Australian Government

Australian Centre for International Agricultural Research

Final report

project

Improved hatchery and grow-out technology for marine finfish aquaculture in the Asia–Pacific region

project number	FIS/2002/077
date published	December 2011

prepared by	Dr Michael A. Rimmer, Senior Research Fellow, Faculty of Veterinary Science, University of Sydney, Australia
co-authors/ contributors/ collaborators	Dr Kevin Williams, CSIRO Marine Research, Australia Dr N.A. Giri, Director, Research Institute for Mariculture, Gondol, Bali, Indonesia Usman, Researcher, Research Institute for Coastal Aquaculture, Maros, South Sulawesi, Indonesia Dr Richard Knuckey and Adam Reynolds, Department of Employment, Economic Development and Innovation, Queensland, Australia Dr Claire Marte, Dr Veronica Alava and Dr Mae Catacutan, Integrated Services for Development of Aquaculture and Fisheries, Iloilo, Philippines, Dr Inneke F.M. Rumengan, Senior Lecturer, Sam Ratulangi University, Manado, North Sulawesi, Indonesia Dr Michael Phillips, Dr Sih-Yang Sim and Simon Wilkinson, Network of Aquaculture Centres in Asia-Pacific, Bangkok, Thailand Dr Le Thanh Luu, Director, Research Institute for Aquaculture No.1, Vietnam
approved by	Dr Chris Barlow, Research Program Manager for Fisheries, ACIAR

final report number	FR2011-32
ISBN	978 1 921962 29 5
published by	ACIAR GPO Box 1571 Canberra ACT 2601 Australia

Contents

1 Acknowledgments

We thank Brian Johnston and Noel W.W. Chan (ACIAR project ADP/2002/105 'Economic and market analysis of the live reef fish food trade in Asia-Pacific') for carrying out the taste evaluation test with mouse grouper in Hong Kong.

We thank Igor Pirozzi and Simon Tabrett for developing and implementing the nutrition workshop, and Usman, Ketut Suwirya and Reza Samsudin for assisting with the presentation of workshop materials. We thank the Director, Dr Endhay Kusnendar Kontara, and the staff of Pusat Riset Perikanan Budidaya (Research Centre for Aquaculture) who assisted with the planning and preparation of the nutrition workshop: Mr Anang Hari, Mrs Iswari Ratna Astuti, Mr Hatim Albasri and Mrs Erfina S., and Dr Geoff Allan and Mrs Helena Heasman for assistance with the workshop program and travel respectively.

We thank Dr Mohammad Murdjani, Pak Syamsul Akbar and Pak Sudjiharno (Directorate General for Aquaculture, Indonesia) for their participation in the project.

We thank Pak Slamet Subyakto and the other staff of BBAP Situbondo for arranging and carrying out the annual Grouper Hatchery Production Technology Training Course.

We thank Mr Nhu Van Can, Director (ARSINC), Vietnam for assistance with arrangements for training RIA1 staff in Australia.

Mike Rimmer wishes to thank the staff of NACA for their assistance in implementing project activities and related travel.

Sih-Yang Sim would like to specifically acknowledge the following organisations that assisted financially, in survey and data collection, field trip arrangements, or in other ways to the activities of Objective 3:

- Network of Aquaculture Centres in Asia-Pacific (NACA).
- Australian Centre for International Agricultural Research (ACIAR).
- Research Institute for Aquaculture No.1, Vietnam.
- Research Institute for Mariculture – Gondol, Bali, Indonesia.
- Brackishwater Aquaculture Development Centre, Situbondo, Indonesia.
- Mariculture Development Centre, Batam, Indonesia.
- Main Centre for Mariculture Development, Lampung, Indonesia.
- Krabi Coastal Research and Development Station, Krabi, Thailand.

2 Executive summary

This project focussed on improving marine finfish aquaculture production in the Asia-Pacific region by focussing on key constraints: improving hatchery technology to improve the availability of seedstock; evaluating the nutritional needs of groupers to support the development of compounded pellet diets; and improving communication and coordination of marine finfish aquaculture research and development activities in the Asia-Pacific region.

Larval rearing

The project developed a range of techniques to improve the larval rearing of marine finfishes, particularly groupers, including:

Demonstrating that the use of nutritional supplements that increase the levels of highly unsaturated fatty acids (HUFAs) in the larval diet lead to improved growth, condition and survival of grouper larvae. Overall, these experiments showed that grouper larvae have a very high requirement for HUFAs, particularly DHA (22:6n-3), but also for ARA (20:4n-6) and EPA (20:5n-3).

Evaluating the capacity of grouper larvae to digest live prey as well as compounded larval diets by describing the development of digestive enzymes during larval development. Our results show that early stage larvae have very low levels of digestive enzymes, and thus limited capacity to digest prey and particularly compounded pellets.

Developing improved techniques for culturing the calanoid copepod *Parvocalanus*. Experiments with feeding *Parvocalanus* to early stage grouper larvae demonstrated dramatic increases in larval survival and growth to day 12.

Cannibalism-related losses during the nursery stage can be reduced by commencing feeding early in the day (i.e. soon after dawn), and maintaining light levels at <600 lux.

Grow-out nutrition

This project and its predecessor project (FIS/97/73) have evaluated the nutritional requirements of groupers, looking at optimal protein, lipid and protein:energy ratios, as well as some minor nutrients such as vitamin C and highly-unsaturated fatty acids (HUFAs).

These results have been adopted by feed manufacturers who are now producing a range of marine finfish feeds. To improve the adoption of project results in Indonesia, the project held a technical workshop in Surabaya in October 2009 to train feed formulators and provide current nutritional information to commercial feed producers in Indonesia.

Communication and technology adoption

This project continued to use the communication methodologies established under FIS/97/73:

- Reporting project outcomes on the NACA web site (www.enaca.org);
- Publishing technical information in printed and electronic (.pdf) versions, including translations into various regional languages;
- A dedicated section on Marine Finfish Aquaculture in the NACA magazine Aquaculture Asia.

These mechanisms have allowed project outcomes to be communicated to countries other than those directly involved in the project, and have supported broader interaction between aquaculture researchers, managers and commercial practitioners in the Asia-Pacific region.

The Regional Grouper Hatchery Production Training Course has been held annually since 2002 in Indonesia. The 2008 course provided a significant milestone with over 100

graduates now having completed hatchery training through this course. Many graduates have gone on to become trainers in their own countries.

3 Background

Aquaculture of high-value marine finfish species is an area of increasing agricultural interest in Southeast Asia. Species such as groupers (Serranidae, Epinephelinae) bring high prices (up to US$70 /kg wholesale) in the live markets of Hong Kong and southern China (McGilvray and Chan 2001). Marine finfish aquaculture is an important contributor to the economies of coastal communities, and aquaculture of high-value species (such as groupers) provides greater benefits to farmers than aquaculture of lower-value species such as milkfish (Yap 2002). However, much of the marine finfish aquaculture in Southeast Asia relies on the capture and grow-out of wild-caught juvenile fish: around 70–85% of cultured groupers are from wild-caught fry. In some areas, the use of hatchery-reared fry is becoming more common. For example, in Indonesia, an estimated 15–25% of cultured groupers are now hatchery-reared, while in Taiwan this proportion may be as high as 70%. However, wild-caught groupers make up the bulk of the seedstock supply in many parts of Southeast Asia, including Vietnam, Thailand and the Philippines. The trade in wild fry is associated with a number of resource management issues, including: overfishing, use of unsustainable harvesting techniques (including cyanide), high levels of mortality; inadequate supply to support the demand of a developing aquaculture industry (Sadovy 2000). To meet the demand for seedstock for aquaculture, and to reduce pressure on wild fisheries, there is a recognised need to develop commercial marine finfish hatcheries throughout the Asia-Pacific region to supply hatchery-reared seedstock.

The need to develop hatchery technology for high-value marine finfish species is a widespread issue throughout the Asia-Pacific region, including Australia. Development of marine finfish aquaculture in Australia has been limited by (amongst several constraints) the lack of seedstock supply – provision of seedstock through harvest fisheries for juvenile fish, which is common throughout Southeast Asia, has not been undertaken because of Australia's strict fisheries management procedures.

The need for compounded (pellet) feeds is also widespread throughout the region. Most marine finfish aquaculture in Southeast Asia is supported by the use of 'trash' fish as the major feed source. Issues regarding the use of trash fish have been identified in detail in several publications (e.g.New 1996) and these include: competition for fishery products with human nutritional requirements and with other agricultural sectors; relatively low efficiency of utilisation of 'trash' fish (FCRs typically range from 8:1 to 16:1 wet basis – equivalent to 2:1–4:1 dry matter basis, compared to 1.0:1–1.8 dry matter basis for pellet diets); and localised pollution due to losses of feed material during feeding (Phillips 1998). Because the use of 'trash' fish for feed is not economic in Australia, the development of marine finfish aquaculture relies on the development of suitable cost-effective feeds. In addition, Australia's strict environmental regulation of aquaculture requires the development of feeds that minimise nutrient release to the environment.

These issues were addressed with considerable success in the previous project (FIS/97/73). However, given the relatively early stage of development of marine finfish aquaculture (compared with more mature agricultural sectors) in the region, and on-going concerns regarding its sustainability, there is a widely recognised need to continue to address these fundamental sustainability issues. Sustainability issues for the marine finfish aquaculture industry in the Asia-Pacific were discussed in detail at the Regional Workshop on Sustainable Marine Finfish Aquaculture for the Asia-Pacific held in HaLong City, Vietnam, 30 September – 4 October 2002. This workshop was funded by ACIAR, the Australian Academies of Technological Sciences and Engineering (through the Department of Science and Technology 'Frontiers of S&T Missions and Workshops' program), and the Government of Vietnam. There were more than 80 participants at the workshop including representatives from Australia, Vietnam, Indonesia, the Philippines, India, China, Hong Kong SAR, Myanmar, Thailand, Malaysia, Brunei Darussalam and Europe, and representatives from a range of regional organisations including NACA,

WorldFish Centre, APEC, FAO, The Nature Conservancy and the Marine Aquarium Council. The topics targeted for this follow-on project are amongst those given a 'high' priority rating at this workshop.

This project follows on from ACIAR project FIS/97/73 Improved hatchery and grow-out technology for grouper aquaculture in the Asia-Pacific region. It has been developed to:

- Incorporate areas of research that were identified in FIS/97/73 as being of significant benefit to improving grouper hatchery and grow-out practices;
- Incorporate areas of research that were identified at the Workshop on Sustainable Marine Finfish Aquaculture for the Asia-Pacific Region, held in HaLong City, Vietnam, 30 September – 4 October 2002, as high-priority research areas;
- Incorporate the recommendations of the formal end-of-project review of FIS/97/73, undertaken by Dr Sagiv Kolkovski (Department of Fisheries, Western Australia);
- Link strongly with other ACIAR marine finfish aquaculture projects, including the proposed projects on '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 Asia-Pacific (ADP/2002/105)'.

4 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.

5 Methodology

Objective 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

1.1.1 Larval nutrition

Marine finfish larvae require high levels of essential fatty acids (EFAs) in the diet because they are unable to bioconvert short chain fatty acids to longer chain n-3 and n-6 fatty acids. In particular, the highly unsaturated fatty acids (HUFAs) eicosapentaenoic acid (20:5n-3, EPA), docosahexaenoic acid (22:6n-3, DHA) and arachidonic acid (20:4n-6, ARA) are essential for survival, growth and good condition of marine fish larvae. Research undertaken as part of FIS/97/73 demonstrated that improving fatty acid nutrition in larval diets improved growth, survival and condition of *E. coioides*. In this project, the same approach was extended to culture of *E. fuscoguttatus* in Indonesia.

This work was undertaken in a structured manner:

- The nutritional composition of prey organisms at partner laboratories was evaluated.
- The levels of HUFAs were increased by evaluating several different commercial supplements.
- The larval requirement for essential fatty acids was evaluated by comparing starved and fed larvae.

Nutritional composition of prey organisms and HUFA supplementation

Live prey organisms cultured at RIM Gondol were sampled: freshwater *Chlorella* sp., *Nannochloropsis oculata*, rotifers *Brachionus altivelis* pre-enriched and enriched with Algamac-3050 and DHA-Selco, and *Artemia* sp. nauplii (INVE brand) enriched with Algamac-3050 and DHA-Selco. Enrichment procedures using Algamac and Selco followed the recommendations of Aquamarine Biofauna (USA) and INVE (Belgium), respectively. The freshwater *Chlorella* sp. was imported from Japan while *N. oculata* was cultured locally using inorganic fertilisers.

Total lipid of samples was extracted as per the techniques described by Alava *et al*. (2004) and analysed (two to three replicate samples) on a Shimadzu GC-17A gas chromatograph.

Larval fatty acid requirement

Tiger grouper reared at RIM Gondol were sampled: newly hatched larvae (NHL), unfed day-3 larvae, day-18 and day-25 larvae reared with live food organisms and Riken (Japan) larval feed then starved for two days. The samples were freeze-dried and analysed for total lipid and fatty acid composition. Total lipid samples were separated into neutral (NL) and polar lipid (PL) using silica cartridges. Fatty acid methyl esters were prepared (three replicate samples) and analysed using a Shimadzu GC 2010 gas chromatograph.

Morphological and histological study of opercular deformities

Hatchery-reared groupers are susceptible to abnormal development, resulting in a range of deformities in juvenile fish. In mouse grouper, a common deformity is unilateral or bilateral opercular deformities that occur with varying severity. Opercular deformities negatively affect biological functions, including respiration which can be impaired due to reduced efficiency of the buccal pump, while the exposed gills are more vulnerable to damage and infection of disease agents, particularly of very young fish. This study presents some observations on the morphology and histology of normal and deformed operculum of mouse grouper juveniles obtained from RIM Gondol, Bali.

Photographs of mouse grouper juveniles with varying severity of opercular deformity were taken. The head region of samples was excised and fixed in 10% buffered formalin for histological processing. The paraffin embedded samples were serially sectioned at 6μm and slides were stained using haematoxylin and eosin for viewing using a stereomicroscope.

Vitamin C feeding trial

In the second part of this study, we investigated whether opercular deformities could be due to a deficiency of essential nutrients. An important component of connective tissues is collagen and its synthesis in fish is enhanced when dietary vitamin C supplement is increased. Vitamin C and n-3 highly unsaturated fatty acids (HUFAs) are important nutrients in diets for marine fish. These were supplemented to the commercial diets used at RIM Gondol for possible repair of operculum deformity in grouper.

Mouse grouper (mean weight 1.6 g) with bilateral opercular deformity were stocked at 20 fish/tank (tank size: 100 L) and fed with five dietary treatments in triplicate for 77 days. The five dietary treatments consisted of two commercial diets (NRD and Otohemi), and Otohemi coated with emulsion preparation of Phosphitan C and/or n-3 HUFA (DHA-Selco). Fish were fed twice daily at satiation level and weighed every week to obtain growth data. At the end of feeding trial, fish were weighed, counted for survival and the number of fish with fully recovered opercula was determined.

1.1.2 Larval digestion

The component of work on larval digestion sought to evaluate how well larval groupers could digest prey of various types at different stages of larval development. It also assessed the capacity of the larvae to digest different feed components. Such information can be used to improve larval rearing practices and improve the performance of inert larval diets.

Enzyme response during initial first feeding stage

Tiger grouper (*E. fuscoguttatus*) and coral trout (*P. leopardus*) larvae reared at NFC Cairns in experimental tanks were reared on a live prey diet in a green water system. Levels of enzyme activity were analysed from daily samples until 10 days post-hatch for both species.

Enzyme response during larval development

Tiger grouper (*E. fuscoguttatus*) and coral trout (*P. leopardus*) larvae reared at NFC Cairns in experimental tanks were reared on a live prey diet (copepods, rotifers and brine shrimp) and inert diets in a green-water system. Levels of enzyme activity were analysed from samples until larval stage was completed (post-metamorphosis) for both species.

Enzyme response to feed type

Tiger grouper larvae reared at Northern Fisheries Centre in experimental tanks on a live prey diet (copepods, rotifers and brine shrimp) and inert diets were subjected to changes in diet composition over a 4 day period. Levels of enzyme activity were analysed from samples before and after dietary changes.

1.1.3 Verification of intensive and semi-intensive hatchery techniques

Larviculture techniques developed through the project were incorporated in Australian larval rearing protocols throughout the life of the project. As discussed later in this report (p.71), Indonesian farmers still purchase fingerlings based primarily on price. Consequently, Indonesian hatcheries focus on reducing production cost, and are reluctant to incorporate techniques that increase capital or operational costs. Because of this, there has been limited uptake of improved hatchery techniques by Indonesian hatcheries.

1.2 Improve the availability and quality of live prey

This component of work aimed to improve larval survival during the early stages of larval rearing by providing adequate quantities of appropriately-sized feed organisms. Groupers are notorious for their small mouth size at first feed compared with many other marine finfish species (Kohno *et al.* 1997), and this component of work aimed to develop techniques to reduce the overall size of rotifers, and to develop culture techniques for alternative live prey organisms of small size, such as copepod nauplii.

1.2.1 SS-strain rotifers

Reduce average rotifer body size by screening

Screening an SS-strain rotifer population to select for the smaller sized rotifers to then scale up to a new population was investigated as a method to reduce the average size of rotifers. To achieve this, the initial rotifer population needs to be synchronous and without egg-bearing rotifers. Previous experiments determined that a synchronous population grown from eggs for 12–17 h at a salinity of 30 ppt post hatch contains rotifers that are approaching full size but not yet fecund (Figure 1). Beyond 17h, some rotifers start to produce eggs.

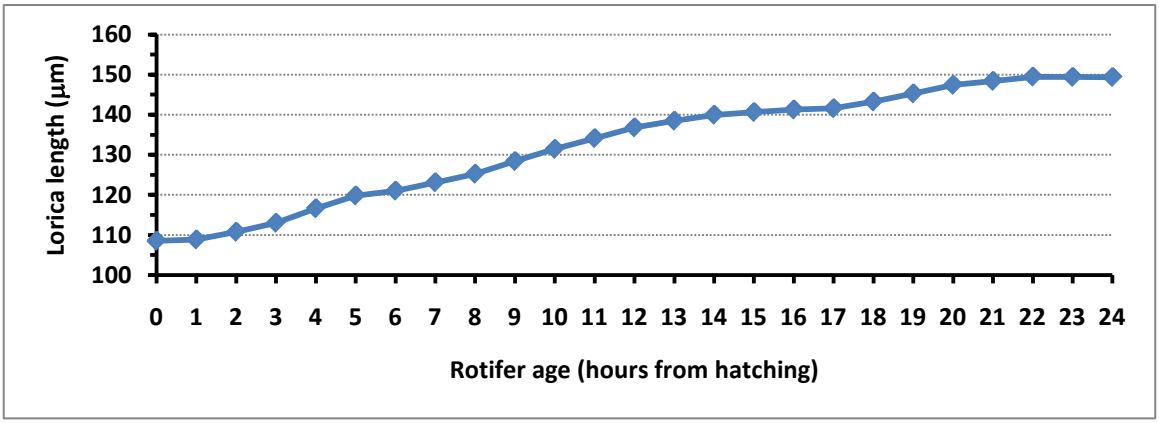


Figure 1 Average lorica length of rotifers cultured at 30 ppt.

A rotifer population was fed for 24 h on Culture Selco HD (high density) to boost the fecundity rate. Rotifers were harvested and 'pulse' blended using a stick-blender. This dislodged the eggs. Rotifers were screened out and the eggs collected onto a 45 µm screen. Some neonates were present and were killed by freshwater washing the eggs. The eggs were allowed to hatch for 2 h and neonates used to set up a synchronous rotifer culture.

The synchronous rotifer culture was fed *Nannochloropsis* and allowed to grow for 12–17 h. It was then screened through a 63 µm screen and small rotifers collected on a 45 µm screen. These, relatively few, rotifers were allowed to grow up for 2 weeks and the average size of egg-bearing rotifers was then measured. The whole cycle was then repeated from collection of eggs to a synchronous culture, collection of small rotifers and growing up to measure the average rotifer size.

Cold-storage of amictic eggs for mass production of SS-strain neonates

Using the methods developed previously for collection of rotifer amictic eggs, an experiment was run to evaluate the possibility to cold-store harvested eggs. Rotifer eggs were collected as detailed in Activity 'Reduce average rotifer body size by screening'. Eggs were distributed amongst 72 x 70 mL plastic jars within a temperature gradient block. The block consisted of 12 columns of increasing temperature with 6 replicate jars in each column. At 24, 48 and 72 h, two jars were sampled from each temperature and the percentage of hatched rotifers calculated. By 48 and 72 h, rotifers at temperatures above 16.5°C had started to reproduce and consequently were not counted. At ~10°C, the percentage of rotifers hatched did not increase with time and this temperature was selected to look at short-term storage of eggs (Figure 2).

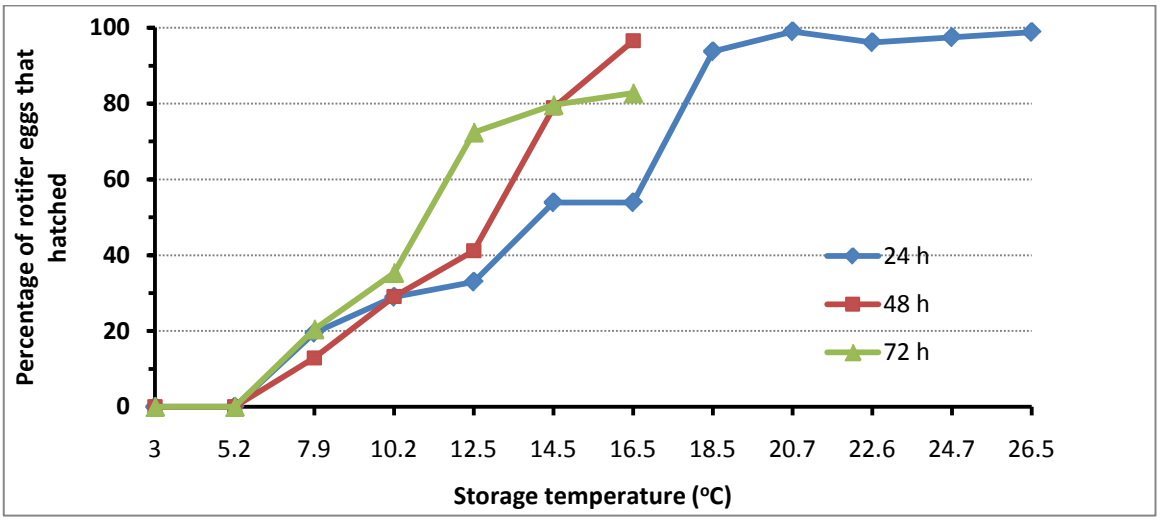


Figure 2 Percentage of hatched rotifer eggs after stored at various temperatures for 24, 48 and 72 hours.

Detect shift in population phenotype as a result of selection pressures

The rotifers used were obtained from Manembo-nembo and Minanga brackish water ponds in North Sulawesi. The rotifers were assessed for size distribution at different salinities (5, 10, 20, and 30 ppt). Each experiment was conducted in a temperature controlled room 28 ± 1°C with three replicates each in a 200 mL container with an initial rotifer density of 50 mL^{-1} and fed *N. oculata*.

Increase the natural variation within a rotifer population through hybridisation of strains and then to select for super-small individuals

Several rotifer strains from North Sulawesi were assessed for natural variation in body size. Rotifers were sampled from six locations across North Sulawesi peninsula (Figure 11; p.50). Three locations (Minanga, Watuliney and Manembo-nembo) were facing the Maluku Sea, and the other locations (Likupang, Tumpaan, Meras) were facing the Sulawesi Sea. Natural size variation among strains was found in range of 120–190 µm. In general, the Watuliney, Likupang, and Tumpaan strains were larger than the Minanga, Manembo-nembo and Meras rotifers (Figure 11).

Hybridisation experiments were initiated by collecting 10 males of Likupang strain to be mated with a single female of Manembo-nembo strain. Fertilized females were cultured using *N. oculata* until resting eggs were produced. The neonates hatched from the eggs (hybrid Li-Ma) were cultured for size measurement.

Assess the use of protozoa as first feed prey for marine finfish larvae

A protozoan contaminant of rotifer cultures was identified as a possible new live feed for finfish larvae. It was small (80 µm) and predominantly free swimming although also sometimes benthic if high nutrient loads were present on the tank bottom. The protozoan was identified as belong to the Hypotrich group.

The protozoan was an intermittent contaminant of the rotifer cultures and during such a period, high numbers of protozoa were transferred to the larval rearing tanks. Although it was not possible to determine if the finfish larvae consumed the protozoa, there was no detectable improvement in the larval survival compared to normal larval runs. It was possible the protozoa were detrimental to the larvae as their numbers increased in the high nutrient load and before flow-through water exchanges flushed them out.

In preliminary experiments, the protozoa was isolated and fed a range of microalgae. However, it failed to thrive and was not stable in culture without rotifers. It is likely that the high nutrient load of the rotifer system and partially digested rotifer faecal matter supports optimal protozoan growth. With changes to high density rotifer culture using formulated diets the protozoa were actively discouraged; consequently, this activity was re-evaluated and cancelled. Instead, effort was focussed on copepod culture where results were indicating a very significant positive benefit to their inclusion in grouper larval diets.

1.2.2 Ultra-small copepod nauplii as first feed prey for marine finfish larvae

Copepod nauplii have been established as a more effective live food source for marine finfish, particularly groupers, than rotifers (Toledo *et al.* 2005). This component of research evaluated the potential to develop culture technologies for copepods, to improve growth and survival of marine finfish in hatcheries.

Evaluation of diets for the cyclopoid copepod, Oithona sp.

The cyclopoid copepod *Oithona* sp. was isolated from a brackishwater pond 30 km east of Manado, North Sulawesi, and cultured at Sam Ratulangi University. Copepods were scaled-up from single reproductive females for use in feeding experiments at 30 ppt seawater containing different species of microalgae (*Nannochloropsis oculata*, *Tetraselmis* sp. and *Isochrysis* sp.).

Culture techniques for Euterpina acutifrons

Observations at RIM Gondol on the population dynamics of the harpacticoid copepod *Euterpina acutifrons* was carried out using 5-L plastic buckets with an initial copepod density 100 ind./L. The microalga *Nannochloropsis* sp. was added to culture media at density of 50,000 cells/mL as a basic feed, to which were added: wheat flour (Treatment A) and minced chicken liver (Treatment B) at a rate of 50 mg/bucket. The additional feeds were provided twice each day with 12 h interval. The morphological stage, number of egg-bearing adults, number of nauplii produced, copepodites and adult copepods were then recorded.

Diet development for the culture of the calanoid copepod, Parvocalanus crassirostris

Feeding experiments on the calanoid copepod *Acartia sinjiensis* had shown it to perform best on a diet dominated by the cryptophyte alga *Proteomonas sulcata*. The need for this more specialised alga and the relatively low densities obtained for adult *Acartia* in mass culture necessitated the need for a copepod that was more amenable to mass culture. Another calanoid copepod, *Parvocalanus crassirostris*, was isolated from estuarine waters off Cairns and had proved stable in culture.

Nine, 250 L conical bottom, fibreglass tanks were filled to 175 L with filtered (1 µm) seawater (34 ‰ salinity, 28°C). Each tank was inoculated with adult *Parvocalanus*. Tanks had constant illumination from overhead cool-white, fluorescent room lighting. Monoalgal diets of *P. sulcata*, *Isochrysis* sp. (T.ISO) and *Tetraselmis* sp. were added at an initial equal ration of 1.3 µg AFDW/mL to each of three replicates. Microalgae were initially added each morning; later, as consumption increased, it was added twice a day. The rate of microalgae addition was recorded and adjusted based on a visual assessment to maintain a minimum feed level of similar colouration to that achieved with the initial 1.3 µg AFDW/mL. Copepod numbers were estimated each day by gently mixing the tank volume and taking a subsample. The volume of the subsample decreased as the copepod density increased but was typically 250–500 mL. The subsample was concentrated to approximately 50 ml and copepods counted in replicate 2 mL volumes.

Determine the fatty acid profile of the calanoid copepod, Parvocalanus crassirostris

For analysis of copepod fatty acid profile, three individual mass cultures of *Parvocalanus* were grown. Two 400 L and one 2,000 L culture were inoculated with copepods and fed only *Isochrysis* sp. (T.ISO). After 8–9 days, the copepod population of each tank was harvested to collect all copepod stages. The harvested population was rinsed with filtered seawater, collected onto a 44 µm screen, and subsamples taken for fatty acid analysis.

Assess nauplii acceptance and benefits to fish larvae as a first-feed prey item

At NFC Cairns, addition of copepods to finfish larval diets is now routinely undertaken. Mass cultures of *Parvocalanus crassirostris* are raised on *Isochrysis* sp. (T.ISO) and harvested when most copepods are late stage copepodites or adults. These copepods are added to the larval tanks on Day 2 along with SS-strain rotifers and a mixture of *Isochrysis* sp. (T.ISO) and *Nannochloropsis*. To assess the impact of adding copepods to the diet, a replicated larval rearing experiment was conducted to test for the effect of increasing the initial dose of copepods in larval rearing of tiger grouper (*E. fuscoguttatus*).

Using a conventional basal diet of SS-strain rotifers, copepods were either not included (0/mL) or added at 4/mL or 10/mL as a single addition on Day 2 post-hatch. No further additions of copepods were made to the cultures, but rotifers were added daily to maintain density. The experiment ran until 12 days post-hatch.

1.2.3 Extension of Acartia culture techniques

Activities undertaken under this objective are summarised on p.57.

1.3 Improve survival of juvenile groupers in the nursery stage

This component of work was designed to evaluate different options for improving the survival of juvenile grouper in the nursery phase, where cannibalism is a major cause of mortality. Activities were grouped into those related to nursery environment, feed management and feed development.

1.3.1 Nursery environment

This aspect of the research evaluated several aspects of the nursery environment that may affect survival of juvenile tiger grouper: tank shape, light intensity and water flow rates.

Tank shape

To investigate the effect of tank shape on the survival rate of tiger grouper, two tank shapes (circular and square) were evaluated. Juveniles were stocked in 300-L tanks at a density of 200 ind./tank and initially fed with live mysid shrimp twice per day. During the first week the fish were fed mixed moist + dry pellets (2:3). In the second week, fish were fed with mixed moist and dry pellets (1:4), and live mysid shrimp were given once each day in the afternoon. From the third week until the end of experiment, fish were fed with dry pellets. The experiment was run for 40 days.

Light intensity

In this experiment, four light treatments were used: (A) control (ambient sunlight, i.e. up to 3,000 lux), and three artificial light treatments: (B) 2000 lux, (C) 600 lux, and (D) 20 lux. Twelve fibreglass tanks (200 L) were used for the study and juvenile tiger grouper (2.5 cm TL) were stocked into each tank at a density of 135 ind./tank. Fish were fed with commercial artificial diet for 30 days.

Water flow rates

Three levels of water current were tested in triplicate: no current (control), 3 mL/min, and 10 mL/min. In this experiment, juvenile tiger grouper (2-2.5 cm TL) were stocked into the rearing tank at a density of 300 ind./tank and reared for four weeks. Fish were fed with artificial feed and mysid shrimp for the first and second week of the experiment, and then with artificial feed and minced trash fish for the last two weeks of experiment.

1.3.2 Feed management

This experiment investigated the influence of the time of day when feeding commenced on growth and survival of juvenile tiger grouper. Two hundred fish with an average weight of 1.5 – 2.0 g were stocked in 300-L tanks and fed with mixed diets. The mixed diets were given starting at 0700, 0900, and at 1100. All treatments were fed until 1800 each day. All fish were fed with live mysid shrimp twice a day for the first week, then with dry pellets six times per day for the remainder of the experiment.

1.3.3 Feed development for late larvae / juveniles

In the first experiment, juvenile tiger grouper were fed with different food combinations: A: artificial feed; B: tiny shrimp then followed by minced trash fish; C: artificial feed + tiny shrimp then followed by minced trash fish; and D: artificial feed with attractant supplementation. The attractant used was a mixture of 3.54 g proline, 2.32 g alanine and 2.07 g inosine monophosphate for 1 kg feed. The experiment was conducted by stocking juveniles (300 fish/tank) into12 fibreglass tanks with a seawater flow rate of 1L/min.

In the second experiment, the effect of attractant addition was investigated by adding it to a moist pellet. The moist pellet was mixed with dry pellet at the ratio of 2:3 in the first week of rearing and 1:4 in the second week of rearing. Juvenile tiger grouper (average weight of 1.5–2.0 g) were stocked into 100-L rearing tanks at a density of 100 ind./tank. Fish were fed with mixed moist diets added with attractant (treatment A) and without attractant (treatment B) six times a day. In the first week, fish were fed with live mysid given in the morning and afternoon and mixed diets 6 times a day. In the second week, fish were fed with only mixed diets and in the third week until the end of experiment, fed with pellet. Rearing was done for 40 days and artificial diets were given every two hours.

In the third experiment, the effect of two different attractants was investigated. The three treatments used were: commercial attractant 1, commercial attractant II, and mysid and minced fish (control). This experiment was conducted in 12 fibreglass tanks into which fish 2.5 cm TL were stocked at a density of 300 ind./tank. Ten grams of the attractant (I or II) mixed with homogenized white egg and CMC was mixed with 1 kg artificial diet before being fed to the fish.

Objective 2 – Develop cost-effective grow-out diets

2.1 Identify ingredients for grouper diets that will reduce formulation cost

This component focussed on determining the nutritive value (digestibility) of alternative feed ingredients and their potential as cheaper protein alternatives to fishmeal in compounded grouper feeds. This work adds to the grouper feeds digestibility database and provides the nutritional basis upon which informed decisions can be made on the nutritive value of these feed ingredients for groupers.

2.1.1 Ingredient digestibility

The apparent digestibility of 9 feed ingredients was evaluated using RICA Maros experimental facilities at Maros and at Aruwange Bay, South Sulawesi. The potential sources of protein available in quantity in Indonesia are: poultry offal, golden snail, green mussel and mysid meal, while yellow and white corn meal, rice bran and sorghum meal are also carbohydrate sources that are in plentiful supply in Indonesia. The proximate nutrient and gross energy composition of the test ingredients are listed in Table 1.

Table 1 Proximate nutrient and gross energy composition of air-dry test feed ingredients used in the digestibility study.

