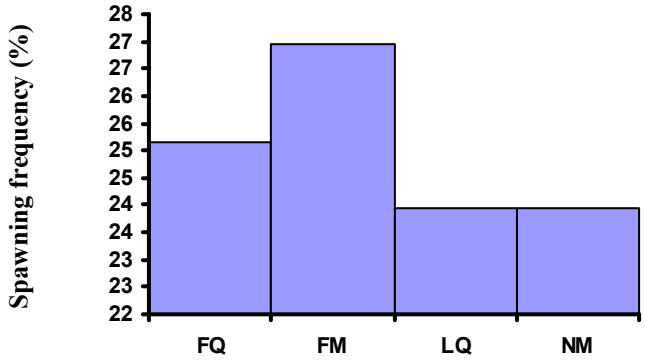


Figure 4. Spawning frequency of estuary cod during 2003 relative to lunar phase. NM = new moon, FQ = first quarter, FM = full moon, LQ = last quarter.

Spawning behaviour

Spawning behaviour involves the male driving the female in tight clockwise circles during ascent to the surface where gamete release occurs. Similar behaviour has been described for *E. akaara* (Okumura *et al.* 2002). Spawning of estuary cod in this study also results in highly variable fertilisation rates that are often very poor although the hatch rates are quite high (>90%). Both direct and video observations of spawning activity have identified multiple attempts at spawning ascents, many of which break off mid water column. When gamete release has been observed, it is as the pair breaks the water surface perhaps indicating that the water depth is inadequate. This apparent requirement of a rapid ascent of a pair to coordinate gamete release has also been observed for *E. marginatus* in the wild (Zabala *et al.* 1997), and *E. akaara* in captivity (Okumura *et al.* 2002).

Summary – Estuary cod

As observed with the flowery cod, substantial population variation in response to concurrent exposure to the same cues has been observed. Variations in behaviour, spawning frequency and fertilisation are common.

- Spawning duration does not appear to be strongly regulated by seasonal cues, but spawning frequency increases over the summer period.
- Spawning occurs over an extended period on both increasing and decreasing temperature and photoperiod phases of controlled photo-thermal regimes.
- There is substantial variation in reproductive and spawning response of different populations to the same cues.
- Constant temperature and photoperiod does not stimulate reproductive development.

Barramundi cod

Introduction

A major constraint to the culture of barramundi cod has been the inconsistency of broodstock reproductive performance. In particular there is a shortage in the supply of mature males due to spontaneous sex reversal of males to females, and the failure of large females to sex change into

males. As with other grouper, barramundi cod are hermaphroditic, starting life as female before maturing as a male at a later stage. Limited understanding of regulatory processors, which control sex change in hermaphroditic species, has meant an inability to control captive spawning in these species. Research into this species at NFC is aimed at improving the performance of captive barramundi cod broodstock, particularly males, and controlling sex change and reversion in captive populations.

Results and Discussion

Reproductive Development

Initially studying the annual reproductive physiology of captive barramundi cod broodstock provided a baseline from which the effects of hormonal manipulations could be compared. A seasonal trend in reproductive development was present in both sexes. Females typically showed a rested state in the winter months (June-August), with only immature oocytes (eggs) dominating ovarian tissue. Seasonal responses in reproductive development of females correlated oocyte maturation to increasing temperature and day-length. Reproductive development peaked over the summer months (December-February), with multiple oocyte stages present as is typical for a sequence spawning species. Steroid levels correlated with the seasonal development of oocytes and an early surge in testosterone during spring, resulted in ovarian activation and tissue development. Subsequent elevation in estradiol coincided with the uptake of yolk by oocytes (vitellogenesis), a process triggered by the livers production of yolk protein in response to estradiol levels. Males demonstrated a longer period of reproductive maturity, with the presence of milting (sperm) males as early as late October and extending through to March. However, no consistent release of milt by any one male was demonstrated during the peak-spawning season. Steroid levels in males also showed a response to seasonal changes, with a significant rise in testosterone and 11 ketotestosterone during the spawning period. In contrast to the females, male steroid levels were highly variable and representative of the variable milt production. In comparison females and males demonstrated distinct steroid profiles during reproductive development, in particular the levels of estradiol, which were present in significantly higher levels in females (Fig. 5).

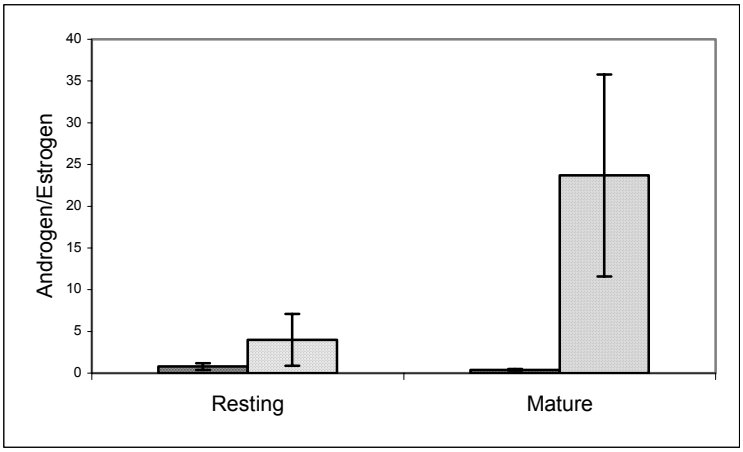


Figure 5 Androgen to estrogen ratio for resting (non-reproductive) and mature (reproductive) barramundi cod broodstock.

Sex change

The incidence of sex change, from male to female (reversion) or female to male (inversion), uniformly showed a reduction in all key sex steroids during a transitional stage. Sex reversal occurred in 45% of male fish and shifted the female sex ratio from 1:1 in 2003 to nearing 1:3 by the end of the study period. The highest frequency of sex reversal during this study occurred in the post-spawning period. In contrast, sex inversion occurred only twice, and in both instances fish were sex-reversed males, which only developed early stage (rested) ovaries.

Hormonal manipulation

Use of a masculinizing steroid (methyltestosterone) demonstrated the successful application of this technique to sex invert females into functioning males. Treated females reduced estradiol synthesis, developing mature testis and production of motile sperm 35 days post treatment.

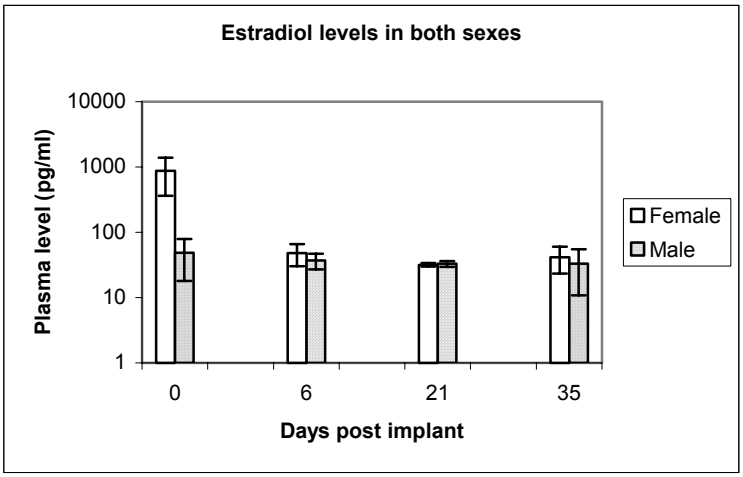


Figure 6 Circulating 17β-estradiol levels in male and female barramundi cod post-implantation with methyltestosterone cholesterol pellets (250 μg/kg body weight).

The stage of reproductive cycle at which the application of masculinizing steroids to females is administered is critical. The administration of methyltestosterone at the same dose in females during gonadal recrudescence required repeated application, in contrast to the single dose in post spawning females. The application of hormonal treatment in pre-spawning females requires further investigation.

Application of slow release implants to induce reproductive behaviour in broodstock demonstrated a positive response to this technique. Application of luteinizing hormone release-hormone over a 3-month period during peak reproductive activity produced a consistent response in female egg release over the new moon phase. Female egg release response culminated with the average release of 0.67 million eggs over a 5 to 7 day period starting between 2 nights prior to, or on the night of, the new moon. While males failed to fertilise eggs released, both sexes showed a positive response in key reproductive indices (sex steroid levels). In addition male milt production was noticeably more consistent than in untreated males or previous baseline male data.

Live prey production and research

Objectives

The live prey component objectives for 2003–2004 were:
1. Improve nutritional value of rotifers.
2. Reduce scale-up time and increase production of rotifers.
3. Increase production of copepods.

Note: Objective 3 includes research funded under the Aquaculture Industry Development Initiative (AIDI).

Improve nutritional value of rotifers

Introduction

Rotifers enriched with polyunsaturated fatty acids (PUFAs) increase survival of finfish larvae. However, high rotifer mortality can occur when using commercial enrichment products according to the manufacturers' recommended dose rates. The recommended enrichment procedures are generic and generally developed for L-strain rotifers cultured under temperate conditions. The high water temperatures in tropical aquaculture, coupled with the high levels of nutrients supplied during enrichment, encourage bacterial growth. This can result in the rapid and severe depletion of dissolved oxygen (DO) with the subsequent death of large numbers of rotifers. Aeration using diffusers and compressed air may not supply enough oxygen to cultures during the crucial period following addition of the enrichment product. To maintain the oxygen levels recommended during enrichment, additional oxygen could be supplied from a pure oxygen source.

The objective of this series of experiments was to improve the nutritional value of SS-strain rotifers (*Brachionus rotundiformis*) by developing enrichment procedures that:
- sustain rotifers during enrichment and maintain a high rate of survival
- produce rotifers with high levels of essential PUFAs
- produce rotifers with ratios of PUFAs desirable for marine finfish larvae
- produce enriched rotifers with a predictable fatty acid profile.

Two commercial enrichment products were assessed:
1. Aquafauna Bio-Marine's Algamac 2000 is a spray dried formulation based on the DHA-rich alga/fungus *Schizochytrium*, and
2. INVE's DHA Protein Selco, is a dry enrichment formulation boosted in DHA with high protein content to improve rotifer survival during enrichment.

Methodology

The experimental tank arrangement used for these experiments is shown graphically in Figure 7. Experimental conditions are summarised below:

Rotifer density	500 or 1000/mL
Tank volume	200 L
Temperature	28 to 30°C
Salinity	28 to 30 ppt

Water treatment	Chlorinate / neutralise seawater (chlorine=0.15 mL/L, thiosulfate (1M)=0.28 mL/L)
DO	Not controlled for treatments with aeration only 6–10 ppm for treatments with addition of oxygen
Replicates	3 replicates per treatment. Replicates run non-concurrently.

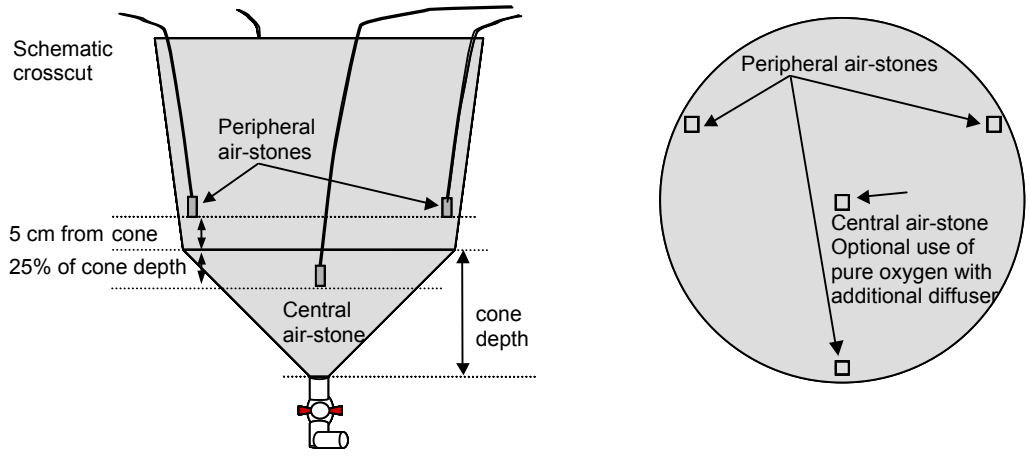


Figure 7 Experimental set-up for rotifer enrichment tanks used in these experiments. When enriching with the addition of pure oxygen, an oxygen diffuser is positioned at the base of the tank below the central air-stone.

Enrichment procedures

Algamac-2000

Preparation and use

1. Hydrate dry Algamac-2000 powder (up to 30 g/L) in freshwater and mix in a blender for one minute.
2. Strain off any excess surface foam.

Transfer harvested, washed rotifers to the enrichment tank filled with treated filtered seawater. Rotifer density is recommended to not exceed 500/mL. Add the prepared Algamac-2000 mixture to the enrichment tank at a rate of 300 mg/10^6 rotifers. Harvest after 12 hours.

DHA Protein Selco

Preparation and use

1. Hydrate dry DHA Protein Selco powder (up to 50 g/L) in freshwater by mixing in a blender for 3 to 5 minutes.
2. Strain off any excess surface foam.

Feed the product in 2 rations, at enrichment start and 4 hours later. If the daily feed rations are stored in the refrigerator, remix before each addition. Harvest after 8 hours. Rotifers can be enriched at densities of 500/mL or 1000/mL (Table 1).

Table 1 Enrichment protocol for DHA Protein Selco at rotifer densities of 500 or 1000/mL.

Feeding Regime		DHA Protein Selco-Separate Enrichment Tank			
Activity	Hours	Rots/mL	ppm	g/10^6 Rots	Comments
Start T0	0	500 1000	125 175	0.25 0.18	= 25 g DHA Selco = 36 g DHA Selco
T4	4	500 1000	125 175	0.25 0.18	= 25 g DHA Selco = 36 g DHA Selco
Harvest	8				

Results

The enrichment tank design is a highly aerated system with 4 large airstones. However, even with this level of aeration the DO level fell in all aeration only, enrichment treatments (Fig.s 8 and 9). Provision of additional oxygen (O_2) kept DO levels in a safe region between 6–10 ppm.

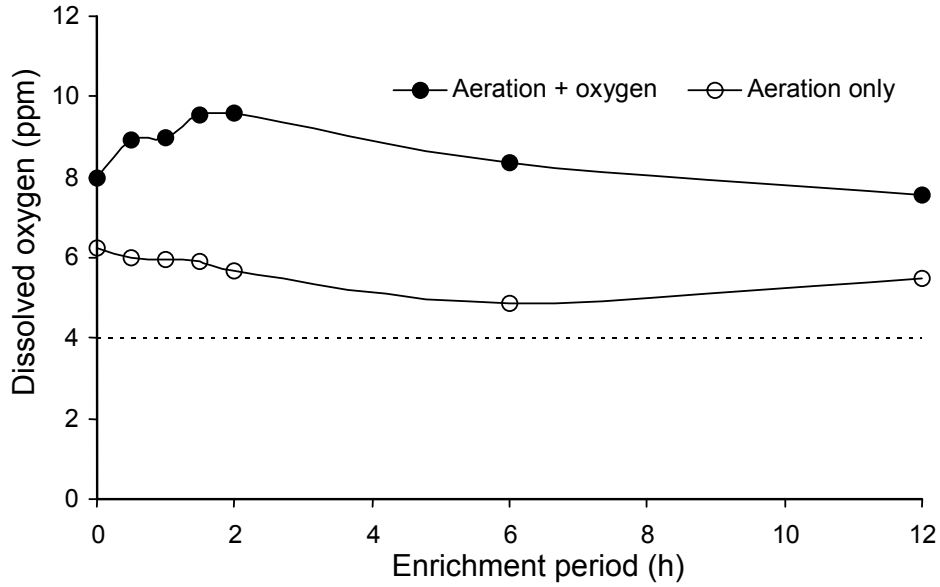


Figure 8 Dissolved oxygen levels in Algamac 2000 enrichment tanks containing rotifers at 500/mL. Single addition of Algamac at T=0. Results are average of 3 replicates. Dotted line is the minimum recommended DO level (4 ppm).

