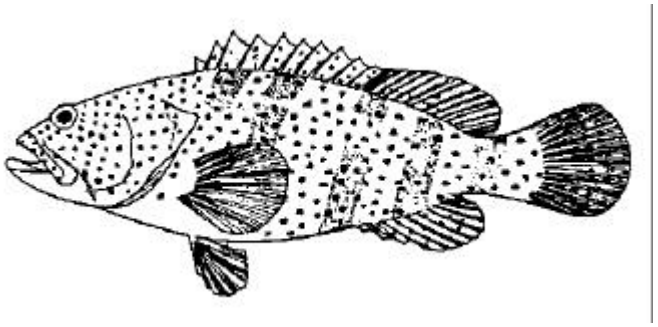
ACIAR Project FIS/97/73

Improved hatchery and grow-out technology for grouper aquaculture in the Asia-Pacific region

Annual Report: July 2000 – June 2001



Prepared by:

M.A. Rimmer[1], K.C. Williams[2], J.D. Toledo[3], K. Sugama[4], T. Ahmad[5], I. Rumengan[6] and M.J. Phillips[7]

[1] *Department of Primary Industries, Agency for Food and Fibre Sciences – Fisheries and Aquaculture, Northern Fisheries Centre, Cairns, Queensland, Australia*
[2] *Commonwealth Scientific and Industrial Research Organisation, Division of Marine Research, Marine Research Laboratories, Cleveland, Queensland, Australia*
[3] *Southeast Asian Fisheries Development Centre, Aquaculture Department, Tigbauan, Iloilo, Philippines*
[4] *Department of Ocean Affairs and Fisheries, Research Institute for Mariculture, Gondol, Bali, Indonesia*
[5] *Department of Ocean Affairs and Fisheries, Research Institute for Coastal Fisheries, Maros, Sulawesi, Indonesia*
[6] *Sam Ratulangi University, Manado, Sulawesi, Indonesia*
[7] *Network of Aquaculture Centres in Asia-Pacific, Bangkok, Thailand*

Executive Summary

Purpose and context of the project

Aquaculture of high value finfish species, such as groupers, is an industry of increasing importance throughout the Asia-Pacific region, including Australia. The development of large and affluent markets for live reef fish, particularly in Hong Kong and southern China, has increased pressure on wildstock resources. In many areas the demand for live reef fish, and the profitability of this trade, has encouraged overfishing and the use of destructive fishing practices, such as the use of sodium cyanide to 'stun' reef fish for capture by divers. Aquaculture of high value reef fish species can potentially supply product to the live reef fish markets, as well as other regional and domestic markets. The development of aquaculture technology for these species will not only support an economically beneficial aquaculture sector, but will also contribute to reducing pressure on wild stocks. Currently, the major bottlenecks to increased aquaculture production of groupers are the generally poor, and highly variable, survival in larviculture, and the limited sources of trash fish for grow-out. The ACIAR project addresess these issues by collaborating with research and development organisations in Indonesia and the Philippines to carry out priority grouper research to improve larviculture and to develop cost-effective grow-out diets of low fish content. An additional objective of the project is to support, through the Network of Aquaculture Centres in Asia-Pacific (NACA), more effective dissemination of research results arising from the project activities, and to promote greater collaboration and information exchange among centres in Asia involved in grouper aquaculture research and development. This objective is being addressed through an interactive grouper web page and an electronic newsletter for dissemination of information

Names of collaborating researchers and institutions

- Dr Mike Rimmer, Department of Primary Industries, Agency for Food and Fibre Sciences – Fisheries and Aquaculture, Northern Fisheries Centre, Cairns, Queensland, Australia.
- Dr Kevin Williams, CSIRO Division of Marine Research, Cleveland, Queensland, Australia.
- Mr Joebert Toledo, South-east Asian Fisheries Development Centre, Aquaculture Department, Iloilo, the Philippines.
- Dr Ketut Sugama[1], Research Institute for Mariculture[2], Gondol, Bali, Indonesia.
- Dr Taufik Ahmad[3], Research Institute for Coastal Fisheries, Maros, Sulawesi, Indonesia.
- Dr Inneke Rumengan, Sam Ratulangi University, Manado, Sulawesi, Indonesia.
- Dr Michael Phillips, Network of Aquaculture Centres in Asia-Pacific, Bangkok, Thailand.

Notes:

1. Dr Ketut Sugama was promoted to Director of Aquaculture for the newly-formed Central Research Institute for Aquaculture in early 2000. He has now moved to Jakarta but remains the nominated project leader for RIM Gondol activities.
2. Gondol has been upgraded from Research Station to Research Institute status, and has been renamed the Research Institute for Mariculture.

3. Due to health problems, Dr Taufik Ahmad has moved to Bogor, but remains the nominated project leader for RICF Maros activities.