Test feed ingredient	Dry matter	Crude protein	Total lipid	Crude fibre	Ash	Gross energy (MJ/kg)
	(%)					
Poultry offal meal	94.5	59.2	16.2	1.8	5.0	22.5
Mysid meal	93.9	57.6	9.1	3.0	14.7	18.8

Golden snail meal	94.3	53.7	4.9	2.6	10.6	18.5
Green mussel meal	92.8	52.9	12.4	1.9	9.0	20.2
Rice bran	93.8	13.7	14.9	5.8	8.8	18.0
Corn meal (yellow)	92.4	10.2	3.8	2.2	1.7	16.8
Corn meal (white)	91.3	10.2	4.6	2.1	1.6	16.8
Sorghum	91.7	9.4	1.4	1.1	1.9	16.4

It was originally planned to do three independent 4×4 latin square experiments to enable 9 feed ingredients to be examined. In reviewing the digestibility work that had been carried out in the previous ACIAR grouper project (FIS/97/73) with humpback grouper, it was decided that only 8 feed ingredients were required to be done in this project and that these could most efficiently be carried out with two 5×5 latin square experiments. Standard substitution procedures were used in these digestibility studies with animal ingredients being substituted in the reference diet at 40% while plant ingredients were substituted at 30%. Chromic oxide was used as the digestibility marker. As the apparent digestibility coefficients for the reference diet in each latin square experiment were statistically not different, the derived coefficients for the 8 test feed ingredients were pooled and analysed by one-way ANOVA. The results are presented in Table 21 (p.60).

2.1.2 Assessment of non-fishmeal protein sources for grouper diets

The use of fish meal and fish oil for diets used to culture carnivorous fish species is widely recognised as a major factor impacting the environmental sustainability of marine fish culture (see, for example, Tacon *et al.* 2010). As with FIS/97/73, this project undertook several experiments to evaluate the potential to use alternative protein sources and reduce dependence on fish meal.

In this study, three ingredients were investigated: poultry offal silage meal, golden snail meal, and fermented blood meal.

Poultry offal silage meal

Poultry offal meal (POM) is an alternative ingredient which has potential to replace fish meal in grouper feeds and is readily available in Indonesia. At a farm scale, poultry waste product can be ensiled with organic acids to produce poultry offal silage meal (POSM) to improve its quality and digestibility. POSM is produced by acidifying chicken viscera and other abattoir waste so as to activate the endogenous proteolytic enzymes in the material. In turn, this results in a partially digested product that is rich in protein, polypeptides and free amino acids (Stone and Hardy 1986). This feeding experiment was conducted to evaluate the effects of replacing fishmeal with POSM in tiger grouper diets.

The poultry viscera was mixed with 3% formic acid and a similar amount of propionic acid and placed in a fermentation jar. The material was fermented for seven days with the material being mixed daily during this period. After seven days, the ensiled product had a pH of 3–4 and this was neutralized by the addition of 1.6% $Ca(OH)_2$ to bring the acidity to a pH of 6–6.5. The POSM was dried and ground prior to being used to prepare test feeds. Typical analysis (% of air-dry product) of the POSM was: crude protein 65.6%, total lipid 18.1%, crude fibre 0.2%, ash 4.7%, nitrogen-free extract 11.4%, and gross energy (MJ/kg) 24.57.

Golden snail meal

Golden snail (*Pomacea* sp) is regarded as a pest in rice culture, but shows potential as replacement for fishmeal in fish diets because its meat contains 50–54 % protein dry weight (Table 1). Golden snails are readily available in Indonesia and its use as a fish feed ingredient might contribute to controlling populations by harvesting. Consequently, the project evaluated the potential of golden snail meal (GSM) as a protein source in grouper diets.

Five test diets containing different level of golden snail meal (0, 10, 20, 30 and 40%) were prepared. The control diet contained fishmeal (5-01-985) as the protein reference, which was partially replaced with GSM. The test diets were isonitrogenous (45% CP) and isoenergetic (20 MJ/kg) moist pellet with 42% water content.

Fermented blood meal

The protein content of blood meal is high, around 72–97 % (Laining *et al.* 2003). However, processing methods affect the quality of blood meal, which in turn, affects the response of the fish. The objective of this study was to evaluate the optimum rate at which fermented blood meal (FBM) could substitute for fish meal in the diet of tiger grouper.

Except for the control diet (FBM0) which contained only fishmeal as the protein source, other test diets contained fermented blood meal at inclusion rates of 7.5% (FBM7.5), 15.0% (FBM15), 22.5% (FBM22.5) and 30.0% (FBM30), substituting for an equivalent amount of fishmeal protein. Fermented blood meal was produced using fresh cattle blood which was homogeneously mixed with a 1:1 propionic and formic acid mixture at 3% of blood total weight. Fermentation was carried out for five days with daily agitation. At the conclusion of fermentation, the acidity of the mixture was pH 3–4 and this was neutralised with 1.6% $Ca(OH)_2$ to obtain a product with a pH of 6.0–6.5. The fermented blood meal was dried and ground to a fine powder. The blood meal product contained 82.7% crude protein (CP), 0.01% total lipid (TL), 2.7% crude fibre (CF), 4.8% ash, and 9.7% nitrogen-free extract (NFE). Chromium oxide at 1% was added to the diet at the expense of wheat flour when used for determining diet digestibility. The test diets were developed to produce isonitrogenous (45% CP) and isoenergetic (20 MJ/kg) moist pellets with 42% water content.

2.2 Compare nutritional requirements of juvenile and market-size groupers

Research on grow-out feeds development in FIS/97/73 focused on determining nutritional requirements of juvenile fish (< 100 g body weight) with *C. altivelis* and *E. coioides* being the species most closely examined. However, anecdotal reports suggest that grouper, particularly tiger grouper, cultured using commercial pellets, exhibit a markedly reduced growth rate after they reach about 200–250 g BW. This component of the project evaluated the basic nutritional requirements of larger (>200 g BW) fish to determine whether the nutritional requirements of the fish change with size.

This information is required to develop cost-effective feed through out the culture period. The objective of the present study was to find the effect of dietary protein and lipid levels on growth performance of tiger grouper upon late-stage grow-out.

2.2.1 Protein and lipid requirements – RIM Gondol

Juvenile tiger grouper (11–100 g BW) require a diet containing 47–50% crude protein and 9% lipid for optimal growth (Giri *et al.* 2004). This experiment examined the protein and lipid requirements for larger (>250 g BW) tiger grouper.

Ten test diets were prepared to contain five levels of protein: 38, 42, 46, 50, and 54%; and two levels of lipid: 9 and 15%. All diets had the same estimated digestible energy of 14.2 MJ/kg. Fish meal, casein, squid liver meal, shrimp head meal and soybean meal were used as the main protein sources. Diets were prepared as dry pellets with a diameter of 12 mm. The feeding experiment was conducted in 30 floating net cages (1×1×1 m^3) in Pegametan Bay, Buleleng Regency, Bali. Forty fish with average weight of 274 g were stocked in each net cage. The experiment was a completely randomized design with two factors (protein and lipid levels) and triplicate cages per treatment. Fish were fed the test diets once daily in the afternoon to satiation for 180 days.

2.2.2 Protein and lipid requirements – RICA Maros

The test diets used in this experiment were moist pellets (41% moisture content) containing (dry matter basis) 46, 49 and 52% protein in combination with dietary lipid levels of 9, 11, and 13%. Protein and lipid contents were adjusted by arranging proportional composition of wheat gluten, casein, fish oil, soybean oil and corn starch in the diets. Diet preparation followed standard procedures: dry ingredients were homogenously mixed and oil ingredients were added prior to trash fish addition. The mixture was homogenously blended into a dough. Moist pellets were made by cold extrusion of the dough through a meat mincer with the die plate being varied to match the size of the fish.

A total of 420 fish were stratified by weight into three groups of average (mean ± SD) weights of 122±4.2, 144±7.1 and 173±10.5g. Five fish from each group were randomly sampled for determination of initial whole body chemical composition. The remaining 405 fish were equally distributed (15 fish/cage) within size groups to 27 net cages of 1×1×2 m^3. Throughout the experiment, diets were carefully fed to satiation twice a day.

2.2.3 Essential fatty acid requirements

This experiment was designed to determine the requirements for fatty acids in the diet of larger (>200 g BW) tiger grouper. Six isonitrogenous and isoenergetic diets containing different amounts of fish oil and soybean oil were used (Table 2). The estimated fatty acid composition of the diets is shown in Table 3.

Table 2 Ingredient composition and proximate analysis of the experimental diets used in Activity 2.2.3. Vitamin mix provided (mg/kg diet): thiamin-HCl 59.2; riboflavin 59.2; Ca-panthothenate 118.5; niacin 23.7; pyridoxine-HCl 47.4; biotin 7.1; folic acid 17.8; inositol 2370; ρ-aminobenzoic acid 59.2; astaxanthin 177.8; menadione 47.4; calciferol 22.5; ∝-tocopherol 237; ascorbic acid 1777.5; cyanocobalamin 1.2; choline-HCl 10971. Trace minerals provided (mg/kg diet): KH_2PO_4 1333; $CaCO_3$ 833; NaH_2PO_4 2050; $FeCl_3.2H_2O$ 553; $ZnSO_4$ 33; $MnSO_4$ 23; $MgSO_4$ 167; $CuSO_4$ 7; KI 0.5; $CoSO_4.7H_2O$ 0.3. Gross energy was calculated from the determined protein, lipid and NFE of the diet using gross energy conversion coefficients of 23.6; 39.5 and 17.2 MJ/kg respectively.

Ingredients	**Test diets (% as used)**					
	A	**B**	**C**	**D**	**E**	**F**
Trash fish	50	50	50	50	50	50
Fish meal	5	5	5	5	5	5
Wheat gluten	10.5	10.5	10.5	10.5	10.5	10.5

Soybean meal	12.5	12.5	12.5	12.5	12.5	12.5
Mysid meal	5	5	5	5	5	5
Rice bran	9.5	9.5	9.5	9.5	9.5	9.5
Vitamin premix	2.0	2.0	2.0	2.0	2.0	2.0
Mineral premix	0.5	0.5	0.5	0.5	0.5	0.5
Fish oil	5	4	3	2	1	0
Soybean oil	0	1	2	3	4	5
Proximate composition (% dry matter):						
- Crude protein	47.0	47.7	47.5	46.9	47.3	47.1
- Total lipid	11.9	11.9	11.4	11.6	12.0	11.8
- Ash	10.8	10.4	10.7	10.9	10.6	10.3
- Crude fibre	3.4	3.1	3.6	3.5	2.9	3.3
- NFE	26.9	26.9	26.8	27.1	27.2	27.5
- Gross energy (MJ/kg)	20.4	20.4	20.3	20.3	20.6	20.5

Table 3 Composition and estimated fatty acid composition of test diets used in this experiment.

Ingredients	**Test diets**					
	A	**B**	**C**	**D**	**E**	**F**
Fish oil (FO)	5%	4%	3%	2%	1%	0%
Soybean oil (SO)	0%	1%	2%	3%	4%	5%
Fatty acid composition (g /kg diet)						
∑ n-6	0.6	1.4	2.1	2.9	3.6	4.4
∑ n-3	2.9	2.7	2.4	2.2	1.9	1.7
C18:2n-6	0.5	1.3	2.0	2.8	3.5	4.3
C18:3n-3	0.2	0.3	0.4	0.4	0.5	0.6
C20:5n-3	1.1	0.9	0.8	0.6	0.5	0.3
C22:6n-3	1.4	1.3	1.1	1.0	0.8	0.7

Tiger grouper were divided into four groups and stocked in 24 sea cages (1×1×2 m^3), providing 4 replicates for each dietary treatment. During the 112-day experimental period, these fishes were fed twice daily to satiation and weighed every four weeks.

2.3 Identify ingredients for grouper diets that will reduce environmental impacts

This component of work was originally planned as a linkage activity with ACIAR project FIS/2003/027 'Planning tools for environmentally sustainable tropical finfish cage culture in Indonesia and northern Australia'. However, ACIAR decided to link FIS/2003/027 with FIS/2002/076 'Land capability assessment and classification for sustainable pond-based aquaculture systems'.

Research results from FIS/2003/027 demonstrated the environmental benefits (reduced nutrient inputs to the local environment) of using pellets versus using 'trash' fish (Alongi *et al.* 2009).

2.4 Improve the uptake of compounded feeds for marine finfish culture at the expense of 'trash' fish use

This component of work evaluated the potential to replace 'trash' fish as a feed for cultured groupers with compounded pellets, by:

- Developing improved feed formulations and feed management strategies to provide advice to farmers, and
- Demonstrating the potential benefits to farmers in 'on-farm' demonstrations.

2.4.1 Effect of feed type and formulation on growth and feed efficiency of tiger grouper

Tiger grouper were fed five test diets comprising three different formulated moist pellets, a dry (commercial) pellet, and trash fish (Table 4). Three groups of juvenile tiger grouper of initial weight of (i) 234±11.3 g, (ii) 268±11.6 g, and (iii) 318±16.6 g were stocked into 1×1×2 m^3 floating net cages at 15 fish per cage. The fish were fed twice daily to satiation for 140 days. After 140 days, six representative fish from each treatment were transported to a seafood restaurant in Hong Kong and used in a product quality test (see Activity 2.5, p.25).

Table 4 Composition of the test diets used in this experiment (% dry matter). NA: information not available.

Ingredient	Moist pellet-1	Moist pellet-2	Moist pellet-3	Dry pellet (commercial)	'Trash' fish
Trash fish	50	25	0	NA	100
Local fish meal	25	30	50	NA	0
Poultry offal meal	0	20	20	NA	0
Shrimp head meal	5	5	5	NA	0
Soybean meal	5	5	15	NA	0
Rice bran	3	3	0	NA	0
Wheat flour	8.48	8.98	6.48	NA	0
Fish oil	1.5	1	1.5	NA	0
Vitamin premix	1.52	1.52	1.52	NA	0

Mineral premix	0.5	0.5	0.5	NA	0.5

2.4.2 Effect of feeding management on productivity and product quality of tiger grouper

This activity was undertaken to develop a better understanding of the impacts of different feeding strategies on productivity and product quality of tiger grouper. Because feed cost is generally the major cost component in fish production (usually at least 50% of production cost), optimisation of feed use will improve farm profitability.

This experiment evaluated two feeding frequencies: once daily and twice daily; and three feeding rates: low, medium and high (Table 5). Juvenile tiger grouper with average initial weight of 55.8g were stocked into 18 units of 1×1×2 m floating net cages at a density of 15 fish per cage. Throughout the 150-day experiment, all fish were carefully fed a dry (commercial) pellet (about 50% CP and 20 MJ/kg) at rates listed in Table 5.

Table 5 Feeding frequency and feeding rate for tiger grouper.

Feeding frequency	**Month**	**Feeding rate (% of fish biomass)**		
		Low (L)	**Medium (M)**	**High (H)**
(F1) Once daily	1	2.5	3.2	4.0
	2	2.2	2.8	3.5
	3	1.9	2.4	3.0
	4	1.6	2.0	2.5
	5	1.3	1.6	2.0
(F2) Twice daily (divided into two equal feeds)	1	2.5	3.2	4.0
	2	2.2	2.8	3.5
	3	1.9	2.4	3.0
	4	1.6	2.0	2.5
	5	1.3	1.6	2.0

2.4.3 Evaluation of pellet diets for grow-out of coral trout

This activity was undertaken to assess the use of dry pellet diets for culture of coral trout (*P. leopardus*). Two test diets were used in this study: pellet prepared by a commercial feed company ('feedmill pellet') and pellet prepared by Gondol Research Institute for Mariculture ('GRIM pellet') (Table 6). The nutrient composition of these test diets is given in Table 7. The feeding experiment was conducted using eight 8 m^3 floating net cages in Pegametan Bay, Buleleng Regency, Bali. One hundred fish of average initial weight 165.5 g were stocked in each net cage. Fish were fed the test diets twice daily to satiation for 6 months. Sampling was carried out every month by weighing all the fish in each cage. At each monthly sampling the fish were dipped in freshwater for 10 minutes to reduce parasite loads.

Table 6 Ingredient composition of the test diet developed by RIM Gondol ('GRIM pellet') and used in Activity 2.4.3.

Ingredients	% as used
Fish meal	55.5
Shrimp head meal	6.0
Soy bean meal	3.0
Squid liver meal	12.0
Wheat flour	14.2
Fish oil	4.7
Vitamin mix	1.3
Mineral mix	1.7
CMC	1.5
Asthaxanthin	0.1

Table 7 Nutrient composition of the two test diets used in Activity 2.4.3.

Nutrient	Feedmill pellet	GRIM pellet
Crude protein	49.1	47.2
Crude lipid	13.1	15.1
Ash	12.0	12.0
Crude fibre	2.0	2.5
Moisture	5.7	6.3

2.4.4 Comparison of commercial pellet diet and 'trash' fish for grow-out of groupers

In this experiment, two diets (trash fish and a commercially-produced dry pellet) were fed to two grouper species: coral trout (*P. leopardus*) and white-spotted grouper (*E. coeruleopunctatus*) cultured in floating net cages. The nutrient composition of the two test diets is listed in Table 8.

Sixteen cages (each 2×2×2 m^3) in Pegametan Bay, Buleleng Regency, Bali, were stocked with either 70 (coral trout) or 150 (white-spotted grouper) at average weight of 210 g and 116.5 g respectively) to constitute a 2 (diet) x 2 (fish species) x 4 (cage replicate) experiment. Fish were fed their respective test diets twice per day for 180 days. To improve fish health and reduce disease incidence, the nets were changed and fish were dipped in freshwater every 15 days for the duration of the experiment.

Table 8 Proximate composition (DM basis) of test diets used in Activity 2.4.4.

Nutrient	Dry pellet	Trash fish
Crude protein	49.1	52.6
Crude fat	18.2	26.9

Ash	15.6	8.9
Crude fibre	5.8	2.9

2.4.5 Field validation – comparison of feed types

This activity was undertaken as an 'on-farm' collaboration with local (South Sulawesi) sea cage farmers. Tiger grouper were reared on three feed types: moist pellet, dry (commercial) pellet, and 'trash' fish.

Three test diets were used in this study:

A. moist pellet containing: 50% trash fish, 25% local fish meal, 5% shrimp head meal, 5% soybean meal, 3% rice bran, 8.5% wheat meal, 1.5% fish oil, 1.5% vitamin premix and 0.5% of mineral premix (47% CP dry matter),
B. commercial pellet (50% CP dry matter), and
C. 'trash' fish (55% CP dry matter).

Two groups of juvenile tiger grouper initial weight (i) 240±22.7g, and (ii) 305±33.6g were selected on the basis of weight and freedom from obvious health defects. Within these groups, groupers were stocked into 2×2×2.5 m^3 floating net cages at a density of 80 and 83 fish per cage for size groups (i) and (ii) respectively. Throughout the experiment, the fish were carefully fed to satiation once a day.

2.5 Identify the impacts of feeds on product quality

This activity was undertaken to better understand the impacts of diet on product (i.e. fish flesh) quality and consumer preference. There have been anecdotal reports that some buyers of live fish pay lower prices for fish fed commercial pellets, and higher prices for fish fed 'trash' fish, because of consumer preference for the latter. This project's evaluation of constraints and limitations of the adoption of artificial feeds (p.73) found that some buyers insist that growers 'finish' the fish on a diet of 'trash' fish for about two weeks prior to harvest. Clearly, this type of market response creates another constraint to the adoption of pellet feeds.

The primary aim of this activity was to incorporate assessment of product quality in ongoing nutrition research, to evaluate the acceptability of compounded diets in regard to product quality.

Tiger grouper

Six fish from each treatment in Activity 2.4.1 (p.22) were tagged and shipped to Hong Kong using the services of a Makassar fish exporter. In Hong Kong a panel evaluated the products for odour, flavour and texture.

Humpback grouper

The acceptability of aquacultured grouper was evaluated in a collaborative activity with ACIAR Project ADP/2002/105 'Economic and market analysis of the live reef fish food trade in Asia-Pacific', in which a taste test of wild-caught and farmed grouper was undertaken with Hong Kong consumers. The taste test set out to compare wild-caught fish with aquacultured fish fed two diets: 'trash' fish and compounded pellets. Both aquacultured samples were supplied by the Gondol Research Institute for Mariculture, Bali.

The taste test was conducted in Hong Kong in November 2005 where a sample of Hong Kong consumers were presented with portions of wild-caught and aquaculture raised live reef fish for comparison. The taste test was a blind one, whereby the tasters did not know whether the portion was wild-caught or aquaculture-raised. In order to make the test as realistic as possible, the cooked fish portions were served in a restaurant well skilled in the preparation of the fish. In this experiment no attempt was made to compare the visual aspects of the live reef fish swimming in the restaurant tanks.

The test was conducted using the scientific principle of a triangular taste test used widely in the food industry to test for differences in food samples. In this test the consumers were presented with three bowls of food and only told that one of the samples was different. They were asked to taste the samples, identify the different one and describe the key food sensory characteristics that made it different. They were also asked to identify which sample they preferred. The research reported here also sought to establish whether such techniques could be successfully applied to the taste assessment of reef fish products.

In the Hong Kong taste test 30 consumers participated: 16 were guests of the Hong Kong Seafood Merchants Association, mostly merchants involved in the trade, and 14 were staff from local seafood restaurants. Each consumer was seated at a separate table and asked not to converse with adjacent tables. They were given a survey questionnaire to answer.

The fish chosen for the test were all of the same species, barramundi cod *Cromileptes altivelis*, commonly known in Hong Kong as humpback grouper. This is a relatively highly priced fish in the Hong Kong market, selling at approximately AUD 100 per kilogram in the restaurants. Unfortunately, a mix up with the transport of the aquacultured fish to the restaurant resulted in both 'trash' fish and pellet -fed samples being combined. Consequently, it was not possible to differentiate between these two samples.

Objective 3 – Facilitate technology adoption

Overall, this objective used a variety of mechanisms to embed research outcomes in aquaculture practices throughout the region.

This objective was addressed by initially undertaking a survey of marine fish hatcheries and farms in Indonesia, Thailand and Vietnam (Activity 3.1). The results of this survey were then used to design some specific interventions to test opportunities to overcome the constraints identified in the survey (Activity 3.2). In parallel, research outcomes were widely disseminated using print and electronic means (activity 3.3), and by incorporation in training courses (Activity 3.4). The project also attempted to strengthen and expand the Asia-Pacific Marine Finfish Aquaculture Network (Activity 3.5) to ensure the sustainability of the networking approach.

3.1 Identify constraints to the uptake of grouper hatchery and grow-out technology in the Asia-Pacific region

Introduction

Grouper aquaculture technology development in some South East Asian countries has improved significantly over the last five years, particularly in Indonesia where there have been some major breakthroughs in larviculture techniques for grouper species since 1999. The technology has been refined over the years and survival rates have increased from <10% to around 50% (Rimmer *et al.* 2004). These improvements, particularly with regards to hatchery techniques and artificial feeds, are the results of cooperative research and

coordination funded by various agencies such as ACIAR, the Japan International Cooperation Agency (JICA), the Network of Aquaculture Centres in Asia-Pacific (NACA), and Indonesia's Research Centre for Aquaculture and Directorate General for Aquaculture (DGA).

This study was conducted under the ACIAR project (FIS/2002/077) 'Improved hatchery and grow-out technology for grouper aquaculture in the Asia-Pacific region' from 2002 to 2007 and from information derived from Sim (2006). Both the hatchery technology and artificial feeds components of the project were investigated and the research has provided considerable understanding of the constraints that limit the uptake of hatchery technology and artificial feeds for grow-out in grouper aquaculture.

Materials and methods

The research methodologies used for the collection of field data included a questionnaire survey (via local extension officers) and face-to-face interviews to verify and collect additional data as required. The research sites chosen for the studies were selected based on the importance of the sites to the grouper aquaculture industry and the accessibility of farms in these sites. The selected sites included:

Phang-nga Bay and Chantaburi, Thailand.

- Cat Ba, Vietnam.
- Lampung, Batam and Situbondo, Indonesia.

Farm level data were collected from the following research sites:

- Indonesia: 34 grow-out farms (cages and pen systems).
- Thailand: 33 farms (pond and cage systems).
- Vietnam: 42 farms (cage system).

Hatchery level data were collected from two locations in Indonesia:

- Situbondo: 16 hatcheries.
- Gondol: 15 hatcheries.

3.2 Where possible, develop responses to overcome identified constraints

Two responses were developed to attempt to address some of the constraints identified in the survey described in Objective 3.1. The first was a workshop on Better Management Practices for Marine Finfish Aquaculture in the Asia-Pacific Region. BMPs have been successful in improving the sustainability of shrimp farming in India and Indonesia, and provide a structure for improving the overall sustainability of marine finfish aquaculture in the region.

The second response was a technical workshop held to provide feed formulators from Indonesian feed companies producing fish feeds with detailed information on fish nutrition, with a focus on marine finfish nutrition. This training targeted a constraint in commercial feed formulation that most formulators are not trained in nutrition and rely on 'least cost' formulation software that inadequately assess nutritional aspects of the formulation.

3.3 Disseminate research outputs widely in the Asia-Pacific region

Research outputs, as well as other non-project-derived information, were disseminated through:

- The NACA web site (www.enaca.org)

  In 2008 the web site was upgraded to a 'portal' format to increase accessibility. The web site displays recent news, publications and project summaries. RSS feeds are

available. Individual news items average from 650 – 1,500 views. Extension manuals are consistently the most popular download.

- E-mail newsletter ('e-news')

  The e-mail newsletter was designed to provide short news items of immediate, but not long-term, interest. It was e-mailed to subscribers approximately monthly. The e-newsletter was discontinued in early 2008 due to resourcing constraints at NACA. News items are now incorporated in the web site news section.

- APMFAN e-Magazine

  This was originally published as a separate electronic (.pdf) file, and included in the NACA magazine 'Aquaculture Asia'. Since 2008 the APMFAN section has been incorporated in both the printed and electronic versions of 'Aquaculture Asia'.

- Technical publications

  Practical guides on aspects of marine finfish aquaculture, and the grouper aquaculture monograph.

3.4 Promote the expansion of sustainable marine finfish aquaculture through 'hands-on' training

Formal training in hatchery production of groupers was undertaken by running a 3-week training course annually from 2005 to 2008 at the Brackishwater Aquaculture Development Centre (BADC) Situbondo.

Training in the use of feeds, particularly farm-made feeds, was done on a demand basis. Details of the training undertaken through the project are provided in Section 7 of this report (p.75).

3.5 Strengthen and expand the research coordination and regional collaboration activities of APMFAN

To strengthen and expand APMFAN, we used the model that NACA has developed for its Fish Health Program, which nominates Regional Resource Centres (RRCs) and Regional Resource Experts (RREs) in member countries. Outcomes of this approach are discussed in Section 7 of this report (p.79).

6 Achievements against activities and outputs/milestones

Objective 1: Improve hatchery production technology for high-value marine finfish

no.	activity	outputs/ milestones	completion date	comments
1.1	Improved hatchery technologies			
1.1.1	Larval nutrition	Fatty acid composition of live prey	Yr 1, m11–Yr2, m6 (May 05 – Dec 05)	Completed Commercially-produced *Chlorella* used at RIM Gondol contained both ARA (20:4n-6) and EPA (20:5n-3) while in *Nannochloropsis* ARA was higher than in *Chlorella*. In order to improve the nutritional content of rotifer and *Artemia* to meet the needs of larval grouper, Algamac or Selco products were used to enrich these live foods. Algamac highly improved the essential n-3 and n-6 highly unsaturated fatty acids (HUFA) of rotifers while Selco elevated these in *Artemia* nauplii.
		Patterns of fatty acid conservation in larvae	Yr 2, m7–12 (Jan 06 – Jun 06)	Completed Newly hatched tiger grouper larvae contained high levels of DHA, ARA and EPA demonstrating the importance of these HUFAs in larval development. The major lipid energy source of unfed day-3 larvae was neutral lipid (NL) while polar lipid (PL), particularly DHA, was more conserved. NL and PL fatty acids of hatchery reared fish increased with age and the pattern of HUFA accumulation was DHA>ARA>EPA. The major lipid energy source of two-day starved day-20 and day-27 larvae was NL and PL respectively, and the pattern of utilisation was MUFA > SFA > PUFA. A higher essentiality of DHA and ARA than EPA was indicated by two-day starved day-20 larvae. In both larval age groups, neutral ARA was highly conserved.
		Response (growth, survival) to different fatty acid composition of live prey	Yr 3, m1–12 (Jul 06 – Jun 07)	Completed This activity focussed on improving the quality of fingerlings produced in hatcheries. Results suggest that increased levels of ascorbic acid AA in the diet of late larval and juvenile grouper *Cromileptes altivelis* reduce deformities. Full recovery from bilateral gill opercular deformity ranged from 10–25% after feeding high quality or supplemented diets for 77 days.

1.1.2	Larval digestion	High-sensitivity enzyme analysis techniques established at RIM Gondol	Yr 2, m1–12 (Jul 05 – Jun 06)	Completed Training at NFC and training and research at RIM Gondol completed. Application of techniques at RIM Gondol limited by the existing plate reader, which cannot utilise high-sensitively (fluorescent) analysis techniques. It can undertake colorimetric analysis but these have reduced sensitivity. High-sensitivity enzyme analysis techniques at NFC Cairns were extended to include lipase, alkaline and acid phosphatase.
		Quantified enzymic responses to initial feeding	Yr 3, m1–6 (Jul 06 – Dec 06)	Completed Experiments demonstrated similar trends in increasing enzyme activity after mouth opening and first feeding in both tiger grouper and coral trout. In addition, low levels of enzyme activity were detected in tiger grouper prior to first feeding. Coral grouper have lower levels of enzyme activity over the first 12 days post hatch indicating potentially increased sensitivity of this species.
		Quantified enzymic response to live vs. artificial larval diets	Yr 3, m7–12; Yr 4, m7–12 (Jan 07 – Jun 07; Jan 08 – Jun 08)	Completed Feeding inert diet only prior to completion of significant developmental changes (i.e. stomach formation) had a negative effect and is reflected in the reduction in feeding (starvation). Consequently, co-feeding of brine shrimp and inert diets is recommended until the onset of metamorphosis in tiger grouper.
1.1.3	Verification of intensive and semi-intensive hatchery techniques	Improved hatchery protocols; improved larval survival to metamorphosis	Yr 2, m1 – Yr 4, m12 (Jul 05 – Jun 08)	Completed Techniques developed through the project, as well as experiences from other participating laboratories, have been incorporated in Australian larval rearing protocols. NFC Cairns has successfully produced fingerlings of four grouper species using these protocols.
1.2	Live prey production			
1.2.1	SS-strain rotifers	Reduce average rotifer body size by at least 20%	Yr 2, m3–12 Yr 4, m1–6 (Sep 05 – Jun 06; Jul 07 – Dec 07)	Completed Synchronous rotifer populations were screened repeatedly to select for smallest individuals. No shift in phenotype (length) of subsequent adult populations was achieved. However, methods developed to mass-harvest amictic rotifer eggs were a positive outcome from these experiments.
		Cold-storage of amictic eggs for mass production of SS-strain neonates	Jun 08 (new additional milestone identified at 06-07 annual project meeting)	Completed A method to collect amictic eggs was developed. A temperature of 10°C was found to be the maximum temperature at which hatching was blocked for up to 3 days. Viability of eggs stored at 10°C decreased rapidly, falling from 95% to 75% in 24 hours. By 2 days the viability had decreased to close to 30% where it remained for up to 4 days before eggs started to disintegrate. The method to collect large numbers of amictic eggs can be used to provide large numbers of neonates for first feeding finfish larvae. However, cold storage of amictic eggs is of little benefit beyond 24 hours.

		Detect shift in population phenotype as a result of selection pressures. Reduce average rotifer body size by at least 20%.	Yr 2, m12 – Yr 4, m1–6 (Jun 06 – Dec 07)	Completed Several rotifer strains from Northern Sulawesi and one from Gorontalo have been assessed for growth and survival at various salinities, temperatures and different algal diets. Phenotype shifted to smaller size when wild rotifers were cultured under controlled conditions.
		Shift normal distribution of rotifer size toward a smaller mean or increase proportion of smaller rotifers.	Yr 3, m1 – Yr 4, m6 (July 06 – Dec 07)	Completed Size variations between isolates from across Sulawesi were not significantly different. This reduces the chance of producing smaller rotifers from hybridisation. Among strains maintained under laboratory conditions, only the one strain (Minanga strain) has successfully undergone sexual reproduction; consequently strain hybridisation experiments could not be undertaken at Sam Ratulangi University as planned. In response, the Minanga strain was cultured at different salinities, but this strain could only produce resting eggs at lower salinities (5, 10 and 20 ppt). The resting eggs were clustered into three size groups of sizes (small, medium and large), and clonal cultured. The medium size group produced a larger proportion of smaller rotifers; up to 25% of population with 110–140 μm body length. The two strains of SS-rotifers Manembo-nembo and Likupang strains were crossed. The fertilized female was cultured in *N. oculata* suspension until produced resting eggs, and the size of resulting neonates was measured. The proportion of larger sized rotifers in the hybrid population decreased, but most of the population (70%) was still within the medium size range (130-150 μm).
		Assess the use of protozoa as first feed prey for marine finfish larvae.	Yr 3, m1 – Yr 4, m12 (Jul 06 – Jun 08)	Cancelled Protozoan is no longer found as a contaminant of rotifer tanks. Use of protozoan has largely been made redundant by success of *Parvocalanus* culture at NFC.

1.2.2	Ultra-small copepod nauplii as first feed prey for marine finfish larvae.	Isolate copepods with ultra-small (<100µm) nauplii. Develop culture techniques. Develop nauplii harvest techniques. Analyse fatty acid composition. Assess nauplii acceptance to fish larvae as a 1st feed prey item.	Yr 2, m6 – Yr 4, m6 (Dec 05 – Dec 07)	Completed Potential copepod species have been isolated in Cairns, Gondol and Manado.. Cultures of *Parvocalanus* are routinely used in larval rearing at NFC Cairns and have replaced *Acartia*. Development of an algal diet for *Parvocalanus* has demonstrated *Isochrysis* sp. (T.ISO) to be a preferred mono-algal diet. This is a simpler diet than that found for *Acartia*. Cultures of *Parvocalanus* have been scaled up to 2000 L using a T.ISO diet. High densities of copepods (14 /mL) were obtained after 5 days of culture. Replicate cultures of *Parvocalanus* were grown and harvested for fatty acid profiling. Copepods were composed of approximately 100 mg FA/g dry sample. DHA (22:6n-3) and palmitic acid (16:1) were dominant and present in almost equal proportions equalling ~40% of fatty acids. The high DHA content corresponded to a DHA:EPA ratio of > 7:1. In a replicated larval rearing experiment testing for the effect of increasing initial dose of copepods in a diet for larval tiger grouper (*E. fuscoguttatus*), survival and all growth parameters were strongly correlated with the initial feed rate of the copepod *Parvocalanus*. Average survival for 0, 4 and 10 copepods/mL treatments were 544, 1609 and 2159 respectively. Not only was survival significantly higher with copepods but also all the growth parameters of total length, body depth, dorsal spine and pelvic spine development. Inclusion of copepods significantly increases survival and vigour of larvae as seen through faster development.
1.2.3	Extension of *Acartia* culture techniques.	Establish culture techniques for the copepod *Acartia* in Vietnam. Improve survival of grouper larvae by incorporation of copepod nauplii into diet.	Yr1 m9 – Yr 3, m12 (Mar 05 – Jun 07)	Completed Two staff from Research Institute for Aquaculture No.1, Mr Cao Van Hanh (Cat Ba hatchery) and Mr Le Anh Tuan (Cua Lo hatchery), undertook two weeks training at NFC Cairns in November 2007. The training involved culture techniques for copepods, as well as culture techniques for super-small (SS-) rotifers on formulated diets.. Dr Gede Sumiarsa (RIM Gondol) visited NFC Cairns for training in copepod culture techniques for a two week period in March 2007.
1.3	Cannibalism			
1.3.1	Tank design	Develop improved nursery tank design that reduces cannibalism in grouper fingerlings	Y2, m1 – Y2, m12 (Jul 05 – Jun 06)	Completed Light intensity 600 lux is optimal. Water flow rates ranging from 3 to 10 $m.min^{-1}$ had no significant effect on grouper survival. Tiger grouper juvenile (0.25 g BW) was stocked in 300-litre capacity tanks of two shapes: round and square, provided with flow-through sea water, at a density of 200 fish/tank. After 40 days, there was no effect of tank shape on growth or survival (24.0 – 24.5%).