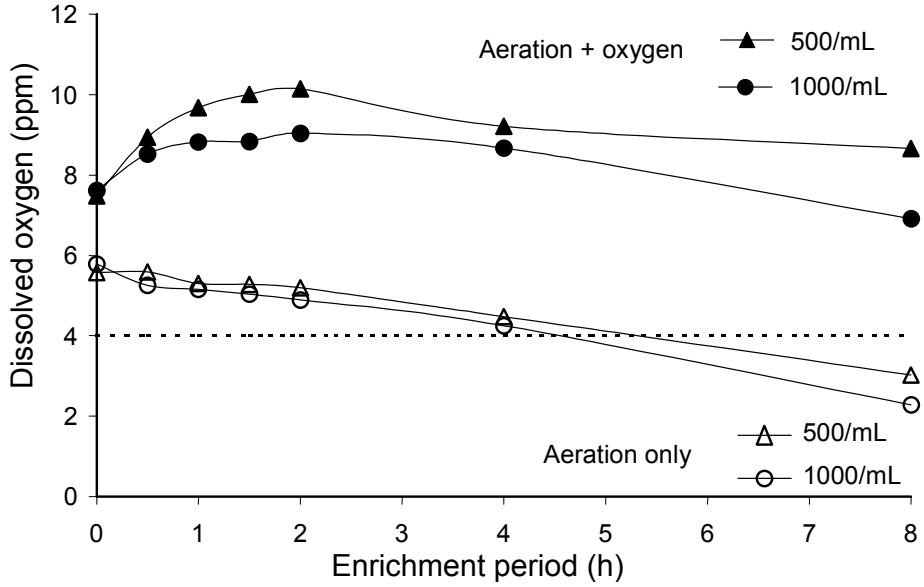


Figure 9 Dissolved oxygen levels in DHA Protein Selco enrichment tanks containing rotifers at 500 and 1000/mL. Two additions, T=0 and T=4. Results are average of 3 replicates. Dotted line is the minimum recommended DO level (4 ppm).

For aeration only enrichments, the DO level was minimal after 6 hours for Algamac-2000 where it approached the critical 4 ppm level before recovering to be about 6 ppm at time of harvest (12 h). However, for DHA Protein Selco the DO level fell below 4 ppm after the second enrichment product addition at T = 4h and was lower for the higher rotifer density.

Survival of rotifers during enrichment was generally good for all treatments (Table 2). It was best for rotifers enriched with Algamac where rotifer density increased while percentage mortality remained low. In comparison, rotifers enriched with DHA Protein Selco decreased in density and percentage mortality increased. In all treatments, addition of O_2 was beneficial to both rotifer density and percentage mortality. Percentage fecundity was unaffected by addition of O_2 but was dependent on enrichment product with DHA Protein Selco enriched rotifers being more fecund after enrichment.

Table 2 Effect of enrichment product (with and without additional O_2) on rotifers at densities up to 1000/mL.

		Change in (variable) over enrichment period		
		Rotifer density	% fecundity	% mortality
Algamac 2000	Aeration only	+ 8%	21% to 3%	2% to 6%
(500 rotifers/mL)	Aeration + O_2	+ 11%	20% to 4%	2% to 7%
DHA Protein Selco	Aeration only	- 8%	13% to 9%	2% to 17%
(500 rotifers/mL)	Aeration + O_2	- 3%	14% to 11%	3% to 12%
DHA Protein Selco	Aeration only	- 16%	17% to 7%	4% to 10%
(1000 rotifers/mL)	Aeration + O_2	- 10%	17% to 7%	4% to 8%

Rotifers enriched with Algamac 2000 (500/mL with O_2) and DHA Protein Selco (1000/mL with O_2) were analysed for their fatty acid profile. Of the PUFA's there are several known to be essential and these are long-chain, highly unsaturated fatty acids (HUFAs). The omega 3 (n-3) HUFAs docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) and the omega-6 (n-6) arachidonic acid (ARA) are considered to be essential fatty acids for many marine finfish. Rotifers enriched with either product had increased levels of essential HUFAs. The final composition of the rotifers reflected the fatty acid profile of the enrichment product with Algamac-2000 enriched rotifers having a higher percentage (% w/w of total fatty acids) of DHA and the omega-6 docosapentaenoic acid (DPA). Rotifers enriched with DHA Protein Selco had the highest level of EPA (Fig. 10).

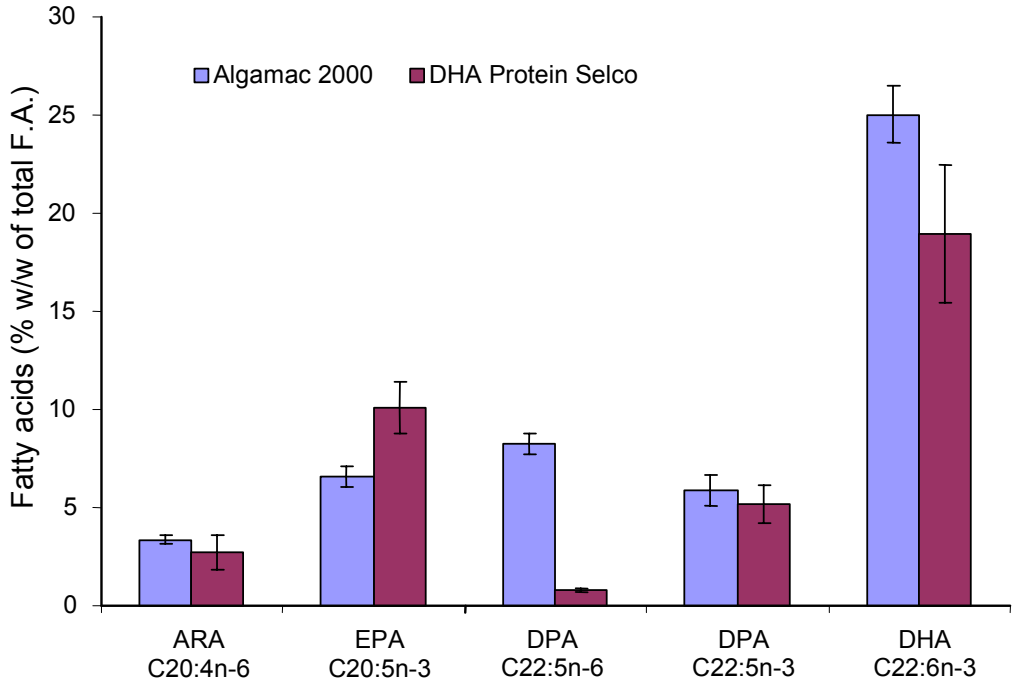


Figure 10 HUFA (% w/w of total fatty acids) content of rotifers enriched with Algamac 2000 and DHA Protein Selco. Error bars are ± SD, n = 3.

For marine finfish, not only the amount of essential HUFA is important but also their ratios. A high ratio (at least 2:1) of DHA:EPA is considered most beneficial. Rotifers enriched with Algamac 2000 had a DHA:EPA double that of those enriched with DHA Protein Selco (Table 3).

Table 3 Ratios of fatty acids and total fat of rotifers enriched with Algamac 2000 or DHA Protein Selco.

	DHA:EPA	DHA:EPA:ARA	Total fat (g/100g)
Algamac 2000	4:1	7:2:1	1.73 ± 0.21
DHA Protein Selco	2:1	7:4:1	2.37 ± 0.32

The total percentage of lipid in rotifers enriched with DHA Protein Selco was on average 37% greater than those enriched with Algamac 2000. This difference does not affect ratios but when taken into account with percentage fatty acid data (Fig. 10) it means that the overall quantity of EPA per rotifer was double in those enriched with DHA Protein Selco while total quantities of DHA, ARA and ΣHUFA were the same for rotifers enriched with either product.

Outcomes

- The set up of the rotifer tank to include a high level of aeration, the position of aeration and a bottom dead-volume has improved rotifer survival even without the use of additional O_2. Inclusion of O_2 has maximised survival particularly at higher rotifer densities. The use of tank design with additional O_2 is recommended for all enrichment procedures.
- Both enrichment products boosted levels of PUFA and in particular essential HUFAs. Although the Algamac product states that a second 12-h enrichment is possible to further boost HUFA levels, our results indicate that without additional O_2, DO levels would fall below the critical level of 4 ppm. Also, the low fecundity level of rotifers after the initial 12-h enrichment indicates that a significant loss of rotifers could occur during a second 12-h enrichment.
- Both enrichment products produced rotifers with a DHA:EPA ratio of at least 2:1. However, Algamac 2000 produced rotifers with a DHA:EPA ratio of 4:1 and it is likely that the higher DHA:EPA ratio is preferable for marine finfish larvae.
- Analysis of the variation in fatty acid enrichment between the 3 replicate runs for each treatment shows that rotifers enriched with Algamac-2000 has a more predictable (lower variation) enriched fatty acid profile.

Recommendations

- Adoption of illustrated enrichment tank design.
- Use of additional O_2 for all enrichment procedures.
- Use of Algamac-2000 for routine enrichment of SS-strain rotifers cultured at NFC.

Enhanced production of SS-strain rotifers

Introduction

Currently at NFC, SS-strain rotifers (*Brachionus rotundiformis*) are raised on the microalga *Nannochloropsis oculata*. To increase production, microalgae is supplemented with bakers yeast. However, continual production of large volumes of algal cultures is labour intensive and rotifer production can collapse if algal cultures fail. Also, when fish spawning events are unpredictable there is a long scale-up period if mass cultures of algae are not continually maintained, just in case of a spawning event.

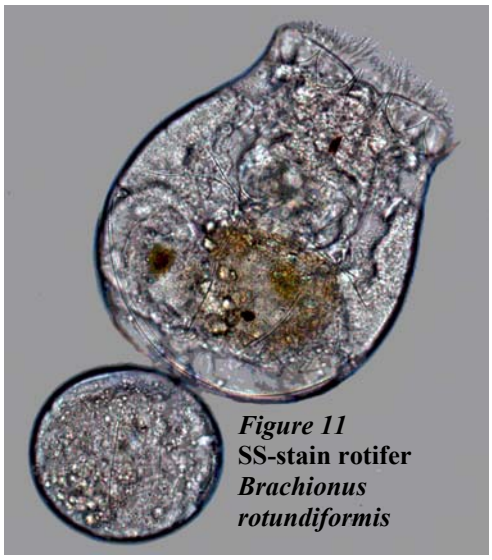


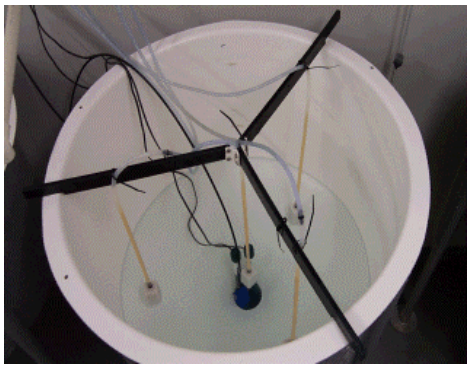
Figure 11
SS-stain rotifer *Brachionus rotundiformis*

To reduce scale-up time, the reliance on algal cultures and to increase our rotifer production capacity we evaluated the commercial INVE formulated diet, Culture Selco 3000. The product is designed for high-density rotifer culture and as a partial replacement for algae. Rotifers can be cultured solely on the diet for up to 10 cycles then a new stock of rotifers raised on algae is required. This is necessary to maintain productivity and to reduce numbers of contaminating ciliates.

The experimental work to reduce scale-up time and increase production of rotifers involved the construction and commissioning of a 4 × 200 L rotifer culture system based on the use of Culture Selco 3000 with a maximum production capacity of 360 x 10^6 rotifers/day. This level of production is over twice the current requirement of the NFC experimental hatchery.

Methodology

Rotifer culture tanks were set up as shown previously for the enrichment tanks (Fig. 7) with an oxygen diffuser always included below the central airstone (Fig. 12).



Rotifer density	500/mL (initial)
Tank volume	200 L
Temperature	28 to 30°C
Salinity	28 to 30 ppt
Light	24 h
Water treatment	1 µm filtration + UV Chlorinate/neutralise
DO	> 4 ppm

Figure 12 Rotifer tank set up for use with Culture Selco 3000 containing three peripheral and one central airstone with a bottom oxygen diffuser and heaters.

Culture Selco 3000 may be used for low, medium or high-density rotifer culture (Table 4). We assessed medium-density culture, as it was the most appropriate

intensity for achieving our desired rotifer production. For medium-density culture in a 200 L tank volume, 100 million rotifers are required. These rotifers need to be of high quality with at least 20% fecundity and free of significant protozoan contamination.

Table 4 Feed protocol for Culture Selco 3000 at low, medium and high-density rotifer culture. Background feed must be supplied at time T=0. Days 1–4 are projected rotifer densities with recommended feed rates.

	Low density			Medium density			High density		
	Background feed 80 ppm			Background feed 100 ppm			Background feed 140 ppm		
Day	Rotifers/mL	g /10^6 rotifers	ppm	Rotifers/mL	g /10^6 rotifers	ppm	Rotifers/mL	g /10^6 rotifers	ppm
0	250	0.7	175	500	0.7	350	1000	0.55	550
1	340	0.55	195	675	0.5	335	1300	0.4	520
2	590	0.45	265	1180	0.5	590	2000	0.4	800
3	850	0.35	300	1500	0.5	750	2600	0.4	1000
4	1050			1800			3200		

Results

Four experimental replicates and two production runs were completed (Fig. 13). The first 2 replicates achieved a final rotifer density of ~800/mL. Although this was higher than the typical density achieved with algae and yeast (500/mL), it was well below that predicted for the product (1800/mL). In these first two replicates, protozoan contamination was high and their numbers increased dramatically following the addition of the Culture Selco 3000. In replicates 3 and 4, intensive initial washing of rotifers greatly reduced protozoan contamination. Rotifer growth in these replicates more closely matched that predicted for the product, exceeding the projected 1800/mL to reach ~2500/mL.