Results / expected results

Larval rearing

Research on pre-feeding larvae at SEAFDEC with *Epinephelus coioides* and at RIM Gondol with *Cromileptes altivelis* has demonstrated that survival of the egg and early larval stages of both species can be improved by optimising environmental variables such as temperature, salinity, aeration, and light levels. These results provide valuable information on optimal incubation conditions for grouper larvae that contribute to an overall improval in larval survival.

Larval nutrition research at SEAFDEC has elucidated patterns of fatty acid conservation in larval grouper (*E. coioides*) which provides an indication of the essential fatty acid requirements of this species. Further work will be aimed at developing larval diets (using enrichment of live prey organisms and larval artificial diets) to provide suitable levels of the identified fatty acids.

Research at SEAFDEC has for the first time described the development of the digestive tract in larval groupers (*E. coioides*) which is fundamental to evaluating the capacity of the larvae to digest both live and artificial feeds. In conjunction with this component, work at NFC has developed highly sensitive fluorescent techniques for assessing the levels of digestive enzymes in the gut of fish larvae. Results have shown that grouper (*E. coioides*) larvae have very low levels of digestive enzymes (e.g. protease) compared to some other species of fish larvae that have been examined (e.g. barramundi *Lates calcarifer*).

Verification trials at SEAFDEC and at RIM Gondol have demonstrated improved larval survival – up to 20% survival to D25 at SEAFDEC (*E. coioides*) and up to 50% survival to D50 (*C. altivelis*) at RIM Gondol. However, the viral disease viral nervous necrosis (VNN) continues to cause major mortalities in hatchery-reared grouper and remains a major limiting factor in successful seed production.

An additional component on selective breeding of SS-strain rotifers (*Brachionus rotundiformis*) was added to the project during 2000–2001. This component will focus on the development of techniques to reduce the overall size of rotifers used for larval rearing of groupers, to provide better efficiencies for grouper hatcheries.

Grow-out diet development

Research to determine the apparent digestibility (AD) of selected and locally available feed ingredients for use in grouper diets has continued at SEAFDEC and RICF Maros. *E. coioides* was used at SEAFDEC while the species used at Maros was *C. altivelis*. At SEAFDEC, the protein of Australian meat and bone meal, tuna fishmeal and gluten was found to be well digested (ADs >76%) whereas the protein digestibility of Australian blood meal was very low (15%). At Maros, oven dried blood meal was found to have a low protein AD (55%), similar to that of rice bran (60%) while better digestibility was observed for soybean meal (67%), shrimp head meal (78%), palm oil cake meal (81%) and local (82%) and imported sardine (93%) fishmeal. Fermentation of blood using organic acids resulted in protein digestibility improving to ADs >84%.

Two nutrient retention growth assay experiments were carried out to examine the protein and protein to energy requirements of *C. altivelis* fingerlings. At Gondol, three protein levels (44, 50 and 56%) were factorially arranged on three lipid levels (6, 9 and 12%) and these diets fed to satiation twice daily to fish (~5 g) for 12 weeks. At CSIRO, five protein levels (serial increments between 41 and 62% DM) were factorially combined with two lipid levels (15 and 24% DM) and the diets fed to satiation twice daily to fish (~12 g) for 8 weeks. In both experiments, fish growth rate improved with increasing protein content of the diet whereas the only response to increasing dietary lipid was an increased deposition of fat without any improvement in growth or food conversion efficiency. The absence of any enhancement of growth upon addition of lipid in the diet differs markedly to the protein sparing response observed with salmonids and different to that observed with Asian seabass where some protein sparing has been observed. These findings need to be confirmed with other grouper species.

In other studies, the suitability of various local and imported protein meals as partial substitutes of fishmeal in practical grouper grow out diets was examined at SEAFDEC and Maros. This work is showing that many terrestrial protein meals have potential as partial replacements for fishmeal in grouper grow-out diets although non-fermented blood meal and shrimp head meal appear to have little value.

Asia-Pacific Grouper Network

Membership of, and interest in, the Asia Pacific Grouper Network continues to grow. The electronic grouper newsletter, developed to facilitate information exchange within the network, has been extremely popular and now has over 230 subscribers. The APGN web site and the ACIAR Grouper Project web site have both been moved to a new server in the US, which allows faster and more reliable access.

Strong linkages have been developed with the APEC Fisheries Working Group and several related activities are being supported by APEC funding, including staff exchanges to promote collaborative research.

Likely direction of future research

Larval rearing

Future work will continue to investigate the digestive physiology of grouper larvae, including development of the digestive tract and ontogeny of enzymes. Additional research on larval nutrition will continue to develop enrichment techniques for live prey organisms that will allow the incorporation of essential fatty acids in the diet, and will examine the effects of these diets on larval growth and survival.