1.3.2	Feed management	Develop cost-effective feed management techniques to reduce cannibalism in grouper fingerlings	Y3, m7 – Y3, m12 (Jan 07 – Jun 07)	Completed Results indicate that fish that commenced feeding earlier in the day (0700) had better survival (24.5%) than fish fed commencing at 0900 (9.2%) or 1100 (7.2%).
1.3.3	Feed development for late larvae / juveniles	Develop cost-effective late larval / weaning diets to reduce cannibalism; induce earlier metamorphosis by diet manipulation	Yr2, m1 – Yr 3, m12 (Jul 05 – Jun 07)	Completed Overall, various trials carried out at RIM Gondol with different diets and attractants have demonstrated that compounded diets can equal or exceed the performance of 'trash' fish diets. However, the results from using various attractants have been inconsistent.

Objective 2: Develop cost-effective grow-out diets

no.	activity	outputs/ milestones	completion date	comments
2.1	Ingredient digestibility, lower-cost feeds	Definition of digestibility of 9 feed ingredients Digestibility of plant by-products as protein and energy sources	Yr 2, m1 to Yr 3, m12 (Jul 05 – Jun 07)	Completed for 4 animal feed ingredients (poultry offal meal, mysid meal, golden snail meal, green mussel meal) and 4 plant feed ingredients (rice bran, maize-yellow, maize-white and sorghum).
		Assess potential of terrestrial meals to replace fishmeal in grouper (*E. fuscoguttatus* and *E. coioides*) diets	Yr 3, m6 to Yr 4, m12 (Dec 06 – Jun 08)	Completed Golden snail meal and fermented blood meal were successfully used to replace fishmeal at relatively low inclusion rates: 20% and 9% respectively. Poultry offal silage meal showed more potential, with satisfactory growth and survival at inclusion rates up to the maximum of 20%.
2.2	Nutrient requirements	Protein:energy and lipid requirements of large (>500 g) groupers	Yr 3, m1–6 (Jul 06 – Dec 06)	Completed RIM Gondol results indicate that dietary levels of 38% protein and 9% lipid is sufficient for growth of larger tiger grouper (>250g). Higher lipid levels result in fat deposition rather than increased growth RICA Maros results indicate that a diet containing 46% protein and 11% lipid is suitable for supporting growth and protein efficiency of tiger grouper. Lipid content of fish fillet increases when lipid content of diet increases.
		Comparative requirements of *C. altivelis* and *E. fuscoguttatus* /*E. coioides* for protein, lipid and essential fatty acids	Yr 2, m6 – Yr 3, m6 (Dec 05 – Dec 06)	Completed Groupers have a high requirement for n-3 fatty acid in the diet, and substitution of fish oil with soybean oil is limited to 3% soybean oil and 2% fish oil; higher levels of soybean oil (which is high in n-6 fatty acids) reduce growth performance in tiger grouper.

2.3	Low-polluting feeds	Reduce nutrient outputs from cage culture using improved feed management practices and high nutrient specification diets	Yr 3, m6–Yr 4, m6 (Dec 06 – Dec 07)	This was based on a planned linkage between FIS/2002/077 and FIS/2003/027 which did not eventuate. Instead, FIS/2003/027 was linked with FIS/2002/076.
2.4	Commercial testing of developed feeds	Demonstrate cost-effectiveness of commercial feeds vs. 'trash' fish	Yr 4, m1–12 (Jul 07 – Jun 08)	Completed Coral trout (*P. leopardus*) fed a commercial pellet feed and a custom-made (RIM-Gondol) feed showed good survival and growth responses. RIM Gondol undertook a comparison of pellet diet and 'trash' fish fed to *P. leopardus* and white-spotted grouper (*E. coeruleopunctatus*). 'Trash' fish outperformed the commercial pellet with both species. Coral trout fed 'trash' fish were substantially larger (554 g) after 180 days than those fed the pellet diet (366 g). Survival of fish fed both diets was similar. Overall, these results suggest that pellet diets at best provide equivalent performance to 'trash' fish, but often perform poorly.
2.5	Effect of feed on product quality	Impact of dietary practices (feeds management and formulation) on chemical (lipid and omega-3 fatty acid) and sensory quality of fish determined	Yr 3, m9 – Yr 4, m12 (Mar 07 – Jun 08)	Completed A trial at RICA Maros with tiger grouper reared from ~ 50 to 250 g using a commercial pellet demonstrated that grouper need be fed only once daily for optimal performance. Slightly restricting the feeding rate to the medium rate used in this study (3.2 – 1.6% biomass) has the advantage of improving the efficiency of feed utilization (and minimizing feed wastage) while not severely reducing growth rate of the fish. This feeding practice may also ensure that a more marketable fish with only a moderate amount of body lipid is produced. Taste tests with *E. fuscoguttatus* and *C. altivelis* in Hong Kong showed that while wild-caught *C. altivelis* were preferred to farmed fish, overall the quality of farmed fish of both species was acceptable to consumers.

Objective 3: Facilitate technology adoption

no.	activity	outputs/ milestones	completion date	comments

3.1	Identify constraints to technology uptake	Selection of research sites in Indonesia, Vietnam, Thailand Research methodologies developed. Baseline data collected.	Yr 2, m1–m6 (Jul – Dec 2005)	Completed Farm level data were collected from: • Indonesia: 34 grow-out farms (cages and pen systems) • Thailand: 33 farms (pond and cage systems) • Vietnam: 42 farms (cage system) Hatchery data were collected from two locations in Indonesia: • Situbondo: 16 hatcheries • Gondol: 15 hatcheries
		Assessment of constraints to technology adoption	Yr 2, m1 – Yr 3, m6 (Jul 2005 – Dec 2006)	Completed Major constraints to uptake of small-scale hatchery and grow-out feeds identified.
3.2	Strategies to assist technology adoption	Implementation of strategies to facilitate technology adoption	Yr 3, m7 – Yr 4, m12 (Jan 2007 – Jun 2006)	Completed Implementation through development of marine finfish BMPs. Process started with workshop in Lampung, November 2007. Nutrition workshop (follow-up to Crawford Fund Masterclass) to target training / information presentation to commercial feed formulators in Indonesia was undertaken in Surabaya in October 2009.
		Data analysis, socio-economic report prepared	Yr 4, m6 – m12 (Dec 2007 – Jun 2008)	Completed
3.3	Extension and communications	Production and distribution of 3 extension manuals	Yr3, m1 – Yr 4, m6 (Jul 2006 – Dec 2007)	Outstanding Three extension manuals have been drafted on: • Hatchery production of tiger grouper (Ketut Sugama) • Nursery culture for groupers (Suko Ismi) • Grow-out (Mike Rimmer)
		Web site maintenance APMFAN e-newsletter published quarterly Marine finfish aquaculture media monitoring e-news published bi-monthly	Yr1, m1 – Yr 4, m12 (Jul 2004 – Jun 2008)	Completed Marine finfish aquaculture articles are incorporated in the 'Marine Finfish' section of the NACA magazine 'Aquaculture Asia', and published on the NACA web site (www.enaca.org). APMFAN e-newsletter and media monitoring were discontinued due to staff losses at NACA.

3.4	Training courses	Regional grouper hatchery training course – CRIA or DGA centres as appropriate	Yr 1, m7–12 Yr 2, m7–12 Yr 3, m7–12 (Jan – Jun 2004, 2005, 2006)	Completed Four training courses have been held at BBAP Situbondo: 18 April – 8 May 2005: 17 participants from 8 countries. 20 November – 9 December 2006: 20 participants from 13 countries. 9–29 July 2007: 16 participants from 8 countries. 5–25 May 2008: 19 participants from 10 countries. APMFAN also organised a marine finfish hatchery training course in Thailand in May 2007 for 6 trainees from Pacific Islands on behalf of SPC.
		Training of YPH staff in farm-made feeds (RICA)	Yr 2, m1–12 (Jul 2005 – Jun 2006)	Completed Five farmers and staff from Yayasan Palu Hijau (Central Sulawesi) attended a short course on grouper feed management at RICA Maros,19–23 September 2005.
3.5	APMFAN strengthening and expansion	Assess regional interest in formal membership of APMFAN	Yr2, m1 – Yr 1, m6 (Jul – Dec 2005)	Completed Following expression of support by NACA member countries, a model based on the NACA Fish Health Program model was developed.
		Formalise institutions and individuals as RRCs and RREs	Yr2, m7 – Yr2, m12 (Jan – Jun 2006)	Completed A total of 11 institutions have been recognised as Regional Resource Centres (RRCs). Institute profiles have been completed for 6 Indonesian institutions. Thirty-one individuals have been recognised as Regional Resource Experts (RREs).
		Attract corporate sponsorship of newsletter and other network activities	Yr 1, m1 – Yr4, m12 (Jul 2004 – Jun 2008)	Completed Support from Skretting Asia, which sponsored the APFMAN web portal, news and communications, and provided scholarships for developing country participants in the APMFAN grouper hatchery training course, lapsed in March 2009. No other sponsors have been engaged.

7 Key results and discussion

Objective 1 – Improve hatchery production technology for high-value marine finfish

1.2 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

1.1.1 Larval nutrition

Nutritional composition of prey organisms and HUFA supplementation

Total lipid and fatty acid composition of *N. oculata* were higher than those of freshwater *Chlorella* sp. (Table 9). *Brachionus* enriched with Algamac-3050 and DHA-Selco contained higher total lipids (6.3 and 5.0%, respectively) than the pre-enriched level (2.0%). Algamac-3050 increased the levels of DHA, ARA and EPA in rotifers while DHA-Selco increased DHA and ARA levels only. The total lipid content in *Artemia* when enriched with DHA-Selco (14.4%) was more than twice as high than when enriched with Algamac-3050 (6%), thus n-3 and n-6 HUFA levels were also higher using DHA-Selco.

Table 9 Lipids and fatty acid composition (% dry weight) of natural food samples from RIM Gondol in March 2008. Under phytoplankton, *Brachionus* and *Artemia* column headings, treatment means with the same superscript are not significantly different at P>0.05.

	Phytoplankton		***Brachionus***			***Artemia***	
FAME	*Chlorella* sp.	*N.oculata*	Pre-enriched	Algamac-3050	DHA-Selco	Algamac-3050	DHA-Selco
20:4n-6 (ARA)	0.06 ^{b}	0.25 ^{a}	0.01 ^{b}	0.19 ^{a}	0.19 ^{a}	0.10 ^{b}	0.18 ^{a}
20:5n-3 (EPA)	0.21 ^{b}	1.03 ^{a}	0.03 ^{b}	0.18 ^{a}	0.20 ^{b}	0.19 ^{b}	1.07 ^{a}
22:6n-3 (DHA)	nd	nd	0.05 ^{c}	0.86 ^{a}	0.20 ^{b}	0.29 ^{b}	1.10 ^{a}
Saturates	0.44 ^{b}	1.58 ^{a}	0.43 ^{c}	2.40 ^{a}	1.08 ^{b}	1.10 ^{b}	3.46 ^{a}
Monounsaturates	0.34 ^{b}	1.68 ^{a}	0.49 ^{c}	0.75 ^{b}	1.28 ^{a}	1.42 ^{b}	3.10 ^{a}
n-3 FA	0.22 ^{b}	1.03 ^{a}	0.15 ^{c}	1.23 ^{a}	0.39 ^{b}	0.63 ^{b}	2.75 ^{a}
n-6 FA	0.10 ^{b}	0.61 ^{a}	0.30 ^{b}	0.38 ^{b}	0.91 ^{a}	1.26 ^{b}	2.13 ^{a}
n-3 HUFA	0.21 ^{b}	1.03 ^{a}	0.14 ^{c}	1.20 ^{a}	0.39 ^{b}	0.51 ^{b}	2.46 ^{a}
n-6 HUFA	0.06 ^{b}	0.25 ^{a}	0.01 ^{c}	0.19 ^{b}	0.42 ^{a}	0.12 ^{b}	0.25 ^{a}
n-3:n-6 FA	2.20 ^{a}	1.69 ^{b}	0.50 ^{b}	3.24 ^{a}	0.43 ^{b}	0.50 ^{b}	1.29 ^{a}
DHA:ARA	nd	nd	5.00 ^{a}	4.53 ^{a}	1.05 ^{b}	2.90 ^{b}	6.11 ^{a}

DHA:EPA	nd	nd	1.67 [b]	4.78 [a]	1.00 [b]	1.53 [a]	1.03 [a]
EPA:ARA	3.50 [b]	4.12 [a]	3.00 [a]	0.95 [b]	0.11 [c]	1.90 [b]	5.94 [a]
Total lipids	1.44 [b]	7.57 [a]	2.00 [c]	5.00 [b]	6.26 [a]	6.02 [b]	14.37 [a]

Larval fatty acid requirement

Newly hatched and unfed day 3 larvae

Tiger grouper newly hatched larvae (NHL) contained DHA> ARA > EPA (about 10, 6.4 and 4.4% of total lipids). Total lipids of NHL (3.91 µg ind^{-1}) declined 53.2% (1.83 µg ind^{-1}) in unfed day-3 larvae (Table 10). The NHL contained higher NL (2.63 µg ind^{-1}) than PL (1.28 µg ind^{-1}). Day-3 larvae utilized more NL (74.5%, 0.67 µg ind^{-1}) than PL (9.4%, 1.16 µg ind^{-1}). Major losses occurred in NL monounsaturated (MUFA, 83.1%), saturated (SFA, 72.6%), and polyunsaturated fatty acids (PUFA, 68.2%) while losses in PL were lower (10.9%, 18.2% and 3.9%, respectively). These losses indicate that NL fatty acids were primarily spent for energy whereas PL fatty acids were generally conserved. Generally, polar and neutral HUFA contents were: DHA>ARA>EPA. The pattern of conservation for NL HUFA was ARA>DHA>EPA while for PL was DHA>ARA>EPA. Among the HUFAs, the polar DHA was most conserved indicating a very high essentiality of this fatty acid for growth and development of early stage tiger grouper larvae.

Table 10 Neutral (NL) and polar lipid (PL) fatty acids in *E. fuscoguttatus* newly hatched larvae (NHL) and unfed day-3 larvae (µg ind^{-1} DW). TL= NL + PL; TL of NHL and day-3 larvae were 3.91 and 1.83 µg ind^{-1} DW, respectively.

	NL		**PL**		**% Conserved**		**% Utilised**	
	NHL	**Day 3**	**NHL**	**Day 3**	**NL**	**PL**	**NL**	**PL**
Lipids	2.63	0.67	1.28	1.16	25.5	90.6	74.5	9.4
Fatty acids:								
SFA	0.95	0.26	0.55	0.49	27.4	89.1	72.6	10.9
MUFA	0.83	0.14	0.22	0.18	16.9	81.8	83.1	18.2
PUFA	0.85	0.27	0.51	0.49	31.8	96.1	68.2	3.9
20:4n-6 (ARA)	0.13	0.07	0.12	0.06	53.8	50.0	46.2	50.0
20:5n-3 (EPA)	0.12	0.02	0.05	0.01	16.7	20.0	83.3	80.0
22:6n-3 (DHA)	0.23	0.07	0.16	0.15	30.4	93.8	69.6	6.2
Ratio:								
DHA:ARA	1.77	1.00	1.33	2.50				
DHA:EPA	1.92	3.50	3.20	15.00				
EPA:ARA	0.92	0.29	0.42	0.17				

Day-18 and day-25 larvae starved for two days

Total lipids increased in fed larvae (day-18 to day-25), from 128.1 to 245.3 µg ind^{-1} and respectively decreased to 51.5 and 102.4 µg ind^{-1} after two days starving (Table 11). Neutral lipids (69.26 and 127.44 µg ind^{-1}) were greater than PL (36.73 and 75.21 µg ind^{-1}) in fed larvae. Saturated, MUFA and PUFA contents of NL and PL of fed larvae increased with age and the pattern of HUFA accumulation was DHA>ARA>EPA. Utilization was MUFA > SFA > PUFA for both starved day-20 and day-27 larvae, but day-20 used more NL while day-27 used more PL. Conserved HUFA of starved day-20 larvae was ARA>DHA>EPA (NL) and DHA>ARA>EPA (PL) while that of starved day-27 larvae was ARA>EPA>DHA (NL) and EPA>DHA>ARA (PL). Among the HUFAs, DHA and ARA were more conserved in starved day-20 larvae. In both larval stages, neutral ARA was highly conserved.

Table 11 Larval dry weight (DW), total (TL), neutral (NL) and polar (PL) lipids and fatty acids (FA) of *E. fuscoguttatus* reared for 18 and 25 days then starved for two days (Starv.).

	Fed	**Starv.**	**Fed**	**Starv.**	**% gain**	**% conserved**		**% loss**	
	Day-18	**Day-20**	**Day-25**	**Day-27**	**18–25**	**18–20**	**25–27**	**18–20**	**25–27**
Larval DW (mg ind^{-1})	1.82	1.20	3.90	2.40	114.3	65.9	61.5	34.1	38.5
TL (µg ind^{-1})	128.10	51.50	245.30	102.40	91.5	40.2	41.7	59.8	58.3
NL (µg ind^{-1})	69.26	24.62	127.44	60.39	84.0	35.6	47.4	64.4	52.6
PL (µg ind^{-1})	36.73	17.65	75.21	24.53	104.8	48.1	32.6	51.9	67.4
Neutral FA (µg ind^{-1})									
SFA	25.02	9.55	46.03	25.52	83.9	38.2	55.4	61.8	44.6
MUFA	21.86	5.14	40.22	11.91	83.9	23.5	29.6	76.5	70.4
PUFA	22.38	9.92	41.19	22.97	84.1	44.3	55.8	55.7	44.2
20:4n-6 (ARA)	3.42	2.57	6.30	5.95	84.2	75.1	94.5	24.9	5.5
20:5n-3 (EPA)	3.16	0.73	5.81	1.70	83.9	23.3	29.3	76.7	70.7
22:6n-3 (DHA)	6.06	2.57	11.15	2.55	83.9	42.5	22.9	57.5	77.1
Ratio:									
DHA:ARA	1.77	1.00	1.77	0.43					
DHA:EPA	1.92	3.52	1.92	1.50					
EPA:ARA	0.92	0.28	0.92	0.29					
Polar FA (mg ind^{-1})									
SFA	15.78	7.46	32.32	10.45	104.8	47.3	32.3	52.7	67.7
MUFA	6.31	2.74	12.93	3.41	104.9	43.4	26.4	56.6	73.6
PUFA	14.63	7.46	29.97	10.68	104.9	50.9	35.6	49.1	64.4
20:4n-6	3.44	0.91	7.05	1.36	104.9	26.5	19.3	73.5	80.7
20:5n-3	1.43	0.15	2.94	1.82	105.6	10.6	61.9	89.4	38.1

22:6n-3	4.59	2.28	9.40	4.09	104.8	49.7	43.5	50.3	56.5
Ratio:									
DHA:ARA	1.33	2.51	1.33	3.01					
DHA:EPA	3.21	15.20	3.20	2.25					
EPA:ARA	0.42	0.16	0.42	1.34					

In summary, newly hatched tiger grouper larvae contained high levels of DHA, ARA and EPA demonstrating the importance of these HUFAs in larval development. The major lipid energy source of unfed day-3 larvae was NL while PL, particularly DHA, was more conserved.

NL and PL fatty acids of hatchery reared fish increased with age and the pattern of HUFA accumulation was DHA>ARA>EPA.

The major lipid energy source of two-day starved day-20 and day-27 larvae was NL and PL respectively, and the pattern of utilisation was MUFA > SFA > PUFA. A higher essentiality of DHA and ARA than EPA was indicated by two-day starved day-20 larvae. In both larval age groups, neutral ARA was highly conserved.

Morphological and histological study of opercular deformities

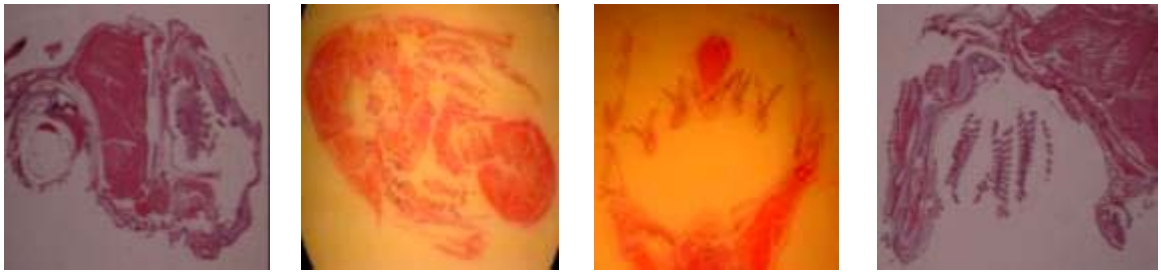
The normal operculum complex protected the gill arches and closed the opercular cavity like a lid. With operculum deformity, the structure of the operculum was variously affected and the gills as well as the gill-chamber showed different degrees of exposure (Figure 3). The deformity expanded ventrally affecting the branchiostegal rays which curled inwards resulting in a shortening of the branchiostegal membrane (Figure 3, C); and in a very severe case – only bent, short, thin, soft, and irregular free edge of the operculum was left (Figure 3, D). The folding which partly or entirely affected the length of the free edge, created a curve of varying degrees.

A B C D

Figure 3 External morphology of the head region of mouse grouper, showing (A) normal operculum; and deoperculation that was (B) slight, (C) severe, and (D) very severe

Histology of the normal operculum showed a regular succession of bone and tissue layers and the gill cavity was completely covered by the operculum complex (Figure 4, A). Operculum deformity that seemed slight (Figure 4, B) and unilateral visually appeared histologically to be bilateral (Figure 4, B). There was loss of structural integrity in both opercula with a folding of the free edge of one operculum into the gill chamber and the free edge of the other operculum bent outwards with epithelial tissue proliferation and fusion (Figure 4, B). The observed changes included a hypoplasia of muscle fibres and a

fusion of tissues between surfaces that come into contact (Figure 4, B, C and D). This deformity affected the operculum bone complex and connective tissues at varied severity.



A B C D

Figure 4 Histology of head region of mouse grouper, showing (A) sagittal view of normal operculum; (B) cross-section of bilateral deformed opercula; (C) cross-section of deformed left and normal right operculum; (D) sagittal view of very severe deoperculation.

Further experiments are needed in order to understand the causal factors behind deoperculation, to verify these observations, and to determine the earliest occurrence of deformities from which an improved hatchery protocol can be derived to minimize or eliminate deformities.

Vitamin C feeding trial

Full recovery from bilateral operculum deformity accounted for 10-25% of the total number of fish stocked per treatment (Table 12). There were different degrees of recovery and some fish did not show any signs of recovery (Figure 5). Despite the deformities, fish fed actively, survived and even gained weight during the experiment. Numerically, the number of fully recovered fish fed Otohemi alone was the highest (Table 12), although this treatment was not significantly different from the other treatments. Treatments with high levels of nutritional supplementation (such as the Otohemi enriched with both Phosphitan C and DHA-Selco) might contain excessive levels of nutrients to become toxic to the fish. Unpublished SEAFDEC AQD studies have shown that phosphated vitamin C at 540 ppm is toxic to juvenile (13–90 g body weight) mangrove red snapper (*Lutjanus argentimaculatus*) (Catacutan *et al.* in press).

Table 12 Recovery, growth and feed performance of mouse grouper fed different diets to improve fingerling quality.

Dietary treatment	**Fish with normal opercula**[*1,2]	**No. of survivors (%)**[*1,2]	**Weight gain (%)**[*2]	**FCR**[*2]
NRD [*3]	11 (18%)	59 (98.3%)	582 ± 45.8	1.82 ± 0.62
Otohemi [*4]	15 (25%)	58 (96.7%)	638 ± 30.6	1.64 ± 0.38
Otohemi + 2000 ppm Phosphitan C [*5]	12 (20%)	59 (98.3%)	669 ± 40.8	1.58 ± 0.40
Otohemi + 6% DHA-Selco [*6]	9 (15%)	60 (100%)	621 ± 51.5	1.72 ± 0.44

Otohemi + 2000 ppm Phosphitan C + 6% DHA-Selco	6 (10%)	55 (91.7%)	610 ± 35.2	1.78 ± 0.36

Notes

*1 Out of 60 fish per treatment.

*2 Mean ± SD of fish with apparently normal opercula and of survival are not shown but all treatments were not significantly different at P>0.05.

*3 NRD: n-3 HUFA 28 mg g^{-1}, vit C 2,000 mg kg^{-1},*4 Otohemi: > 12% lipid. *5 Phosphitan C (93% ascorbyl-2-monophosphate or 875 ppm ascorbic acid). *6DHA-Selco: vit A 1,500,000 IU kg^{-1}, vit B3 150,000 IU kg^{-1}, vit E 3,600 mg kg^{-1}, vit C 800 mg kg^{-1}, n-3 HUFA min 200 mg g^{-1} dwt, and DHA/EPA min 2.5.

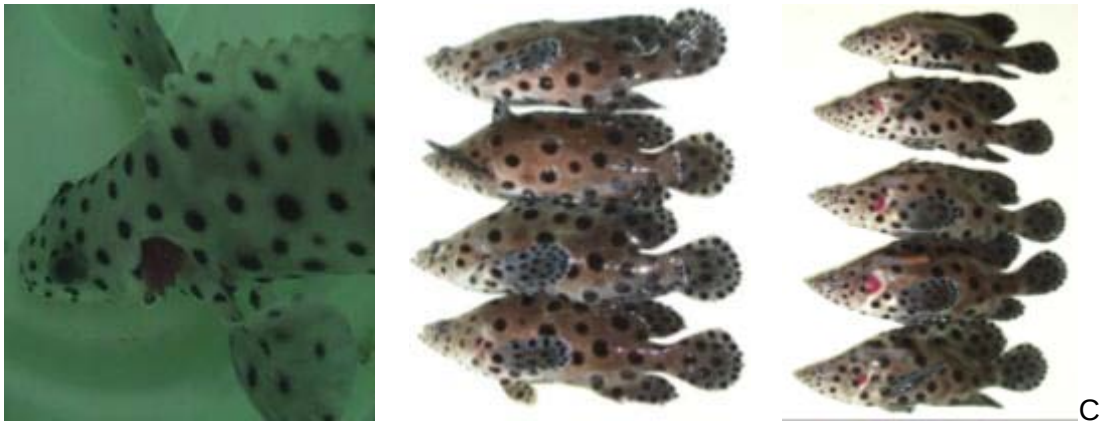


Figure 5 Mouse grouper (A) with operculum deformity; (B) fully recovered; (C) partially recovered or did not recover.

The two commercial diets used in this study were the usual fish nursery feeds used at RIM Gondol. Although the occurrence of operculum deformity in mouse grouper at RIM Gondol was highly variable in occurrence and severity, it is likely that the incidence would have been higher if high-quality feeds were not used. Mouse grouper is a high value aquaculture species and studies on the prevention and healing of operculum deformity are worth pursuing.

1.1.2 Larval digestion

Transfer of high sensitivity enzyme analysis technology

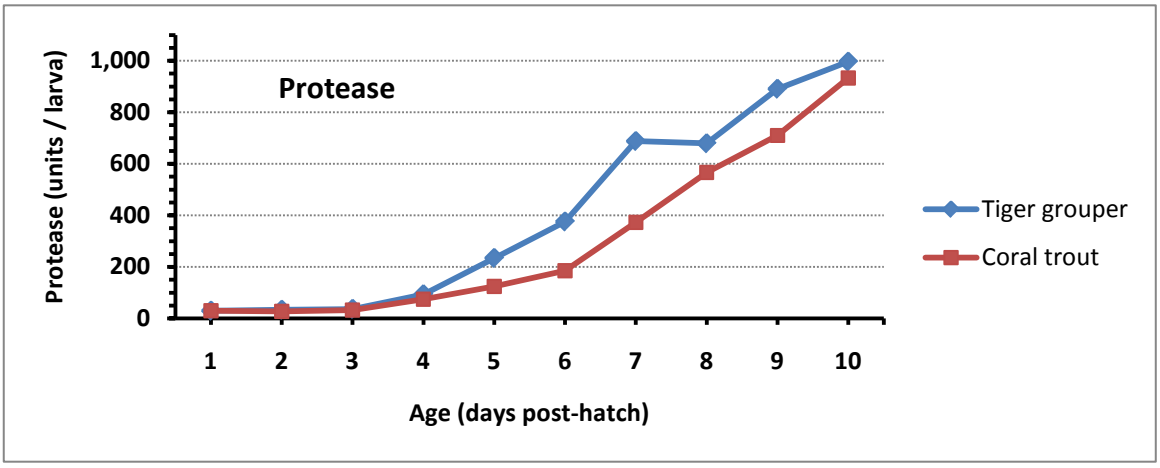
Training of RIM Gondol staff members in use of fluorescence detection for enzyme activity was undertaken at NFC Cairns. Subsequent training of RIM Gondol staff in sample protocols and preparation of crude enzyme extract occurred during visits by NFC Cairns staff to RIM Gondol during the project. However, the training was limited in extent because the RIM Gondol microplate reader was unable to use fluorescence detection techniques.

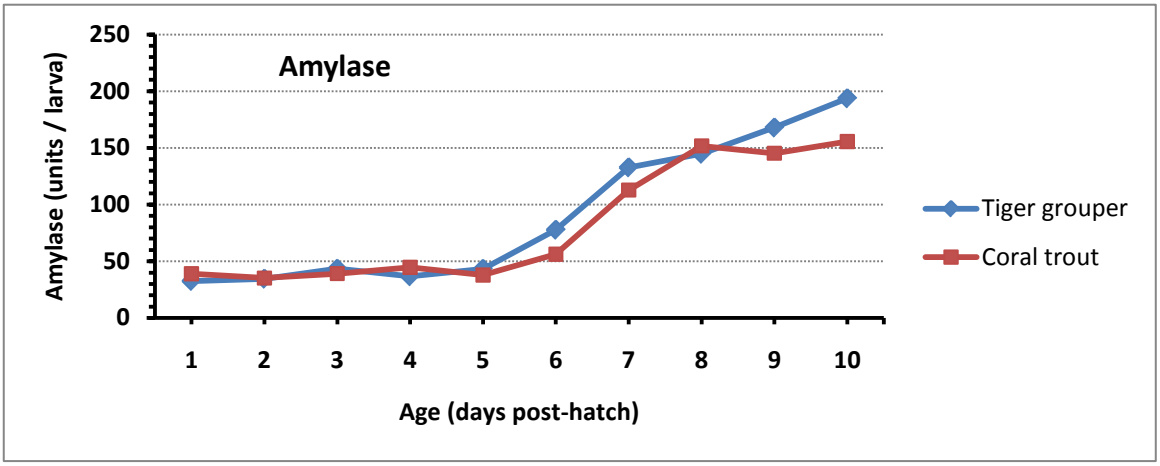
Modification of colorimetric techniques to microplate scale proved unsuccessful. While enzyme activity in 4–5 day old larvae are at lower measurable levels, preparation of crude extract for microplate assay requires significantly higher number of larvae sampled (approximately 500 to 1000) to provide a response within detectable limits. In comparison the fluorescent technology require only 30 larvae for a detectable level.

Enzyme response during initial first feeding stage

Protease and amylase activity is present in both tiger grouper and coral trout prior to mouth opening (Figure 6), which is presumably indicative of preparation for first feeding and a shift from endogenous to exogenous nutrition. However initial enzyme activity is very low compared to levels when the larvae commence feeding. Significant increases in protease and amylase activity were correlated with increases in consumption during days 5, 6, and 7 post-hatch (Figure 6). Lipase activity is present in tiger grouper prior to first feeding; however no lipase activity could be detected in coral trout prior to day 5 post-hatch (Figure 6).

While both tiger grouper and coral trout demonstrate significant increasing levels of enzyme activity after first feeding through to day 10 post-hatch, coral trout generally shows a lower enzymatic capacity compared to tiger grouper (Figure 6). This is reflected in the increased difficulty in rearing coral grouper larvae, with slower growth rates, and lower survival as early as day 10 post-hatch.