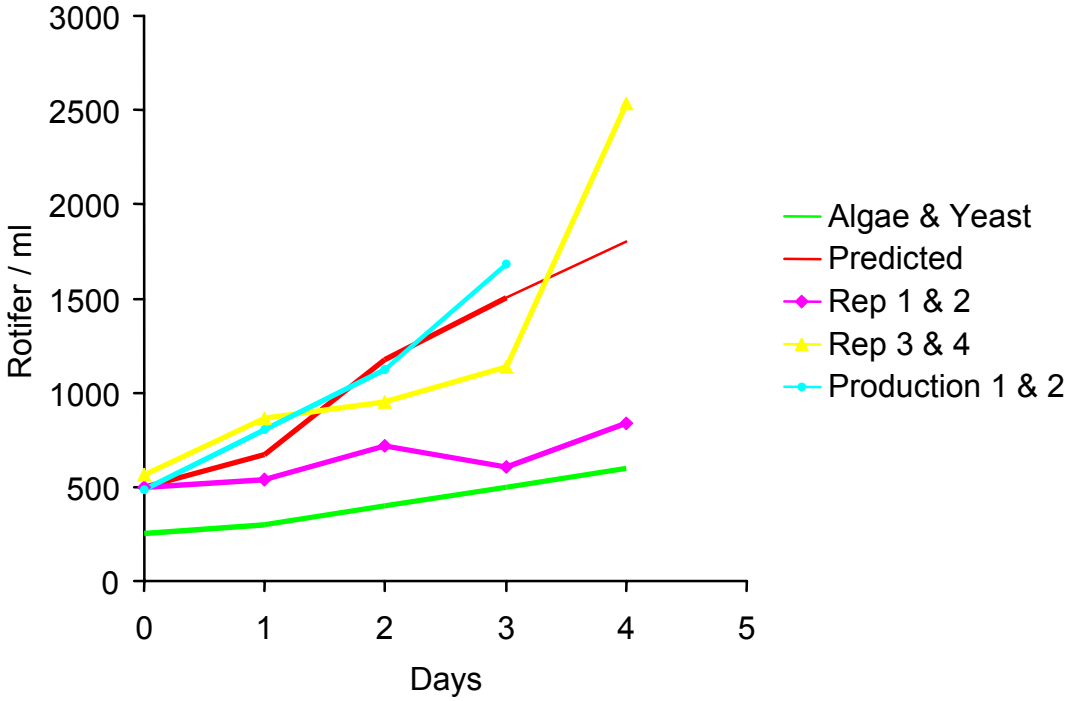


Figure 13 Rotifer production using Culture Selco 3000 in 4 replicates and 2 production runs. For comparison, the predicted production and that achieved using algae and yeast is included.

The Culture Selco 3000 system was put into practice during a production run to supply rotifers for fish larvae. Three tanks were run: two were inoculated at 500 rotifers/mL (medium density) and the third at 250 rotifers/mL (low density). Production from the medium-density cultures exceeded the predicted level and achieved almost 1700 rotifers/mL after day 3 where they were harvested for enrichment (Fig. 13). Production from the low-density culture was 600 rotifers/mL by day 4, well below the estimated density of 1050/mL.

Outcomes

Based on our results, 4 × 200 L rotifer culture tanks using the medium-density culture regime would be predicted to produce 360 x 10^6 rotifers/day. Our results have shown that such a system could produce at least 500 x 10^6 rotifers/day and that the desired production could be achieved after day 3 rather than (as predicted) day 4. This would mean that 3 rather than 4 operational tanks would be required.

The higher than predicted production may have occurred because the recommended feed rates apply for rotifers regardless of strain and this would provide a greater feed quantity for the smaller SS-strain rotifers. It is likely that the feed rate could be reduced for culturing SS-strain rotifers. This would have the benefits of:

- reducing production costs,
- increasing culture stability by maintaining higher water quality,
- reducing the capacity of protozoa to proliferate.

The low-density rotifer culture performed worse than expected. This may indicate that this density is too low for SS-strain rotifers and it takes too long for the rotifer

biomass to condition the culture water. This may give contaminants an opportunity to establish or for water quality to deteriorate and adversely effect rotifer productivity.

Recommendations

- The medium-density, Culture Selco 3000 system to be adopted as a method to reduce labour required to produce rotifers and to increase the capacity of the rotifer culture facility at NFC.
- Feed rates should be fine tuned for SS-strain rotifers by gradually reducing feed levels.

Increased production of copepods

(Note: includes research activities funded under the Aquaculture Industry Development Initiative)

Introduction

Copepod production is limited when compared to other live foods such as rotifers or *Artemia*. This limitation is a result of copepod biology where high-density culture is not natural. This may be due to diet restrictions, cannibalism and the physical damage they sustain from repeated collisions when very active copepods are held at high density.

To increase production of copepod nauplii for use as first feed for grouper larvae, we evaluated:

- diet optimisation
- scale-up of existing culture technology
- cold storage of eggs and nauplii
- alternative species of copepod to *Acartia sinjiensis* (Fig. 14).

Figure 14 Select developmental stages of the calanoid copepod, *Acartia sinjiensis*.

Diet development

A series of previous experiments has demonstrated the cryptophyte, *Rhodomonas* sp. to be an excellent mono-algal diet for the growth and development of *A. sinjiensis*. It supports significantly faster growth and development of both nauplii and copepodid stages than other commonly mariculture microalgae species. However, *Rhodomonas* is more difficult to grow than these microalgae. *Rhodomonas* cultures are prone to crashing so a replacement species was sourced. A related cryptophyte, Cryptomonad (CS 412) was chosen. It has the advantages of being readily obtainable from the CSIRO Collection of Living Microalgae, it's an indigenous species (Fitzroy Is.) and has proven stable in culture.

Methodology

Copepods

Acartia eggs produced over 24 hours were collected, rinsed with fresh water to kill any hatched nauplii and the eggs incubated for 2 hours at 28°C. Nauplii (N1) that hatched during this period were used as the inoculum for the experiment. Thirty nauplii were isolated using a wide-bore 1 mL pipette and put into each 1 L Schott bottles containing 500 mL of seawater (28°C, 30 ppt).

Algal concentration varies between species because the algal sizes vary. The ash-free-dry-weight (AFDW) of the algae are *Rhodomonas* 72 pg/cell, Cryptomonad 33 pg/cell. Calculated feed volumes were added to each bottle and volumes made up to 1 L.

Experimental apparatus and environmental conditions

- The experiments were undertaken in a controlled environment room (28 ± 2°C) using the plankton wheel that was set to hold up to 16 × 1L Schott bottles. The wheel revolved at 0.5 rpm.
- T=0 was at ~2–3 pm and the experiments ran for 72 hours.
- Copepods were fed at T=0, 24 and 48 h. At T=24 and 48 h, 90% of culture volume was slowly removed using a siphon with a 53 µm intake filter to screen out copepods.
- Volumes of algal cultures required to provide the correct cell concentration were calculated after converting the culture absorbance (678 nm) reading to a cell count (cells/mL) using. For low cell concentration treatments that required only small volumes of algae, the culture was diluted 1:10.

Samples and analysis

- At completion of each experiment, all surviving copepods were collected and preserved in formalin. Each copepod was counted (survival data), photographed and measured and a developmental index assigned (Fig. 15). Development scores were averaged for each treatment.

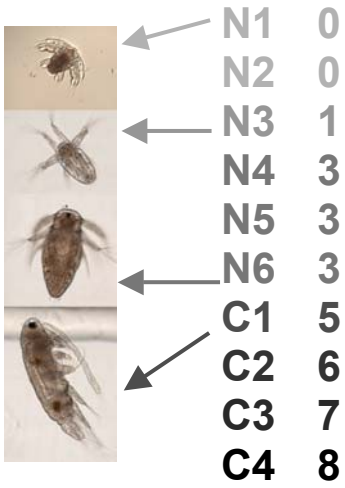


Figure 15 Images (to scale) of selected developmental stages of *A. sinjiensis* nauplii and copepodids with assigned score for each stage.

Results

When copepods were fed *Rhodomonas* or Cryptomonad on an equal AFDW basis, there was no significant difference in copepod growth or development rate for all feed levels (Fig. 16). Copepods developed rapidly and were in good condition when fed either alga.

Combining data for *Rhodomonas* and Cryptomonad from this experiment with *Rhodomonas* data from previous experiments better defined the optimal feed rate for *A. sinjiensis*. This is where development is limited by time and not feed concentration and occurred at a feed concentration of 1.13 µg AFDW/mL.

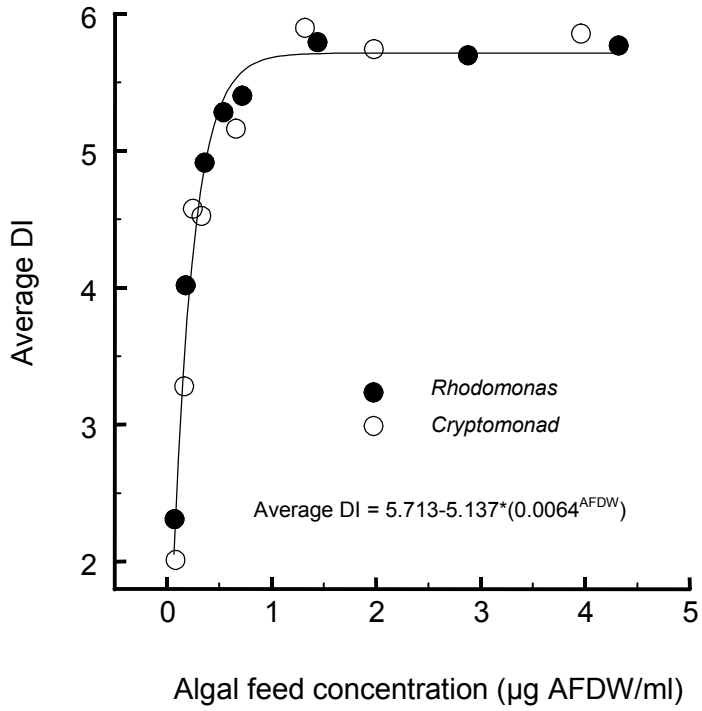


Figure 16 Growth response (average developmental index) of *A. sinjiensis* fed with increasing concentrations of either *Rhodomonas* or Cryptomonad (CS-412).

Outcomes

- When fed on an equal AFDW ration, Cryptomonad CS-412 is as nutritious as *Rhodomonas* for *A. sinjiensis*.
- Minimum feed concentration for maximal (99%) growth was adjusted from 1.34 to 1.13 µg AFDW (*Rhodomonas* or Cryptomonad)/mL.

Recommendation

- Cryptomonad CS-412 replace *Rhodomonas* in the diet for *A. sinjiensis* cultured at NFC.

Scale-up of existing culture technology

Introduction

Currently, *Acartia* are cultured in 400 L hemispherical tanks (Fig. 17). The maximal production of ~ 0.5 x 10^6 eggs-nauplii/day can be maintained for 7 to 10 days. Operation of the tanks is relatively labour intensive considering their small volume. The major labour component is servicing of tank equipment and collection of eggs and nauplii. Labour on a larger tank would be similar for the current tanks so productivity could be increased with a relatively small increase in labour.

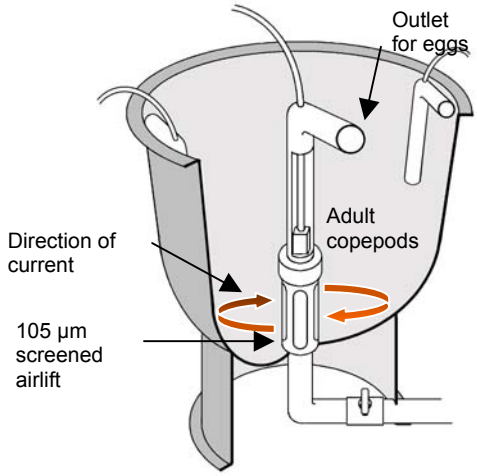


Figure 17 *Acartia* culture tank set-up. Adults are screened off from the centre airlift that collects eggs and nauplii by emptying into a floating screened (53 µm) collection vessel.

The experimental work to scale-up existing *Acartia* culture technology involved:

- Adapt current culture methods for 400 L hemispherical tanks to 1200 L conical bottom tanks and measure productivity of 1200 L tank.
- Determine daily *Acartia* production for 400 L tanks in new facility.
- Quantify change in productivity with increasing culture volume.
- Determine the potential and the required culture volume to supply the hatchery with *Acartia* nauplii at a feeding density of 2/mL.

Methods

The harvesting equipment and tank furniture used in the 400 L hemispherical tanks was scaled up for use in 1200 L (Fig's. 18 and 19).

Figure 18 (left) Centre airlifts with stainless steel screens (105 µm) to exclude copepodids and adults.

Figure 19 (below) Floating, screened (53 µm) egg-nauplii collection vessels.

Results

Although there were problems with handling the larger collection vessel for the 1200 L tank and clogging of the screen with faecal pellets, the production per unit volume was comparable to the current 400 L tanks (Fig. 20).

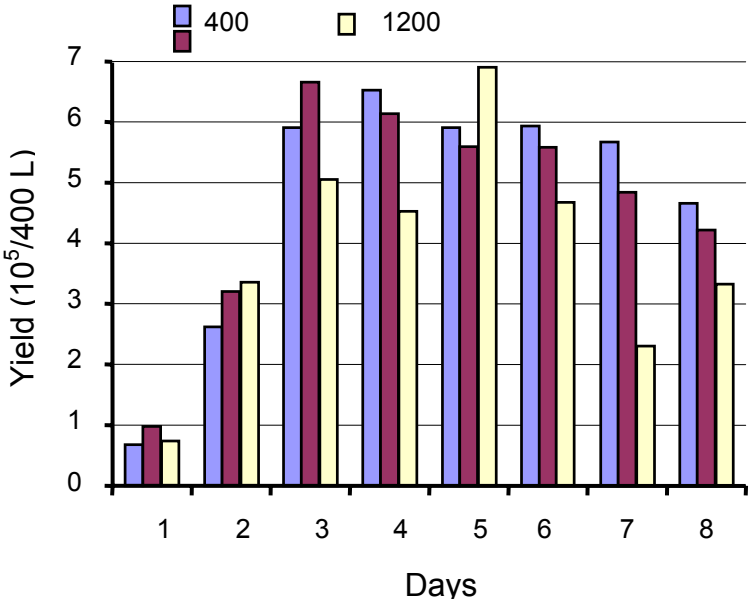


Figure 20 Copepod production (eggs + nauplii) per unit volume (400 L) for 2 × 400 L and 1 × 1200 L tank.

The average daily yield (eggs + nauplii) from day 2 to day 6 was 5.4 x 10^5/day for each 400 L tank and 4.5 x 10^5/day/400 L of the 1200 L tank. The 1200 L tank was 83% as productive per unit volume as the 400 l tanks. Over the 8 days, 16.3 x 10^6 egg + nauplii were harvested from the tanks.

Outcomes

- Average daily yield from days 2 to 6 was 5.4 x 10^5 eggs + nauplii/day. Total production from days 2 to 6 was 16.3 x 10^6 eggs + nauplii/day. Modifications to tank equipment are still required to optimise recovery and handling of eggs and nauplii.
- Yield from the 1200 L culture was 83% (per unit volume) that of the 400 L tanks. With some modifications to the 1200 L tank, this percentage is likely to approach 100%.
- Based on these results, 10,000 L of *Acartia* culture would be required to supply the hatchery at a feed rate of 2 nauplii/mL (12 × 280 L larval rearing tanks). This would require 11.2 g AFDW of algae per day. This is equivalent to approximately 150 L of mature Cryptomonad culture.

Recommendations

- Increase capacity for production of algal species required for copepod diet, in particular Cryptomonad sp. CS-412 and *Isochrysis* sp. (T. ISO).
- Design and construct mass culture system for *Acartia*: two 5000 L culture tanks with existing tanks used to provide seed populations.
- Improve tank equipment to maximise recovery of copepod eggs and nauplii.