Larval rearing methods will continue to be refined to improve larval survival and growth. The impacts of these improvements will be evaluated using the economic models developed for this project.

Grow-out diet development

The focus of the research will remain largely unchanged with work being carried out to determine the AD of ingredients and to examine the usefulness of alternative

terrestrial feed ingredients as fishmeal substitutes. Studies are planned to examine fermented blood products, dehulled lupin meal and meat and bone meal as partial substitutes of fishmeal in practical diets for grouper grow-out. Further research on how dietary lipid is metabolised in *C. altivelis* and other grouper species are planned to see if better use can be made of dietary lipid as an energy source and to spare dietary protein.

Asia-Pacific Grouper Network

The activities of the Asia-Pacific Grouper Network will be continued, particularly in conjunction with the APEC Collaborative Grouper R&D Network project. Regional workshops will continue to be held at regular intervals, and this series will incorporate the ACIAR end-of-project workshop which is planned to be held in Singapore in September 2002. NACA will continue to coordinate the overall grouper R&D program, based on the outline developed in this project.

The Electronic Grouper Newsletter will be continued, since this is an increasingly popular mechanism for information dissemination. The ACIAR project web site and the NACA grouper web site will be expanded.

APEC has committed to support additional small research topics of relevance to the ACIAR project, including the development of the grouper virus research project, and additional work aimed improving research collaboration and extending the results to farmers and project seeking to improve coastal livelihoods through aquaculture.

Key to abbreviations and acronyms

AAHRI	Aquatic Animal Health Research Institute (Bangkok, Thailand)
AIAT	Assessment Institute for Agricultural Technology
ACIAR	Australian Centre for International Agricultural Research
AFFA	Agriculture, Forestry and Fisheries Australia
AFFS – F&A	Agency for Food and Fibre Sciences – Fisheries and Aquaculture (DPI)
AIMS	Australian Institute for Marine Science
APD	apparent protein digestibility
APEC	Asia-Pacific Economic Cooperation
APGN	Asia-Pacific Grouper Network
ARA	arachidonic acid (20:4n-6)
ARC	Australian Research Council
AusAID	Australian Agency for International Development
BOBP	Bay of Bengal Program
CARD	Capacity-Building for Agriculture and Rural Development
CRD	completely randomised design
CRIA	Central Research Institute for Aquaculture (Indonesia)
CSIRO	Commonwealth Scientific and Industrial Research Organisation
DFID	Department for International Development (United Kingdom)
DHA	docosahexaenoic acid (22:6n-3)
DKP	Departemen Kelautan dan Perikanan (Department for Ocean Affairs and Fisheries – Indonesia)
DPI	Department of Primary Industries (Queensland)
EPA	eicosapentaenoic acid (20:5n-3)
FAO	Food and Agriculture Organisation of the United Nations
FWG	Fisheries Working Group (APEC)
GC	gas chromatograph
HUFA	highly unsaturated fatty acids
JCU	James Cook University of North Queensland
NACA	Network of Aquaculture Centres in Asia-Pacific
NICA	National Institute of Coastal Aquaculture (Songkla, Thailand)
NFC	Northern Fisheries Centre (Cairns, Queensland, Australia)
PSRC	Port Stephens Research Centre (NSW Fisheries)
PUFA	polyunsaturated fatty acids
R&D	research and development
RICF	Research Institute for Coastal Fisheries (Maros, Sulawesi, Indonesia)
RIM	Research Institute for Mariculture (Gondol, Bali, Indonesia)
S- / SS-	small / super-small strain rotifer
SEAFDEC AQD	South-east Asian Fisheries Development Centre, Aquaculture Department (Tigbauan, Philippines)
TNC	The Nature Conservancy
TVP	Technology Verification Program (SEAFDEC)
UoF	University of Fisheries (Nha Trang, Vietnam)

Progress of Research Work

Project Objectives

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

1. Larval rearing of groupers

The objective of this component of the research is to improve growth and survival of groupers during the hatchery phase.

The research is concentrating on developing a better understanding of the capacity of grouper larvae to digest various live prey organisms, and the nutritional composition that must be provided by live prey. This information is being used to assess the suitability of different live prey organisms at different stages of the larval rearing process, and to develop improved nutritional profiles for live prey organisms. Direct enhancement of larval nutrition, using artificial diets, is also being examined. These results will be integrated with other studies on environmental factors affecting grouper larvae to develop an improved methodology for larval rearing of groupers.

2. Diet development for on-growing of grouper

The objective of this component is to develop compounded feeds for grouper grow-out that have low environmental impact, have a low content of fishery resource, and are as cost-effective for the on-growing of grouper as the alternative of using trash fish.