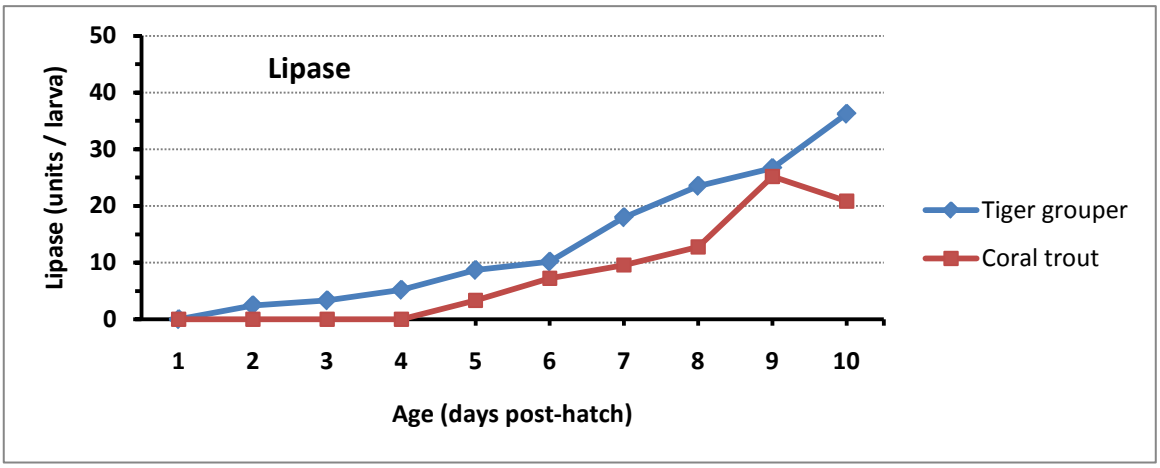


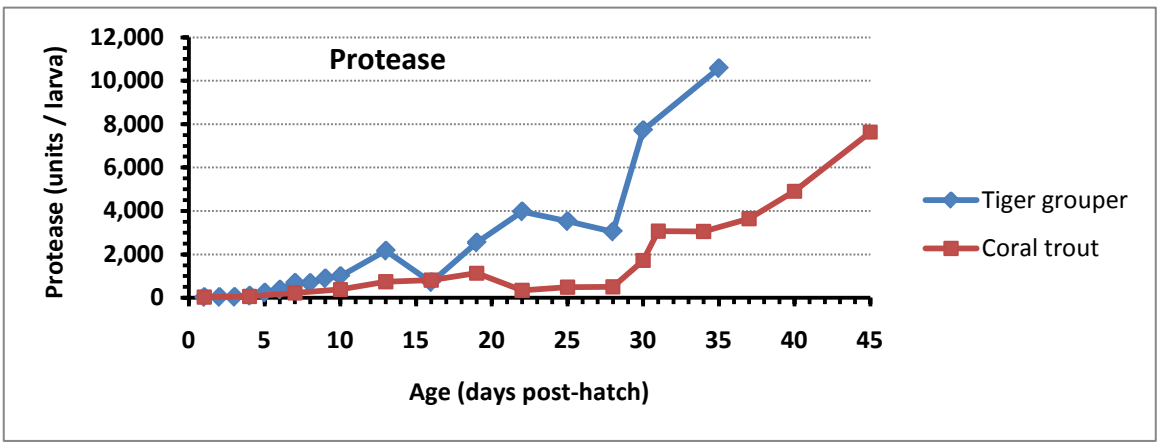
Figure 6 Levels of protease, amylase and lipase in tiger grouper (*E. fuscoguttatus*) and coral trout (*P. leopardus*) during early stages of development.

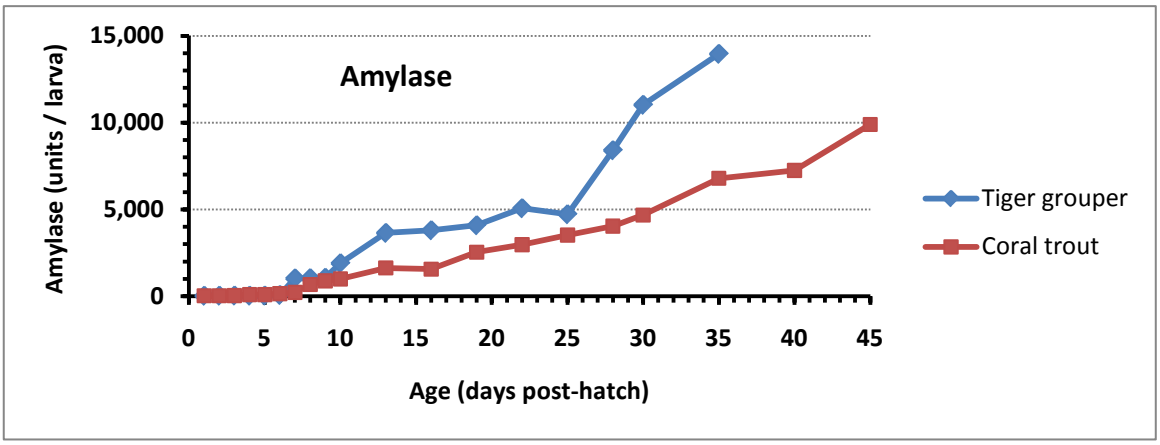
Enzyme responses during larval development

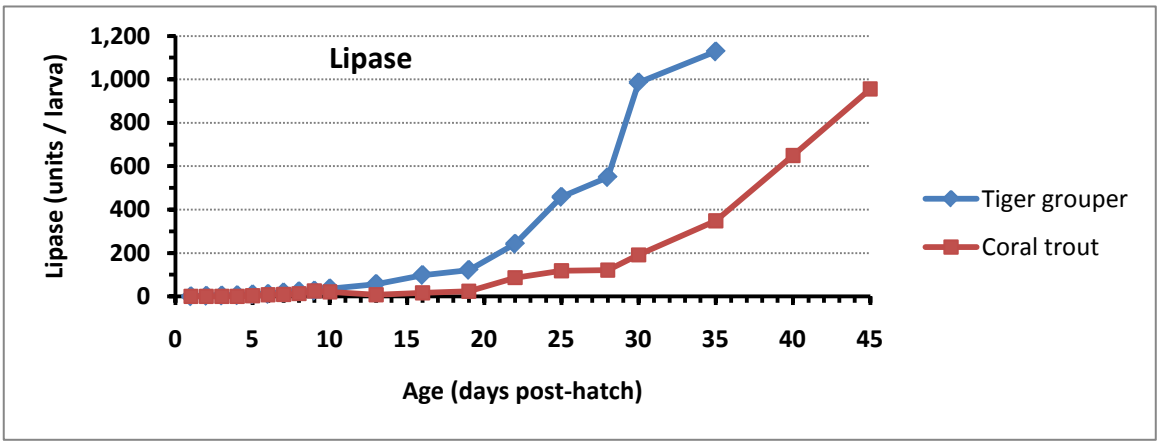
Tiger grouper demonstrated a weak capacity in enzyme activity during the endogenous nutrition stage, prior to day 3 post-hatch and the on-set of first feeding. Enzyme activity increased significantly once larvae began exogenous nutrition, and continued to increase through to metamorphosis (Figure 7). Significant increase in the levels of all enzymes, particularly protease and amylase, occur after the formation of the stomach and completion of digestive tract maturity between days 24 and 26 post-hatch.

The onset of metamorphosis at day 28–30 post-hatch and increase in enzyme activity is associated with the rapid uptake of inert diets.

Coral trout demonstrated a weaker capacity in enzyme activity during endogenous nutrition stage, as also seen in tiger grouper prior to day 3 post-hatch and at the on-set of first feeding enzyme levels are low. While activity of all enzymes increases once larvae being exogenous nutrition, protease and lipase only have a gradual increase until day 25-30 post-hatch before rising significantly through to metamorphosis. Increases in enzymes (protease, amylase, and lipase) demonstrate a different rate compared to tiger grouper after day 10 post-hatch. The completion of stomach formation (day 30–33 post-hatch) and delayed onset of metamorphosis is indicative of the slower digestive tract development and lower enzyme activity in coral trout.







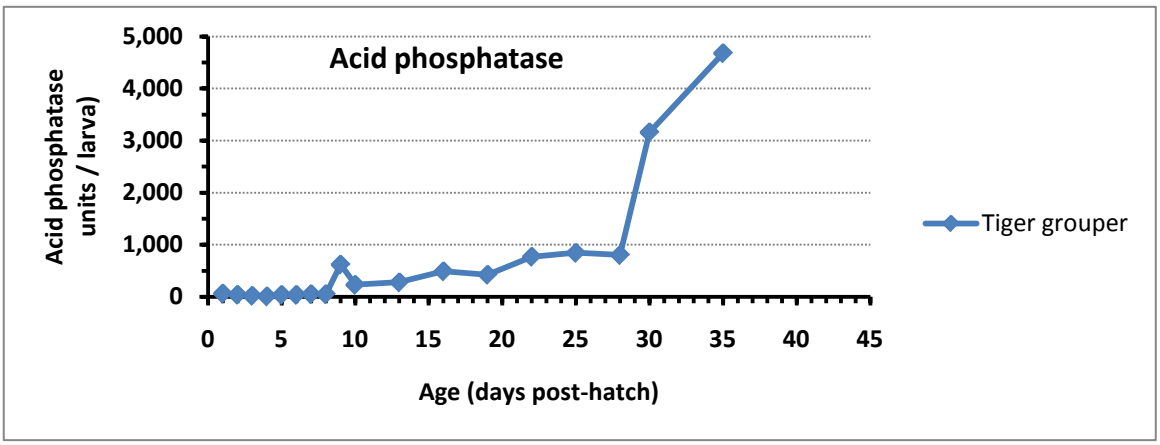


Figure 7 Levels of protease, amylase, and lipase in tiger grouper (*E. fuscoguttatus*) and coral trout (*P. leopardus*), and acid phosphatase in tiger grouper, during larval development.

Enzyme response to feed type

Enzyme activity in response to the addition of rotifer or brine shrimp was not significant between treatments and comparable with the activity levels measured in the longitudinal study. However grouper larvae showed a decrease in enzyme activity in response to the withdrawal of live prey at day 20 post-hatch. Feeding inert diet only prior to completion of significant developmental changes (i.e. stomach formation) had a negative effect and is reflected in the reduction in feeding (starvation). Hence co-feeding of brine shrimp and inert diets is still recommended until the onset of metamorphosis in tiger grouper.

In contrast to the use of other live prey organisms, tiger grouper larvae demonstrated a significant difference in enzymic (protease) response when copepod nauplii were incorporated in the larval diet. The use of copepods (in addition to rotifers) at levels of 4 and 12 ml^{-1} significantly increased protease activity by 25.8% and 23.3% respectively by day 9 post-hatch (Figure 8). A species such as coral trout, which has a reduced enzyme capacity during its early development stage, would particularly benefit from the use of copepods.

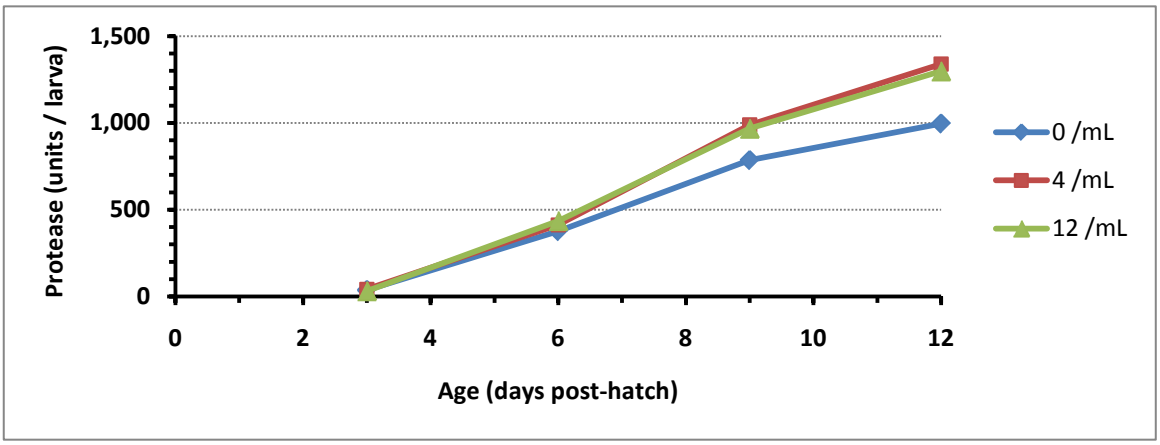


Figure 8 Enzymic (protease) response to the addition of copepods in the diet for early stage larval tiger grouper *E. fuscoguttatus*.

1.1.3 Verification of intensive and semi-intensive hatchery techniques

Hatchery techniques developed through the project were incorporated into hatchery protocols developed at NFC Cairns. In addition, interaction between researchers and staff from participating laboratories enabled the exchange of experiences which led to changes in practice in a variety of aspects of grouper larviculture. Specifically, the following aspects of larval rearing were changed or incorporated either as a result of experimental work carried out under the ACIAR project, or as a result of experiences with collaborating laboratories:

Aspect / practice	**Original**	**Current**	**Direct / Indirect**
Broodstock sex ratio	1-2 ♂ /tank	Increase 2-3 ♂ /tank	Indirect
Egg handling		At defined development stages	Direct (FIS/97/73)
Larval rearing tank colour	Dark blue	Yellow	Indirect
Use of copepods	None or limited	Routinely using *Parvocalanus*	
Nutritional supplementation of live prey	Based on *L. calcarifer* data; unsuitable for groupers	Using products better targeting nutritional requirements of grouper larvae	Direct
Use of squid oil to prevent surface tension mortality	No	Yes	Indirect

The incorporation of these techniques into NFC Cairns larval rearing protocols has led to the first production in Australia of fingerlings of three grouper species: *E. fuscogutattus*, *P. leopardus* and *E. lanceolatus*.

There is potential to improve the uptake of technologies by Indonesian hatcheries by operating a demonstration hatchery and working directly with more progressive private sector groups, such as ABILINDO. This is discussed further in Section 9.2 Recommendations (p. 90).

1.2 Improve the availability and quality of live prey

1.2.1 SS-strain rotifers

Reduce average rotifer body size by screening

The initial average size of egg-bearing rotifers was 148 µm body length and 113 µm body width. Following the screening process the average size of rotifers remained unchanged at 146 µm body length and 120 µm body width.

This selection process did not reduce the body size of rotifers. However, the method to obtain a synchronous rotifer population is useful for collecting large numbers of rotifer eggs for hatching to feed first feeding larvae, to use to initiate a contaminant free culture or to investigate cold storage of the eggs.

Cold-storage of amictic eggs for mass production of SS-strain neonates

This initial experiment did not measure the viability of eggs stored at the cool temperatures. A second experiment in which multiple samples of rotifers eggs were stored for increasing periods at 10°C was run to determine the viability of cold-stored eggs. Eggs were stored for varying periods at 10°C and then replicate samples were removed and allowed to equilibrate to 28°C where they were held for 24 h to allow eggs to hatch. After this time, rotifers and eggs were counted and a percentage hatch rate calculated (Figure 9).

From Figure 2, eggs stored at 10°C will have around 30% hatched neonates after storage from 24 to 72 h. This background hatching rate did not increase over the storage time and is likely to have occurred within the first few hours as the temperature dropped and those eggs that were close to hatching did so. In Figure 9, a measured hatch rate of 30% following the 24 h post-storage incubation would therefore indicate that stored eggs are non-viable.

The initial hatch rate was 95% but this declined to 75% after 24 hours of cold-storage. Viability then fell rapidly to be effectively zero (the ~30% minimum) by the end of 2 days storage (Figure 9). During the first 4 days, the total number of eggs and neonates recovered from replicates for each sample point was consistent at 73 ± 9. This indicates that eggs and neonates remained intact and able to be accounted for. However, by the final sample time (7 days storage, not shown in Figure 9) only an average of 24 eggs/neonates was recovered in the replicate samples. Nauplii and eggs had started to disintegrate.

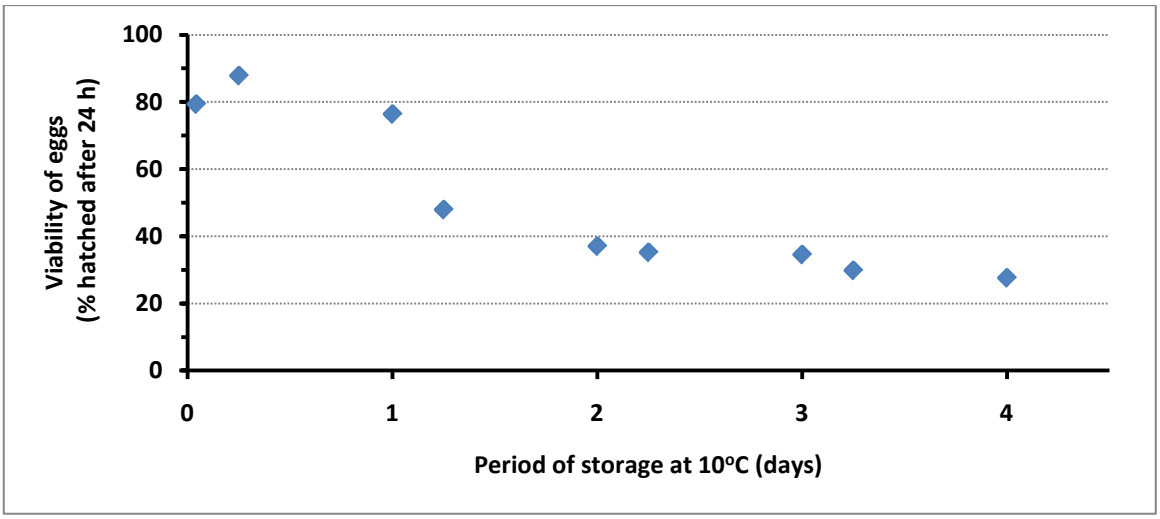
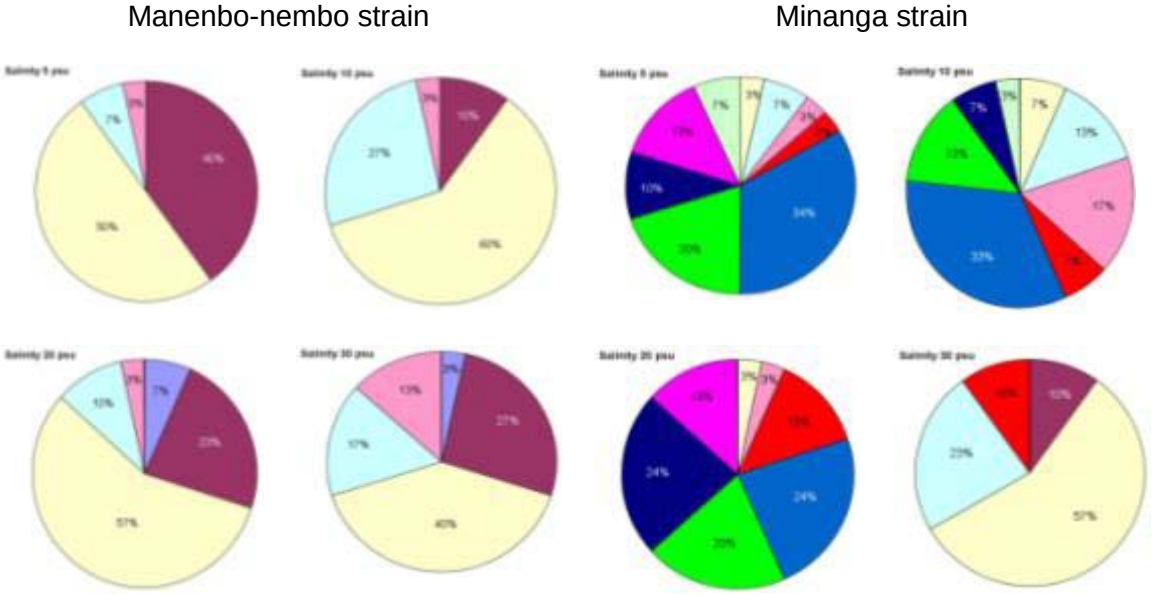


Figure 9 Viability of amictic rotifer eggs stored at 10°C for up to 4 days.

This method to collect large numbers of amictic eggs can be used to provide large numbers of newly hatched neonates for first feeding finfish larvae. However, cold storage of amictic eggs is of no benefit beyond 24 hours. After this time, unhatched eggs are non-viable and the apparent 30% hatch rate is what had occurred during the initial chilling down process.

Detect shift in population phenotype as a result of selection pressures

There was no significant difference in body size at the different salinity levels for each strain. However, the size difference between the two strains was significant with the Minanga strain being bigger than the Manembo-nembo strain when grown at all the tested salinity levels (Figure 10). The body length of Minanga strain is in range of 120–160 µm with about 10–40% of population with the size <130 µm. The Minanga strain had an average body length of 130–220 µm. When cultured at 30 ppt a small proportion of population (10%) were smaller than 130 µm.



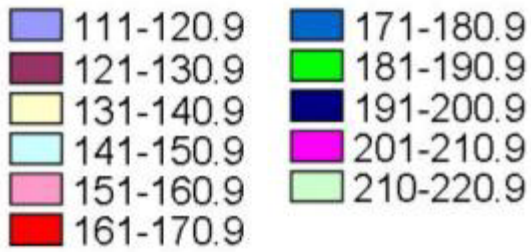
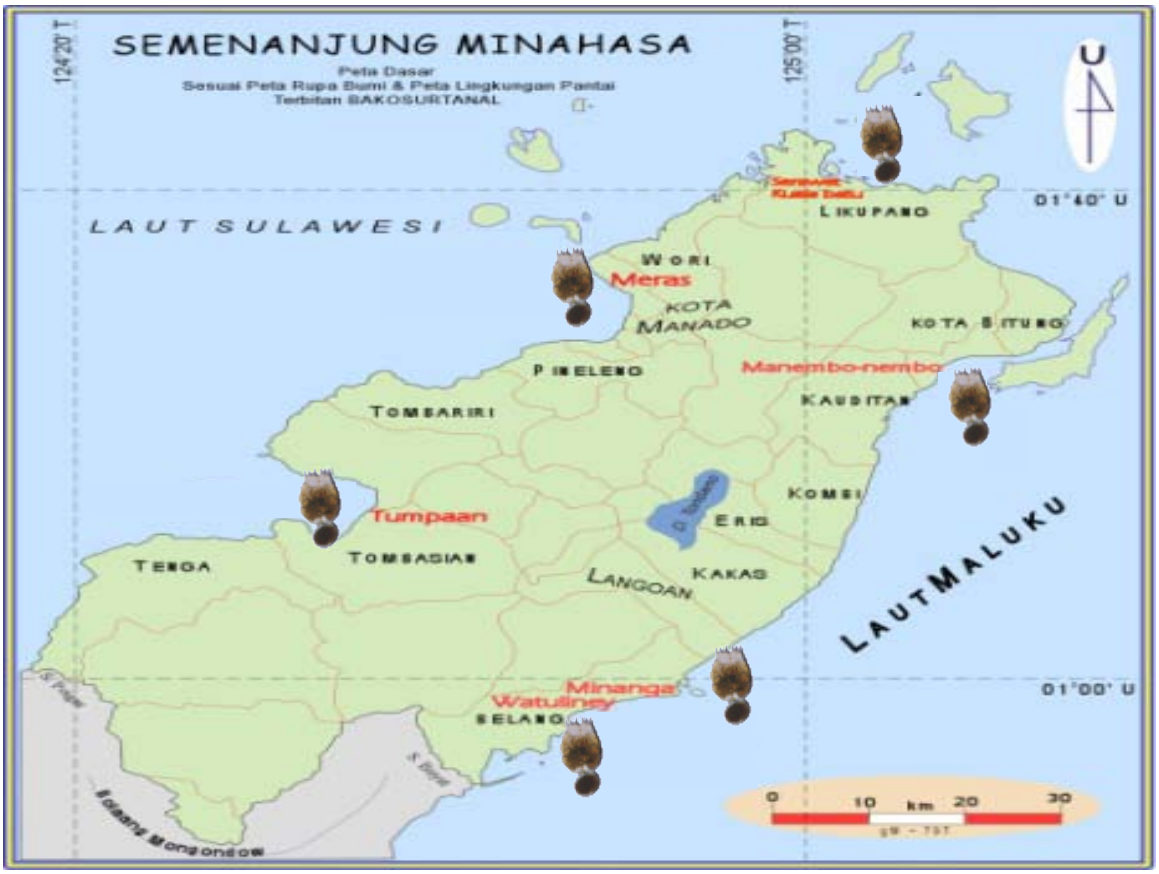


Figure 10 Size distribution of populations of Manembo-nembo and Minaga strain rotifers cultured at 5, 10, 20 and 300 ppt salinity.

Increase the natural variation within a rotifer population through hybridisation of strains and then to select for super-small individuals

A shift in the phenotype to smaller body size occurred when wild rotifers were cultured under controlled conditions. This was demonstrated using synchronous cultures of rotifers from Manembo-nembo and Likupang (Fig 1B). The two strains were then hybridised.



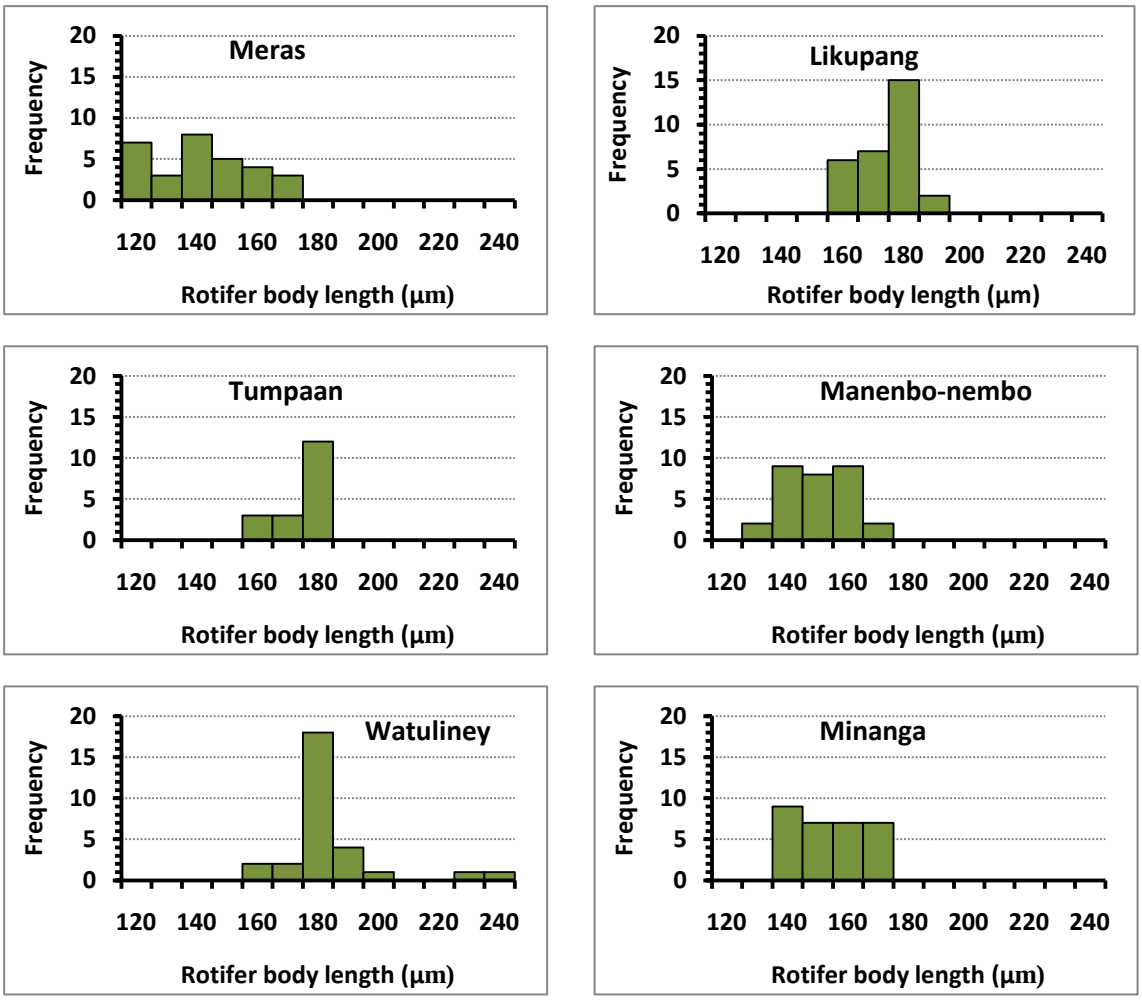
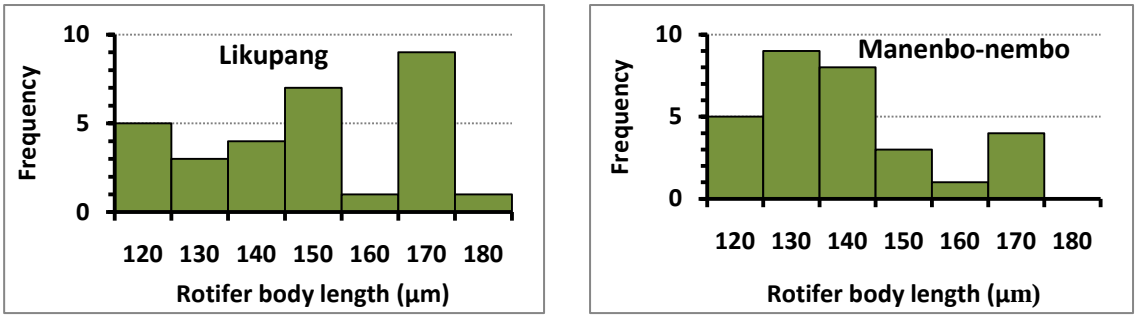


Figure 11 Map of North Sulawesi showing sampling locations for various rotifer strains, and frequency distributions of each strain.

As shown in Figure 12, when the Likupang strain with a greater proportion of larger rotifer in population is crossed to Manembo-nembo strain (females) with a greater proportion of smaller rotifer in population, the resulting hybrids had a similar proportion of smaller group (<130 um in lorica length), but an increase of medium group (lorica length, 130-150 um, 70%) compared to both Likupang and Manembo-nembo, and a decrease of larger group (lorica length, >150 um, 27%) compared to Likupang (Figure 12).



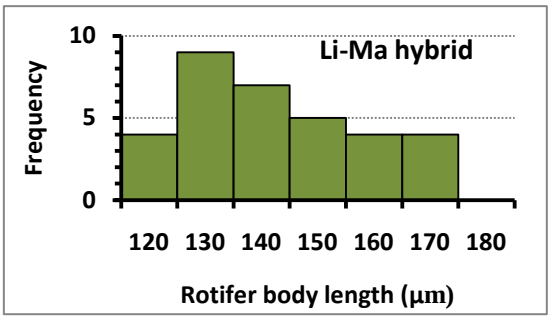


Figure 12 Frequency distributions of body length of rotifers of Likupang strain and Manembo-nembo strain and their hybrid Li-Ma.

1.2.2 Ultra-small copepod nauplii as first feed prey for marine finfish larvae

Evaluation of diets for the cyclopoid copepod, Oithona sp.

As shown in Figure 13, there was no difference in the number of individuals in populations fed on the three species of microalga. Copepods in all treatments started to reproduce after 20 days, as indicated by the sharp increase in the number of individuals in the population. However, by comparing the proportion of nauplii, copepods and adults number in the populations (Figure 14), it can be seen that copepods fed on *N. oculata* developed faster than those fed on the other two microalgae. Naupliar stages of copepods fed on *N. oculata* took less than 8 days to develop, and about 50% of them developed into adults after 16 days (Figure 14).

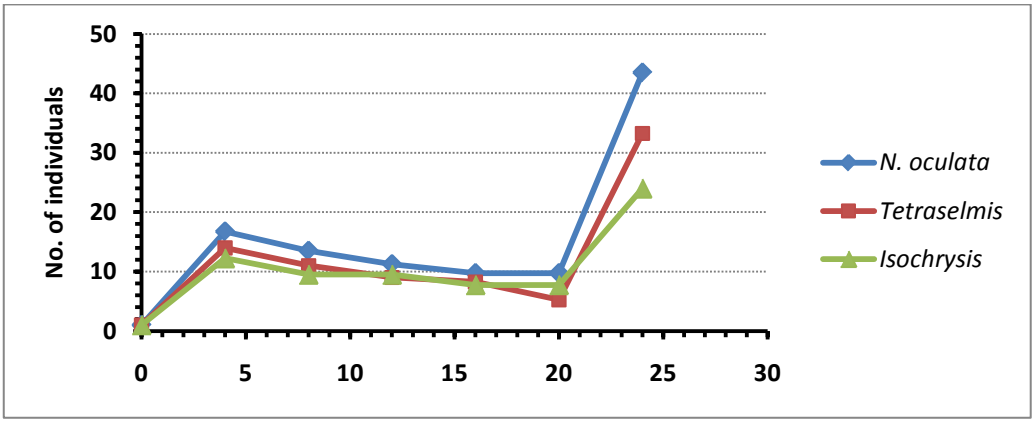


Figure 13 Population growth of Oithona sp. cultures fed three different microalgae diets: *Nannochloropsis oculata*, *Tetraselmis* sp. and *Isochrysis* sp.

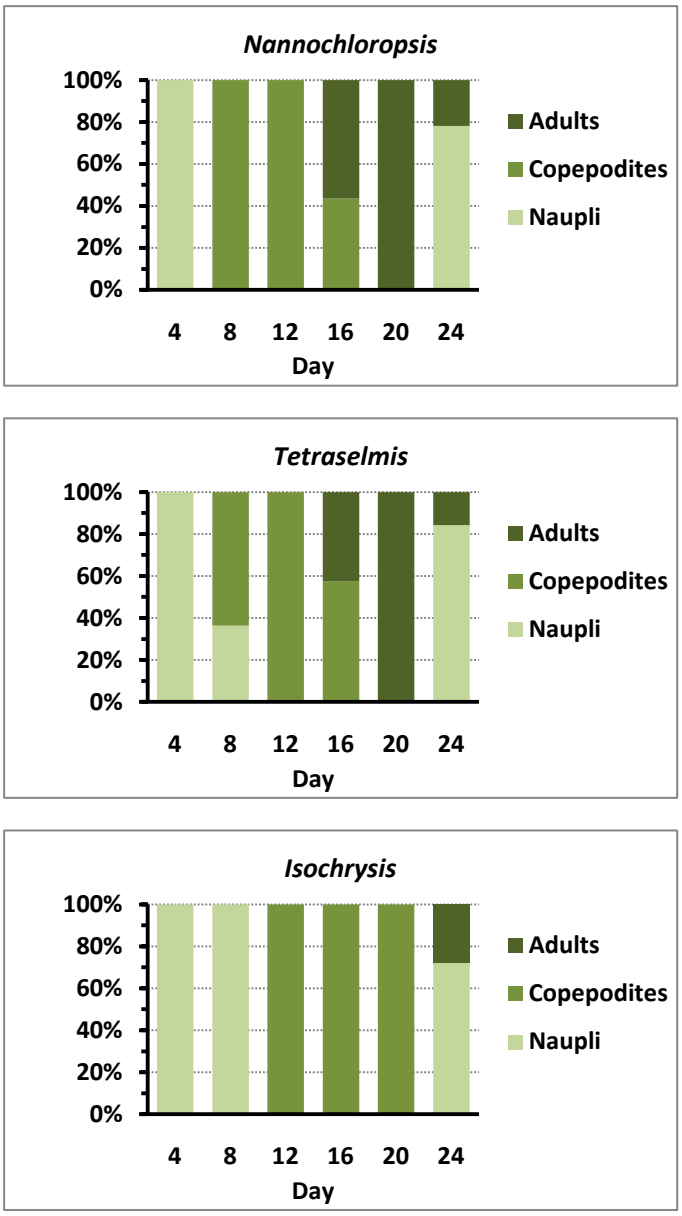


Figure 14 Proportion of nauplii, copepodids and adults in *Oithona* sp. cultures fed different microalgal diets.