Cold storage of eggs and nauplii

Introduction

Although copepods are generally of high nutritional value to all stages of fish larvae, they are of particular importance to first feeding larvae. Newly hatched N1 and the early development stages (N2 – N3) are of particular importance to finfish larvae with a small mouth gape. To increase the availability of these early stage nauplii during the critical first feeding period of larviculture, cold storage of eggs and nauplii was investigated.

The objectives of this work were to:

- determine a maximum temperature at which hatching was effectively inhibited for 72 h.
- measure survival (% hatch after 24 h at 28°C) of eggs stored for up to 21 days at the selected temperature.
- determine a maximum temperature where nauplii survival remained high while development was effectively blocked for 72 h.

Methodology

Eggs and N1 nauplii were placed in a temperature gradient ranging from ~1°C to 28°C (Fig. 21). Samples of eggs were withdrawn during a 72 h period to measure the percentage hatch over the experimental temperature range. Following determination of the temperature that inhibited hatching of eggs, an extended cold-storage experiment was conducted to measure survival of eggs over time. Samples of nauplii were taken at 24, 48 & 72 h to measure growth and survival of nauplii.

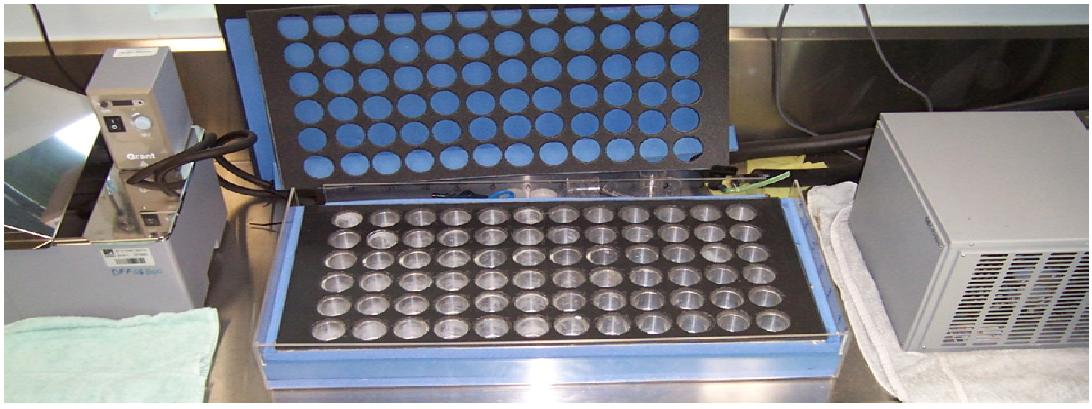


Figure 21 Temperature gradient block consisting of 12 columns (12 temperatures) and 6 rows (6 replicate temperatures). Refrigerated and heated water baths are attached to opposite ends of the block.

Cold storage of eggs

Over 72 h, hatching of eggs followed the relationship:

$$\% \text{ Hatch (72 h)} = -4.2 + 113.8/(1 + e^{-0.1989 (x-20.2)}) \qquad r^2 = 0.92$$

28°C = 90% hatch
10°C = 9% hatch
4°C = 0.2% hatch

Two temperatures (4°C & 10°C) were selected for further evaluation. Four degrees was chosen because it is normal refrigeration temperature and hatching was inhibited. Ten degrees was chosen because it was the warmest temperature where hatching was largely inhibited. An experiment to test the long-term viability of eggs stored at these two temperatures was undertaken using refrigerators. Although results are not presented here, there was too much temperature variation using standard refrigerators. Survival was poor for eggs stored at 10°C while eggs died at 4°C. A second experiment conducted only at 10°C was undertaken with more intensive sampling. Temperature was accurately maintained at 10°C by using a temperature-controlled water-bath. Aliquots of eggs were removed over 21 days and warmed to 28°C and viable eggs allowed to hatch over a 24 h period. Hatch success was calculated as a percentage of total recovered (eggs and nauplii) and of initial number of eggs in the sample. Over 72 h of cold storage, viability averaged 90% expressed as either % recovered or of initial number (Fig. 22).

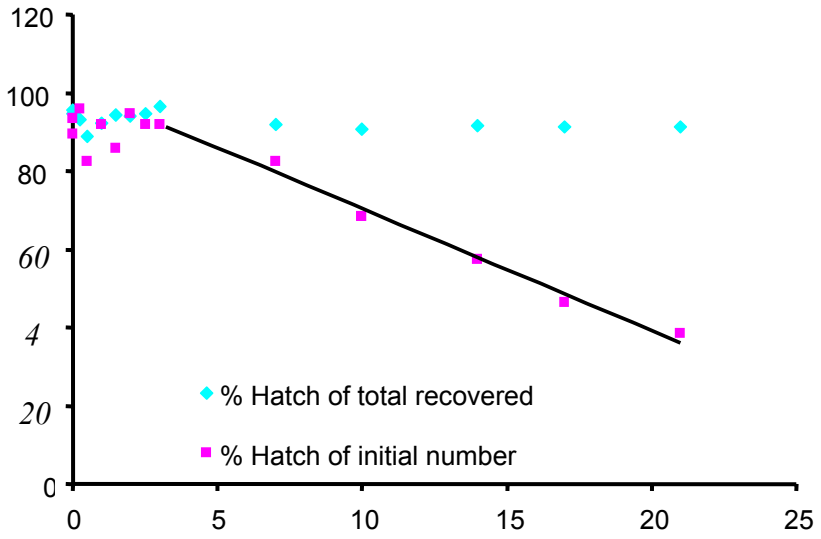


Figure 22 Hatch rate of *Acartia sinjiensis* eggs that have been cold-stored at 10°C for up to 21 days. Hatch rate expressed as either percentage of total recovered (eggs + nauplii) or of initial number of eggs. Data points are an average of 5 replicates.

After 72 h eggs began to disintegrate, either while under cold-storage or during the 24 h hatching period at 28°C. This is evident by the linear decline in % hatch (intitial number of eggs) compared to that calculated from total recovered. However, even after 7 days 80% of cold-stored eggs were recovered as hatched nauplii.

Cold storage of nauplii

Development of *A. sinjiensis* nauplii stored for 72 h at temperatures from 1.5°C to 22°C was highly dependent on temperature (Fig. 23).

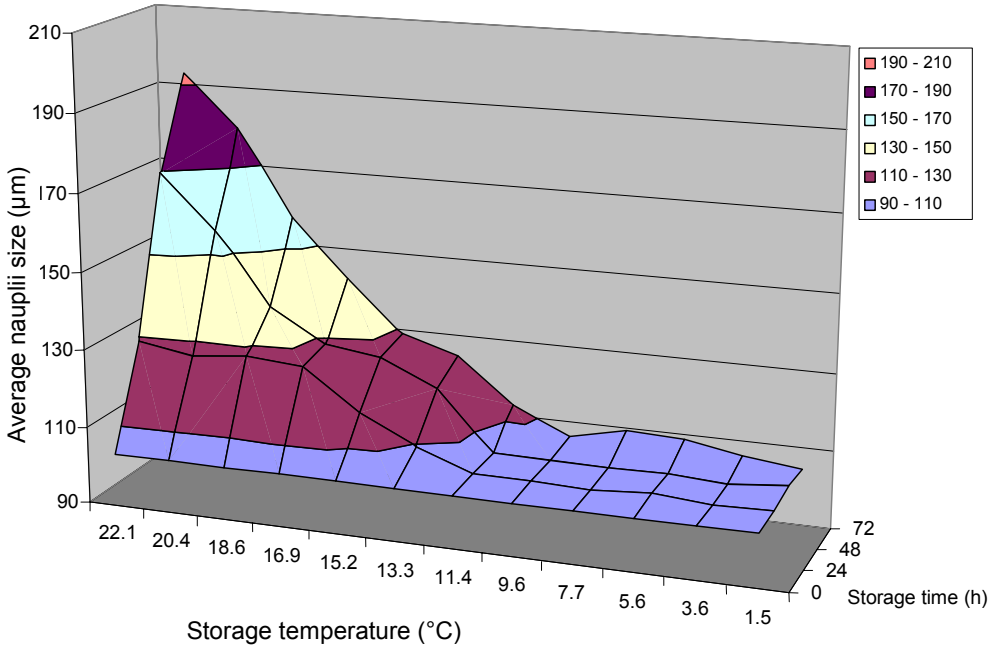


Figure 23 Development of *A. sinjiensis* nauplii over a 72 h period at temperatures ranging from 1.5°C to ~22°C. Data points are averages of 3 replicate experiments with duplicate samples per experiment.

Nauplii stored at 22°C were on average around 190 µm after 72 h, equivalent to an N5 development stage. This compares to a size of ~450 µm (copepodid stage C1 to C2) for copepods raised at an ambient temperature of 28°C. As the storage temperature decreased, development slowed until at ~10°C there was almost no increase in the average size of nauplii over 72 h. There was a change in development rate as copepods reached 130 µm indicated by a flattening of the graph in this region. This coincides with nauplii developing through to exogenous feeding stages (N3 and above).

Survival of nauplii was also dependent to storage temperature (Fig. 24). At temperatures above ~6°C survival after 72 h was close to 100%. However, below ~6°C survival decreased rapidly and was zero at the coldest temperature (1.5°C).

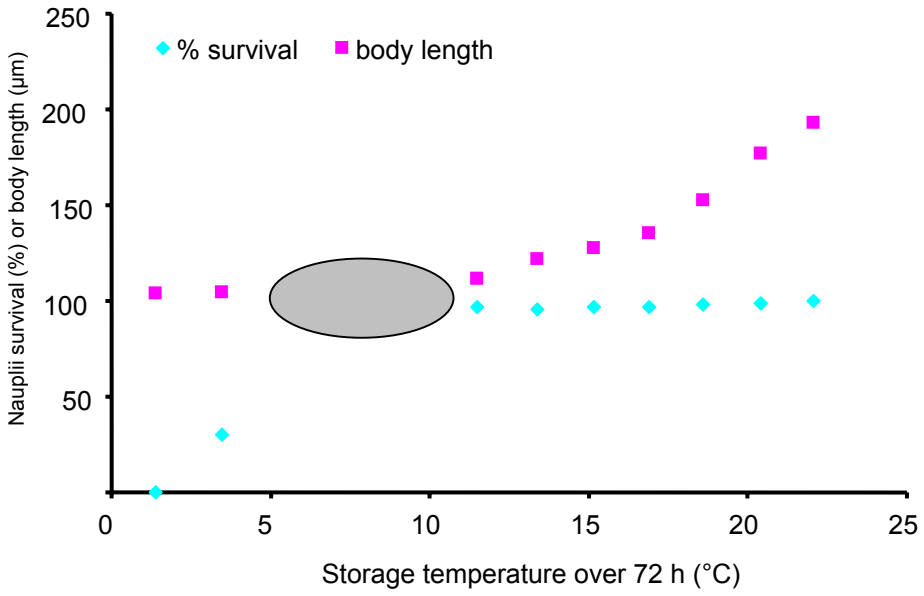


Figure 24 Growth and survival of *A. sinjiensis* nauplii stored for 72 h over a temperature range. Temperatures within the oval are where survival is high but development is minimal.

Outcomes

Hatching of *A. sinjiensis* eggs was effectively (at least 90%) inhibited for 72 h at storage temperatures ≤ 10°C.

Eggs may be cold stored at 10°C for 72 h with no loss of viability while those stored for 7 days retain 80% viability. Storage at 4°C results in the rapid loss of viability of eggs.

Nauplii (N1) stored at 10°C for 72 h remain the same size (development is blocked) while survival is unaffected.

Recommendations

Scale-up copepod production to be able to supply the hatchery with high numbers of nauplii for rigorous feeding experiments.

- Upgrade copepod production to 2 × 5000 L tanks.
- Upgrade algal production for new tanks.
- Incorporate cold storage of eggs and nauplii into management of tanks.
- Production per tank could = 5×10^6 / day.
- With cold storage stockpiling, up to 20×10^6 nauplii per 5000 L tank could be available for the crucial first feeding stage of finfish larvae.

Larval rearing

Objectives

The larval rearing component objectives for 2003–2004 were:
1. Achieve consistent first feeding
2. Consistent survival past D6
3. Increase survival to D10

Introduction

Both flowery cod and estuary cod larval rearing at NFC during 2003 has resulted in consistent mortalities of almost 100% by day 3 post-hatching. The mortality pattern suggests either egg quality or unsuitable physical culture parameters. Kohno (1998) considers that there is a causal relationship between early mortality levels and the biological characteristics of larval fish. The author states that grouper larvae are poorly suited to early survival having neither large amounts of endogenous nutrition nor a high exogenous feeding capacity. Ordonio-Aguilar *et al*. (1995) concluded that *E. coioides* larvae are difficult to rear due to their poor endogenous nutritional reserves at feeding onset and their poor initial feeding ability.

Previous work has indicated that light intensity influences the early development stages of estuary cod larvae. This trial assessed three light intensities to identify a more optimal light intensity range to maximise survival of the early larval rearing phase for both grouper species.

Estuary cod

Methodology

The effect of light intensity on survival and condition of pre-feeding larvae was evaluated. Fertilised eggs were collected from spontaneous spawning of F1 brood stock held under a simulated 365-day photo-thermal regime at the Northern Fisheries Centre, Cairns. Spawning occurred in the late afternoon and eggs were collected overnight via the tank outlet into an external tank with a collecting screen. Buoyant, fertilised eggs were incubated at a density of 500 eggs.L^{-1} and hatching occurred late in the afternoon (D0). Estuary cod larvae were obtained from three cohorts (Cohort 1: 18 August 2003; Cohort 2: 21 August 2003; Cohort 3: 24 August 2003) for light intensity trials.

Three light intensities of 0 (dark); 1 – 1.5 (low); and 12 - 17 (high) µmol photons.m^{-2}.s^{-1} were randomly assigned to thirty static experimental chambers with illumination provided by two fluorescent lights suspended above the chambers. Light intensity was measured at the surface of each chamber with a Biospherical Instruments QSL-100/101 light meter. Thirty larvae were transferred into each of the chambers. Temperature was maintained at 26–27ºC and salinity at 34–35ppt during each experiment.

Larvae were left undisturbed until the morning of D3 when surviving larvae from each chamber were counted and fixed in 10% neutral buffered formalin for 48 hours and then stored in 70% ethanol. Fixed larvae were measured using a microscope and ocular micrometer read to the nearest 0.01mm to determine standard length (mm) (SL: length from the rostral tip to the end of the notochord), body depth (height from dorsal to ventral finfold, anterior to the anal opening), and oil globule volume (mm^3) using $V = 4/3\pi r^3$ (volume of a sphere, where *r* is the oil globule radius) (Avila and Juario 1987).

Results

Survival rates did not differ significantly between light intensities (Fig. 25). However, both standard length and body depth were significantly greater at lower light levels. Significant cohort

variation occurred highlighting the importance of cohort replication to qualify experimental outcomes.

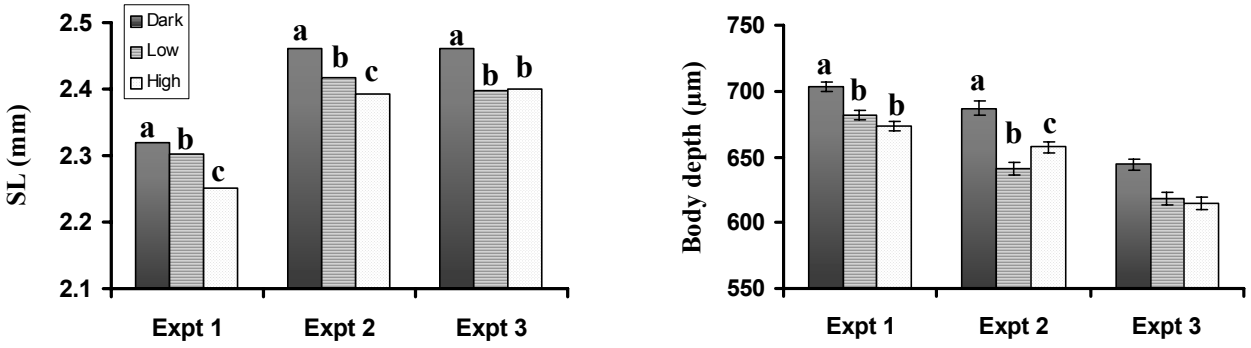


Figure 25 Standard length and body depth of larval estuary cod exposed to three light intensities (dark 0, low 1 – 1.5, and high 12 - 17µmol.s^{-1}.m^{-2}).

Flowery cod

The impact of light intensity on survival, growth and feeding incidence of flowery cod was assessed across multiple cohorts. A relatively narrow range of light intensity was chosen based on previous work indicating a preference of early stage larvae for lower light levels.

Methodology

A blocked design of three light intensity treatments with four replicates was used to assess the impact of light intensity on first feeding incidence and growth (Cohort 1); and on survival (Cohort 2) of larval flowery cod. Twelve, 300L larval rearing tanks were stocked with 40 eggs.L^{-1} on D0 (day of hatching). Cohort 1 was fed SS-rotifers whilst Cohort 2, used to assess survival prior to first feed, was unfed.

Results/Discussion

Cohort 1

Morphology

Larval survival during this trial was poor and inconsistent with 100% mortality occurring by the morning of D5. Standard length, yolk volume, body depth and eye diameter did not differ significantly between light intensities. The utilization rate of oil globule between treatments varied with age, on day 1 decreasing as light intensity decreased. On D3, oil globule volume was significantly larger at the low compared to the high light intensity.

Feeding incidence

Although feeding was established on D3 and D4, larvae did not appear to be strong feeders with the number of prey ingested per larva not exceeding 4 rotifers/larva.

The percentage of larvae feeding did not differ significantly between light intensities (the analysis of feeding data was influenced by the order of sampling). Although feeding incidence on D3 was greater in the medium and high light levels, fewer larvae fed at these light levels on D4, whilst there was an increase in the feeding incidence of larvae under low light levels (Fig. 26).

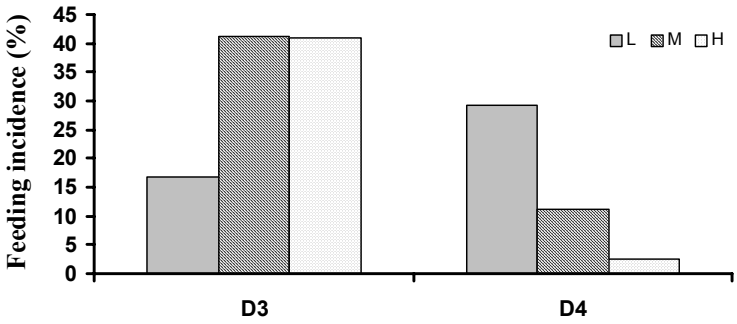


Figure 26 Feeding incidence of larval flowery cod exposed to three light intensities (low - 0.05-0.085, medium - 1.2 – 1.75 and high - 6 - 10µmol.s^{-1}.m^{-2}).

Cohort 2

Larval survival at D3 tended to increase with decreasing light intensity (0.05 – 10 µmol photons.m^{-2}.s^{-1}) although this result was not significantly different (Fig. 27, $p>0.05$). This trial needs to be replicated across cohorts to clarify these survival data. Body depth also increased significantly with decreasing light intensity and significantly smaller oil globule volumes were recorded for larvae under the highest light intensity (Fig. 27).

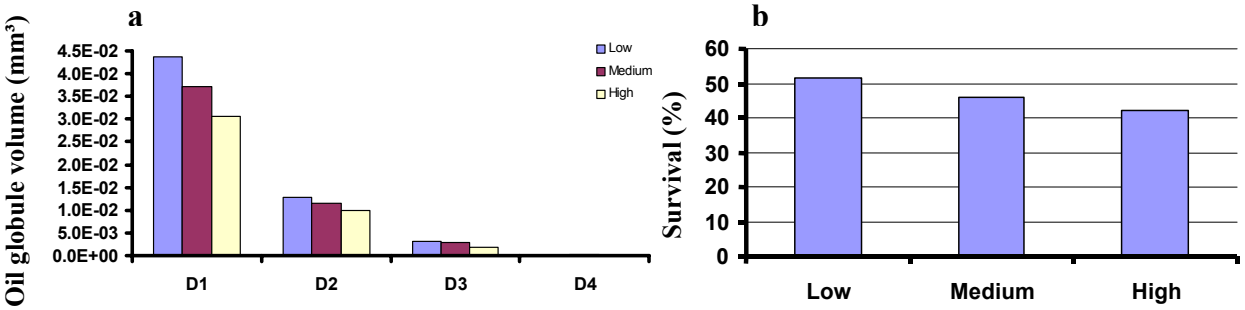


Figure 27 (a) Oil globule volume and (b) survival of day 3 larval flowery cod exposed to three light intensities (low - 0.05-0.085, medium - 1.2 – 1.75 and high - 6 - 10µmol.s^{-1}.m^{-2}).

Conclusions

Generally, outcomes of light intensity experiments indicate that lower light ranges are preferable for the pre- and early feeding stages of larvae. Morphological data suggest that energy expenditure is used for growth rather than increased swimming activity at the lower light levels tested, however the variability between cohorts require that this data is interpreted with caution until replicated further. In addition, larval survival and condition during more extensive feeding trials are required to fully assess the feeding ability in response to light intensity.

All experimental work during 2003/2004 was impeded by high and inconsistent mortalities, with 100% mortality occurring by day 5 in all trials for both species. Significant cohort variation was also identified as a factor, highlighting the importance of cohort replication to qualify experimental outcomes.

Given the difficulties experienced in obtaining consistent survival to D4/5 post-hatching with both grouper species, the primary larval rearing objective has focussed on identifying basic physical parameters to increase early survival. A range of issues associated with inconsistent survival (including surface-associated mortality) has since been identified and will be assessed during the 2004/2005 season.

Rearing these highly sensitive larval grouper species in small-scale intensive recirculation systems has necessitated the adaptation of existing culture methods used overseas. A combination of modifications to rearing protocols and system design has recently increased larval survival, with

consistent and high survival of pre- and early feeding stage larvae achieved across multiple cohorts. This new rearing protocol will be assessed in feeding trials in the coming (2004—2005) season. In addition, a recent trial utilizing a large-scale mesocosm culture system has resulted in Australia's first production of juvenile flowery cod. This large-scale culture used copepod (*Acartia sinjiensis*) nauplii as an early feeding prey source in addition to SS-strain rotifers *Brachionus rotundiformis*. This trial will be repeated this season in order to confirm results and allow for refinement of the methodology.

Reef Fish Aquaculture Grow-out

Background

At the 6 November 2003 meeting of the AFFS Aquaculture R&D Committee, the issue of availability of grow-out sites for reef fish aquaculture was tabled and discussed. There is a perception within industry that the increasingly onerous restrictions on aquaculture development in Queensland will severely restrict the application of reef fish aquaculture technology in the state.

The concern regarding availability of sites for reef fish aquaculture reflects industry concern regarding the broader issue of aquaculture development in Queensland. The recent productivity commission report notes that 'New South Wales, Queensland and Western Australia have made limited progress with marine aquaculture planning. This may constrain marine aquaculture, or result in ad hoc approvals for individual sites, and conflicts over resource use' (Productivity Commission 2004).

Discussion of this issue between DPI&F and State Development and Innovation (SD&I) staff suggested that a collaborative approach to investigating potential mechanisms to identify grow-out sites for reef fish aquaculture was a useful first step in responding to this expressed industry concern.

Subsequently, a meeting of representatives of DPI&F (AFFS–F&A, QFS), SD&I, GBRMPA and QEPA was held in Cairns on 10 February 2004.

Cairns workshop

The workshop discussed options for reef fish aquaculture grow-out in regard to the following production systems:

- Sea cages
- Coastal ponds (including cages in ponds)
- Recirculation systems

and discussed mechanisms to:

- identify the most likely production systems for reef fish aquaculture in northern Queensland;
- identify potential sites, as well as constraints to site identification / use;
- identify specific projects to address development issues (e.g. bioeconomic modelling of production systems).

Participants

Mike Rimmer, DPI&F (AFFS–F&A), NFC Cairns
Richard Knuckey, DPI&F (AFFS–F&A), NFC Cairns
Chris Robertson, DPI&F (QFS), NFC Cairns
Phil Hales, DPI&F (QFS), NFC Cairns
Kathy Rankin, SD&I Cairns
Andrew Broadbent, SD&I Brisbane
Brian Prove, SD&I Townsville
Leigh Gray, GBRMPA
John Dunn, GBRMPA
Paula Tomkins, GBRMPA
Brynn Mathews, QEPA Cairns

Outcomes

The workshop concluded that there was greater likelihood of some production systems being less contentious than others, and that these production systems should be the focus of ongoing research and industry development activities. In particular, sea cages within the Great Barrier Reef Marine Park are likely to be highly contentious issues and are likely to face widespread community opposition, particularly from environmental lobbying groups.

Based on this perspective, it is appropriate to focus reef fish aquaculture development on production systems and areas that are likely to have a greater probability of acceptance. A subjective assessment of Queensland regions and potential reef fish aquaculture grow-out systems is listed below.

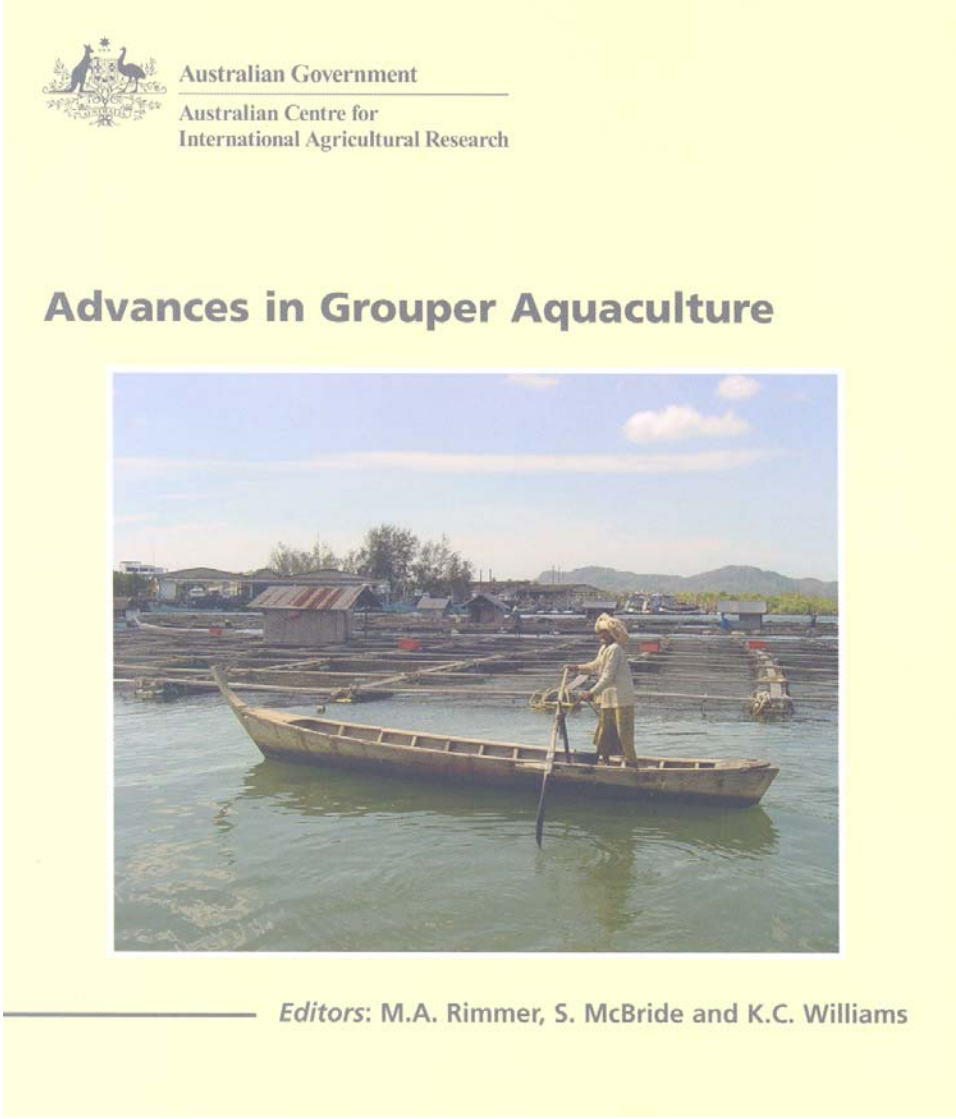
Table 5 Priorities for development of reef fish aquaculture grow-out systems within broad regions of Queensland. Letters represent High, Medium and Low priorities for development in each region. RAS: recirculating aquaculture systems.

	Sea cages	**Coastal ponds**	**RAS**
Eastern Queensland (within GBRMP)	L	H	H
Eastern Queensland (outside GBRMP)	M	H	H
Torres Strait	H	L	L
Gulf of Carpentaria	M	M	L

Associated Projects

This section provides an overview of ongoing research, development and extension activities closely associated with the Reef Fish Aquaculture R&D Project. Funding from the Australian Centre for International Agricultural Research (ACIAR) has supported the development of a portfolio of closely-related projects that cover a substantial proportion of the food and fibre chain for marine finfish aquaculture production, ranging from production technology, through grow-out technology and the alleviation of environmental impacts, to the market chain.

For brevity, these projects are summarised in this report. Additional details of these projects are available through the Asia-Pacific Marine Finfish Aquaculture Network website maintained by the Network of Aquaculture Centres in Asia-Pacific (NACA): www.enaca.org



Improved hatchery and grow-out technology for grouper aquaculture in the Asia-Pacific region *(ACIAR project FIS/97/73)*

Project outline

Aquaculture of high value finfish species, such as groupers, is an industry of increasing importance throughout the Asia-Pacific region, including Australia. The development of large and affluent markets for live reef fish, particularly in Hong Kong and southern China, has increased pressure on wildstock resources. In many areas the demand for live reef fish, and the profitability of this trade, has encouraged overfishing and the use of destructive fishing practices, such as the use of sodium cyanide to 'stun' reef fish for capture by divers. Aquaculture of high value reef fish species can potentially supply product to the live reef fish markets, as well as other regional and domestic markets. The development of aquaculture technology for these species will not only support an economically beneficial aquaculture sector, but will also contribute to reducing pressure on wild stocks. Currently, the major bottlenecks to increased aquaculture production of groupers are the generally poor, and highly variable, survival in larviculture, and the limited sources of 'trash' fish (widely used in Asia as a feed source for finfish aquaculture) for grow-out.