Culture techniques for Euterpina acutifrons

Morphological observations confirmed that the harpacticoid copepod *Euterpina acutifrons* has developmental stages from nauplius (60-80 µm) to copepodite of 3 days and from copepodite to adult of 5-6 days. The population pattern of every stage of *E. acutifrons* for both treatments is shown in Figure 15. Figure 15 shows that there was no difference in population patterns between the two treatments, suggesting that population dynamic cycle of *E. acutifrons* was independent of the type of additional feed.

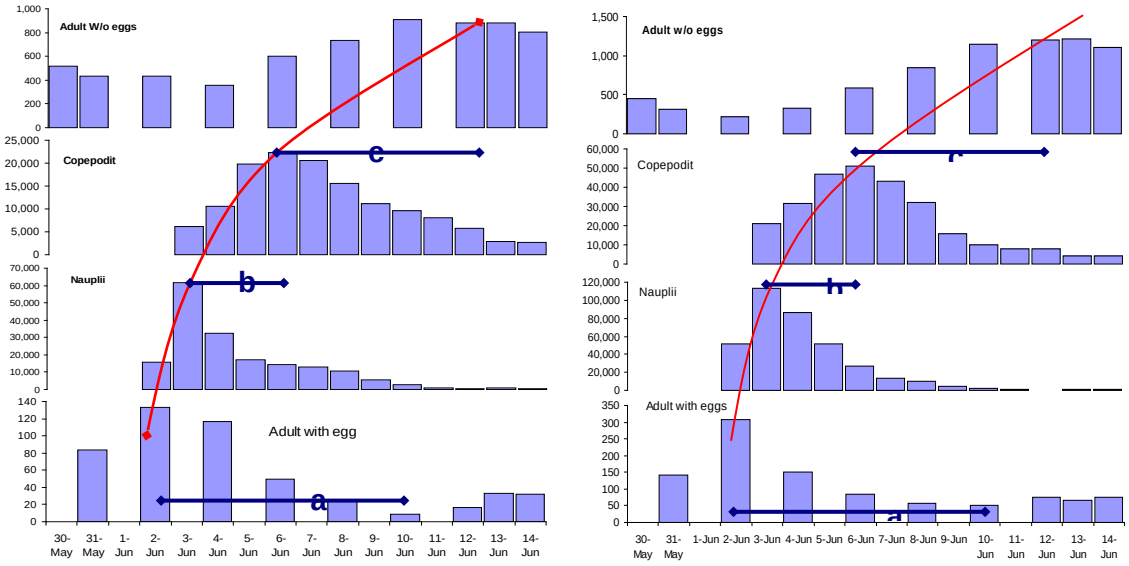


Figure 15 Population parameters for the harpacticoid copepod *Euterpina acutifrons* cultured on wheat flour (Treatment A) and minced chicken liver (Treatment B) at RIM Gondol.

The differences in both treatments were seen in the population growth. In Treatment A the highest number of copepod-bearing-egg was only 133 ind., nauplii production up to 62,833 ind. and number of copepodites was 22,333 ind., all of which were lower than in Treatment B in which the highest copepod-egg was 308 ind., nauplii production up to 113,333 ind. and number of copepodites was 51,167 ind. The survival rate of naupliar-to-copepodite stage in Treatment A was lower (36%) than that in Treatment B (45%). However, the survival rate of copepodite-to-adult stage in Treatment A was higher (4%) than that in Treatment B (2%), suggesting that the type of additional feed influenced the survival rate.

This experiment indicated that the kind of feed provided (wheat flour vs. minced chicken liver) did not influence population pattern (quality) but affected population growth. Data analyses of survival rate showed that Treatment B was superior to that Treatment A at naupliar and copepodite stages.

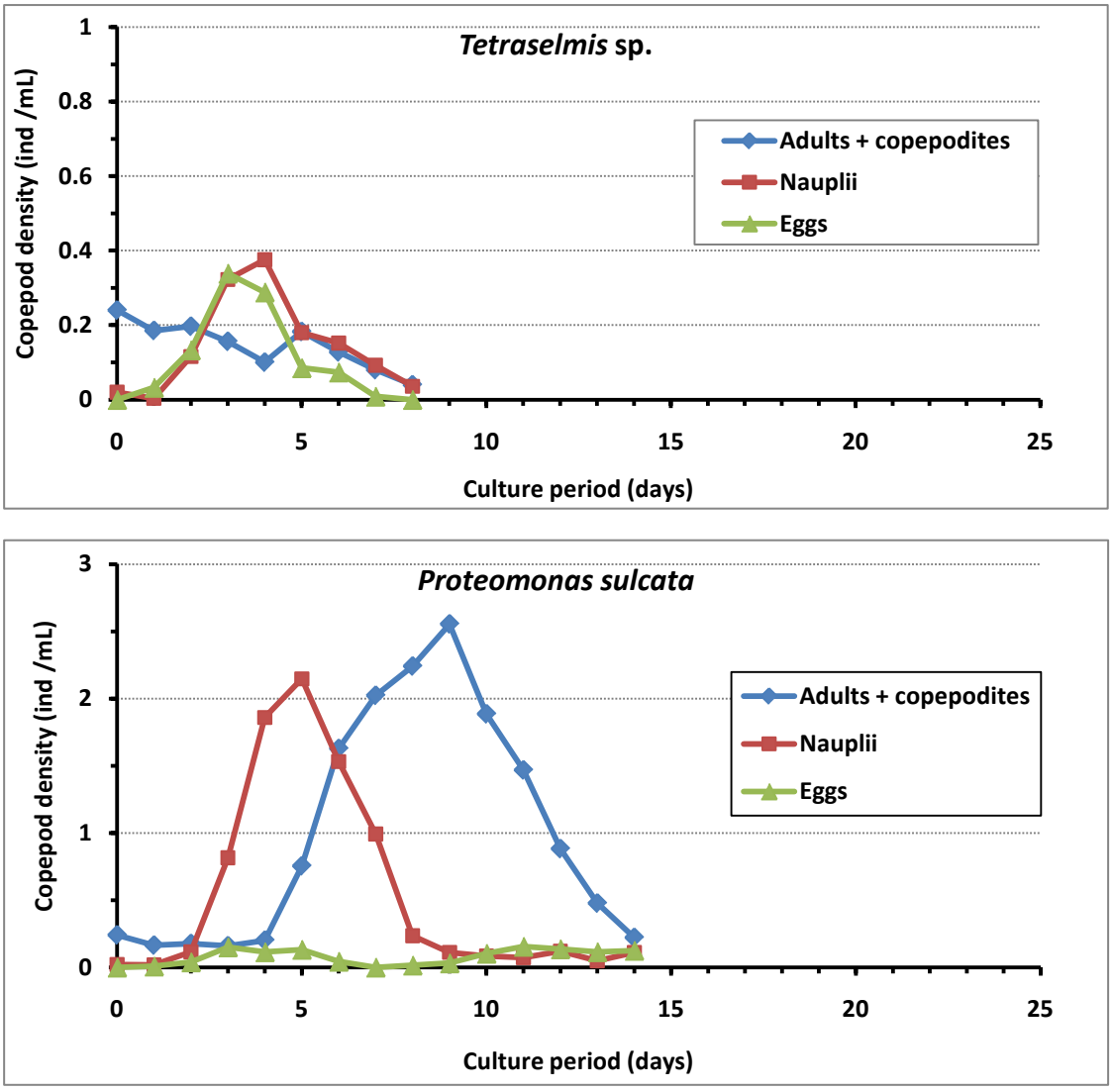
Diet development for the culture of the calanoid copepod, Parvocalanus crassirostris

The growth responses of the copepods fed the three monoalgal diets were similar in times for key development stages but very different in the size of the response and its duration. Copepods fed *Tetraselmis* sp. produced eggs for 5 days (days 1–6) with peak production on day 3 (Figure 16). A similar response was seen in copepod cultures fed the other monoalgal diets and should have resulted in a spike in nauplii numbers as the eggs hatched. Although this did occur, the response was very weak as nauplii increased from day 2 to peak on day 4 at only ~0.4/mL.

Copepods fed *P. sulcata* grew in a similar fashion to those fed *Tetraselmis* sp. except that their level of response was much more pronounced and sustained (Figure 16). Initial egg production occurred for 4 days (days 2–6) and peaked on day 3. This was followed by increasing nauplii numbers as eggs hatched. Nauplii numbers peaked on day 5 then rapidly decreased as egg production declined and nauplii moulted through to copepodites. Initial copepod numbers declined over the first 3–4 days before increasing rapidly to peak

on day 9 as stocks were replenished from developing nauplii. This new, maturing population of copepods started to produce eggs from day 9 until day 15. However, there was no secondary peak in nauplii numbers that would have been expected as the eggs hatched.

Copepods fed a monoalgal diet of *Isochrysis* sp. (T.ISO) initially grew in a similar manner to those fed *P. sulcata* or *Tetraselmis* sp. However, they differed in the timing for peak production and in the magnitude of the growth response and number of growth cycles (Figure 16). Initial egg production occurred for 5 days (days 2–7). As eggs hatched, nauplii numbers increased to peak on day 6 at 3.6/mL. Copepodite numbers started to increase from day 4 to peak at 1.6/mL on day 10. This first cycle was similar to the pattern seen in copepods cultured on *P. sulcata* except that the peak production occurred one day later. However, the copepods cultured on *Isochrysis* sp. (T.ISO) also demonstrated a second growth cycle that achieved a maximal total copepod density of 15/mL on day 19.



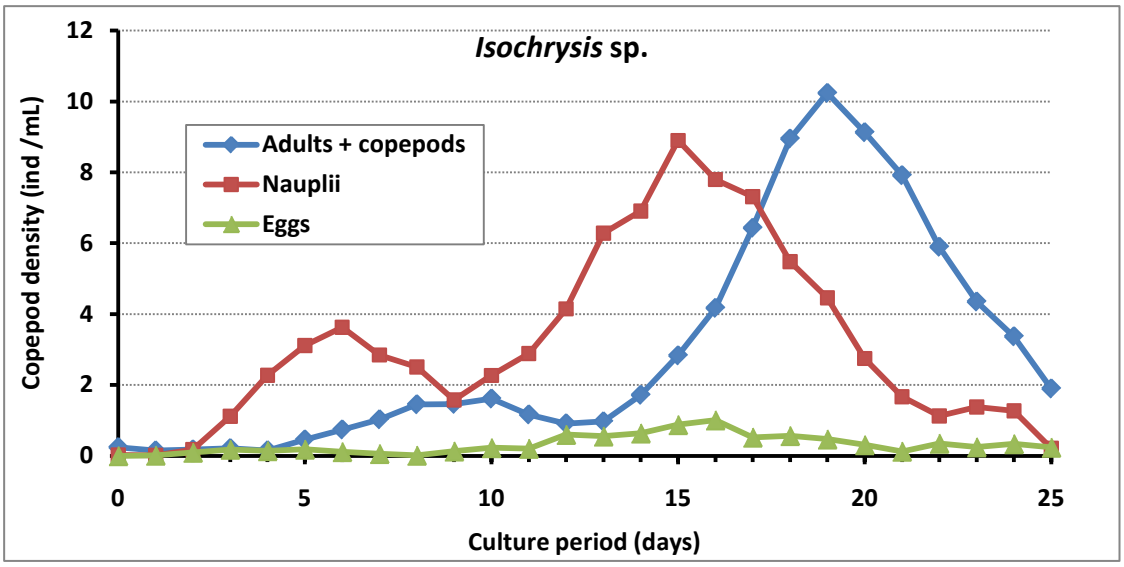


Figure 16 Production of adults and copepodites, nauplii, and eggs of *Parvocalanus* cultured using three different microalgal species.

In summary, *Parvocalanus* can be successfully cultured on a mono-algal diet of *Isochrysis* (T.ISO) which is a much simpler diet than the mixed algal diet needed by *Acartia*. *Parvocalanus* cultures can also reach much higher densities of ~15/mL. *Parvocalanus* is now routinely cultured at NFC in 2,000 L volumes using only T.ISO. These are batch harvested to collect a mixed-stage population which is fed to finfish larvae. The results of feeding trials with grouper are described below.

Determine the fatty acid profile of the calanoid copepod, Parvocalanus crassirostris

The total lipid content of mixed stages of *Parvocalanus* was 105 mg/g DW (Table 13). Polyunsaturated fatty acids (PUFA) dominated the fatty acid composition accounting for 51% of total lipid. Of these, docosahexaenoic acid (DHA: 22:6n-3) was the major PUFA comprising 20.5% of total lipid. Other significant PUFAs were linoleic (18:2n-6), α-linoleic (ALA: 18:3n-3) and stearidonic (SDA: 18:4n-3) acids with 9.6%, 7.3% and 6.7% of total lipid, respectively. Of the other essential PUFAs, arachidonic acid (ARA: 20:4n-6) was lowest with 0.4% of TL followed by eicosapentaenoic acid (EPA: 20:5n-3) with 2.6% of TL. The DHA/EPA ratio was high at 7.9:1. Saturated fatty acids were the next dominant lipid group and accounted for 33.4% of total lipid. Palmitic (16:0), myristic (14:0) and stearic (18:0) acids were the major saturates accounted for 18.8%, 8.8% and 3.7% of total lipid, respectively. The more minor, monounsaturated fatty acids averaged 15.5% of TL with oleic (18:1n-9) acid being the major MUFA at 8% of total lipid. The major fatty acids were the same across the three replicates. Greatest variability occurred in the PUFAs where linoleic, ALA and in particular, DHA all had higher coefficients of variation. Variability of analysis within multiple samples of each replicate was very minor. The bulk of the variability within the PUFAs occurred because of much lower levels of linoleic and ALA and higher levels of DHA in one replicate.

Table 13 Fatty acid composition of the calanoid copepod *Parvocalanus crassirostris* fed a mono-algal diet of *Isochrysis*.

Fatty acid (% of total)	**Mean ± SD**	**Fatty acid (% of total)**	**Mean ± SD**
Saturates		*Polyunsaturates*	
14	8.80 ± 0.63	18:2n-6	9.61 ± 3.28
15	0.83 ± 0.53	18:3n-6	0.86 ± 0.27
16	18.81 ± 1.00	18:3n-3	7.27 ± 2.68
17	1.17 ± 1.22	18:4n-3	6.72 ± 1.00
18	3.66 ± 0.67	20:2n-6	0.34 ± 0.09
22	0.17 ± 0.01	20:4n-6	0.38 ± 0.00
Total	33.44 ± 2.78	20:3n-3	0.76 ± 0.32
		20:5n-3	2.60 ± 1.17
Monounsaturates		22:5n-6	1.68 ± 0.37
16:1n-7	4.96 ± 1.61	22:5n-3	0.25 ± 0.11
18:1n-9	7.95 ± 0.51	22:6n-3	20.51 ± 6.07
18:1n-7	1.87 ± 0.49	Total	50.90 ± 0.48
20:1n-9	0.15 ± 0.01		
22:1n-9	0.16 ± 0.02		
24:1n-9	0.37 ± 0.02		
Total	15.46 ± 2.38		
Other			
DHA:EPA	7.9:1		
Total (µg·mg^{-1} DW)	105.3 ± 10.79		

The very high DHA content, presence of the other essential PUFAs, and the small size of the nauplii, indicate *Parvocalanus* has potential as a larval diet component for tropical marine finfish such as groupers.

Assess nauplii acceptance and benefits to fish larvae as a first-feed prey item

Larval survival (Figure 17) was strongly correlated with the initial feed rate of the copepod with survival being significantly greater with inclusion of copepods with rotifers as opposed to a rotifer only diet. The average surviving numbers of larvae in treatments of 0, 4 and 10 copepods/mL addition was 544, 1609 and 2159 respectively. The latter two treatments represent an increase in survival of 171% and 259% respectively over the control (no copepod) treatment.

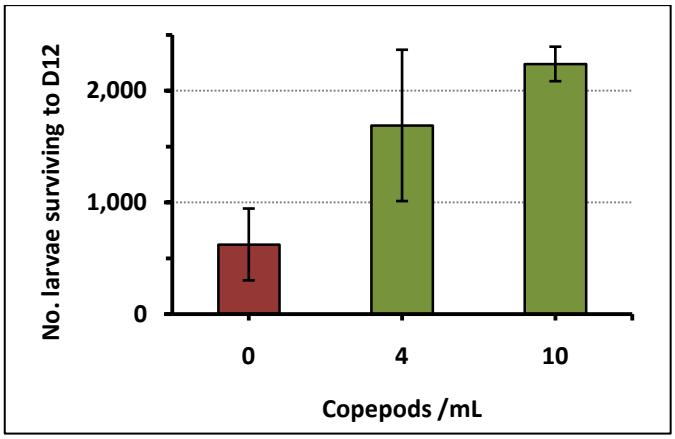


Figure 17 Survival of tiger grouper reared using different initial concentrations of the calanoid copepod *Parvocalanus*.

As well as measuring survival, the growth parameters of total length, body depth, dorsal spine and pelvic spine development were determined (Table 14). Larvae start feeding on Day 3 and by Day 6 significantly greater growth (body length and depth) was measured in larvae that had copepods included in their diet. These larvae continued to develop faster than those receiving rotifers only, even though there were no subsequent additions of copepods to the tanks. By the end of the experiment (Day 12), all treatments were significantly different from each other.

Table 14 Survival and growth data for tiger grouper larvae fed on different initial concentrations of the calanoid copepod *Parvocalanus* to 12 days post-hatch. Figures in brackets represent relative improvements in survival and growth compared with the control.

Copepod density (ind. /mL)	Average survival	Total length (mm)	Body depth (mm)
0	624	4.94	1.24
4	1,689 (171%)	5.69 (15%)	1.32 (15%)
10	2,239 (259%)	6.30 (27%)	1.43 (25%)

In summary, inclusion of the calanoid copepod *Parvocalanus* in the larval diet significantly increased survival and growth of tiger grouper larvae to Day 12 post-hatch.

1.2.3 Extension of Acartia culture techniques

Indonesia

Dr Gede Sumiarsa (RIM Gondol) visited NFC Cairns for training in copepod culture techniques for a two week period in March 2007.

Vietnam

Two staff from Research Institute for Aquaculture No.1, Mr Cao Van Hanh (Cat Ba hatchery) and Mr Le Anh Tuan (Cua Lo hatchery), undertook two weeks training at

Northern Fisheries Centre Cairns in November 2007. The training involved culture techniques for the copepods *Acartia* and *Parvocalanus*, as well as culture techniques for super-small (SS-) rotifers on formulated diets.

India

Under the Asia-Pacific Marine Finfish Aquaculture Network project 2007-01 'Development of sustainable marine finfish aquaculture in the Andaman and Nicobar Islands region of India', training in copepod production was provided to staff of the Rajiv Gandhi Centre for Aquaculture hatchery at Kodiyaghat, Andaman and Nicobar Islands, August – September 2009.

1.3 Improve survival of juvenile groupers in the nursery stage

1.3.1 Nursery environment

Tank shape

Both circular and square tanks demonstrated similar growth and survival rates for tiger grouper (Table 15). Based on this result, it appears that tank shape has no effect on cannibalism in tiger grouper.

Table 15 Growth and survival rate of tiger grouper juveniles reared in circular and square tanks. Similar superscript letters in each column indicate no significant difference (P>0.05).

Treatments	Initial weight (g)	Weight gain (g)	Survival (%)
Circular tank	0.25 ± 0.05[a]	6.33 ± 0.06[a]	24.5 ± 5.00[a]
Square tank	0.26 ± 0.03[a]	6.38 ± 0.10[a]	24.0 ± 2.29[a]

Light intensity

The different light intensities used in this study did not have any significant effect on growth (measured as weight gain and total length) of the juvenile tiger grouper (Table 16). However, fish reared at lower light levels (<600 lux) demonstrated higher survival (72 – 74%) than those reared at higher light levels (Table 16).

Table 16 The influence of light intensity on survival rate, weight gain, and total length of tiger grouper juvenile reared for 30 days. Values in the same column followed by a similar superscript letter are not significantly different (P>0.05).

Light intensity	Survival (%)	Weight gain (g)	Total length (cm)
Ambient (30–3000 Lux)	57.8[a]	9.6[a]	7.5[a]
2000 Lux	62.7[ab]	9.4[a]	7.5[a]
600 Lux	74.3[c]	9.5[a]	7.5[a]
20 Lux	72.1[bc]	9.4[a]	7.4[a]

Water flow rates

The experiment on water flow rates showed that the flow rates used had no significant effect on growth or survival of juvenile tiger grouper.

1.3.2 Feed management

Although survival rate in this experiment was very low, the results indicate that fish that commenced feeding earlier in the day (0700) had higher survival than those that commenced feeding at 0900 or 1100 (Table 17).

Table 17 Effects of different feeding commencement times on growth and survival rate (SR) of juvenile tiger grouper. Values in each column with the same superscript letter are not significantly different (P>0.05).

Treatments (commence feeding time)	Initial weight (g)	Weight gain (g)	SR (%)
0700	0.24 ± 0.05[a]	6.34 ± 0.04[a]	24.5 ± 5.00[b]
0900	0.25 ± 0.03[a]	6.83 ± 0.09[a]	9.2 ± 1.89[a]
1100	0.27 ± 0.06[a]	6.93 ± 0.18[a]	7.2 ± 0.76[a]

1.3.3 Feed development for late larvae / juveniles

The different feed combinations used in the first experiment did not give any significant effect on weight gain and total length but did have an effect on the survival rate of juvenile tiger grouper (Table 18). Addition of attractant to the artificial feed significantly improved the survival rate from 48% to 72% (Table 18). The higher survival was comparable with treatment B (tiny shrimp + minced fish).

Table 18 Effects of different dietary treatments on survival rate (SR), weight gain (WG) and total length (TL) of nursery stage juvenile tiger grouper. Values in the same column followed by the same superscript letter are not significantly different (P>0.05).

Treatment	SR (%)	WG (g)	TL (cm)
A. Artificial feed	48.0[a]	5.9[a]	6.8[a]
B. Tiny shrimp, then minced fish	71.2[b]	6.6[a]	7.3[a]
C. Artificial feed + tiny shrimp, then minced fish	43.6[a]	5.9[a]	7.0[a]
D. Artificial feed with attractant	71.8[b]	5.9[a]	6.9[a]

In the second experiment, the addition of attractants to moist diets had no significant effect on growth or survival of juvenile tiger grouper (Table 19).

Table 19 Effects of attractant addition to moist pellets fed to juvenile tiger grouper. Values with similar superscript letters in each column are not significantly different (P>0/05).

Treatments	Initial weight (g)	Weight gain (g)	SR (%)
Moist pellet with attractant (A)	0.24 ± 0.05[a]	6.02 ± 0.26[a]	26.0 ± 2.08[a]
Moist pellet without attractant (B)	0.25 ± 0.03[a]	6.23 ± 0.36[a]	22.0 ± 9.17[a]

The third experiment showed that juvenile tiger grouper fed with Attractant I had higher survival than those fed Attractant 2, but this was not significantly different from the control group (Table 20). There was no significant difference in growth between treatments (Table 20).

Table 20 Survival and growth of juvenile tiger grouper fed three test diets. Values in the same column with the same superscript letter are not significantly different (P>0.05).

Treatment	Survival (%)	Weight gain (g)	Total length (cm)
Attractant 1	77.2[a]	6.0[a]	6.0[a]
Attractant 2	51.3[b]	5.8[a]	5.8[a]
Control	82.0[a]	6.5[a]	6.9[a]

Objective 2 – Develop cost-effective grow-out diets

2.1 Identify ingredients for grouper diets that will reduce formulation cost

2.1.1 Ingredient digestibility

Experimental results are summarised in Table 21. The study showed that tiger grouper efficiently digest animal feed ingredients, indicating that the ingredients have potential to be used as dietary replacements for fish meal. Some caution is advised for golden snail meal since its overall digestibility was inexplicably poor, perhaps indicating that unknown factors may be affecting its nutritional value. While the plant meals were not easily digested, nevertheless they are an integral part of compounded diets and data on their digestibility are important when formulating diets to satisfy the animal's requirements for digestible nutrients. However, steam conditioning of these meals prior to pelleting or using hot extrusion manufacturing procedures may increase their digestibility for tiger grouper.

Table 21 Dry matter (DM), crude protein (CP), total lipid (TL) and gross energy (GE) apparent digestibility coefficients of the test feed ingredients. Within columns, means without a common letter differ (P<0.05).

Test feed ingredient	DM	CP	TL	GE
	Apparent digestibility coefficient (%)			
Poultry offal meal	65.4[AB]	84.0[A]	84.1[AB]	80.5[A]

Mysid meal	69.9^{A}	88.9^{A}	93.9^{A}	82.8^{A}
Golden snail meal	59.4^{B}	76.9^{A}	62.0^{C}	70.8^{B}
Green mussel meal	66.0^{A}	83.4^{A}	88.9^{A}	78.3^{A}
Rice bran	36.3^{D}	43.7^{B}	65.8^{BC}	39.1^{D}
Maize – yellow	47.8^{C}	41.9^{B}	30.4^{D}	41.3^{CD}
Maize – white	48.8^{C}	41.3^{B}	30.9^{D}	41.7^{CD}
Sorghum	52.5^{C}	52.1^{B}	23.7^{D}	45.1^{C}

The digestibility of a range of ingredients for grouper diets is summarised in Appendix 1 (p.100).

2.1.2 Assessment of non-fishmeal protein sources for grouper diets

Poultry offal silage meal

Four dietary inclusion levels of POSM at 5, 10, 15, and 20% substitution of fish meal were compared with the fishmeal based control diet (0% POSM). Tiger grouper juveniles with three blocks of initial (± SD) size of 46.3 ±5.2, 62.5 ±7.4 and 82.9 ±10.5 g were fed the experimental diets (moist pellet) at satiation twice daily for 20 weeks in experimental cages (1×1×2 m^3).

The addition of POSM at dietary inclusion rates of 20% (equivalent to a 39% replacement of fish meal protein) was well accepted by the fish without any adverse effects on fish productivity. Fish grew well on all diets with biomass gains averaging 303% of initial weight. Partial replacement of fish meal with POSM did not affect the composition of the carcass in our experiment as treatments had no significant effect on body protein or total lipid content. High and relatively similar protein and lipid digestibility coefficients among treatments, 89.4–92.3% and 91.2–95.9% respectively, indicate high digestibility of the protein and lipid of POSM.

Golden snail meal

Tiger grouper juveniles with average individual weight was 27.1±1.38 g were randomly stocked into 15 cages (each cage 1×1×2 m^3) at density of 16 fish/cage. Careful feeding was carried out twice a day to satiation; the fish were sampled every other week.

Based on growth performance of the fish in each treatment, the diets met the minimum nutrient requirement for the test fish. The fish exhibited similar growth patterns, except for the fish feed 40% GSM which grew a bit slower. Specific growth rate, weight gain, and protein retention of the fish feed 40% GSM diet were significantly lower ($P<0.05$) than the fish fed 0–10% GSM diets. A similar pattern was seen in protein efficiency of 40% compared with 0–20% GSM diets. Feed consumption did not differ ($P>0.05$) between treatments.

In this experiment, the diet containing 34.2% fishmeal and 10% GSM meal provided the highest SGR, weight gain, feed efficiency, and protein efficiency ratio amongst the treatments. GSM contains less methionine than fishmeal and less than is found in tiger grouper flesh. Slow growth of the fish fed the diet containing 40% GSM might be due to the low content of methionine in the test diet. In comparison, the better performance of the 10% GSM test diet may reflect a better amino acid balance in the diet due to the inclusion of fishmeal.

Carcass proximate analyses confirmed no difference between protein and lipid content amongst treatments, indicating that GSM inclusion at all the tested levels did not affect carcass protein and lipid.

Fermented blood meal

Tiger grouper juvenile with initial weight of 31.1 ± 2.1 g, were stocked into 15 units of 1×1×2 m floating net cages at a density of 20 fish per cage. The fish were carefully fed the test diet twice each day to satiation for 140 days. Digestibility of the test diet was determined indirectly by reference to the digestibility marker chromium oxide (Cr_2O_3).

Fermented blood meal contained relatively less methionine, cysteine and isoleucine than fishmeal or whole body tiger grouper protein. Blood meal therefore is not a complete source of dietary protein for fish but could be a useful supplementary source of protein if combined with feed ingredients that contain high amounts of methionine and isoleucine. The fish fed moist pellet containing 22.5 % or more of fermented blood meal grew significantly slower ($P<0.05$) than fish fed the control diet. Fish fed the 30% fermented blood meal diet grew 47% slower than fish fed the control diet. Specific growth rate, weight gain, feed efficiency and protein efficiency ratio of fish fed diets containing up to 15% FBM diets were not significantly different ($P>0.5$) and all were higher than those fed 22.5–30.0% FBM diets. However, there was a significant curvilinear decline in overall fish performance with increasing inclusion of FPM, most notably for weight gain, feed efficiency and feed consumption. Based on regression analysis, the asymptote where fish growth deteriorates as a function of FBM inclusion was determined to be 8.9%. Feed consumption was lower for all diets containing FBM. Unpalatability of the diets containing FBM is the most likely reason for the reduced fish growth associated with increasing dietary inclusion of FBM.

Fermented blood meal added to the diet at less than 9% does not adversely affect tiger grouper growth. However, including more than 9% fermented blood meal as a replacement for fish meal protein is likely to depress fish performance, most likely due to the unpalatability of the diet and consequent reduced feed consumption. Depending on relative ingredients costs, it may be economic to include more than 9% fermented blood meal in the diet, with the lower fish productivity being compensated for by the lower feed cost.

2.2 Compare nutritional requirements of juvenile and market-size groupers

2.2.1 Protein and lipid requirements – RIM Gondol

Protein levels ranging from 38% to 54% and lipid levels 9–15% did not have a significant effect on tiger grouper growth or survival (Table 22), suggesting that 38% protein and 9% lipid is adequate to support growth and survival of larger (>250 g) tiger grouper. Fish fed with the 15% lipid diet had higher carcass lipid content than those fed the 9% lipid diet (Figure 18).

Table 22 Weight gain, survival and feed conversion ratio (FCR) of tiger grouper fed experimental diet for 180 days. Initial fish weight = 274.7 g. Weight gain = (final weight – initial weight) x 100/initial weight. Feed conversion ratio (FCR) = dry weight feed (g)/wet weight gain (g). Values in the column followed by the same superscript are not significantly different ($P>0.05$).

Dietary factor	Dietary level (%)	Weight gain (%)[2]	Survival (%)	FCR[3]

Protein	38	127.2[a]	72.7[a]	1.82[a]
	42	122.6[a]	79.4[a]	1.66[ab]
	46	117.8[a]	70.3[a]	1.81[a]
	50	134.4[a]	74.1[a]	1.63[ab]
	54	128.1[a]	76.1[a]	1.48[b]
Lipid	9	131.7[x]	74.3[x]	1.62[a]
	15	120.3[x]	74.7[x]	1.74[a]

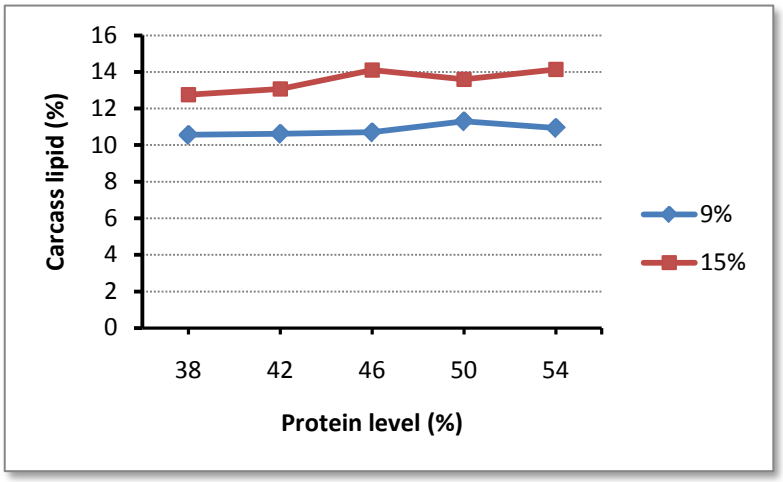


Figure 18 Carcass lipid content of tiger grouper fed test diets with 9% and 15% lipid.

2.2.2 Protein and lipid requirements – RICA Maros

The results of this experiment showed no significant interaction between the levels of protein and lipid in the diet and productivity responses of the fish. However, there were marked but independent effects of dietary protein and lipid with fish weight gain and feed efficiency improving with increasing dietary protein and lipid. Specific growth rate and survival rate however, were not significantly affected while protein efficiency ratio declined with increasing dietary protein but improved with increasing dietary lipid.

Whole body dry matter and lipid composition of the fish were not affected by dietary protein but increased with increasing dietary lipid level. The increased adiposity of the fish increased linearly with dietary lipid, confirming that much of the dietary intake of lipid was used for fat storage rather than being mobilised as a ready available energy source.

Overall in this experiment, the fastest growth and most economical feed efficiency occurred when fish from 120 to 600 g were fed a diet containing 49% protein and 11–13% lipid.

2.2.3 Essential fatty acid requirements

Survival was the same across all treatments. Specific growth rate and weight gain were higher in diets A, B, C and D (Table 23). Feed efficiency (FE), protein efficiency ratio (PER) and nitrogen retention (NRtn) were generally higher in diets A, B, C and D than in diets E and F. The whole body composition of fish was not significantly ($P>0.05$) for all treatments.

Table 23 Growth performance and feed utilization of tiger grouper fed diets containing different levels of essential fatty acids (Table 3, p.21). Means in the same row followed by a common superscript letter are not significantly different (P>0.05).

Variables	Test diets					
	A	B	C	D	E	F
SGR (%/day)	0.72±0.03^{a}	0.72±0.06^{a}	0.71±0.04^{ab}	0.69±0.02^{ab}	0.66±0.01^{b}	0.67±0.01^{b}
WG (%)	124.6±6.5^{a}	125.4±15^{a}	120.6±9.4^{ab}	116.9±4.6^{ab}	110.2±2.6^{b}	110.9±2.5^{b}
FE	0.65±0.06^{a}	0.66±0.07^{a}	0.64±0.06^{ab}	0.62±0.03^{ab}	0.59±0.03^{b}	0.58±0.04^{b}
PER	1.39±0.12^{ab}	1.40±0.15^{a}	1.36±0.13^{abc}	1.33±0.07^{abc}	1.25±0.07^{bc}	1.24±0.09^{c}
NRtn (%)	25.7±1.4^{a}	25.2±1.7^{ab}	24.5±2.0^{ab}	24.6±1.8^{ab}	24.1±1.3^{ab}	23.2±1.7^{b}
Survival (%)	97.9±4.15^{a}	100±0^{a}	100±0^{a}	100±0^{a}	97.9±4.15^{a}	96.2±4.47^{a}

Overall, the results showed a trend that decreasing levels of n-3 lipids or increasing levels of n-6 lipids in the diet slightly reduces the growth performance of the fish. Similar growth performance was obtained with diets ranging from 5% fish oil and 0% soybean oil to 2% fish oil and 3% soybean oil. However, higher levels of soybean oil substitution decreased fish performance.