The ACIAR project *Improved hatchery and grow-out technology for grouper aquaculture in the Asia-Pacific region* addressed these issues by collaborating with research and development organisations in Indonesia and the Philippines to carry out priority grouper research to improve larviculture and to develop cost-effective grow-out diets of low fish content. An additional objective of the project is to support, through the Network of Aquaculture Centres in Asia-Pacific (NACA), more effective dissemination of research results arising from the project activities, and to promote greater collaboration and information exchange among centres in Asia involved in grouper aquaculture research and development.

Objectives

The overall objective of the ACIAR project is to increase grouper production in the Asia-Pacific area by developing improved hatchery and grow-out technology. The project has three major components:

Larval rearing of groupers
Improve growth and survival of groupers during the hatchery phase.

Diet development for on-growing of grouper
Develop compounded feeds for grouper grow-out that have low environmental impact, have a low content of fishery resource, and are as cost-effective for the on-growing of grouper as the alternative of using trash fish.

Support for the Grouper Aquaculture Research and Development Program
'Value add' existing grouper aquaculture R&D efforts in the Asia-Pacific region by improving communication and promoting collaborative research between regional laboratories and agencies.

Current status

The project was reviewed in September – October 2002 and was subsequently extended for 12 months, to 31 December 2003. A follow-on project (FIS/2002/077) has been developed and will commence 1 July 2004.

Results

Larval rearing

The larval rearing component of the project investigated a range of aspects of grouper larviculture to improve survival, as well as the consistency of production, in the hatchery phase. Grouper larvae are small and fragile with small reserves of endogenous nutrition and low initial feeding rates (Ordonio-Aguilar *et al.* 1995). This combination of factors is considered to be a fundamental cause of the high mortalities and delayed development observed during larviculture (Kohno *et al.* 1997).

An essential pre-requisite for successful marine finfish larviculture is to maximise survival and condition of the larvae prior to the commencement of exogenous feeding. Optimal environmental parameters during egg incubation and rearing of pre-feeding larvae were established for *E. coioides* and *C. altivelis* (Table 6).

The nutritional value of the live prey used to feed grouper larvae is a major determinant of larval growth and survival. *E. coioides* larvae conserve the fatty acids eicosapentaenoic acid (EPA, 20:5n-3), arachidonic acid (ARA, 20:4n-6) and docosahexaenoic acid (DHA, 22:6n-3), indicating the essentiality of these fatty acids. These fatty acids are particularly conserved in the phospholipid fraction, whereas neutral lipids are primarily used as an energy source for developing larvae. The levels of EPA, ARA and DHA can be enhanced in rotifers and brine shrimp using various enrichment products, and nutritional enhancement of live prey results in improved growth, survival and pigmentation of *E. coioides* larvae.

A better understanding of the nutritional physiology of grouper larvae was established through this project. The histological study of the digestive tract in *E. coioides* and descriptions of the ontogeny and of digestive enzymes in *E. coioides* and *C. altivelis* provide a basis for assessing the digestive capacities at different developmental stages. The low activities of digestive enzymes and the rudimentary structure of the digestive tract indicated that grouper larvae have a low digestive capacity prior to 10 days post hatch. As the structural complexity of the stomach progressed between 10 and 16 days post hatch, fluctuations in trypsin and total protease activities were observed suggesting a change in digestive physiology. The appearance of gastric glands coincided with a general increase in enzyme activities indicating an increase in the digestive capacity of the larvae preceding metamorphosis. It is also apparent that there are differences between grouper species in the emergence of digestive enzymes implying there may be different capabilities between genera for digesting the major macronutrients.

The small mouth size and limited physical abilities of grouper larvae has limited the suitability of traditional live prey organisms for early feeding. Successful larval rearing relies on the use of smaller prey organisms, such as copepod nauplii and the 'super-small' (SS) -strain rotifer. Selection for smaller rotifers tends to select females that reproduce at a smaller size, but which still grow to a normal size. Larger

proportions of smaller rotifers suitable for first-feeding grouper larvae are obtained when the rotifers are fed a microalgal diet of small particle size, such as *Stichococcus*.

Copepod nauplii show considerable potential as an alternative live prey for larval rearing of marine finfish because they are in many cases smaller than super-small (SS)-strain rotifers and are of superior nutritional value to rotifers (McKinnon *et al.* 2003). Copepods are also a useful supplement to brine shrimp during the later stages of larval rearing, because of their better nutritional profile. The addition of copepods in the semi-intensive larval rearing of *E. coioides* also improved survival. The high total protease activity found in copepod nauplii in comparison to rotifers, suggests that they are more digestible by early stage grouper larvae and may partly explain this improved survival.

With improvements in larval nutrition and husbandry techniques, survival has now increased from around 3% to 20–40% for *E. coioides* and from <10% to 30–50% for *C. altivelis*. This has moved larval rearing technology for groupers into the realm of commercial viability. That this technology is commercially viable has been well demonstrated in Indonesia where there are now (2003) an estimated 67 hatcheries (52 'backyard', six medium and nine large hatcheries) producing grouper fingerlings for the food fish and ornamental markets. Estimated production of grouper fingerlings from Indonesian hatcheries in 2002 was 3,350,200 fish (5–10 cm total length) comprising 2,656,200 *E. fuscoguttatus*, 697,800 *C. altivelis*, and 2,200 *E. coioides* (Sugama 2003).

Despite this success, the viral disease viral nervous necrosis (VNN) continues to impact the survival of grouper larvae in hatcheries. VNN-related mortality in *E. coioides* was dramatically reduced using the protocols developed through this project, and particularly by increasing the levels of HUFA's in the live prey fed to grouper larvae. However, the prevalence and transmission mechanisms of the virus are poorly known in tropical marine finfish aquaculture, and further research is necessary to better understand the disease and to develop methods of control.

Marine finfish hatcheries provide important socio-economic contributions to coastal communities. The socio-economic assessment of 'backyard' hatcheries in Bali, Indonesia, showed that hatcheries are important sources of employment and economic benefit in northern Bali. Economic features of these hatcheries include: high profitability ($6,300 – $100,000 per annum), high internal rates of return (>12%), positive benefit-cost ratios (1.3 – 3.1), and rapid payback of capital cost (often with one year). These hatcheries are important sources of employment for local people, including women, either directly or in associated industries such as fish brokerage. A feature of these hatcheries is that they may switch between different species as commodity prices fluctuate; thus, the industry as a whole is relatively robust to market fluctuations.

The impacts of this project were spread more widely than the participating countries by the development of the Asia-Pacific Grouper Network, and its successor, the Asia-Pacific Marine Finfish Aquaculture Network, coordinated by NACA. A regular training course at the Gondol Research Institute for Mariculture, Bali, Indonesia, has trained aquaculture researchers and industry practitioners from several countries in the Asia-Pacific region. This training has contributed directly to successful production of

grouper fingerlings in Vietnam, Thailand, Malaysia and Australia as well as in Indonesia.

Table 6 Environmental conditions for incubation of eggs and early larvae of two grouper species for optimal survival

Stage	Tank	Water exchange (%/day)	Aeration	Salinity (ppt)	Light Intensity
E. coioides					
Eggs (400/L)	4L	0	Moderate (100mL/min)	32–42	
Larvae, early stage	40L	0	Gentle (0.62–1.25 mL/min)	16–24	500–700 lx
C. altivelis					
Eggs (500/L)	Incubation	200 (1)	High (600 mL/min) (2)	34–35	
Larvae, early stage	5 tonne	0	Gentle and evenly distributed	34–35	1000–1500 lx at water surface

Notes
1. determined in a 100L tank.
2. determined in a 4L tank.

Grow-out diet development

The overall goal of the project's grow-out diet development research was to develop compounded pelleted grouper feeds as a more sustainable, lower-polluting and cost-effective alternative to the feeding of fresh fishery bycatch (or 'trash' fish). The grouper species studied in this research were humpback or polka dot grouper, *Cromileptes altivelis*, tiger or flowery grouper, *Epinephelus fuscoguttatus* and the gold spot or estuary cod, *Epinephelus coioides*. The research approach was to define the requirements of groupers for the key nutrients that largely determine the rate at which fish grow, determine the nutritive value of locally available marine and terrestrial feed ingredients and examine the extent to which high cost marine protein feed ingredients could be replaced using cheaper and more renewable terrestrial protein feed ingredients.

The crude protein (CP) requirement of humpback grouper and tiger grouper was met with diets that contained not less than 44% dry matter (DM) digestible CP (about 50% on an as-fed CP basis). Increasing the lipid content of the diet beyond about 9–10% did not improve fish growth rates but instead reduced the fish's appetite and resulted in higher rates of fat deposition in the fish. Adding dietary lipid in the form of coconut oil as a rich source of medium chain fatty acids (C 10–14) resulted in an accelerated

rate of lipid oxidation in humpback grouper compared to diets in which the lipid was provided as long chain (C 18+) fatty acids. However, this led to a profound depression of the fish's appetite and a profound decline in the fish's growth rate. Growth rate and survival of sea-caged humpback groupers were improved when diets were supplemented with up to 150 mg/kg of vitamin C as the heat-stable form of L-ascorbyl-2-monophosphate-Na-Ca. This benefit of vitamin C supplementation was most apparent following heavy flood rains, which caused a marked deterioration in water quality (increased turbidity and reduced dissolved oxygen content) around the cages. The dietary requirement for the essential omega-3 highly unsaturated fatty acids (n-3 HUFA) was examined for humpback grouper and tiger grouper. Increasing the supplementation rate up to 1–1.5% of the diet resulted in improved fish growth rates and better survival. In studies examining the capacity of humpback grouper to utilize different types of carbohydrate as energy sources, best results were achieved using glucose while starch and sucrose were the least effective.

These nutrient requirement studies indicate that juvenile groupers require diets that are high in digestible CP (around 45%), moderately low in lipid (around 10%) and contain not less than 1.0% and preferably 1.5% of n-3 HUFA. Addition of at least 100 mg of a heat stable form of vitamin C per kg of diet is recommended and this should be increased to 150 mg/kg if stressful culture conditions are likely to occur.

The apparent digestibility of a comprehensive range of ingredients available in the Philippines and Indonesia was determined for gold spot grouper and humpback grouper, respectively. The CP in both marine and terrestrial animal meals was well digested (above 76%) by both grouper species with the exception of oven-dried blood meal, which was poorly digested (55%). The protein digestibility of plant products was more variable (from 43 to 100%) with high fibre meals such as rice bran and lucaena (ipil-ipil) meal being poorly digested. The DM digestibility of the meals was adversely affected by the amounts of ash and fibre they contained. A collation of the DM and CP apparent digestibility values of the tested ingredients is presented in Table 7.

In studies examining the ability of terrestrial protein meals to substitute for fishmeal in formulated feeds for juvenile gold spot grouper, a 4:1 combination of meat meal and ring-dried blood meal, respectively was able to replace up to 80% of fishmeal protein in the diet without adverse effects on growth, feed conversion or survival of the fish. Other terrestrial protein meals such as cowpea, corn gluten, lucaena (ipil-ipil) meal and soybean meal were less successful as fishmeal replacements. With humpback grouper, growth rate and feed conversion deteriorated markedly when shrimp head meal was used at inclusion rates above 10% as a replacement for fishmeal protein.

In laboratory and field cage studies, a practical low-cost dry pelleted diet was formulated on a digestible nutrient basis to meet the requirements of juvenile gold spot grouper and compared with feeding either a commercial pellet diet or fresh fishery bycatch. In both studies, fish fed the project formulation diet survived and grew as well as those fed the fresh bycatch. In the laboratory study, fish fed the commercial pellet diet grew significantly slower and converted feed less efficiently than those fed either the project diet or fresh bycatch. The analysis of the commercial pellet diet showed a sub-optimal specification. When the commercial mill adjusted the

formulation to meet these specifications, fish fed that diet in the field study performed as well as those fed either the project diet or fresh bycatch.

The research carried out in the project has conclusively shown that juvenile groupers will readily accept pelleted dry diets. Diets formulated to meet the fish's requirements of digestible nutrients and not containing excessive amounts of plant protein meals will enable juvenile groupers to grow as well as those fed fresh fishery bycatch.

Table 7 The dry matter (DM) and crude protein (CP) apparent digestibility (AD) of selected feed ingredients determined for gold spot grouper in the Philippines and for humpback grouper in Indonesia. All data are mean ± standard deviation.

Feed ingredient	Gold spot grouper		Humpback grouper	
	DMAD[1]	CPAD[1]	DMAD[1]	CPAD[1]
Marine product				
Fishmeal (Chilean 65% CP)	83.6 ±3.09	98.0 ± 0.72		
Fishmeal (mixed 45% CP)	59.1 ±1.23	82.4 ± 1.99	59.1 ± 1.23	82.4 ± 1.99
Fishmeal (sardine 65% CP)			87.2 ± 2.53	92.5 ± 1.40
Fishmeal (tuna 50% CP)	75.4 ± 3.61	76.2 ± 1.92		
Fishmeal (white 69% CP)	89.2 ± 1.69	98.6 ± 0.31		
Shrimp meal (Acetes 72% CP)	76.0 ± 4.00	95.0 ± 0.72		
Shrimp head meal (50% CP)			58.8 ± 3.33	78.0 ± 1.32
Squid meal (71% CP)	99.4 ± 0.95	94.2 ± 0.21		
Terrestrial animal product				
Blood meal (Australian ring 84% CP)				
Blood meal (oven dried 84% CP)			48.1 ± 0.85	55.2 ± 1.35
Blood meal (formic 87% CP)			67.9 ± 1.63	87.5 ± 0.55
Blood meal (propionic 84% CP)			61.7 ± 2.60	84.2 ± 0.69
Meat meal (Australian 44% CP)	60.8 ± 0.80	98.9 ± 1.32		
Meat meal (Philippine 45% CP)	77.7 ± 0.09	83.8 ± 1.66		
Meat solubles (73% CP)	99.3 ± 0.45	97.6 ± 0.08		
Poultry feather meal (67% CP)	74.3 ± 3.06	81.8 ± 2.58		
Plant product				
Corn germ meal (8% CP)	85.2 ± 2.81	82.9 ± 4.71		
Corn gluten meal (56% CP)	94.0 ± 2.03	99.5 ± 0.65		
Cowpea meal (white 24% CP)	74.2 ± 3.14	93.5 ± 1.22		
Lucaena (ipil-ipil) meal (19% CP)	56.0 ± 0.04	78.8 ± 2.64		
Lupin albus meal (26% CP)	54.1 ± 1.24	97.5 ± 3.65		
Palm oil cake meal (11% CP)			45.3 ± 2.37	80.5 ± 1.30
Rice bran meal (11-14% CP)	68.5 ± 7.02	42.7 ± 5.38	22.2 ± 1.52	59.5 ± 1.41
Soybean concentrate (54% CP)	76.3 ± 4.88	85.5 ± 0.40		
Soybean meal (full-fat 41% CP)			54.8 ± 2.72	67.2 ± 1.29
Soybean meal (solvent 51% CP)	75.7 ± 1.69	96.0 ± 0.13		
Wheat flour (9% CP)	72.8 ± 0.85	82.9 ± 1.26		

Communication, coordination, extension

One of the constraints to the development of sustainable grouper aquaculture in the Asia-Pacific region has been the uncoordinated nature of the substantial regional research effort that has taken place over the last two decades. Researchers and practitioners felt they were working in isolation and were unaware of the many similar lines of research being undertaken by other laboratories.