2.3 Identify ingredients for grouper diets that will reduce environmental impacts

This component of work was not undertaken – refer discussion in Section 5 of this report (p.22).

2.4 Improve the uptake of compounded feeds for marine finfish culture at the expense of 'trash' fish use

2.4.1 Effect of feed type and formulation on growth and feed efficiency of tiger grouper growout

Fish fed with moist pellet-1, commercial pellet and 'trash' fish demonstrated relatively similar responses in terms of specific growth rate, weight gain, feed efficiency, protein efficiency ratio, protein retention and lipid retention (Table 24), whereas the lowest specific growth rate, weight gain, feed efficiency, protein efficiency ratio, protein retention and lipid retention occurred in the fish fed moist pellet-3 followed by moist pellet-2. Survival rate was not significantly different between the treatments (Table 24).

Table 24 Growth performance and feed utilization of tiger grouper fed different test diets. Means in the same row with the same superscript letter are not significantly different (P>0.05).

Variable	**Test diet**				
	Moist pellet-	**Moist pellet-**	**Moist**	**Commercial**	**'Trash' fish**

	1	2	pellet-3	pellet	
Specific growth rate (%/d)	0.68±0.05[a]	0.66±0.07[a]	0.60±0.07[b]	0.67±0.06[a]	0.69±0.06[a]
Weight gain (%)	158.2±19.0[a]	151.5±23.9[a]	132.2±22.6[b]	156.0±20.2[a]	162.2±23.7[a]
Feed conversion ratio (DM feed basis)	1.85±0.11[ab]	2.19±0.20[b]	2.89±0.45[c]	1.83±0.12[ab]	1.66±0.08[a]
Feed conversion ratio (as-fed basis)	3.30±0.20[b]	4.13±0.38[b]	5.90±0.91[c]	1.99±0.13[a]	6.64±0.30[c]
PER	1.14±0.07[a]	0.94±0.09[b]	0.70±0.01[c]	1.11±0.07[a]	1.09±0.05[a]
N retention (%)	21.0±0.5[a]	18.2±2.3[b]	13.7±0.1[c]	21.3±1,0[a]	20.3±0.7[ab]
Survival rate (%)	98.0±3.87[a]	93.3±6.65[a]	80.0±11.55[a]	95.5±3.87[a]	91.1±3.81[a]

The different FCRs shown in Table 24 reflect not only the ability of the fish to effectively utilise the nutrients in the diet, but also the moisture content of the diets. After compensating for moisture content, the FCRs for fish fed 'trash' fish, dry pellet and moist pellet-1 were best, and were all higher than the FCR for the treatment moist pellet-3.

Whole body composition of the fish showed that dry matter, crude protein, lipid, crude fibre and ash contents of test fish were relatively similar amongst the treatments.

A comparison of feed costs based on the test feeds used in this experiment, and taking into account the FCRs obtained in the experiment, showed that the lowest feed cost to produce 1 kg of grouper was for fish fed moist pellet-2 and the commercial pellet; the highest feed cost was for fish fed trash fish (Table 25). However, these calculations are limited in application: calculation of the moist pellet cost was based only on ingredient cost without taking into account the cost of making the pellets; whereas the cost of the commercial pellet includes manufacturing, shipping and other costs. Considering pellet production cost, practical use, feed stability and environmental impacts, it is likely that the commercial pellet was economically as good as other diets trialled in this experiment.

Table 25 Comparison of feed costs based on feed conversion ratios achieved from test diets used in this experiment, calculated to produce 1 kg of tiger grouper.

Variable	Test diet				
	Moist pellet-1	**Moist pellet-2**	**Moist pellet-3**	**Commercial pellet**	**'Trash' fish**
Feed conversion ratio (as-fed basis)	3.30	4.13	5.90	1.99	6.64
Feed price (Rp/kg as feed)	7,175	4,985	3,860	12,000	5,000
Feed cost to produce 1 kg of tiger grouper (Rp/kg fish)	23,678	20,588	22,774	23,880	33,200

2.4.2 Effect of feeding management on productivity and product quality of tiger grouper

There was no significant difference between the main effects of feeding rate and feeding frequency for any of the production traits (Table 26). Significant independent effects were observed in that FE, PER and nitrogen retention (NRtn) became worse as feeding rate or feeding frequency increased. SGR improved as feeding rate increased but was unaffected by feeding frequency.

It is interesting to note that growth rate was highest for fish fed at the highest feeding rate but this feeding rate resulted in the worst nutrient utilisation efficiency. Reducing the amount of feed fed did improve nutrient utilisation efficiency but at the expense of fish growth rate. However, it can be speculated that any further restriction in feeding rate beyond that used in this study would probably cause a worsening of nutrient utilization efficiency as nutrient supply fell to or below maintenance requirements.

Table 26 Initial and final weight and productivity responses of tiger grouper fed at different frequencies and rates for 150 days. Within main effects and response traits, means with a similar superscript letter do not differ (P>0.05). See Table 5 (p.23) for experimental design and treatment abbreviations.

Trait	Feed rate[1]			Feed frequency[2]	
	L	M	H	F1	F2
Survival (%)	72.2^{A}	78.9^{A}	77.8^{A}	78.5^{X}	74.1^{X}
SGR (%/d)	0.89^{A}	0.96^{B}	0.96^{B}	0.93^{X}	0.93^{X}
FE (g gain/g feed)	0.60^{A}	0.55^{AB}	0.47^{B}	0.59^{X}	0.49^{Y}
PER (g gain/g CP feed)	1.19^{A}	1.10^{AB}	0.94^{B}	1.16^{X}	0.98^{Y}
NRtn (%)	19.3^{A}	17.6^{AB}	14.9^{B}	18.8^{X}	15.6^{Y}

Whole body composition of the fish was not significantly affected by either feeding rate or feeding frequency. There was a slight trend for dry matter content to increase and for ash content to decrease as feeding rate increased, while feeding frequency had no effect on final body composition. Based on the results of the present study with fish reared from ~50 to 250 g, it is concluded that tiger grouper need to be fed only once daily for optimal performance. Slightly restricting the feeding rate to the medium rate used in this study had the advantage of improving the efficiency of feed utilisation (and minimizing feed wastage) while not severely reducing growth rate of the fish. This feeding practice may also ensure that a more marketable fish with only a moderate amount of body lipid is produced.

2.4.3 Evaluation of pellet diets for grow-out of coral trout

Weight gain, daily growth rate, and FCR of coral trout fed with the feedmill pellet were slightly higher than those fed with the GRIM pellet (Table 27, Figure 19). This is most likely because the feedmill pellet was prepared using an extruder, which produces a better quality pellet in terms of physical performance and digestibility.

Table 27 Growth performance, feed conversion ratio (FCR), and survival rate of coral trout grouper fed with the test diets. DGR: daily growth rate.

Parameters	GRIM pellet	Feedmill pellet
Initial Weight (g)	162	167
Final Weight (g)	411	422
Weight gain (g)	249	255
Survival rate (%)	46	49
Rearing period (day)	180	180
DGR (g/day)	1.38	1.41
FCR	1.55	1.45

Figure 19 shows the growth performance of the coral trout fed with the two test diets. While the growth performance of the fish fed the feedmill pellet is slightly better than those fed the GRIM pellet, there is little practical difference between the two treatments. This suggests that dry pellets generally are suitable and well accepted by coral trout for grow-out.

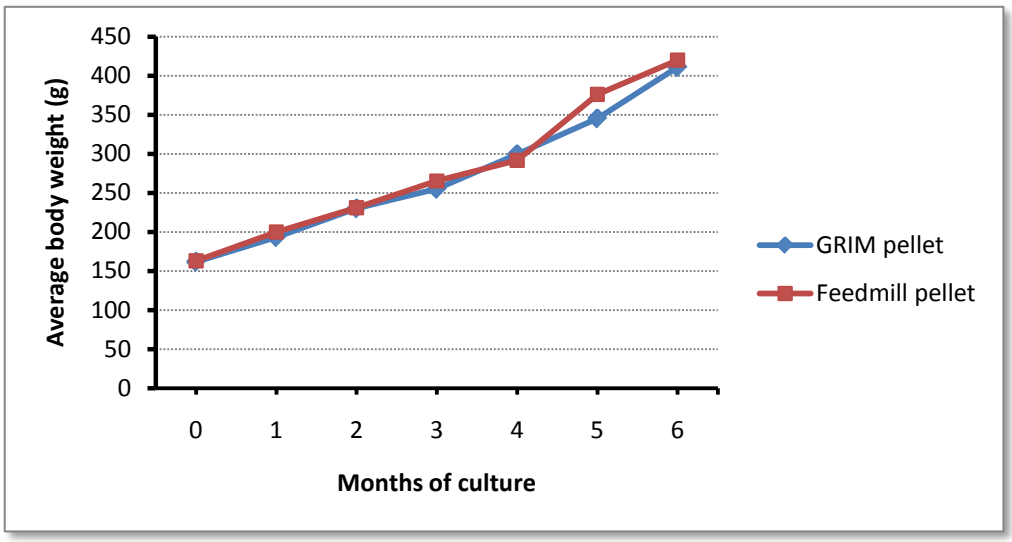


Figure 19 Growth of coral trout fed an experimental pellet diet (GRIM pellet) and a commercially produced pellet diet.

2.4.4 Comparison of commercial pellet diet and 'trash' fish for grow-out of groupers

Figure 20 shows that growth of both coral trout and white-spotted grouper was faster on the 'trash' fish diet. Daily growth rate of both species was higher, and final size was larger, in fish fed the 'trash' fish diet (Table 28). Survival rates were similar between treatments (Table 28).

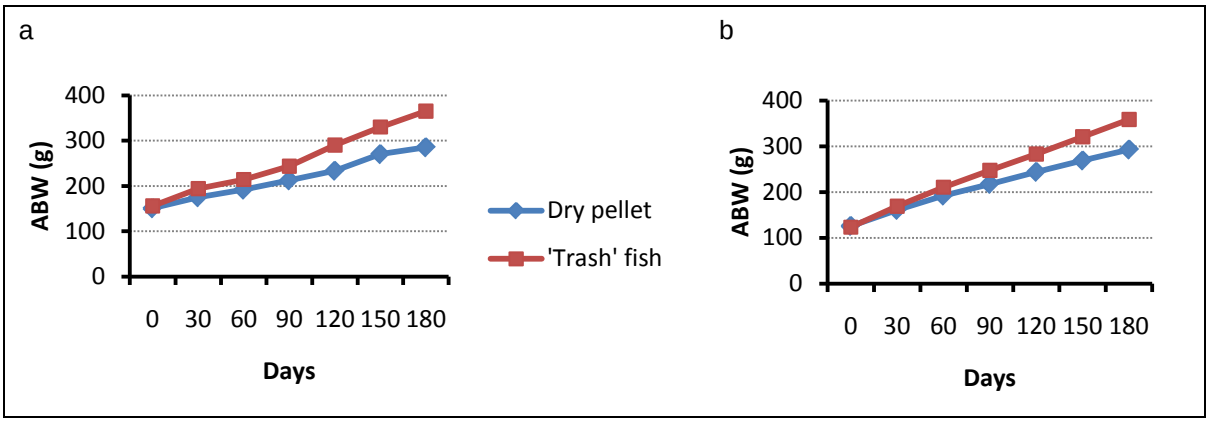


Figure 20 Growth of (a) coral trout and (b) white-spotted grouper fed dry pellet and 'trash' fish diets.

Table 28 Weight, daily growth rate (DGR), food conversion ratio (FCR), and survival rate (SR) of coral trout and white-spotted grouper fed with test diets for 180 days. Pellet diets FCRs are DM basis; 'trash' fish FCRs are wet-basis.

Parameter	Coral trout		White-spotted grouper	
	Dry pellet	'Trash' fish	Dry pellet	'Trash' fish
Initial weight (g)	217 ± 15	202 ± 2	123 ± 19	114 ± 27
Final weight (g)	391 ± 40	554 ± 69	278 ± 5	316 ± 24
DGR (g/day)	0.96 ± 0.14	2.11 ± 0.20	0.80 ± 0.08	1.04 ± 0.05
FCR ('as fed' basis)	2.2± 0.1*[1]	5.8 ± 1.2*[2]	1.9 ± 0.1	4.5 ± 0.3
FCR (DM basis)	–	1.8 ± 0.3	–	1.5 ± 0.2
SR (%)	72 ± 10	75 ± 9	82 ± 22	96 ± 7

However, a comparison of the contribution of the two feeds to production costs demonstrates that the 'trash' fish was much more expensive than pellet feed (Table 29).

Table 29 Cost of pellet diet and 'trash' fish feed used in Activity 2.4.4. and the feeds' relative contribution to production costs. Feed preparation costs for white-spotted grouper are higher than those for coral trout because more feed was required to produce white-spotted grouper.

Parameter	Coral trout		White-spotted grouper	
	Dry pellet	'Trash' fish	Dry pellet	'Trash' fish
Total feed (kg)	19,415	177,388	34,670	187,167
Feed price (IDR)	14,000	6,000	14,000	6,000
Feed cost preparation (IDR)	0	88,694	0	93,583
Total feed cost (IDR)	271,810	1,153,019	485,380	1,216,583
Production cost per kg fish (IDR)	59,909	79,365	30,582	53,192

2.4.5 Field validation – comparison of feed types

After 140 days, all treatments had similar specific growth rate (0.56–0.57 %/d), weight gain (118.5–120.9%), protein efficiency ratio (0.82–1.16) and survival rate (94.0–98.0%) (Table 30).

Although the growth performance was almost identical for all treatments, the feed conversion ratio (as fed basis) was better for fish fed commercial pellet (2.62) followed by fish fed moist pellet (4.63) and worst for fish fed 'trash' fish (6.26). However, on a dry matter basis, the fish fed 'trash' fish had a better feed conversion ratio (1.57) compared to the fish fed commercial pellet (2.41) and moist pellet (2.59) diets (Table 30).

An evaluation of the feed costs based on cost of ingredients and FCR indicated that all three test feeds were relatively similar in cost (Table 30). However, the 'costs' included in these calculations differ between the diets: calculation of the cost of the moist pellet diet was based only on the combined cost of each of the ingredients used to make the diet (i.e. no manufacturing cost was included) whereas the cost of the commercial pellet includes the cost of ingredients, manufacturing, shipping and the need for the manufacturer to make a profit. A more rigorous assessment of feed costs is likely to show that the commercial diet provides a lower grouper production cost than the other diets.

Table 30 Feed costs based on feed conversion ration and estimated cost of feed, calculated to produce 1 kg of tiger grouper.

Variable	Test diet		
	Moist pellet	**Commercial pellet**	**Trash fish**
Feed conversion ratio (as-fed basis)	4.63	2.62	6.26
Feed conversion ratio (DM basis)	2.59	2.41	1.57
Feed price (Rp/kg as feed)	7,175	12,000	5,000
Feed cost to produce 1 kg of tiger grouper (Rp/kg fish)	33,220	31,440	31,300

Generally, tiger grouper that are not weaned to dry pellet feed in the hatchery adapt very slowly to dry pellets. This and the previous experiment demonstrated that tiger grouper that are not yet weaned to dry pellets can more easily be weaned from 'trash' fish to dry pellets by using moist pellets as an intermediate diet.

Overall, this component of research has provided more information on the various factors that impact on farmer adoption of pellet feeds. In some cases, pellet feeds do not perform as well as 'trash' fish in terms of fish growth. While pellets can be more cost-effective, the 'true' costs of 'trash' fish may be obscured; for example, the cost of preparation of 'trash' fish (removing heads, fins and intestines) is rarely calculated by farmers. As part of this project, attempts were made to improve pellet performance by holding a nutrition workshop with Indonesian feed companies; this is described in the report section on communication and dissemination activities (p.88).

2.5 Identify the impacts of feeds on product quality

Tiger grouper

As a follow up to the experiment described in Activity 2.4.1 (p.22, p.64), RICA Maros sent samples of tiger grouper fed on five test diets (see Table 4, p.22 for test diet composition) to Hong Kong for consumer evaluation. There were no differences in carcass body composition between treatments. Overall, panellists preferred fish fed moist pellet-2 (Table 31) and felt that this product had better meat quality than grouper from Taiwan and Thailand. However, the meat quality of fish fed most pellet-1, commercial pellets and 'trash' fish were generally well accepted by the panellists (Table 31).

Table 31 Flesh quality attributes for tiger grouper fed five test diets and evaluated in Hong Kong.

Variables	Test diets				
	Moist pellet-1	**Moist pellet-2**	**Moist pellet-3**	**Commercial pellet**	**'Trash' fish**
Odour	• fresh • seaweedy	• strong • fresh • seaweedy	• fishy	• light fresh • seaweedy	• fishy
Flavour	• light fresh • sweet	• strong fresh • sweet	• loss of flavour	• some loss of sweetness	• loss of flavour
Texture	• slightly dry	• very moist and smooth	• very dry and tough	• moist and smooth	• dry and tough

Humpback grouper

Of the 30 consumers who participated in the taste test, just over 50% (16) were able to correctly identify the odd sample on the plates. However, only 11 were able to correctly determine which bowl was the wild-caught sample and which was the aquaculture sample. The wild fish samples were described as having better freshness, smoothness, and stronger in flavour and 'mouthfeel' texture. Skin of wild fish samples was more elastic and not so tough. On the other hand, cultured fish samples were described as more 'fishy' in taste and the flesh as 'less smooth'. The skin was tougher, darker and thinner. The texture of flesh was somewhat flaky, rather tough and the flavour was not as strong as the wild fish samples.

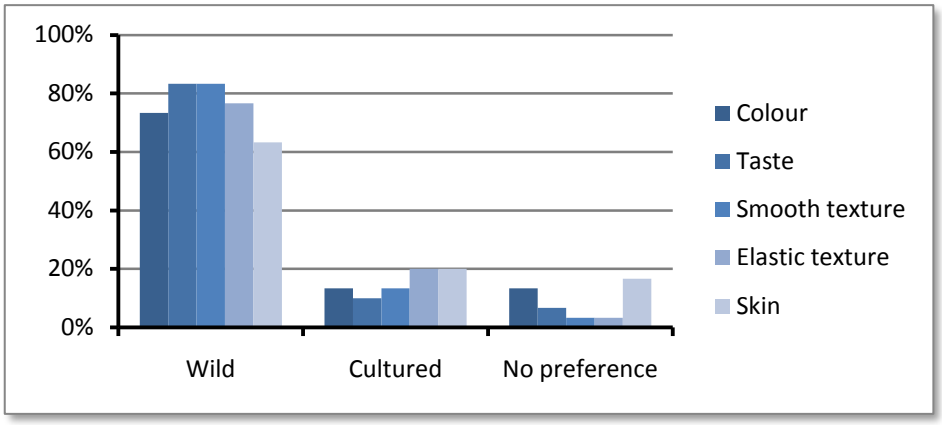


Figure 21 Overall preference for wild or cultured humpback grouper, and 'no preference' responses, for five taste attributes amongst Hong Kong consumers; from Chan (2007).

There was a definite preference for the colour, taste and texture of the wild-caught product among the participants with over 70% preferring the wild-caught product on these criteria (Figure 21). However, the aquaculture product was also found to be highly acceptable to the consumers. This result may have been confounded by the differential size of the two sample groups (the wild-caught fish being larger and older, which is known to influence flesh texture). This bodes well for the widespread commercial acceptance of the aquaculture product in the future. However, further research into the effects of pellet diets on consumer acceptance is essential to better evaluate the impacts of different diets on market attributes.

A more detailed description of this research has been published by Chan (2007).

Objective 3 Facilitate technology adoption

This objective was addressed by initially undertaking a survey of marine fish hatcheries and farms in Indonesia, Thailand and Vietnam (Activity 3.1). The results of this survey were then used to design some specific interventions to test opportunities to overcome the constraints identified in the survey (Activity 3.2).

3.1 Identify constraints to the uptake of grouper hatchery and grow-out technology in the Asia-Pacific region

Constraints to technology uptake applicable to this project were identified from several sources including Sim (2006) and field visits under ACIAR project funding from 2002 to 2007.

3.1 Identify constraints to technology uptake

3.1.1 Hatchery technology

The backyard grouper hatchery technology development facilitated through ACIAR projects has been widely adopted in Indonesia. In 1999, only five hatcheries in Indonesia produced grouper fingerlings and by 2004 the number had increased to 147. Hatcheries are no longer confined to Bali but have spread across to Lampung, East Java, and new investments in hatchery production are also in development in Riau. The technology was first successful in producing *Cromileptes altivelis* in 1998, followed by *Epinephelus fuscoguttatus* in 2001. Since then this technology has been applied to other marine finfish

including grouper species such as *E. coioides*, *E. polyphekadion*, *E. coeruleopunctatus* , *Plectropomus leopardus*, and the golden trevally, *Gnathanodon speciosus*.

The simplicity, flexibility and economic viability of the hatchery technology has facilitated its uptake and spread within Indonesia. Increasingly, the technology has also been applied in other countries, in part through the Asia-Pacific Marine Finfish Aquaculture Network grouper hatchery production training course conducted annually in Indonesia since 2002. A total of 101 participants from 22 countries have been trained since the course began. The technology is simple and not mechanically complicated so a technician or owner who runs a hatchery does not necessarily need to be highly educated or technically skilled. The flexibility of the technology also enables the hatchery systems to switch between fish species or between fish and shrimp. The low investment and operating costs required enable fishermen or other small players with limited finances to participate in the hatchery business either in the form of employment or by running their own.

Economic analysis carried out by Sim (2006) has found that grouper hatchery production is a lucrative business even at low survival rates (i.e. 3% from eggs to fingerlings) if costs and the sale price remains stable at US$0.10 per fingerling (2-3 cm). The operational costs are similar for all grouper species except for *C. altivelis*, where the cost of producing fertilized eggs is higher. This has been confirmed in a separate study (Siar *et al.* 2002), which also found small scale hatcheries operating in Indonesia to be profitable.

3.1.2 Constraints and limitations of hatchery technology uptake

The further expansion of the grouper fingerling hatchery industry is presently inhibited by several constraints:

Availability of good quality fertilized eggs

Supplies of good quality fertilized eggs are only available from government centres and a limited number of large-scale hatcheries. As the cost of maintaining broodstock is very high so many small- and medium-scale hatcheries cannot afford to keep their own broodstock. Therefore, small- and medium-scale hatcheries have limited opportunity to access good quality eggs and have to take what is available to them. This in turn reduces hatchery production and economic returns. Poor disease control by eggs suppliers also increases the risks for small-scale hatchery operators and subsequently, for farmers.

Infrastructure

Good sites for hatchery operation are normally located in rural/remote areas where the marine water is clean and unpolluted, but such areas typically have poor supporting infrastructure such as roads, power and freshwater supplies, which limit hatchery development in these areas. Accessibility to fertilized eggs is more difficult and technical support from government centres is also limited.

Price and markets

The spawning pattern of most grouper species follows the lunar cycle and therefore fingerling production tends to occur at the same time. This causes a sudden “flood” in the supply of fingerlings, thus creating more competition and driving the price lower. On the other hand, when there is a shortage of fertilized eggs due to cold weather or poor broodstock condition, the fingerling price will increase due to limited supply. The combination of success in the development of hatchery technology, the spawning pattern of grouper species and the limited supplier of fertilized eggs has created a problem in price and markets for hatchery operators.

Many hatchery operators, particularly small- and medium-scale hatcheries in Indonesia have indicated that lack of access to overseas markets is a significant constraint for them. This issue needs to be carefully examined. Direct sale to overseas markets may provide

higher profit margins but the risks associated with export are also higher, particularly with regard to product and financial risks, as well as the risk of disease transfer between countries.

Price orientation

Most small scale hatchery operators are price oriented. They tend to focus on high priced species rather than working on reduction of their production costs. For example, they will choose to produce higher priced grouper species such as humpback grouper or tiger grouper rather than the orange-spotted grouper, which normally fetches a lower market price (both fingerlings and table fish).

Small scale hatcheries also tend to switch to other species that offer them better profit margin and shorter culture period. Many small scale hatcheries in Bali and Situbondo, for example, produce milkfish fry or shrimp post larvae (PL) if the prices for both species are better. These production patterns create problems in consistency of supply thus leading to grouper fingerling supply problems for grow-out farmers.

3.2.1 Artificial feed uptake

Studies conducted in Indonesia, Thailand and Vietnam have shown that the uptake of artificial feed usage in grouper farming is still limited.

In Indonesia, a total of 34 grow out farms (cages and pens) were surveyed through various field visits. Some farmers have adopted artificial feed at the nursery stage, however at grow-out stage the feeds have not delivered good results. Therefore, the usage of artificial feed in grouper farming particularly at grow-out stage is limited. Farmers still believe that feeding trash fish during grow-out provides a faster growth rate at a lower cost. It is estimated that less than 20% of the farmers surveyed are using artificial feed during the grow-out stage.

In Thailand and Vietnam, a total of 33 and 42 grouper farms, respectively were surveyed. No artificial feeds were observed in use in either country.

3.2.2 Constraints and limitations of the adoption of artificial feeds

The studies and surveyed conducted in Indonesia, Thailand and Vietnam have indicated the following constraints and limitations to the adoption of artificial feeds for grouper farmers.

Price

Economic analysis by Sim (2006) has shown that trash fish is still more economically viable for farmers. The average FCR for artificial feed is about 2.64 at a feed cost of around US$1.00/kg. The trash fish price level during the study period averaged around US$0.20/kg. The equilibrium level for trash fish to the artificial feed is FCR at 13.2. Therefore if farmers can maintain trash fish FCR below 13:1, feed cost savings can be achieved. Although the saving is fairly small it is a significant amount for small-scale farmers.

Most farmers are also price oriented, preferring to use feeds that are cheaper and provide good results, as opposed to focusing on cost reduction through good feed and feeding management to reduce wastage.

Accessibility

Artificial feeds are not easily available to small scale farmers particularly those that are living in rural or remote locations, which is the case for many small scale farmers in Asia.

Although some of these farmers may have access to artificial feeds they are normally at a much higher price due to transportation costs and small quantity per order.

In addition, trash fish may be the most viable option for these farmers and it is also associated with their traditional practices and culture because these farmers are also fishers. Traditional farmers may lack the cash or access to credit to invest in purchase of artificial feeds.

Performance

Based on the farms surveyed and field visit in various locations, farmers have indicated that the growth rate from feeding trash fish is better that that obtained with artificial feeds. Some farmers have reported good outcomes with artificial feeds at nursery stage (approximately three month) but no farmers surveyed reported good results with artificial feeds during grow-out stage, in addition to the price differential with trash fish. Farmers perceive that the quality of artificial feeds may not be adequate to meet the nutritional requirements of groupers. Many farmers are therefore reluctant to use artificial feeds in grow-out and continue to use trash fish. During the survey period, some of the commercial feeds observed in use in the grouper farms were 'generic' feeds that were claimed to be suitable for all marine species. However, different species of marine finfish have different nutritional requirements, for example seabass and grouper require different level of fat content, and the use of generic feeds may have contributed to the poor performance of artificial feeds as reported by farmers.

Some farmers have indicated that they are willing to switch to artificial feeds if the feed can provide them good results at a competitive cost.

Buyer requirements

Discussions with farmers during the surveys and field visits to various locations also provided insight into some of the problems associated with pressure from buyers. Some buyers specifically requested farmers to feed their grouper with 'trash' fish for at least two weeks prior to shipment.

Farmers were told by exporters that the grouper that are fed with trash fish are more tolerant to handling and transport over long distances. The exporters claimed that grouper that are fed artificial feeds tend to be more easily stressed leading to high mortality and losses during long journey transportation, particularly by boat.

Discussion and recommendations

Hatchery technology uptake

Hatchery technology is very well adopted by small-scale operators in Indonesia so the constraints are more associated with access to good quality eggs, infrastructure and markets. These constraints are not directly associated with technology issues so they are difficult to address in the R&D component. The possible strategies to tackle these limitations and constraints are subject to local government policy and hatchery operators or owners attitudes toward maintaining stable supplies at cost effective level.

To increase the availability and distribution of good quality eggs the establishment of national broodstock and egg production centres should be considered to cater for increasing number of hatcheries established in the region.

Educating small scale hatchery operators on the need to maintain consistent supplies and to adopt better management practices to improve survival rate and quality of seed production would be beneficial. This would be a better way to improve the cost effectiveness of hatchery operations than trying to cut corners at the expense of producing high quality seed with better survival rates. The move to industrialize marine finfish hatchery technology can probably help to improve this situation. However, shifting the

focus and attitudes of hatchery operators away from their present low cost and low input operations may take years to achieve, if not prove impossible.

Feeds uptake

As noted above, primary analysis done by Sim (2006) has shown that trash fish is more economical for small-scale farmers. Many R&D trials at the experimental level continue to indicate that the FCR for artificial feed for grouper farming is less than 2. However, discussions with farmers continue to indicate that FCR for commercial feeds under field conditions is greater than 2 and in some cases as high as 3. Therefore it is apparent that there are some gaps between the R&D trials and the farm level performance of the artificial feeds. Whether these gaps are due to problems associated with the honesty of the commercial feed producers, poor feeding and feed management by farmers, or whether more R&D is needed to improve artificial feeds is not known.

Farm level or extension level trials should be conducted for commercial feeds in order to verify and resolve the perceived gap between the R&D outputs and farm level performance. If trials can be proven to be in line with the R&D outputs then the results can be demonstrated to the small scale farmers and/or farm management practices may need to be improved. The trials should focus on the feed and feeding management and measure the biological and economic performance of the commercial feeds.

The possibility of the three groups (farmers, commercial feed companies and R&D centres) working together for such an experiment and research should be explored. However, to conduct such an experiment will require a long period of delegated processes including grow out period for grouper species ranging from 8 to 18 months. Identification of suitable sites and farms is vital and firm commitments from farmers to ensure proper feed and feeding management is mandatory for such an experiment.

3.2 Where possible, develop responses to overcome identified constraints

As noted in Section 5 of this report (p.10), two specific responses were developed to attempt to address some of the constraints identified in the survey described in Objective 3.1. The first was a workshop on Better Management Practices for Marine Finfish Aquaculture in the Asia-Pacific Region; the second was a nutrition workshop targeting commercial feed companies in Indonesia.

Marine finfish BMPs workshop

NACA and the Directorate General of Aquaculture (DGA) of Indonesia in conjunction with ACIAR through project FIS/2002/077 held a workshop on the 'Development of Better Management Practices for Marine Finfish Aquaculture in the Asia-Pacific region', in Lampung, Indonesia, 7–10 November 2007. The workshop was held to begin the process of developing Better Management Practices (BMPs) for the marine finfish aquaculture sector, which is growing rapidly in Asia. The rapid expansion of marine finfish aquaculture, and concerns regarding its environmental sustainability, has led to the development of several accreditation / certification schemes, and the proposed development of others. NACA and ACIAR are concerned that many current schemes effectively disadvantage small-scale farmers, who provide the bulk of production in Asia. The development of a BMPs-based approach is intended to allow small-scale farmers to adopt practices that will better support their participation in more formal accreditation / certification schemes in the future, and facilitate market access by small-scale farmers in the face of increasing consumer demands for environmental and social responsibility in aquaculture.

The four-day workshop was attended by 60 participants from Australia, Cambodia, China, France, India, Indonesia, Myanmar, Norway, Philippines, Thailand and Vietnam. The workshop was strongly supported by the private sector, with about half the participants coming from private industry, including representatives of farmer organizations and feed companies. Industry participants were supportive of the need to develop BMPs for marine finfish aquaculture as a way to enhance the sustainability of their industry. The workshop was also attended by representatives of environmental and other NGOs.

Workshop outputs included a review of the current status of marine finfish aquaculture in the Asia-Pacific region, including a description of the marine finfish aquaculture sector and the constraints that were identified in the various country presentations. The workshop, through four working groups, drafted implementation guidelines for the development of BMPs for marine finfish aquaculture, based on the 'International Principles for Responsible Shrimp Farming' structure. The development of marine finfish aquaculture BMPs will continue to be facilitated by NACA through an open and transparent process to ensure that all stakeholders have input to the development of the BMPs.

Nutrition workshop

A three-day workshop on fish nutrition was held at the Santika Hotel, Surabaya, 20–22 October 2009, as an activity under FIS/2002/077. Indonesian feed companies were invited to send technical representatives, particularly feed formulators, to the workshop to update their knowledge of fish nutrition and to hear the results from research carried out under FIS/2002/077 as well as other research undertaken by the Indonesian Agency for Marine Affairs and Fisheries Research. Technical training in fish nutrition was provided by Mr Igor Pirozzi (NSW Department of Primary Industries) and Mr Simon Tabrett (CSIRO).

Between 16 and 9 participants attended the workshop with numbers declining over the three days of the workshop. Feedback on the workshop was generally positive. Several participants requested more practical activities in the workshop; however, it is difficult to see how these can be integrated into a workshop intended for commercial feed companies.

With regard to marine finfish aquaculture, the point was made that this is a relatively small sector in Indonesia. The feed companies are focussed on the larger markets, for freshwater finfish and shrimp, rather than marine finfish. Because of the relatively small demand for marine finfish feeds, the use of 'trash' fish is not regarded by the feed companies as a critical issue. While the marine finfish sector remains relatively small (compared with freshwater fish and shrimp), there appears to be relatively little interest in developing marine finfish feeds to replace 'trash' fish.

3.3 Disseminate research outputs widely in the Asia-Pacific region

A total of 38 editions of the eNewsletter were produced. As noted above, news items are now incorporated in the NACA web site.

Reporting year	APMFAN eMagazine	APMFAN eNewsletter
2004–05	Four issues (Nos. 1–4)	26 issues published to 30 June 2005
2005–06	Four issues (Nos. 5–8)	Five issues (Nos. 27 – 31)
2006–07	Three issues (Nos. 9-11)	Seven issues (Nos. 32-38)
2007–08	Three issues (Nos. 9–11)	Seven issues (Nos. 32–38)

Extension publications

Two practical guides on hatchery technology and feeds were produced:

> Sim, S.Y., Rimmer, M.A., Toledo, J.D., Sugama, K., Rumengan, I., Williams, K. and Phillips, M.J. (2005). *A Guide to Small-Scale Marine Finfish Hatchery Technology.* Network of Aquaculture Centres in Asia-Pacific, Bangkok, Thailand. 17 pp.
>
> Sim, S.Y., Rimmer, M.A., Williams, K., Toledo, J.D., Sugama, K., Rumengan, I. and Phillips, M.J. (2005). *A Practical Guide to Feeds and Feed Management for Cultured Groupers*. Network of Aquaculture Centres in Asia-Pacific, Bangkok, Thailand. 18 pp.