In response to the identified need to improve communication and coordination of research effort, the Asia-Pacific Grouper Network was established in 1998 at a grouper aquaculture workshop held in Bangkok, Thailand. The network is coordinated by the Network of Aquaculture Centres in Asia-Pacific (NACA) and has received support from the Australian Centre for International Agricultural Research (ACIAR) and the Asia-Pacific Economic Cooperation (APEC), through its Fisheries Working Group.

Recognizing the importance of marine fish farming in the Asia-Pacific region, senior government representatives at the NACA 13th Governing Council Meeting in 2002 absorbed the grouper network into NACA's core programme, to ensure its long-term sustainability. The coverage of the network was also expanded to include other species such as sea bass, snapper, cobia, tuna and marine ornamentals and the name was changed to the Asia-Pacific Marine Finfish Aquaculture Network (APMFAN).

The overall objective of the network is to promote cooperation to support responsible development of marine finfish aquaculture within the Asia-Pacific region. Network activities are particularly directed at development of marine finfish aquaculture that:

- provides an alternative source of income and employment for coastal people, especially those currently engaging in destructive fishing practices;
- provides a quality alternative source of fish to wild-caught species, including fish fingerlings, that may be captured using destructive fishing techniques;
- contributes to protection of endangered reefs and reef fish from the pressures of illegal fishing practices through responsible aquaculture development;
- promotes environmentally sustainable marine fish culture practices by addressing environmental constraints to marine fish culture associated with present practices, such as feed and fingerling supply; and
- promotes diversification of marine fish culture species appropriate to local economies and markets.

With such diverse and complex problems there is a need to share knowledge and experience to assist in finding solutions. The network provides the platform for cooperation in the Asia-Pacific region where aquaculture specialists can work with government agencies, non-government organisations, the private sector, communities and markets to ensure that aquaculture is integrated into broader objectives of conservation and poverty alleviation in coastal areas.

Communication

Facilitating communication between researchers, managers and industry is a central platform for the APMFAN.

Electronic communication

The communication strategies adopted by the network reflect the rise of internet-based communication methods, particularly e-mail and the World Wide Web. The use of electronic communication strategies allows rapid and widespread dissemination of information at relatively low cost.

The network produces two e-newsletters:

- A fortnightly e-news service with brief items on recent developments in marine finfish aquaculture, and
- A quarterly newsletter that covers research and development issues in more depth, including invited contributions from network participants.

The APMFAN web site (www.enaca.org/grouper/) provide an information resource on marine finfish aquaculture, including archived articles from technical experts throughout the Asia-Pacific region, workshop proceedings and presentations, and contact details for those wishing to obtain more information about the subject.

Workshops

Workshops have proven to be an ideal forum for facilitating exchange of ideas and experiences between grouper aquaculture researchers, aquaculture managers and industry. The high level of regional interest in marine finfish aquaculture has supported workshops at various centres throughout the region, including Thailand, Australia, Indonesia, the Philippines and Vietnam. This ability to utilise network resources to hold workshops in different locations has allowed many local representatives to participate, who would otherwise find it difficult to attend.

A major feature of the workshops has been the development of individual projects to support the network's research, development and extension program (see below). For example, the network workshop held in Hat Yai, Thailand, in April 1999 identified a number of needs for enhancing the sustainability of grouper aquaculture in the region with particular emphasis on grouper viral diseases. Based on these recommendations, network participants developed several projects that were subsequently funded by APEC, including:

- the publication of a husbandry and health manual for grouper, coordinated by the Southeast Fisheries Development Centre's Aquaculture Department; and
- the development of a regional research program on grouper virus transmission and vaccine development, assisted by the fish health section of the Asian Fisheries Society and the Aquatic Animal Health Research Institute, Thailand.

Publications

An excellent example of the strength of the networking approach to developing extension information is the Husbandry and Health Manual for Grouper. Access to network participants provided the coordinating agency, SEAFDEC AQD, with information and experience from grouper aquaculture researchers and practitioners throughout the Asia-Pacific region. Following publication of the original English version, network participants provided translation into local languages: Filipino, Indonesian, Mandarin, Thai and Vietnamese. The result was a high-quality publication of direct application to farmers in the major grouper farming countries of Southeast Asia.

Staff exchanges

To encourage cooperation and information exchange amongst APMFAN partners, the network has supported staff exchanges between participating institutions (funded by both ACIAR and APEC). These exchanges have supported the development of human resources, provided a basis for capacity building, and ensured the transfer of new technology on various aspects of grouper culture to participating economies.

Research, development and extension coordination

A major focus of APMFAN has been to provide a structure to help coordinate the overall research effort within the region. This approach has been used to minimise overlap and prevent duplication of research effort on marine finfish aquaculture.

To achieve this, APMFAN has developed a program / project structure, where individual projects contribute to a program of activities. The structure of the APMFAN program is:

1 Production technology
   - 1.1 Broodstock
   - 1.2 Larviculture
   - 1.3 Nursery
   - 1.4 Grow-out
   - 1.5 Post-harvest

2 Environment
3 Marketing and Trade
4 Food safety and certification
5 Socio-economics and coastal livelihoods
6 Fish health
7 Training and extension

The network works with institutions and projects operating throughout the region undertaking research, development and extension activities on these different components in a complementary and structured way, sharing experiences through the network, and, where possible, integrating activities between network partners.

The program structure facilitates gap analyses to identify research needs. For example, while there was a relatively high level of effort focussed on developing production technology for groupers and other high-value marine finfish, there had been relatively little work done on the socio-economic aspects of marine finfish aquaculture. Identification of this gap in the program allowed the development of a socio-economic study of Indonesian marine finfish hatcheries carried out by staff of SEAFDEC AQD, DPI&F and NACA and funded by APEC and ACIAR (Siar *et al.* 2002). This socio-economic assessment indicated that these hatcheries are an important source of employment and economic benefits in northern Bali, and that the continued development of the marine finfish hatchery sector can provide valuable livelihoods for coastal communities.

Technology uptake

APMFAN has a strong focus on 'hands-on' training to facilitate technology uptake by farmers. An example of this is the Regional Grouper Hatchery Production Course, run at the Gondol Research Institute for Mariculture, Bali, Indonesia, for the last 3 years. The Gondol course provides hands-on training for a limited number (~15) of

participants at a centre renowned for its excellence in developing production technology for marine finfish, particularly groupers.

The success of the course is evident from the results that have been achieved by course participants. In Thailand, Indonesia, Vietnam, Malaysia and Australia course graduates have been able to apply the techniques learnt from the training and have successfully produced grouper fingerlings, including *Epinephelus coioides*, *E. fuscoguttatus* and *Cromileptes altivelis*. Further courses are planned based on these successes.

Other network partners have also incorporated recent research results into their training courses. For example, the Aquaculture Department of the Southeast Asian Fisheries Development Centre has incorporated recent technological improvements in grouper hatchery production into their regular Marine Finfish Hatchery course, and Department of Primary Industries, Queensland, has run a series of workshops for farmers interested in grouper aquaculture in Australia. The Gondol Research Institute for Mariculture has run several courses in Indonesia for local farmers and fisheries officers.

Through these training courses, APMFAN has spread the impact of the network's research outcomes, including those of the ACIAR project, beyond the agencies that are formally involved in the project, and has provided direct technology transfer to farmers.

Additional information

Details of the outcomes of ACIAR project FIS/97/73 can be found on the APMFAN website which is accessible through the NACA website: www.enaca.org. Details of research outcomes will be available in late 2004 in the form of a monograph in the ACIAR Technical Series 'Advances in Grouper Aquaculture'.

Project personnel

- Dr Mike Rimmer, Department of Primary Industries, Animal Sciences – Profitable Aquaculture Systems, Northern Fisheries Centre, Cairns, Queensland, Australia.
- Dr Kevin Williams, CSIRO Division of Marine Research, Cleveland, Queensland, Australia.
- Dr Joebert Toledo, South-east Asian Fisheries Development Centre, Aquaculture Department, Iloilo, the Philippines.
- Dr Ketut Sugama / Dr Adi Hanafi, Research Institute for Mariculture, Gondol, Bali, Indonesia.
- Dr Taufik Ahmad / Mr Muharijadi Atmomarsono, Research Institute for Coastal Fisheries, Maros, Sulawesi, Indonesia.
- Dr Inneke Rumengan, Sam Ratulangi University, Manado, Sulawesi, Indonesia.
- Dr Michael Phillips, Network of Aquaculture Centres in Asia-Pacific, Bangkok, Thailand.

Contact details

Project Leader in Australia:
Dr Mike Rimmer
Northern Fisheries Centre
PO Box 5396, Cairns 4870, Queensland, Australia
Tel: 07 4035-0109; Fax: 07 4035-6703
Email: mike.rimmer@dpi.qld.gov.au

IGF-I, IGF-II and IGF-IR expression as molecular markers for egg quality in mullet and grouper

Project outline

This project forms the research component of a Masters of Applied Science degree for Ms Josette Bangcaya (Southeast Asian Fisheries Development Centre Aquaculture Department, Tigbauan, Philippines) at the Queensland University of Technology. Ms Bangcaya was awarded a John Allwright Fellowship (ACIAR) in conjunction with ACIAR project FIS/97/73 *Improved hatchery and grow-out technology for grouper aquaculture in the Asia-Pacific region*.

Abstract

Common measures of egg quality have been survival to specific developmental stages, higher hatching rate of fertilized eggs and final production of fry. Determinants of egg quality are variable among and between teleost species and no common unified criteria have been established. Maternally inherited genes influence egg quality and early embryo development is partially programmed by the messenger ribonucleic acid (mRNA). Among the genes, the insulin family is important for growth functions and presence of their transcripts in the ovary, oocytes and embryos implies their involvement during the reproductive process and their relevance to egg quality. The insulin-like growth factor (IGF) system has three components, the ligands IGF-I and II, the IGFBP (insulin-like growth factor binding proteins) and the IGF receptors that mediate biological activity of the ligands. Vitellogenin (Vtg) is the major source of nutrients for the developing embryo and elevated levels in the female fish plasma signals gonadal development preceding spawning. In oviparous fish where the developing embryo is dependent on the stored food in the yolk, vitellogenin level in the egg could indicate its capability to support embryonic growth.

This study aimed to develop molecular tools, specifically probes for IGF-I, IGF-II and IGF-IR, for the evaluation of fish egg quality. These probes would be used to determine expression levels of IGF-I, IGF-II and IGF-IR during egg development to assess their potential as molecular indicators for egg quality. In addition, this study also aimed to establish enzyme-linked immunoassay (ELISA) for quantifying Vtg in fish eggs and determine if differences in Vtg levels could be linked to fertilization and hatching success.

Through reverse-transcription polymerase chain reaction (RT-PCR) putative complementary deoxyribonucleic acid (cDNA) fragments of IGF-I, IGF-II and IGF-IR were cloned and sequenced from mullet (*Mugil cephalus*) and grouper (*Epinephelus coioides*). The relative expression ratio of the three genes in the eggs of mullet and grouper were assayed by quantitative PCR (QPCR) and calculated using the Pfaffl method. Levels of vitellogenin in different batches of mullet eggs were quantified by ELISA.

Spawned eggs of grouper were grouped into low (<60%) or high (>60%) fertilisation rate and the fertilized eggs that were incubated until hatching were grouped into medium (<90%) or high (>90%) hatching rate. Samples were categorized into sinking eggs, late embryo and hatched larvae. Relative expression ratio of IGF-II was significantly high ($P<0.01$) compared to IGF-I and IGF-IR in all samples examined.

All three genes were strongly expressed in sinking eggs compared to either late embryo or hatched larvae. However, there was no significant interaction effect between the genes and the samples analysed. Mullet samples all came from a high fertilisation rate and high hatching rate group and were categorized into sinking, multicell stage, blastula, gastrula, late embryo and hatched larvae. There was a significant interaction effect ($P<0.01$) between gene and stage, showing that genes are differentially expressed during embryonic development. IGF-II was strongly expressed relative to the other genes in all stages examined and was highest during the gastrula stage.

Vtg levels were examined in mullet oocytes and egg samples that were grouped into 4; oocytes from females that subsequently spawned, had fertilized eggs which hatched (Group A); oocytes from females that did not spawn, therefore no fertilisation and no hatching (Group B), eggs that were stripped, artificially fertilized but no hatching (Group C) and; eggs that was spawned, assumed to be fertilized but did not hatch (Group D). Group A showed a trend of higher Vtg levels than the other three but this result was not statistically significant.

Improved hatchery and grow-out technology for marine finfish aquaculture in the Asia-Pacific region *(ACIAR project FIS/2002/077)*

Project summary

Marine finfish aquaculture is an important contributor to the economies of coastal communities throughout the Asia-Pacific region. Although production of hatchery-reared fingerlings is increasing, much of the seedstock supply for this sector continues to be dependent on capture of wild fry and fingerlings, which limits fingerling availability and contributes to over-harvesting. Grow-out operations use 'trash' fish, which results in localised pollution and competes with other needs for fishery products. The previous project *Improved hatchery and grow-out technology for marine finfish aquaculture in the Asia-Pacific region* (FIS/97/73) made substantial improvements to the sustainability of marine finfish aquaculture in the Asia-Pacific region. This follow-on project will continue lines of research that demonstrated maximum benefits in the earlier project, and will continue to support the synergies that were developed between partner agencies in the earlier project through collaborative research activities.

The follow-on project will focus on improving survival of hatchery-reared high-value marine finfish larvae, and increasing the reliability of hatchery production. Larval rearing technologies will be expanded to other high-value species such as coral trout (*Plectropomus* spp.). The grow-out diet development component of the project will focus on promoting uptake of compounded pellet diets at the expense of 'trash' fish use. Research activities will focus on identifying ingredients likely to lower diet cost and reduce environmental impacts (nutrient outputs).

A third component of the project will evaluate the socio-economic constraints to uptake of the technologies (hatchery production, artificial diets) and develop strategies to overcome these constraints. The communication and coordination strategies developed under FIS/97/73 will be continued and the Asia-Pacific Marine Finfish Aquaculture Network will be strengthened and expanded through a process of formalisation of participating agencies and individuals. Enhanced industry involvement in the network will be encouraged, and long-term network sustainability will be enhanced, by accessing corporate sponsorship of network activities.

These outputs will contribute to the development of sustainable marine finfish aquaculture in the Asia-Pacific region by increasing the supply of hatchery-reared fingerlings to support increasing demand for high-value marine finfish species for aquaculture. The use of compounded grow-out diets will reduce 'trash' fish utilisation and reduce pollution associated with the use of 'trash' fish as a feed source. The project will link closely with two other ACIAR projects: *Environmental impacts of cage aquaculture in Indonesia and Australia* (FIS/2003/027) and *Economic and Market Analysis of the Live Reef Fish Food Trade in the Asia-Pacific* (ADP/2003/022).