Both guides were translated into Thai by Thai Department of Fisheries staff, into Indonesian by Ministry of Marine Affairs and Fisheries Indonesia staff, and one (hatchery guide) into Vietnamese through the DANIDA-funded Support for Marine and brackishwater Aquaculture (SUMA) project.

Grouper aquaculture monograph publication

The results of the predecessor project (FIS/97/73 'Improved hatchery and grow-out technology for grouper aquaculture in the Asia-Pacific region') were published as an ACIAR monograph:

> Rimmer, M.A., McBride, S. and Williams, K.C. (2004). *Advances in Grouper Aquaculture*. ACIAR Monograph 110. Australian Centre for International Agricultural Research, Canberra. 137 pp.

The monograph was made available in both hard copy and electronic (.pdf) versions, the latter on the ACIAR web site (www.aciar.gov.au). Both hard copy and electronic versions have proven very popular. Data on distribution of the hard copy and electronic versions of the monograph and the guides are listed in Table 32.

Table 32 Data on distribution of the ACIAR monograph *Advances in Grouper Aquaculture*.

Publication	Hard copies distributed (NACA only)	Electronic version downloads (via NACA web site only)
ACIAR Monograph 110 Advances in Grouper Aquaculture	1,750	534 (forwarded to ACIAR website for download)
A Guide to Small-Scale Marine Finfish Hatchery Technology	>200	2,285
A Practical Guide to Feeds and Feed Management for Cultured Groupers	>200	1,560

Symposia and workshops

In conjunction with the annual project meeting held in Makassar, South Sulawesi, Indonesia, the project team organised a public Symposium on Marine Finfish Aquaculture, which was held at the Quality Hotel, Makassar, on 27 July 2008. The objective of the symposium was to present the outcomes of our research to researchers from government and universities, to Dinas Kelautan dan Perikanan and to the private sector.

A total of 61 participants (including presenters) attended the symposium, including representatives from:

- Private sector, including feed companies;
- Balai Budidaya Air Payau Takalar;
- Dinas Kelautan dan Perikanan;
- Universities (UNHAS, UMI);
- Mars Symbioscience and Mitra Bahari (Sea Partnership).

Overall, the meeting was successful in presenting the results of FIS/2002/077 to a broader audience. The meeting was also successful in promoting interaction between different agencies, including National and Provincial agencies, local universities and private sector.

3.4 Promote the expansion of sustainable marine finfish aquaculture through 'hands-on' training

Hatchery training

The Regional Grouper Hatchery Production Training Course originally started at RIM Gondol in 2002 under ACIAR project FIS/97/73, and has been held annually since 2005 at the Brackishwater Aquaculture Development Centre (BADC) Situbondo. The training course has been very well supported by the Directorate-General of Aquaculture (DGA), Ministry of Marine Affairs and Fisheries, Indonesia. The objective of the training course is to train government and private-sector representatives from throughout the region in grouper hatchery production technology, particularly incorporating research outcomes from FIS/2002/077. Table 33 summarises the number of participants and the countries represented in each training course.

Table 33 Participation in the annual grouper hatchery production training course held at Brackishwater Aquaculture Development Centre Situbondo, Indonesia. The 2006 and 2008 courses each included two participants from BADC Ujung Batee, funded under ACIAR project FIS/2006/002 'Aceh Aquaculture Rehabilitation Project'.

Dates	Participants	Countries
18 April – 8 May 2005	17	8: Australia, Brunei Darussalam, Indonesia, Malaysia, Maldives, Marshall Islands, Singapore, Vietnam
20 November – 9 December 2006	20	12: Australia, China – Hong Kong SAR, India, Indonesia*, Malaysia, Maldives, Myanmar, Qatar, Saudi Arabia, Singapore, Thailand, Vietnam
9 – 29 July 2007	16	8: Australia, China – Hong Kong SAR, Palau, Philippines, Singapore, Sri Lanka, Thailand, Vietnam
5 – 25 May 2008	19	10: Australia, China – Hong Kong SAR, Indonesia*, India, Iran, Malaysia, Oman, Thailand, Trinidad and Tobago, Vietnam

The 2008 course provided a significant milestone with over 100 graduates now having completed hatchery training through the RIM Gondol and BADC Situbondo courses.

APMFAN also organised a marine finfish hatchery training course for the Secretariat for the Pacific Community (SPC) for a group of six trainees from Pacific Islands Countries (Fiji, French Polynesia, New Caledonia, Papua New Guinea) in May 2007 at Krabi Coastal Fisheries Research and Development Centre (Krabi CFRDC) of DOF Thailand.

Farmer training

Five farmers and staff from Yayasan Palu Hijau (Central Sulawesi) undertook a short course on grouper feed management at RICA Maros,19–23 September 2005. In May 2009, the YPH representative (Ms Abigail Moore) indicated that efforts to have local Dinas Kelautan dan Perikanan implement this training had not been successful. However, the material has been incorporated in the local university curriculum and Ms Moore is confident that this will provide a long-term impact because Dinas recruits from local graduates.

RICA Maros provided short-term training for farmers from Sengata Regency (Kalimantan Timur Province) on feed preparation and feeding management.

Five farmers from Mappi Regency, Papua Province, undertook 5 days training in grouper grow-out in floating net cages at RICA Maros.

Usman and Neltje Palinggi (Research Institute for Coastal Aquaculture, Maros, South Sulawesi) provided training in farm-made feeds for marine and brackishwater finfish aquaculture in Aceh, 20–24 January 2009, in collaboration with the Aceh Aquaculture Rehabilitation Project (FIS/2006/002). Training was undertaken at four sites: BBAP Ujung Batee; Pidie district (Desa Pusong); Bireuen district (Samalanga BPP office); and Aceh Utara district (AARP BMPs demonstration ponds at Syamtalira Bayu). A total of 12 BBAP Ujung Batee staff, 56 farmers, 14 Dinas Kelautan dan Perikanan staff, 2 SUPM Ladong staff, and 4 FAO ARC project staff participated in this training:

RIM Gondol provided training in grouper hatchery and nursery culture to five staff from Balai Benih Ikan Pantai (BBIP) Nias, North Sumatra, and two staff from BBIP Simeulue, Nanggroe Aceh Darussalam in June – July 2008 and October – November 2008. This training was supported by the Asian Development Bank's Earthquake and Tsunami Emergency Support Project.

NFC Cairns provided on-farm training to two farmers on culture techniques for grouper with particular reference to methods of cage culture and fish grading. Also, training was provided to a barramundi hatchery operator on the culture of tiger grouper. This training provided the first fingerlings of tiger grouper produced by a commercial operator in Australia.

3.5 Strengthen and expand the research coordination and regional collaboration activities of APMFAN

The Regional Resource Centres / Regional Resource Experts approach is based on that used by NACA for its Fish Health Program. However, replication of this approach for APMFAN has been largely unsuccessful. Many countries (e.g. Australia) simply ignored the call for nominations of RRCs / RREs. Most RREs are not active in supporting the network.

Regional Resource Centres (RRCs)

At the 18th NACA Governing Council Meeting (GC 18) in Bali, 2007, four institutes were approved as Regional Resource Centres for the Asia-Pacific Marine Finfish Aquaculture Network:

1. Research Centre for Mariculture, Gondol, Indonesia.
2. National Brackishwater Aquaculture Development Centre, Situbondo, Indonesia.
3. Mariculture Development Centre, Batam, Indonesia.
4. Rajiv Gandhi Centre for Aquaculture, India.

In response to the recommendations of GC 18, NACA wrote to member governments requesting them to nominate additional RRCs and Regional Resource Experts (RREs) for the network. An additional seven RRCs were endorsed by the GC 19 in 2008:

5. Main Centre for Mariculture Development, Lampung, Indonesia.
6. Main Centre for Brackishwater Aquaculture Development Centre, Jepara, Indonesia.
7. Mariculture Development Centre, Lombok, Indonesia.
8. Central Marine Fisheries Research Institute, Kochi, India.
9. South Iran Aquaculture Research Centre, Iran.
10. National Integrated Fisheries Technology Development Centre, Philippines.
11. Krabi Coastal Fisheries Research and Development Centre, Thailand.

Regional Resource Experts (RREs)

Member countries of NACA were requested to nominate RREs who were internationally accepted as experts in grouper / marine finfish aquaculture, and who would actively contribute to the network. Nominations were formally accepted / rejected by the NACA Governing Council. Terms of reference for consideration / acceptance as an RRE are:

- Available to answer technical questions related to their field of expertise.
- Provide a contact point for national / provincial interest in the development of sustainable marine finfish aquaculture.
- Assist network in development of extension materials and related documents
- Support distribution of extension materials locally.
- Provide an annual summary of developments in their field of expertise.
- Assist the development of NACA's annual work program in regard to marine finfish aquaculture activities.

Currently, there are formally accepted RREs from India (7), Indonesia (6), Iran (6), Hong Kong SAR (1), Malaysia (10), Philippines (1).

Development of institutional profiles

Institutional profiles have been developed for six Indonesian DGA centres (MCMD Lampung, MDC Batam, BADC Situbondo, MDC Lombok, MDC Ambon and BADC Takalar) based on data gathered during visits by Dr Sih Yang Sim in 2006 and 2007. These profiles are available on the NACA web site (www.enaca.org).

8 Impacts

8.1 Scientific impacts – now and in 5 years

8.1.1 Larval rearing

The scientific impacts arising from this project are considerable, and make an important contribution to our overall knowledge of hatchery production technology for marine finfish.

Larval nutrition

The project has evaluated the nutritional requirements for *E. fuscoguttatus*, which follows on from research in FIS/97/73 that defined the nutritional requirements for *E. coioides*. The enhancement of live prey organisms used for grouper larval rearing improves survival and growth of grouper larvae. In addition, dietary supplementation improves overall fish health and condition, obviating the 'day-25 syndrome' which was previously a major cause of mortality in grouper larval rearing.

An area of research in the project with potentially substantial impact over the next 5+ years, is the demonstration of improved survival using copepods as a live feed for grouper larvae. Copepods have long been recognised as being a valuable addition to the diets of marine finfish larvae, but this research has clearly demonstrated that they significantly and substantially improve survival: in our case by 259%. Now that *Parvocalanus* can be cultured in hatcheries, there is tremendous potential for the application of copepods in larval rearing of a range of marine finfish species.

Larval development

The research on the development of larval enzymes is the first time that a biotechnical approach has been taken to understanding the digestive processes of grouper larvae. This has provided considerable knowledge of larval enzyme ontogeny, which is particularly important for developing and applying inert larval diets.

This approach – looking in more detail at the digestive ability of marine fish larvae – is gradually becoming established worldwide as a useful approach to developing larval rearing techniques. The techniques are established at NFC Cairns, at SEAFDEC AQD (Philippines) and at RIM Gondol, and in the latter case are now being applied to other projects and other fish species (see Capacity Building Impacts discussion below).

Larval biology

Although not a direct output of this project, FIS/2002/077 facilitated collaboration between James Cook University and RIM Gondol to evaluate transgenerational isotope labelling in groupers, and provided logistical and technical assistance to the experiments. This work confirmed that larval groupers can be labelled using stable isotopes injected into the female parent, allowing tracking of the dispersal of offspring (Williamson *et al.* 2009). This technique has many potential applications in fisheries ecology and management, for example, in demonstrating the effectiveness of no-take areas as recruitment sources.

Nursery culture

Although considerably more research needs to be undertaken on the causes of deformities in groupers, this project has shown that many deformities can be reversed by the use of high-quality diets. Project results have also provided important information on opportunities to replace fish meal with alternative protein sources in grouper diets.

Research results have provided important information and techniques for reducing cannibalism amongst juvenile tiger groupers. In addition, this research has developed techniques for weaning juvenile tiger grouper to compounded feeds.

Grow-out nutrition

This project and its predecessor project (FIS/97/73) have made a valuable contribution to our knowledge of the nutritional requirements of groupers, particularly the mouse grouper (*C. altivelis*), the green grouper (*E. coioides*) and the tiger grouper (*E. fuscoguttatus*).

Research results have been adopted by commercial feed manufacturers.

Communication of results

The communication mechanisms developed by the project were used to distribute scientific results to other researchers, industry and aquaculture practitioners around the world. For example, information on research and development outcomes has been distributed worldwide to more than 1,000 subscribers to the Marine Finfish e-News and e-Magazine. Consequently, the results of the project have impacted a much broader audience than the participating countries.

8.2 Capacity impacts – now and in 5 years

Research capacity

RIM Gondol staff were trained in high-sensitivity enzyme analysis techniques through training in Australia, as well as on-site training at RIM Gondol. This training substantially increased the capacity of RIM Gondol staff to undertake analyses of larval digestive enzymes. This enhanced capacity is being utilised for other projects at RIM Gondol. For example, Regina Melianawati and Retno Andamari undertook another study to evaluate digestive enzymes in larval coral trout (*P. leopardus*). Ms Melianawati received training in enzyme analysis techniques at Gadjah Mada University, but acknowledged Ketut Suwirya's assistance in the enzyme analyses done at RIM Gondol.

DPI&F technical staff were also trained in enzyme analysis techniques at NFC Cairns during 2005–06.

Hatchery technology

DPI&F technical staff were trained in marine finfish larval rearing techniques during a visit to RIM Gondol in April 2006. These training outcomes were successfully transferred to NFC Cairns with resultant improvements in hatchery production. These techniques have also been extended to commercial hatcheries in Australia.

Nutrition and feed development

This project and its predecessor project have substantially increased capacity of staff at RICA Maros and to a lesser extent at RIM Gondol, to undertake research into fish nutrition and feed development. During this project, RICA Maros staff published five scientific papers in the Indonesian Journal of Aquaculture (see Section 10 References, p.93).

Usman (RICA Maros) attended the Aquaculture Nutrition Master Class, 7–19 August 2006 in Bangkok, Thailand. Participation in this Master Class improved Usman's knowledge of fish nutrition and increased his capacity to undertake nutrition research.

Project training courses

As noted above, the Regional Grouper Hatchery Production Training Course operated by the Asia-Pacific Marine Finfish Aquaculture Network, through NACA, has now trained in excess of 100 hatchery practitioners from 22 countries.

This training course has been effective in 'training of trainers'. For example, Mr Samart Detsathit of Krabi CFRDC was trained in the 2002 training course at RIM Gondol under the APEC Staff Exchange Program. Mr Detsathit succeeded in producing tiger grouper after his return to Krabi CFRDC and he is now one of the main trainers for the marine finfish hatchery training course in Krabi CFRDC.

APMFAN also organised a marine finfish hatchery training course for the Secretariat for the Pacific Community (SPC) for a group of six trainees from Pacific Islands Countries (Fiji, French Polynesia, New Caledonia, Papua New Guinea) in May 2007 at Krabi Coastal Fisheries Research and Development Centre (Krabi CFRDC) of DOF Thailand.

Participation in the annual Regional Grouper Hatchery Production Training Course by Brackishwater Aquaculture Development Centre (BADC) Situbondo staff has improved their training skills and improved their English language skills.

8.3 Community impacts – now and in 5 years

Increased aquaculture production

Larval rearing techniques used in Indonesia are providing the basis for development of larval rearing techniques in Australia. Larval rearing techniques used at RIM Gondol have been adapted for use at one large commercial hatchery in Queensland and at DPI&F's Northern Fisheries Centre, Cairns.

Results generated by the ACIAR projects are being accessed by the Queensland aquaculture industry in regard to diet, stocking density and fish husbandry.

Indonesian hatcheries have already begun producing fingerlings of *P. leopardus* for aquaculture, and it is likely that hatchery production of this species will expand over the next 5 years due to demand for this high-value species.

Extension and training

The publications ' A Guide to Small-scale Marine Finfish Hatchery Technology' and 'A Practical Guide to Feeds and Feeding for Cultured Groupers' have been translated into Thai by Department of Fisheries staff. Both guides have been used by Thai Department of Fisheries staff for training, including a training course at Krabi Coastal Fisheries Research and Development Centre from 30 June to 2 July 2005 on basic farm operation of better management practices. Twelve sea cage farmers attended this course. CFRDC supported training facilities, staff, dormitory and technology, and NACA supported training materials and meals.

Both Thai version guides were also used in tsunami relief operations in Thailand, being disseminated to farmers in the fish farming communities of Koh Yao Noi (Phang-nga Province) and Koh Lanta Noi (Krabi Province), Andaman coast (where NACA worked on rehabilitation of tsunami-affected fish farms). Two hundred farmers were targeted for training courses in Phang-nga Province under an FAO – NACA tsunami rehabilitation project.

The publication 'A Guide to Small-scale Marine Finfish Hatchery Technology' has been translated into Vietnamese through the Support to Marine Aquaculture Development (SUMA) program and about 1,000 copies have been disseminated to fish farmers throughout Vietnam.

The Indonesian language version of the publication 'A Practical Guide to Feeds and Feeding for Cultured Groupers' has been used to train farmers in Aceh in making farm-made feeds to reduce their use of 'trash' fish, and to overcome local shortages in 'trash' fish supply.

Participating laboratories have implemented training incorporating project results, to farmers and local agencies in Indonesia and Australia:

- Five farmers and staff from Yayasan Palu Hijau (Central Sulawesi) undertook a short course on grouper feed management at RICA Maros,19–23 September 2005. In May 2009, the YPH representative (Ms Abigail Moore) indicated that efforts to have local Dinas Kelautan dan Perikanan implement this training had not been successful. However, the material has been incorporated in the local university curriculum and Ms Moore is confident that this will provide a long-term impact because Dinas recruits from local graduates.
- RICA Maros provided short-term training for farmers from Sengata Regency (Kalimantan Timur Province) on feed preparation and feeding management.
- Five farmers from Mappi Regency, Papua Province, undertook 5 days training in grouper grow-out in floating net cages at RICA Maros.
- RIM Gondol provided training in grouper hatchery and nursery culture to five staff from Balai Benih Ikan Pantai (BBIP) Nias, North Sumatra, and two staff from BBIP Simeulue, Nanggroe Aceh Darussalam in June – July 2008 and October – November 2008. This training was supported by the Asian Development Bank's Earthquake and Tsunami Emergency Support Project.
- NFC Cairns provided on-farm training to two farmers on culture techniques for grouper with particular reference to methods of cage culture and fish grading. Also, training was provided to a barramundi hatchery operator on the culture of tiger grouper. This training provided the first fingerlings of tiger grouper produced by a commercial operator in Australia.

8.3.1 Economic impacts

Increased hatchery production

Technology refinements in marine finfish larval rearing have improved larval survival from 7–20% to 34–40%. There has been substantial adoption of these technologies by the private sector in Indonesia, particularly small-scale ('backyard') hatcheries: at present 480 units of 'backyard' hatcheries in northern Bali (from a total of 1,980 units) are producing grouper fingerlings. In addition, 9 large-scale hatcheries are producing grouper fingerlings.

Small-scale marine finfish hatcheries have also developed around Tigbauan in the Philippines. Several hatcheries are operated by SEAFDEC AQD staff or ex-staff members.

The SS-rotifers of Manembo-nembo strain were supplied by Sam Ratulangi University to a private hatchery in Lampung, Sumatra, for mass production to feed grouper larvae. This rotifer strain has also been used in a local hatchery in Tatelu, North Sulawesi, as live prey for tilapia larvae, but this hatchery lacks facilities for mass rotifer culture.

NFC Cairns has successfully and repeatedly cultured *P. leopardus* which has generated interest within the private industry and government for its capacity to provide economic benefits for Queensland. The success with this species is providing weight to discussions on possible sea-cage aquaculture in Queensland. Results from ACIAR project FIS/2003/027 *Planning tools for environmentally sustainable tropical finfish cage culture in Indonesia and northern Australia* will also add value to this process.

One large commercial hatchery in Queensland is producing barramundi cod (*Cromileptes altivelis*) fingerlings using techniques based on those developed by RIM Gondol. Due to constraints in developing grow-out sites in Queensland, this company is undertaking its grow-out operations in sea cages in the Republic of the Marshall Islands.

Increased grouper production in Indonesia and Australia

Improvements in hatchery production technology for marine finfish have driven production increases: production of market sized groupers in Indonesia increased from 2,100 tonnes in 2000 to 8,200 tonnes in 2006 due to the increased availability of hatchery-reared fingerlings.

Several Australian prawn farms that have diversified to include grouper culture have started to harvest and sell live and fresh-chilled grouper into the domestic restaurant market. The grouper fingerlings were supplied to the farms by NFC Cairns. Acceptance of the live fish product has been positive and prices strong. Australian production is expected to be about 15 tonnes in 2010.

Draft BMPs developed at the Lampung workshop on 'Development of Better Management Practices for Marine Finfish Aquaculture in the Asia-Pacific Region' are being used by Indonesian Mariculture Association members as a 'check list' to improve their farm performance.

8.3.2 Social impacts

Marine finfish hatchery production in Indonesia has created additional employment for people living in coastal areas: there are now 5,080 people directly involved in hatchery production in northern Bali.

Northern Fisheries Centre, Cairns, is producing fingerlings of flowery cod / tiger grouper for grow-out in ponds on Queensland prawn farms. This is intended to provide an alternative production commodity for prawn farms adversely affected by the cheap imported prawns that have, over the past few years, provided significant competition for Australian farmed prawns.

8.3.3 Environmental impacts

Training of farmers and others in feed management and farm-made feeds (Central Sulawesi, East Kalimantan, Papua and Aceh) will reduce reliance on the use of 'trash' fish as a feed source for marine finfish production.

This project, and its predecessor (FIS/97/73), have made a major contribution to our knowledge of grouper nutrition, including the potential to replace fish meal with alternative protein sources. Fish meal and fish oil use in aquaculture are widely regarded as major issues adversely affecting industry sustainability, and mechanisms to address these issues (such as reducing the amount of fish meal in diets) will be critical in the immediate future to develop new markets for expanding aquaculture production.

In Indonesia, aquaculture of high-value marine finfish such as groupers has contributed to the reduction in Illegal fishing (including explosive and cyanide fishing), assisting in the conservation of coral reefs.

8.4 Communication and dissemination activities

Annual project meetings

2005

The first annual meeting of ACIAR project FIS2002/077 was held at the Research Institute for Aquaculture No.1, Bac Ninh, Vietnam, on 4–5 June 2005. Representatives of all project laboratories attended the meeting, as well as representatives from NACA Vietnam, DANIDA (SUMA) and the Centre for Rural Progress.

Project progress was reviewed and the workplan for the next 12 months discussed, particularly in light of delays in project implementation and funding provision.

2006

No project meeting was held in 2006 due to the project leader's transfer from Queensland DPI&F to James Cook University and the associated administrative changes in the project, and his relocation to Aceh to implement the Aceh Aquaculture Rehabilitation Project (FIS/2006/002).

2007

The second annual project meeting for ACIAR project FIS/2002/077 was held at the Grand Majesty Hotel, Batam, 26–28 June 2007. The meeting was kindly hosted by the Director of Balai Budidaya Laut Batam, Mr Syamsul Akbar.

Participants provided detailed presentations (available as .pdf versions) on project progress and upcoming activities. Overall, project progress was assessed as adequate, although some problems with the spawning performance of broodstock at Gondol RIM (particularly *P. leopardus*) caused some delays in project activities. Where possible, project activities have switched to other species to avoid further delays.

Details of presentations and project outcomes are included in the report on this workshop. The project team visited BBL Batam, and also a private marine finfish cage farm at Batam.

2008

The third annual project meeting for ACIAR project FIS/2002/077 'Improved hatchery and grow-out technology for marine finfish aquaculture in the Asia-Pacific region' was held at the Sahid Makassar Hotel, Makassar, South Sulawesi, 22–25 July 2008. The meeting was officially opened by the new Director of the Agency for Marine and Fisheries Research, Mr Gellwynn Yusuf, introduced by the new Director for Seed Production in the Directorate-General of Aquaculture, Prof. Dr Ketut Sugama.

Progress of project activities was discussed in relation to the project milestones table. Main discussion points are summarised in the meeting report, and overall progress incorporated in this annual report.

The meeting agreed to undertake a number of planned or additional activities to address outstanding or incomplete project objectives. Based on the need to complete a number of project activities to satisfactorily address the project objectives, it was agreed that Mike Rimmer would request from ACIAR a 12-month extension to the project timeframe.

Following the project meeting, the project team visited the RICA Maros experimental cages at Aruwange Bay, Barru Regency, and the adjacent shrimp hatchery of BBAP Takalar.

In addition to the project meeting, the project team held a one-day symposium to report project results to a wider audience of researchers, government officers and industry. Details of the symposium are provided below (p.87).

2010

The final project workshop was held at Research Institute for Mariculture (RIM) Gondol, 16–20 February 2010. The workshop was primarily focussed on providing detailed information to the project reviewers, Dr Stewart Fielder (Australia) and Mr Wajan Sudja (Indonesia).

PowerPoint summaries of project activities and outcomes, as well as training and extension activities, were given by the research team. Both formal and informal discussion between the research team and the reviewers was encouraged.

Three field trips were also organised including tours of the RIM Gondol hatchery research and production facilities, the RIM Gondol seacage research facility and a visit to several commercial backyard grouper hatcheries. This provided the reviewers, especially Dr Fielder who had not visited the sites previously, with a better appreciation of experimental facilities and the scope of industry development and possible areas for further development.

On the final day of the meeting, Chris Barlow invited the reviewers to wrap-up the meeting by providing a brief overview of the review outcomes and recommendations, and to flag potential future activities needed to continue development of marine finfish aquaculture in the Asia-Pacific region.

Symposia and workshops

In conjunction with the 2008 annual project meeting, the project team organised a public Symposium on Marine Finfish Aquaculture, which was held at the Sahid Makassar Hotel, Makassar, on 27 July 2008 (Table 34). The objective of the symposium was to present the outcomes of our research to researchers from government and universities, to Dinas Kelautan dan Perikanan and to the private sector.

A total of 61 participants (including presenters) attended the symposium, including representatives from:

- Private sector, including feed companies;
- Balai Budidaya Air Payau Takalar;
- Dinas Kelautan dan Perikanan;
- Universities (UNHAS, UMI);
- Mars Symbioscience and Konsorsium Mitra Bahari (Sea Partnership Consortium).

Overall, the meeting was successful in presenting the results of FIS/2002/077 to a broader audience. The meeting was also successful in promoting interaction between different agencies, including National and Provincial agencies, local universities and private sector.

Table 34 Program for Symposium on Marine Finfish Aquaculture, held at the Sahid Makassar Hotel, Makassar, 27 July 2008.

Time	Presentation	Presenter(s)
0900 – 0930	Welcome • Introduction to project	Prof. Dr Ketut Sugama, Dr Rachmansyah, Dr Mike Rimmer
0930 – 1030	Larval rearing • Overview of techniques • Larval nutrition • Application: small-scale hatcheries	Mrs Suko Ismi, Dr Veronica Alava
1030 – 1100	*Coffee*	
1100 – 1145	Live feed production • Rotifers • Copepods	Mrs Reiny Tumbol, Dr Richard Knuckey
1145 – 1230	Nursery culture and weaning • Weaning techniques • Reducing cannibalism • Reducing deformities	Mr Ketut Suwirya, Dr Mae Catacutan

1230 – 1400	*Lunch*	
1400 – 1445	Feed development – RICA Maros • Moist pellets • Feed validation ('trash' fish, moist pellets, dry pellets)	Mr Usman
1445 – 1530	Feed development – RIM Gondol • Comparison of 'trash' fish and test diets • Trials using commercial pellets	Mr Tatam Sutarmat
1530 – 1615	Evaluation of carrying capacity for sea cage culture	Dr Helmar Halide, Dr Rachmansyah
1615 – 1630	Close	Prof. Dr Ketut Sugama

Workshops

Development of Better Management Practices for Marine Finfish Aquaculture in the Asia-Pacific Region, Lampung, Indonesia, 7–10 November 2007

NACA and the Directorate General of Aquaculture (DGA) of Indonesia in conjunction with the Australia Centre for International Agricultural Research (ACIAR) held a workshop on the 'Development of Better Management Practices for Marine Finfish Aquaculture in the Asia-Pacific region', in Lampung, Indonesia, 7–10 November 2007. The workshop was held to begin the process of developing Better Management Practices (BMPs) for the marine finfish aquaculture sector, which is growing rapidly in Asia. The rapid expansion of marine finfish aquaculture, and concerns regarding its environmental sustainability, has led to the development of several accreditation / certification schemes, and the proposed development of others. NACA and ACIAR are concerned that many current schemes effectively disadvantage small-scale farmers, who provide the bulk of production in Asia. The development of a BMPs-based approach is intended to allow small-scale farmers to adopt practices that will better support their participation in more formal accreditation / certification schemes in the future, and facilitate market access by small-scale farmers in the face of increasing consumer demands for environmental and social responsibility in aquaculture.

The 4-day workshop was undertaken as part of the ACIAR-funded project 'Improved hatchery and grow-out technology for marine finfish aquaculture in the Asia-Pacific region' (FIS/2002/077). The workshop was attended by 60 participants from Australia, Cambodia, China, France, India, Indonesia, Myanmar, Norway, Philippines, Thailand and Vietnam. The workshop was strongly supported by the private sector, with about half the participants coming from private industry, including representatives of farmer organizations and feed companies. Industry participants were supportive of the need to develop BMPs for marine finfish aquaculture as a way to enhance the sustainability of their industry. The workshop was also attended by representatives of environmental and other NGOs.

Workshop outputs included a review of the current status of marine finfish aquaculture in the Asia-Pacific region, including a description of the marine finfish aquaculture sector and the constraints that were identified in the various country presentations. The workshop, through four working groups, drafted implementation guidelines for the development of BMPs for marine finfish aquaculture, based on the 'International Principles for Responsible Shrimp Farming' structure. The development of marine finfish aquaculture BMPs will continue to be facilitated by NACA through an open and transparent process to ensure that all stakeholders have input to the development of the BMPs.

Nutrition workshop, Surabaya, 20–22 October 2009

A three-day workshop on fish nutrition was held at the Santika Hotel, Surabaya, 20–22 October 2009, as an activity under ACIAR project FIS/2002/077 Improved hatchery and grow-out technology for marine finfish aquaculture in the Asia-Pacific region. Indonesian feed companies were invited to send technical representatives, particularly feed formulators, to the workshop to update their knowledge of fish nutrition and to hear the results from research carried out under FIS/2002/077 as well as other research undertaken by the Indonesian Agency for Marine Affairs and Fisheries.

Between 16 and 9 participants attended the workshop with number declining over the three days of the workshop. Feedback on the workshop was generally positive. Several participants requested more practical activities in the workshop; however, it is difficult to see how these can be integrated into a workshop intended for commercial feed companies.

With regard to marine finfish aquaculture, the point was made that this is a relatively small sector in Indonesia. The feed companies are focussed on the larger markets, for freshwater finfish and shrimp, rather than marine finfish. Because of the relatively small demand for marine finfish feeds, the use of ‘trash’ fish is not regarded by the feed companies as a critical issue. While the marine finfish sector remains relatively small (compared with freshwater fish and shrimp), there appears to be relatively little interest in developing marine finfish feeds to replace ‘trash’ fish.

One potential follow-up from the workshop is development of a more accessible list of ingredients, with information on proximate composition, digestibility, and including potential negative inclusion information such as anti-nutritional factors, etc. to assist feed formulators to decide which ingredients can be used in various diets.

9 Conclusions and recommendations

9.1 Conclusions

Overall, this project successfully improved technologies for larval rearing and grow-out for marine finfish aquaculture. Attempts to reduce the average size of rotifer populations had only limited success. Based on these results, further research on rotifers for small-mouthed fish larvae should concentrate on isolating and developing culture techniques for rotifers smaller than *Brachionus*, such as *Proales* (Wullur *et al.* 2009).

This project, together with FIS/97/73, has demonstrated that improving the nutritional composition of live feeds by increasing the levels of HUFAs increases survival and improves the condition of grouper larvae.

An area of research that shows tremendous potential is the use of copepods as a feed for larval finfish. This project demonstrated that the inclusion of *Parvocalanus* in the larval diet dramatically improved survival during early larval development. The results from this project indicate that there is substantial potential to improve survival, growth and condition of hatchery-reared marine finfish larvae through the use of copepods as a food source.

As noted in this report, the uptake of these technologies has been limited, particularly in Indonesia. The major reason for this is that many farmers purchase fingerlings primarily based on price rather than quality. To improve technology uptake, we propose developing a demonstration hatchery to facilitate the incorporation of project results in larval rearing protocols.

This project and its predecessor (FIS/97/73) have defined many of the main nutritional requirements of groupers. While this information is available to commercial feed companies, the performance of pellet diets remains, at best, comparable to that of 'trash' fish. However, in many cases the performance of 'trash' fish exceeds that of pellet feeds. In the case of small-scale farmers, where harvesting 'trash' fish may be a farm activity, feed supply is an opportunity cost and not a cash cost. Consequently, uptake of compounded pellet diets is likely to be better on medium and large-scale farms than on smallholder farms. One solution to improve the uptake of compounded feeds in marine finfish culture in Indonesia is proposed in the 'Recommendations' section below.

9.2 Recommendations

9.2.1 Future research and development for marine finfish aquaculture

ACIAR has supported two large projects on grouper / marine finfish aquaculture and these have made significant strides in moving the industry forward. However, the current project identified a number of areas where technology adoption was slow. Future research should focus closely on adoption of the technologies that have already been developed through FIS/97/73 and FIS/2002/077, while implementing research in areas where there is a strong need, such as fish health.

Larval rearing

Future work should focus on the implementation of research results from FIS/2002/077 and FIS/97/73 in hatcheries as well as the implementation of improved biosecurity systems and protocols, with evaluation of the cost-benefits of this 'best practice' approach. We propose that a demonstration hatchery be operated at RIM Gondol (an existing hatchery has been identified as being ideal for this purpose) which would operate half as a 'traditional small-scale' hatchery and half as a 'best practice' hatchery. This would allow a controlled evaluation of the costs and benefits of improved hatchery practices. Following

this, the hatchery would be operated as a demonstration facility to demonstrate improved hatchery practices to local hatchery operators. This activity would link to commercial hatcheries that collaborate with ABILINDO (Indonesian Mariculture Association).

Feeds

The use of pellet feeds contributes to fish health by providing a nutritionally complete diet, thus improving overall fish 'condition' and reduces the incidence of parasitic infestations in cultured fish (Rückert *et al.* 2009). Aside from sustainability arguments, the use of compounded feeds is part of any 'best practice' culture package. In addition, immunostimulants may be added to feed if research data indicate significant enhancement of fish health – currently this benefit is anecdotal, and their inclusion may increase the cost of feed without real benefit.

However, further feeds work requires support and cooperation from feed companies. As part of the review of FIS/2002/077 undertaken in Bali in February 2010, Mr Wajan Sudja (Secretary-General of the Indonesian Mariculture Association [ABILINDO]) proposed that future work on pellet feeds for marine finfish, particularly grouper, should involve a three-way arrangement between commercial growers (through ABILINDO), commercial feed companies and researchers. This would allow the farmers to exert pressure on the feed companies to improve their products, and researchers to advise both the feed companies and the growers regarding feed composition and use.

Fish health

Future R&D on marine finfish health should build on the ACIAR-funded SRA FIS/2005/137 'Control of nodaviral disease in tropical marine finfish hatcheries: enhanced biosecurity through the application of contemporary biotechnology, epidemiology and pathobiology'. While nodavirus remains a significant problem in hatcheries, there are a range of other diseases in marine finfish culture that are poorly understood, including parasite infestations (Rückert *et al.* 2009). The overall profitability of the industry could potentially benefit from improved survival through reducing disease-related mortality.

A component of R&D on fish health could be integrated with the topics outlined above to provide a project 'package' of practices covering disease prevention (best practices to improve fish health, vaccination), disease diagnosis and disease treatment.

10 References

10.1 References cited in report

Alava, V.R., Priolo, F.M.P., Toledo, J.D., Rodriguez, J.C., Quinitio, G.F., Sa-an, A.C., de la Pena, M.R. and Caturao, R.C. (2004). Lipid nutrition studies on grouper (*Epinephelus coioides*) larvae. In: Rimmer, M.A*., et al.* (eds.). *Advances in Grouper Aquaculture. ACIAR Monograph 110*. pp.·47-52. Australian Centre for International Agricultural Research, Canberra.

Alongi, D.M., McKinnon, A.D., Brinkman, R., Trott, L.A., Undu, M.C., Muawanah and Rachmansyah (2009). The fate of organic matter derived from small-scale fish cage aquaculture in coastal waters of Sulawesi and Sumatra, Indonesia. Aquaculture 295**,** 60-75.

Catacutan, M.R., G.E., P., Doyola-Solis, E., Teshima, S.-I. and Ishikawa, M. (in press). Growth and feed efficiencies in the mangrove feed snapper, *Lutjanus argentimaculatus* (Forsskal 1775), fed practical diets supplemented with L-ascorbyl-2-monophosphate-Mg. The Israeli Journal of Aquaculture - Bamidgeh.

Chan, N.W.W. (2007). Survey and taste test for live reef-fish in Hong Kong. In: Johnston, B. (ed.) *Economics and market analysis of the live reef-fish trade in the Asia-Pacific region. ACIAR Working Paper No. 63*. pp.·45-59. Australian Centre for International Agricultural Research, Canberra.

Giri, N.A., Suwirya, K. and Marzuqi, M. (2004). Optimum level of dietary protein and lipid for rearing juvenile tiger grouper (*Epinephelus fuscoguttatus*). In: Rimmer, M.A*., et al.* (eds.). *Advances in Grouper Aquaculture. ACIAR Monograph 110*. pp.·92-94. Australian Centre for International Agricultural Research, Canberra.

Kohno, H., Ordonio-Aguilar, R., Ohno, A. and Taki, Y. (1997). Why is grouper larval rearing difficult?: an approach from the development of the feeding apparatus in early stage larvae of the grouper, *Epinephelus coioides*. Ichthyological Research 44**,** 267-274.

Laining, A., Rachmansyah, Ahmad, T. and Williams, K. (2003). Apparent digestibility of selected feed ingredients for humpback grouper, *Cromileptes altivelis*. Aquaculture 218**,** 529-538.

McGilvray, F. and Chan, T.T.C. (2001). *The Trade in Live Reef Food Fish: A Hong Kong Perspective.* International Marine Life Alliance, Hong Kong. 16 pp.

New, M.B. (1996). Responsible use of aquaculture feeds. Aquaculture Asia Magazine 1 (1)**,** 3-15.

Phillips, M.J. (1998). Chapter 2 - Tropical Mariculture and Coastal Environmental Integrity. In: De Silva, S.S. (ed.) *Tropical Mariculture*. pp.·17-69. Academic Press, London.

Rimmer, M.A., McBride, S. and Williams, K.C. (2004). *Advances in Grouper Aquaculture. ACIAR Monograph 110.* Australian Centre for International Agricultural Research, Canberra. 137 pp.

Rückert, S., Klimpel, S., Al-Quraishy, S., Mehlhorn, H. and Palm, H. (2009). Transmission of fish parasites into grouper mariculture (Serranidae: *Epinephelus coioides* (Hamilton, 1822)) in Lampung Bay, Indonesia. Parasitology Research 104**,** 523-532.

Sadovy, Y. (2000). Regional survey of fry/fingerling supply and current practices for grouper mariculture: evaluating current status and long-term prospects for grouper mariculture in Southeast Asia, 120 pp. University of Hong Kong, Hong Kong SAR, China.

Siar, S.V., Johnston, W.L. and Sim, S.Y. (2002). *Study on Economics and Socio-economics of Small-scale Marine Fish Hatcheries and Nurseries, with Special Reference*

to Grouper Systems in Bali, Indonesia. 36 pp. Report prepared under APEC Project FWG 01/2001 - 'Collaborative APEC Grouper Research and Development Network'. Asia-Pacific Marine Finfish Aquaculture Network Publication 2/2002. Network of Aquaculture Centres in Asia-Pacific, Bangkok, Thailand. 36 pp.

Sim, S.Y. (2006). *Grouper aquaculture in three Asian countries. PhD thesis, Deakin University, Australia*. 254 pp.

Stone, F.E. and Hardy, R.W. (1986). Nutritional value of acid stabilised silage and liquefied fish protein. Journal of the Science of Food and Agriculture 37**,** 797-803.

Tacon, A.G.J., Metian, M., Turchini, G.M. and De Silva, S.S. (2010). Responsible aquaculture and trophic level implications to global fish supply. Reviews in Fisheries Science 18**,** 94 - 105.

Toledo, J.D., Golez, M. and Ohno, A. (2005). Studies on the use of copepods in the semi-intensive seed production of grouper *Epinephelus coioides*. In: Lee, C.-S*., et al.* (eds.). *Copepods in Aquaculture. Proceedings of a Workshop on Culture of Copepods and Applications to Marine Finfish Larval Rearing, Honolulu, HI (USA), 5-8 May 2003*. pp.·169-182. Blackwell, Oxford.

Williamson, D., Jones, G. and Thorrold, S. (2009). An experimental evaluation of transgenerational isotope labelling in a coral reef grouper. Marine Biology 156**,** 2517-2525.

Wullur, S., Sakakura, Y. and Hagiwara, A. (2009). The minute monogonont rotifer *Proales similis* de Beauchamp: culture and feeding to small mouth marine fish larvae. Aquaculture 293**,** 62-67.

Yap, W.G. (2002). Role of mariculture in securing food supply and reducing poverty in the Philippines. In *World Aquaculture 2002 International Aquaculture Conference and Exposition*, 864 pp. World Aquaculture Society, Beijing, China.

10.2 List of publications produced by project

Note: not all of the listed publications are directly on outcomes of the ACIAR research; some are on related fields of research which were not directly supported by the project, e.g. Josette Gonzaga's paper arising from her JAF research which was associated with FIS/2002/077. Some, particularly some scientific papers, were 'carried over' from the results of FIS/97/73. However, all the publications listed have been nominated as 'project publications'.

Scientific papers

Eusebio, P.S., Coloso, R.M. and Mamauag, R.E.P. (2004). Apparent digestibility of selected ingredients in diets for juvenile grouper, *Epinephelus coioides* (Hamilton). Aquaculture Research 35, 1261–1269.

Gonzaga, J., Anderson, A., Richardson, N., Nocillado, J. and Elizur, A. (2010). Cloning of IGF-I, IGF-II and IGF-IR cDNAs in mullet (*Mugil cephalus*) and grouper (*Epinephelus coioides*): molecular markers for egg quality in marine fish. Asian Journal of Biological Sciences 3, 55–67.

Marzuqi, M., Suwirya, K. and Giri, N.A. (2006). Pattern of lipid and essential fatty acid changes in early development of tiger grouper, *Epinephelus fuscoguttatus* larvae. Indonesian Aquaculture Journal 1(2), 129–134.

Nguyen, T.T.T., Davy, F.B., Rimmer, M.A. and De Silva, S.S. (2009). Use and exchange of genetic resources of emerging species for aquaculture and other purposes. Reviews in Aquaculture 1, 260-274.

Nishida, S. and Rumengan, I.F.M. (2005). A new species of *Pseudodiaptomus* (Copepoda: Calanoida: Pseudodiaptomidae) from the coastal waters of Sulawesi, Indonesia. Plankton Biology and Ecology 52(1): 27–32.

Rachmansyah, Usman, Kabangnga, N. and Makmur (2006). Utilization of poultry offal meal silage in tiger grouper, *Epinephelus fuscoguttatus* diets. Jurnal Riset Akuakultur 1(1):87–96.

Rumengan, I.F.M., Sulung, M., Lantiunga,Z. and Kekenusa, J. (2007). Morphometry of SS-strain rotifer *Brachionus rotundiformis* originated from Minanga and Watuliney brackishwater ponds cultured at different salinities. Jurnal Riset Akuakultur (2)2, 221–229 (in Indonesian).

Sagala, S.L., Ismi, S. and Giri, N.A. (2010). The effect of vitamin C (L-ascorbyl monophosphate-Mg) on the deformity performance of humpback grouper (*Cromileptes altivelis*) larvae. Indonesian Aquaculture Journal 5, 29-36.

Smith, D.M., Williams, I.H., Williams, K.C., Barclay, M.C., Venables, W.N. (2005). Oxidation of medium-chain and long-chain fatty acids by polka dot grouper *Cromileptes altivelis*. Aquaculture Nutrition 11, 41–48.

Sumilat, D.A., Mamesah, J.A.B. and Rumengan, I.F.M. (2003). Preliminary study on the local strain of *Chlorella*-like microalga isolated from a hydrothermal coastal water in Likupang, North Sulawesi. Ekoton 3(1): 7–13.

Tuan, L.A. and Williams, K.C. (2007). Optimum dietary protein and lipid specifications for juvenile malabar grouper (*Epinephelus malabaricus*). Aquaculture 267, 129–138.

Usman, Rachmansyah and Kamaruddin (2006). Substitution of fish meal with golden snail meal (*Pomacea* sp) in diets for grow-out of tiger grouper (*Epinephelus fuscoguttatus*). Jurnal Riset Akuakultur 1(2): 161–170.

Usman, Rachmansyah, Kamaruddin, Lante, S. and Ahmad, T. (2006). Replacement of fish meal with poultry offal meal in diets for grow-out of humpback grouper (*Cromileptes altivelis*). Indonesian Aquaculture Journal 1(1): 45–52.

Usman, Kamaruddin, Palinggi, N.N. Rachmansyah and Ahmad, T. (2007). Fermented blood meal use for tiger grouper, *Epinephelus fuscoguttatus* grow-out diet. Indonesian Aquaculture Journal 2(1), 7–13.

Usman, Rachmansyah, Laining, A., Ahmad, T. and Williams, K.C. (2005). Optimum dietary protein and lipid specifications for grow-out of humpback grouper *Cromileptes altivelis* (Valenciennes). Aquaculture Research 36, 1285–1292.

Usman, Rachmansyah, Makmur and Ahmad, T. (2005). Substitution of fish meal with soybean meal in humpback grouper, *Cromileptes altivelis* juvenile diets supplemented with phytase. Indonesian Fisheries Research Journal 11, 73–80.

Williams, K.C. (2009). A review of feeding practices and nutritional requirements of postlarval groupers. Aquaculture 292, 141–152.

Williams, K.C., Irvin, S. and Barclay, M. (2004). Polka dot grouper *Cromileptes altivelis* fingerlings require high protein and moderate lipid diets for optimal growth and nutrient retention. Aquaculture Nutrition 10, 125-134.

Wullur, S., Rumengan, I.F.M. and Manu, G. (2004). Fecundity of *Apocyclops* sp in culture media containing microalga, *Nannochloropsi oculata*. Journal of the Faculty of Fisheries and Marine Science, Sam Ratulangi University 27(1): 95–100.

Yoshinaga, T., Minegishi, Y., Rumengan, I.F.M., Kaneko, G., Furukawa, S., Yanagawa, Y., Tsukamoto, K. and Watabe, S. (2004). Molecular phylogeny of the rotifers with two Indonesian *Brachionus* lineages. Coastal Marine Science 29(1): 45–56

While the research described in the following publication was not directly associated with the ACIAR project, facilitation of access to RIM Gondol as well as technical and logistical support was provided by the ACIAR project team:

Williamson, D., Jones, G. and Thorrold, S. 2009. An experimental evaluation of transgenerational isotope labelling in a coral reef grouper. Marine Biology 156, 2517–2525.

Books, book chapters

Rimmer, M.A. and McBride, S. (2008). Grouper Aquaculture in Australia. pp. 177–188. In: 'The Aquaculture of Groupers'. Liao, I.C. and Leaño, E.M. (eds). Asian Fisheries Society, World Aquaculture Society, Fisheries Society of Taiwan, National Taiwan Ocean University. 241 pp.

Rimmer, M.A. and Ponia, B. (2007). A Review of Cage Aquaculture: Oceania. pp. 208–231. In: Halwart, M., Soto, D. and Arthur, J.R. (eds). 'Cage Aquaculture – Regional Reviews and Global Overview'. FAO Fisheries Technical Paper No 498. Food and Agriculture Organisation of the United Nations, Rome. 241 pp.

Sim, S.Y., Rimmer, M.A., Toledo, J.D., Sugama, K., Rumengan, I., Williams, K. and Phillips, M.J. (2005). A Guide to Small-Scale Marine Finfish Hatchery Technology. Network of Aquaculture Centres in Asia-Pacific, Bangkok, Thailand. 17 pp.

Sim, S.Y., Rimmer, M.A., Williams, K., Toledo, J.D., Sugama, K., Rumengan, I. and Phillips, M.J. (2005). A Practical Guide to Feeds and Feed Management for Cultured Groupers. Network of Aquaculture Centres in Asia-Pacific, Bangkok, Thailand. 18 pp.

Conference and workshop proceedings

Chan, N.W.W. (2007). Survey and taste test for live reef-fish in Hong Kong. In: Johnston B. (ed.). Economics and market analysis of the live reef-fish trade in the Asia–Pacific region. ACIAR Working Paper No. 63. pp. 45–59.

Kamaruddin, Usman and Rachmansyah (2007). The effects of additional tryptophan in diets on cannibalisms, survival rate and growth of tiger grouper juvenile, *Epinephelus fuscoguttatus*. Technology Development of Aquaculture, pp. 301-306. (In Indonesian).

Phillips, M.J., Rimmer, M. and Sim, S.Y. (2005). Trends in coastal aquaculture and effect on feed demand in the Asian region. Paper presented on behalf of the Network of Aquaculture Centres in Asia-Pacific (NACA) and Asia-Pacific Marine Finfish Aquaculture Network at the Regional Workshop on Low Value and 'Trash Fish' in the Asia-Pacific Region, Hanoi, Vietnam, 7–9 June 2005. Published by FAO / Asia-Pacific Fishery Commission, Bangkok, Thailand (available at www.apfic.org).

Rimmer, M.A. (2008). Regional review on existing major mariculture species and farming technologies. pp. 105–125. In: Lovatelli, A., Phillips, M.J., Arthur, J.R. and Yamamoto, K. (eds). FAO / NACA Regional Workshop on the Future of Mariculture: a Regional Approach for Responsible Development in the Asia-Pacific Region. Guangzhou, China, 7–11 March 2006. FAO Fisheries Proceedings. No. 11. Food and Agriculture Organisation of the United Nations, Rome. 325 pp.

Rimmer, M.A., Phillips, M.J. and Sim, S.Y. (2006). Aquaculture of groupers in Asia and the Pacific. In: Johnston, B. and Yeeting, B. (eds.). Economics and marketing of the live reef fish trade in Asia-Pacific. ACIAR Working Paper No. 60. Australian Centre for International Agricultural Research, Canberra. pp. 116–134.

Rimmer, M.A., Phillips, M.J. and Yamamoto, K. (2007). Recent developments in aquaculture of groupers in the Asia–Pacific region. In: Johnston B. (ed.). Economics and

market analysis of the live reef-fish trade in the Asia–Pacific region. ACIAR Working Paper No. 63. pp. 77–84.

Rimmer, M.A. and Svennevig, N. (2009). The growing market for marine finfish – global needs. In: Souvenir of the Indian International Aquaculture Exposition INDAQUA-2009, Bhubaneswar, Orissa, India, 21–23 January 2009. pp. 67–70.

Setiadharma, T., Setiawati, K. M. and Giri, N.A. (2006). Effect of different tank color on cannibalism activity of juvenile tiger grouper *Epinephelus fuscoguttatus*. Prosiding Konferensi Nasional Akuakultur 2005. Masyarakat Akuakultur Indonesia. Semarang, 23–25 November 2005.

Setiadharma, T., Setiawati, K.M. and Giri, N.A. (2006). Effect of water depth on cannibalism activity and performance of tiger grouper juveniles. Prosiding Seminar Nasional Pengembangan Teknologi Budidaya Perikanan. Denpasar, Bali, 21–23 November 2006.

Setiawati, K.M., Setiadharma, T., Giri, N.A. and Sutarmat, T. (2006). The effect of water current on growth and survival of juvenile tiger grouper. Prosiding Seminar Nasional Perikanan Indonesia 2006. Sekolah Tinggi Perikanan. Jakarta, 12-13 Desember 2006.

Setiawati, K.M., Hanafi, A. and Giri, N.A. (2005). Effectiveness of additional attractant in diet for reducing cannibalism activity on juvenile tiger grouper *Epinephelus fuscoguttatus*. Prosiding Seminar Agribisnis Rusnas Kerapu. Jakarta, 12 August 2005.

Setiawati, K.M. (2006). Effect of light intensity on cannibalism activity of tiger grouper juveniles. Prosiding Seminar Nasional Pengembangan Teknologi Budidaya Perikanan. Denpasar, Bali, 21–23 November 2006.

Sugama, K., Hanafi, A. and Rimmer, M. 2005. Hatchery technology on the breeding and fry production of marine finfish in Indonesia. pp 112-115 In: Kolkovski, S., Rimmer, M.A., Battaglene, S.L. & Van Barneveld, R. eds. The second hatchery feeds and technology workshop, Novatel Century, Sydney, Australia, 30 September - 1 October 2004.

Usman, Kamaruddin and Makmur (2007). Biological performance of tiger grouper, *Epinephelus fuscoguttatus*, fed diet containing different levels of carophyll pink (astaxantin). Technology Development of Aquaculture, pp. 295-300. (In Indonesian).

Williams, K.C. and Rimmer, M.A. (2005). The future of feeds and feeding of marine finfish in the Asia-Pacific region: the need to develop alternative aquaculture feeds. Paper presented on behalf of the Network of Aquaculture Centres in Asia-Pacific (NACA) and Asia-Pacific Marine Finfish Aquaculture Network at the Regional Workshop on Low Value and 'Trash Fish' in the Asia-Pacific Region, Hanoi, Vietnam, 7–9 June 2005. Published by FAO / Asia-Pacific Fishery Commission, Bangkok, Thailand (available at www.apfic.org).

Yamamoto, K. (2007). The role of the Network of Aquaculture Centres in Asia–Pacific (NACA) in addressing food safety and trade issues in cultured seafood. In: Johnston B. (ed.). Economics and market analysis of the live reef-fish trade in the Asia–Pacific region. ACIAR Working Paper No. 63. pp. 85–90.

Yamin, M., Palinggi,N.N., Usman and Rachmansyah (2007). Protease activity in intestine with different size of tiger grouper. Proceedings of The 3rd Marine National Seminar, Surabaya, 24 April 2007, pp. 40-44. (In Indonesian).

Yamin, M., Usman, Kamaruddin and Rachmansyah (2007). Fermentation of shrimp by-product meal by chitinase enzyme from isolate of *Aeromonas* sp. (G) with different water content. Proceeding of The Fourth of Research Result National Seminar of Marine and Fisheries, Gadjah Mada University, Yogyakarta, 28 July 2007. (In Indonesian).

Articles

Anon (2009). Aquaculture course hatches its 100th graduate. Partners Magazine, Nov. 2008 – Feb. 2009, p.29.

Chan, N.W.W. and Johnston, B. (2007). Applying the triangle taste test to wild and cultured humpback grouper (*Cromileptes altivelis*) in the Hong Kong market. SPC Live Reef Fish Information Bulletin 17, 31–35.

Corby, A., Sadler, J. and Knuckey, R. (2008). Flowery cod for restaurants. Queensland Aquaculture News 31, 5.

Cox, E., Fry, P. and Johnston, A. (2006) Mesocosm technology advances grouper culture in northern Australia. Aquaculture Asia Magazine 11(1), 34–36.

Cox, E. Fry, P., Johnston, A. and Rimmer, M. (2004). First Australian production of flowery cod juveniles. Queensland Aquaculture News 25, 11.

Giri, N.A., Suwirya, K., Sutarmat, T. and Marzuqi, M. (2007). Effect of dietary protein and lipid level on growth performance of tiger grouper *Epinephelus fuscoguttatus* during late-stage grow-out. Aquaculture Asia 12(4), 39.

Komarudin, U., Rimmer, M.A., Islahuttaman, Zaifuddin and Bahrawi, S. (2010). Grouper nursing in Aceh, Indonesia. Aquaculture Asia-Pacific 6, 21-25.

Look, G. (2006). Skretting scholars take new knowledge home after NACA hatchery course. Aquaculture Asia Magazine 11(4), 24–25.

McBride, S. (2005). Australian success with barramundi cod. Aquaculture Asia Magazine 10(3), 23.

Rachmansyah, Usman, Palinggi, N.N. and Williams, K. (2009). Formulated feed for tiger grouper grow-out. Aquaculture Asia Magazine 14(2), 30-35.

Reynolds, A. and Knuckey, R. (2008). Australia's first production of juvenile coral trout. Queensland Aquaculture News 31, 1–2.

Rimmer, M. (2008). Marine finfish aquaculture developments at 'Indonesian Aquaculture 2007'. Aquaculture Asia Magazine 13(1), 45–46.

Rimmer, M. (2008). Production update – marine finfish aquaculture in the Asia-Pacific region. Aquaculture Asia Magazine 13(1), 46–49.

Rimmer, M. (2008). Production update – marine finfish aquaculture in the Asia-Pacific region. Aquaculture Asia Magazine 13(3), 44–46.

Rimmer, M., Kistle, S. and Muldoon, G. (2004). Assessment of the use of coded wire tags (CWT) to trace coral trout (*Plectropomus leopardus*) in the live reef food fish trade. Aquaculture Asia Magazine 9(4), 38–39.

Rimmer, M. and Phillips, M.J. (2008). Marine, brackishwater finfish culture in Indonesia – Aceh industry recovering from 2004 tsunami. Global Aquaculture Advocate 11(2), 54–57.

Rimmer, M., Sim, S.Y., Sugama, K. and Phillips, M. (2004). Alternatives for reef fishing – can aquaculture replace unsustainable fisheries. Global Aquaculture Advocate 7(1), 44–45.

Rimmer, M., Sugama, K., Sim, S.Y. and Phillips, M. 2004. Alternatives for reef fishing - can aquaculture replace unsustainable fisheries? Global Aquaculture Advocate 7, 44-45.

Rimper, J., Kaswadji, K., Widigdo, B., Sugiri, N., and Rumengan I.F.M. (2008). Body size of rotifers (*Brachionus rotundiformis*) from estuaries in North Sulawesi. Aquaculture Asia Magazine 13(1), 49-50.

Rumengan, I.F.M. (2007). Biotechnological prospectus of rotifer *Brachionus rotundiformis*. Squalen 2(1), 17–21.

Sim, S.Y. (2004). First breeding success of Napoleon wrasse and coral trout. Aquaculture Asia Magazine 9(2), 33–34.

Sim, S.Y. (2004). Regional developments and update. Aquaculture Asia Magazine 9(2), 36–38.

Sim, S.Y. (2004). Some insights into the live marine food fish markets in the region. Aquaculture Asia Magazine 9(3), 31–33.

Sim, S.Y. (2005). Influence of economics conditions of importing nations and unforseen global events on grouper markets. Aquaculture Asia Magazine 10(4), 23–31.

Sim, S.Y. (2006). Marine finfish market information and aquaculture development trends in selected locations in Indonesia and Malaysia. Aquaculture Asia Magazine 11(4), 18–22.

Sim, S.Y., Kongkeo, H. and Rimmer M. (2006). Brief overview of recent grouper breeding development in Thailand. Aquaculture Asia Magazine 2005 (3), 24–26.

Sim, S.Y., Montaldi, P., Montaldi, A. and Kongkeo, H. (2004). Farming practices, market chains and prices of marine finfish in Malaysia. Aquaculture Asia Magazine 9(3), 33–39.

Sim, S.Y., Montaldi, P., Montaldi, A. and Kongkeo, H. (2004). Grouper farming, market chains and prices of marine finfish in Indonesia. Aquaculture Asia Magazine 9(3), 39–42.

Sim, S.Y., Montaldi, P., Montaldi, A. and Kongkeo, H. (2004). Marine finfish markets in Hong Kong. Aquaculture Asia Magazine 9(3), 42–47.

Sim, S.Y., Suwirya, K. and Rimmer, M. (2007). Update: Marine finfish research and development at the Research Institute for Mariculture, Gondol, Bali, Indonesia. Aquaculture Asia Magazine 12(3), 34–35.

Sim, S.Y. and Williams, K. (2005). Feeds and feeding practices at farm level for marine finfish aquaculture in the Asia-Pacific region. Aquaculture Asia Magazine 10(1), 25–28.

Suwirya, K. (2004). Spawning and larval rearing of coral trout at Gondol. Aquaculture Asia Magazine 9(3), 48.

Suwirya, K. (2005). Spawning and larval rearing of coral trout at Gondol. SPC Live Reef Fish Information Bulletin 13, 45.

Suwirya, K. and Giri, N.A. (2005). Feed development and application for juvenile grouper. Aquaculture Asia Magazine 10(1), 34–35, 46.

Teitlebaum, A. (2007). Marine fish hatchery training for aquaculture technicians from the South Pacific Region, in Krabi CFRDC, Thailand. Aquaculture Asia Magazine 12(3), 36.

Thai, N.Q. (2005). Report on grouper hatchery training course in Indonesia. Aquaculture Asia Magazine 10(4), 34.

Tuan, L.A. (2005). Best practices for using trash fish in the culture of juvenile Malabar grouper, *Epinephelus malabaricus*. Aquaculture Asia Magazine 10(1), 40–41.

Usman, Rachmansyah, Laining, A. and Ahmad, T. (2005). Grouper grow-out feeds research at Maros Research Institute for Coastal Aquaculture, South Sulawesi, Indonesia. Aquaculture Asia Magazine 10(1), 42–45.

Usman, Rachmansyah, Palinggi, N.N. and Ahmad, T. (2007). Utilisation of local feed ingredients in tiger grouper grow-out moist diets. Aquaculture Asia Magazine 12(2), 33–36.

Williams, K.C. (2005). 'Groupers'. AquaFeeds: Formulation and Beyond 2(2), 20–24.

Williams, K.C., Smith, D.M., Williams, I.H., Irvin, S.H. and Barclay, M. (2005). ACIAR grouper grow-out feeds program and related CSIRO research. Aquaculture Asia Magazine 10(1), 29–33.

Reports and miscellaneous publications

Anon (2005). Report of the Study Program on Marine Aquaculture and Seafood Markets in Southern China (Guangdong and Hong Kong), July 4–15, 2005. 16 pp.

Anon (2006). End-of-training report to The Crawford Fund for training of Mr Ketut Suwirya and Mr Tatam Sutarmat (Research Institute for Mariculture Gondol, Indonesia) in marine fish aquaculture in Queensland and Tasmania, 26 February – 5 March 2006. 5 pp.

Anon (2007). Workshop Report – Development of Better Management Practices for Marine Finfish Aquaculture in the Asia-Pacific Region, Hotel Indra Puri, Lampung, Indonesia, 7–10 November 2007. 50 pp.

CABI (2005). CAB International Aquaculture Compendium: cultured aquatic animal data sheets for: *Cromileptes altivelis*, *Epinephelus akaara*, *E. coioides*, *E. fuscoguttatus*, *E. lanceolatus* by M. Rimmer. http://www.cabicompendium.org/ac/

FAO 2006. Cultured Aquatic Species Information Programme – *Lates calcarifer*. Text by Rimmer, M.A. In: FAO Fisheries and Aquaculture Department [online]. Rome.

Rimmer, M. and Kistle, S. (2004). Assessment of the use of coded wire tags (CWT) to trace coral trout (*Plectropomus leopardus*) in the live reef food fish trade. Report to the Asia-Pacific Economic Cooperation (APEC) endorsed project to develop Industry Standards for the Live Reef Food Fish Trade – a collaborative project of the Marine Aquarium Council and The Nature Conservancy. 6 pp.

Rimmer, M., Pirozzi, I. and Tebrett, S. (2009). Report on Fish Nutrition Workshop held at Santika Hotel, Surabaya, Indonesia, 20–22 October 2009. 14 pp.

Sim, S.Y. (2005). Report of the Grouper Hatchery Production Training Course 2005, Brackishwater Aquaculture Development Centre (BADC) – Situbondo, Indonesia, April 18 – May 8, 2005. 32 pp.

Sim, S.Y. (2006). The Report of the 4th Regional Grouper Hatchery Production Training Course 2006. Brackishwater Aquaculture Development Center (BADC) – Situbondo, Indonesia, Nov 20 – December 9, 2006. 32 pp.

Sim, S.Y. (2007). The Report of the 5th Regional Grouper Hatchery Production Training Course 2007, Brackishwater Aquaculture Development Center (BADC) – Situbondo, Indonesia, 9–29 July, 2007. 31 pp.

Teitelbaum, A. (2007). Report on 1st SPC Marine Finfish Hatchery Training Krabi CFRDC, Thailand, 14 May – 1 June 2007. 41 pp.

11 Appendixes

11.1 Appendix 1: Digestibility coefficients of some feed ingredients in two grouper species

Aquaculture expansion has increased the demand for fish feeds which mainly depend on fishmeal as the major dietary components due to their ideal nutritional quality. Due to rising demand, high cost and uncertain availability, studies have been carried out to partially or completely substitute fish meal in fish diets by other protein sources. The nutritional value of an ingredient is determined by its nutritional contents and nutrient availability, and therefore nutrient digestibility is an important part in the evaluation of feed ingredients. Published information on the apparent digestibility coefficients of dry matter, crude protein, crude fat and gross energy of various feedstuffs for groupers are listed here.

Species	Feedstuff	Feed Number	Dry matter	Crude Protein	Crude Fat	Gross Energy
Cromileptes altivelis [a]	Shrimp head meal	5-04-226	58.5	78.0		63.6
	Soybean meal	5-14-005	54.8	67.2		51.1
	Palm oil cake meal	5-04-487	45.3	80.5		40.4
	Dried blood meal	5-00-380	48.1	55.2		nd
	Blood meal (formic acid preserved)		67.9	87.5		nd
	Blood meal (propionic acid preserved)		61.7	84.2		nd
	Local sardine meal	5-02-015	87.2	92.5		85.2
	Local mixed-fish meal		59.1	82.4		77.2
	Rice bran meal	4-03-928	22.2	59.5		44.3
Epinephelus coioides [b]	Chilean Fish meal		83.6	98.0		
	Shrimp meal		76.0	95.0		
	Soybean meal defatted		75.7	96.0		
	White fish meal		89.2	98.8		
	White cowpea meal		74.2	93.5		
	Ipil-ipil leaf meal		56.0	78.8		
	Squid meal		99.4	94.2		
	Meat & bone meal		60.8	98.9		
	Blood meal (Australia)		36.9	15.4		
	Corn gluten meal		94.0	99.5		
	Tuna fish meal		75.4	76.2		
	Wheat flour		72.8	82.9		
	Corn germ meal		85.1	82.9		
	Lupin seed meal		54.1	97.5		

	Poultry feather meal		74.3	81.8		
Epinephelus coioides [c]	White fish meal	5-02-009	78.9	89.8	94.5	93.3
	Brown fish meal	5-01-985	79.1	87.3	92.4	89.5
	Soybean meal	5-04-604	69.9	84.0	93.1	70.5
	Peanut meal	5-03-650	73.7	80.8	90.7	73.1
	Yeast	7-05-527	57.7	61.1	82.8	51.7

[a] With reference diet and 1% Chromic Oxide (Laining et al. 2003; Laining et al. 2004).

[b] With reference diet at 70-30% and 1% Chromic Oxide (Eusebio et al. 2004a, Eusebio et al. 2004b).

[c] With reference diet at 70-30% and 0.5% Chromic Oxide (Lin et al. 2004).

12 References

Eusebio, P., Coloso, R.M. and Mamauag, R.E.P. (2004a). Apparent digestibility of selected feed ingredients in diets for grouper (*Epinephelus coioides*). In: Rimmer, M.A., McBride, S. and Williams, K.C. (eds.). Advances in Grouper Aquaculture. ACIAR Monograph 110. pp. 75-78. Australian Centre for International Agricultural Research, Canberra.

Eusebio, P., Coloso, R.M. and Mamauag, R.E.P. (2004b). Apparent digestibility of selected feed ingredients in diets for grouper (*Epinephelus coioides*) Hamilton. Aquaculture Research 35, 1261-1269

Laining, A, Rachmansyah, Ahmad, T. and Williams, K.C. (2003). Apparent digestibility coefficients of several feed ingredients for humpback grouper (*Cromileptes altivelis*). Aquaculture 218, 529-538.

Laining, A, Rachmansyah, Ahmad, T. and Williams, K.C. (2004). Apparent digestibility of selected local feed ingredients for humpback grouper (*Cromileptes altivelis*). In: Rimmer, M.A., McBride, S. and Williams, K.C. (eds.). Advances in Grouper Aquaculture. ACIAR Monograph 110. pp. 85-87. Australian Centre for International Agricultural Research, Canberra.

Lin, H., Liu, Y., Tian, L., Wang, J., Zheng, W., Huang, J. and Chen, P. (2004). Apparent digestibility coefficients of various feed ingredients for grouper, *Epinephelus coioides*. Journal of the World Aquaculture Society 35, 134-142.