Objectives

The overall objective of the project is to enhance the sustainability of marine finfish aquaculture in the Asia-Pacific region by improving hatchery production technology and facilitating the uptake of compounded feeds for grow-out.

Within this overall aim, specific objectives and their related sub-objectives are to:

1. Improve hatchery production technology for high-value marine finfish
   1.1. Improve survival and reliability of production of high-value marine finfish, focussing on *Epinephelus coioides*, *E. fuscoguttatus*, *Cromileptes altivelis*, and *Plectropomus* spp., in hatcheries through improvements in larval rearing technologies.
   1.2. Improve the availability and quality of live prey to support 1.1.
   1.3. Improve survival of juvenile groupers in the nursery stage.

2. Develop cost-effective grow-out diets
   2.1. Identify ingredients for grouper diets that will reduce formulation cost.
   2.2. Compare nutritional requirements of juvenile and market-size groupers.
   2.3. Identify ingredients for grouper diets that will reduce environmental impacts.
   2.4. Improve the uptake of compounded feeds for marine finfish culture at the expense of 'trash' fish use.
   2.5. Identify the impacts of feeds on product quality.

3. Facilitate technology adoption
   3.1. Identify constraints to uptake of technologies developed under the project.
   3.2. Where possible, develop responses to overcome identified constraints.
   3.3. Disseminate research outputs widely in the Asia-Pacific region.
   3.4. Promote the expansion of sustainable marine finfish aquaculture through 'hands-on' training.
   3.5. Strengthen and expand the research coordination and regional collaboration activities of the Asia-Pacific Marine Finfish Aquaculture Network.

Current status

This project commences 1 July 2004.

Project personnel

- Dr Mike Rimmer, Department of Primary Industries, Animal Sciences – Profitable Aquaculture Systems, Northern Fisheries Centre, Cairns, Queensland, Australia.
- Dr Kevin Williams, CSIRO Division of Marine Research, Cleveland, Queensland, Australia.
- Dr Joebert Toledo, South-east Asian Fisheries Development Centre, Aquaculture Department, Iloilo, the Philippines.
- Dr Ketut Sugama, Central Research Institute for Aquaculture, Jakarta, Indonesia.
- Dr Nyoman Adiasmara Giri, Research Institute for Mariculture, Gondol, Bali, Indonesia.
- Mr Usman, Research Institute for Coastal Fisheries, Maros, Sulawesi, Indonesia.
- Dr Inneke Rumengan, Sam Ratulangi University, Manado, Sulawesi, Indonesia.
- Dr Le Thanh Luu, Research Institute for Aquaculture No.1, Bac Ninh, Vietnam.
- Dr Michael Phillips, Network of Aquaculture Centres in Asia-Pacific, Bangkok, Thailand.

Contact details

Project Leader in Australia:
Dr Mike Rimmer
Northern Fisheries Centre
PO Box 5396, Cairns 4870, Queensland, Australia
Tel: 07 4035-0109; Fax: 07 4035-6703
Email: mike.rimmer@dpi.qld.gov.au

Economic and Market Analysis of the Live Reef Fish Food Trade in the Asia-Pacific *(ACIAR project ADP/2003/022)*

Project summary

The LRFFT is already substantive in the Asia-Pacific region, now involving over 20 countries, with the principal market based in Hong Kong, China and southern Peoples Republic of China. Recent estimates value the trade at approximately $US 350 million, with the majority of this value at the retail end of the market.

There are a number of important economics, social and environmental issues involving future development of the trade that would benefit form research. These include both supply and demand issues. On the supply side, the sustainability of the industry is in doubt due to over-exploitation and the use of destructive fishing practices in some supplying countries (e.g. cyanide fishing and the targeting of spawning aggregations). The major supplying countries are important partner countries for ACIAR; including Australia, Thailand, Indonesia, Philippines, Vietnam, and potentially Papua New Guinea, Fiji and the Solomon Islands. The Australian live reef fishery is the only one that regulates for over-fishing and enforces bans on destructive fishing behaviour.

On the demand side, the future market potential for wild-caught and cultured live reef product is largely unknown. Demand analysis to include the impact of income and population growth in Hong Kong, China and southern PRC, and consumer preferences for different fish attributes (such as colour, rarity and taste) would be beneficial to developing country (most of whom are small-scale/subsistence fishers) and Australian fishers. Such information would also be of value to ACIAR projects (managed by Barney Smith) research aquaculture technologies in the Asia-Pacific region.

This project aims to provide a thorough market analysis of the LRFFT to encourage sustainable economic development of the trade. Key research objectives are:

1. To quantify short and long-term demand of live reef fish in Hong Kong, China and southern PRC sourced from Asia-Pacific developing countries
2. To quantify short and long-term supply of live reef fish from wild-caught and aquaculture production sourced from Asia-Pacific developing countries
3. To measure the key cost and risk components of the marketing chain
4. To quantify future changes in supply and demand for live reef fish arising from new technology, management practices and economic growth, and to identify the beneficiaries of these developments
5. To identify the highly-valued product attributes (e.g. colour, taste, texture) of wild-caught and aquaculture live reef product
6. To identify possible policy options to improve market performance
7. To build capacity in economic assessment through the Asia-Pacific to provide and coordinate economic research and disseminate information on the trade utilising the existing live reef fish research and development networks (APMFAN, SPC, WorldFish)

The research team comprises Dr Brian Johnston as project leader, Dr Elizabeth Petersen, Mr Geoffrey Muldoon, Dr Mahfuzuddin Ahmed and Mr Being Yeeting. A

key research outputs from this study will include a partial equilibrium model of the live reef food fish trade in Asia-Pacific, integrating the empirical modelling of supply and demand, and marketing costs of the trade. From this model, policy options for improving market performance of the industry will be analysed (including options for fishery regulation) with identification of the beneficiaries of market improvements. Dr Petersen will be largely responsible for completing these tasks in collaboration with Dr Ahmed, Mr Yeeting and partner country collaborators. Another key research output will be a spreadsheet model of market chain data for fishery managers in Australia and at least two SE Asian and two Pacific supplying countries to enable them to assess future viability of live reef capture fisheries. Mr Muldoon will be largely responsible for completing these tasks in collaboration with Being Yeeting and other country partners.

The information from these outputs will feed into a number of forums over the two years. These include research workshops to be held by the project with key collaborators in the region, workshops held by the ACIAR Mariculture Grouper project, the APEC working group on standards, the annual conference of the Australian Agricultural and Resource Economics Society (AARES) and biennial international conference of the International Institute of Fisheries Economics and Trade (IIFET). There are also good extension opportunities for the outputs of the project through the Secretariat of the Pacific Community (SPC) Live Reef Fish extension network.

Objectives

The overall aim of the project is to enhance the sustainable economic development of the live reef food fish trade, through economic analysis of policy options for improved market performance. Specific objectives are:

1. To quantify short and long-term demand of live reef fish in Hong Kong, China and southern PRC sourced from Asia-Pacific, including developing countries
2. To quantify short and long-term supply of live reef fish from wild-caught and aquaculture production sourced from Asia-Pacific, including developing countries
3. To measure the key cost and risk components of the marketing chain
4. To quantify future changes in supply and demand for live reef fish arising from new technology, management practices and economic growth, and to identify the beneficiaries of these developments
5. To identify the highly-valued product attributes (e.g. colour, taste, texture) of wild-caught and aquaculture live reef product
6. To identify possible policy options to improve market performance
7. To build capacity in economic assessment through the Asia-Pacific to provide and coordinate economic research and disseminate information on the trade utilising the existing live reef fish research and development networks (APMFAN, SPC, WorldFish)

Current status

This project commences 1 July 2004.

Project personnel

- Dr Brian Johnston, Australian National University, Canberra, Australian Capital Territory, Australia.
- Dr Elizabeth Petersen, University of Western Australia, Bateman, Western Australia, Australia.
- Dr Geoffrey Muldoon, Cooperative Research Centre for Sustainable Use of the Great Barrier Reef, Townsville, Queensland, Australia.

Contact details

Project Leader in Australia:
Dr Brian Johnston
Visiting Senior Fellow
The Australian National University
Tel: 02 6258-1556; Fax: 02 6258-9770
E-mail: njvj@austarmetro.com.au

Minimizing environmental effects of finfish grow-out cages in Indonesia and Australia *(ACIAR project FIS/2003/027)*

Project outline

The environmental effects of fish cage culture are poorly understood in the tropics, though there is a very substantial literature from temperate areas, primarily concerning the effects of salmonid farming in North America and the U.K. The extent of nutrient impacts originating from fish farming is determined by coastal hydrography and geomorphology, with the effects more apparent in semi-enclosed waterways. In the Northern Territory, there is a 1000 tonne fish cage system at Port Hurd, Bathurst Island for the grow-out of barramundi. The success of this operation, and government imperatives to expand aquaculture will inevitably result in industry expansion which will need to develop on an ecologically sustainable basis.

In South East Asia fish cage farming is at least as productive as pond culture of shrimp, and is growing rapidly, and most nations share Australia's concerns in maintaining appropriate environmental standards. Indonesia, in particular is seeing dramatic growth in marine fish culture that has already attracted the attention of environmental non-government organizations. We propose to collect and synthesize environmental information from farms in Australia and Indonesia and to use this to develop tools and farming protocols to underpin ESD-based industry development. Information will be collected from research based experimental fish farms and ground-truthed at operational farms.

The tropical environments of Indonesia and Northern Australia are obviously dissimilar to European systems in a number of ways (e.g. tidal regimes, sediment types, water chemistry and rainfall regimes). Our preliminary work will identify the key variables in tropical systems that can be utilized in existing planning tools for industry development and management, such as the guidelines for the development of fish cage culture in the Mediterranean (MERAMED; www.meramed.com). The major outcome of the project will be the improved management of tropical fish cage culture in a broad spectrum of environments. Collaboration, exchange of personnel and sharing of resources will facilitate the growth of scientific capacity in each partner country. Community benefits will include the minimisation of the environmental impacts of cage culture and a firm Ecologically Sustainable Development framework for future cage farming development. The latter will enable proactive management and a strategic approach to sea cage culture developments by both countries.

This proposal plans to link AIMS' expertise on coastal marine ecology in the tropics to research on the environmental effects of fish cages being undertaken in Indonesia at the Maros Institute and at Gondol. For the Australian component of the work, the project will directly involve the staff of NTDBIRD (NT Department of Business, Industry and Resource Development) and NTDIPE (NT Department of Infrastructure, Planning and Environment). For the Indonesian component of the work, Dr Jes Sammut (UNSW) will develop planning tools based upon the environmental work conducted by AIMS and Indonesian partners. Adoption pathways for the results of this project will be primarily through licencing and management agencies in Indonesia (Ministry of Agriculture and Fisheries) and Australia (NTDBIRD). This project will work in close cooperation with the grouper project led by Dr Mike

Rimmer and feed into GIS infrastructure developed by Dr Sammut's acid-sulphate soils project, both of which are ACIAR projects. Dr Sammut is also developing a new project proposal for land classification for shrimp and polyculture systems, which will link to this proposed work. In Australia this information will enable tropical cage farming operations to be developed within the ESD framework for Aquaculture that is currently being jointly developed by the aquaculture industry and Government institutions. Progress of the project during its operation and the outputs resulting from the project will be advertised on the NACA website.

Objectives

1. Develop best practice guidelines for siting and best environmental practice of tropical marine fish cage culture, compatible for both northern Australian and Indonesian environments, including the provision of planning and mapping outputs.

2. Adapt/develop an appropriate model to determine carrying capacity of generic environments for fish cage culture, including external factors. Carrying capacity will be determined by quantifying impact in terms of production intensity.

3. Ensure transfer of outputs to Indonesian management agencies and adoption of appropriate protocols for managing industry expansion.

4. Build capacity and provide training for Indonesian collaborators.

Current status

This project is currently under development and planned for commencement in January 2005.

Project personnel

- Dr David McKinnon, Australian Institute of Marine Science, Townsville, Queensland, Australia.
- Dr Jesmond Sammut, University of New South Wales, Sydney, New South Wales, Australia.
- Dr Adi Hanafi, Research Institute for Mariculture, Gondol, Bali, Indonesia.
- Dr Rachmansyah, Research Institute for Coastal Fisheries, Maros, Sulawesi, Indonesia.

Contact details

Project Leader in Australia:
Dr David McKinnon
Australian Institute of Marine Science
PMB No.3, Townsville MC 4810, Queensland, Australia
Tel: 07 4753-4292; Fax: 07 4753-4429
Email: d.mckinnon@aims.gov.au

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Appendix 1 – Abbreviations and Acronyms

AARES	Australian Agricultural and Resource Economics Society
ACIAR	Australian Centre for International Agricultural Research
AD	apparent digestibility
AFDW	ash-free dry weight
AFFS	Agency for Food and Fibre Sciences (DPI&F)
AFFS – F&A	AFFS – Fisheries and Aquaculture
AIDI	Aquaculture Industry Development Initiative
AIMS	Australian Institute of Marine Science
APEC	Asia-Pacific Economic Cooperation
APMFAN	Asia-Pacific Marine Finfish Aquaculture Network
ARA	arachidonic acid (20:4n-6)
C*n*	copepodite stage
cDNA	complementary deoxyribonucleic acid
CP	crude protein
CSIRO	Commonwealth Scientific and Industrial Research Organisation
D*n*	day number (D0 = day of hatching)
DHA	docosahexaenoic acid (22:6n-3)
DM	dry matter
DO	dissolved oxygen
DPA	docosapentaenoic acid (22:5n-6)
DPI&F	Department of Primary Industries and Fisheries (Queensland)
ELISA	enzyme linked immunosorbent assay
EPA	eicosapentaenoic acid (20:5n-3)
ESD	Ecologically Sustainable Development
GBRMPA	Great Barrier Reef Marine Park Authority
GIS	Geographic Information System
HUFA	highly unsaturated fatty acid
IIFET	International Institute of Fisheries Economics and Trade
IGF	insulin-like growth factor
IGFBP	insulin-like growth factor binding proteins
L-strain	large strain rotifer (*Brachionus plicatilis*)
LRFFT	Live Reef Food Fish Trade
mRNA	messenger ribonucleic acid
N*n*	naupliar stage
NACA	Network of Aquaculture Centres in Asia-Pacific
NFC	Northern Fisheries Centre
NTDBIRD	NT Department of Business, Industry and Resource Development
NTDIPE	NT Department of Infrastructure, Planning and Environment
PUFA	polyunsaturated fatty acid
QEPA	Queensland Environmental Protection Agency
QFS	Queensland Fisheries Service (DPI&F)
QPCR	quantiative polymerase chain reaction
RAS	recirculating aquaculture system
R&D	research and development

S- / SS-	small strain / super-small strain rotifers (*B. rotundiformis*)
SD&I	State Development and Innovation (Queensland)
SEAFDEC AQD	Southeast Asian Fisheries Development Centre, Aquaculture Department (Tigbauan, Philippines)
SPC	Secretariat for the Pacific Community
UNSW	University of New South Wales
Vtg	vitellogenin

Appendix 2 – Publications

Project publications

Scientific Papers

McKinnon, A.D., Duggan, S., Nichols, P.D., Rimmer, M.A., Semmens, G. and Robino, B. (2003). The potential of tropical paracalanid copepods as live feeds in aquaculture. Aquaculture 223, 89–106.

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Publications from associated projects

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