This is being addressed in a structured way, acquiring nutritional information on feeds available for diet manufacture, characterising the requirements of groupers for key nutrients and demonstrating the cost effectiveness of the compounded feeds. The research plan recognises that grow-out nutrition work in Australia can only be done subsequent to the successful larval rearing of the fry but this constraint does not apply for the overseas collaborators where collection of fry from the wild is permitted.

3. Support for the Grouper Aquaculture Research and Development Program

The objective of this component is to 'value add' existing grouper aquaculture R&D efforts in the Asia-Pacific region by improving communication and promoting collaborative research between regional laboratories and agencies.

NACA, in cooperation with participating institutions, has prepared a cooperative grouper aquaculture research and development program based on the recommendations and specific research detailed in the proceedings of the Grouper Aquaculture Workshop held in Bangkok in April 1998, and more recent workshops held in Hat Yai (Thailand) and Medan (Indonesia). The program will be circulated to respective institutions to seek institutional support and commitment. NACA, in cooperation with participating institutions, will continue to seek funding support for specific projects under the Grouper Aquaculture Research and Development Program, with particular

emphasis on the development of collaborative research and development projects. NACA is facilitating enhanced communication amongst grouper aquaculture researchers by pursuing reports of research findings from participating institutions, and compiling and publishing this information in regional aquaculture magazines, and on the NACA grouper web site.

Research

Adherence to timetable / staff engaged

The timetable has generally been maintained at all institutions with the following exceptions:

DPI

Delays in constructing the new Aquaculture and Stock Enhancement Facility in Cairns have restricted the available facilities for experimental work on groupers. In particular, the relatively small number of grouper broodstock at Northern Fisheries Centre has constrained the availability of eggs and larvae for larviculture experiments. Consequently, we have not been able to go ahead with the larval rearing experiments planned at NFC.

To compensate, additional funding was sought and received for Dr Shannon McBride (DPI project biologist) to visit RIM Gondol and SEAFDEC in April 2001. The objective of this visit was for Shannon to initiate and participate in collaborative research utilising grouper larvae being reared at both partner laboratories. The trip was very successful, and additional collaborative experiments are currently underway.

CSIRO

The lack of availability of grouper fingerlings in Australia has required the importation of fingerlings from the Research Institute for Mariculture in Gondol, Bali, Indonesia, under quarantine restrictions. This has caused minor delays in regard to some of the planned grow-out nutrition research.

SEAFDEC AQD

Delays in obtaining chemicals in the Philippines has led to some delays in a few activities, most notably those related to documenting the development of the digestive system and the ontogeny of digestive enzymes. In particular, the difficulty in obtaining knives for the SEAFDEC cryotome has delayed the commencement of the work on the localisation of digestive enzymes in grouper larvae.

None of these delays have budgetary implications for the project.

With the additional funding provided for selective breeding of SS-strain rotifers, an ACIAR-funded research assistant, Mr Erly Kaligis, has been appointed at Sam Ratulangi University, Manado, Sulawesi, Indonesia. He is currently running experiments to determine the optimal feed density, salinity and development rate fully of the NFC SS-strain rotifer.

Methodology and Principal Experiments / Analyses

1 Project administration

1.1 Project meetings

The second project meeting was held Cairns, Queensland, Australia, on 24–25 July 2000. The project meeting was attended by representatives from all the participating research institutions, and by representatives of other agencies who are involved in collaborative research which interacts with the ACIAR Grouper Project.

Names	Country	Agency / Centre
Mike Rimmer, Elizabeth Cox, Richard Knuckey, Shannon McBride, Abigail Elizur, Bill Johnston	Australia	DPI (Northern Fisheries Centre and Bribie Island Aquaculture Research Centre)
Kevin Williams, Simon Irvin	Australia	CSIRO Division of Marine Research
Ketut Sugama, Adiasmara Giri	Indonesia	CRIFI –Research Station for Coastal Fisheries, Gondol, Bali
Taufik Ahmad	Indonesia	CRIFI – Research Institute for Coastal Fisheries, Maros, Sulawesi
Joebert Toledo	Philippines	SEAFDEC Aquaculture Department, Iloilo, Philippines
Jerome Bosmans	Australia	NT DPIF, Darwin Aquaculture Centre
Peter Appleford	Australia	James Cook University, Aquaculture Department
David McKinnon	Australia	Australian Institute of Marine Science
Nguyen Dinh Mao, Le Anh Tuan	Vietnam	University of Fisheries, Nha Trang, Vietnam
Cathy Hair	Solomon Islands	ICLARM

In conjunction with the ACIAR Grouper Project meeting, DPI hosted a Reef Fish Aquaculture Symposium in Cairns on 26 July 2000. The symposium was opened by the Queensland Minister for Primary Industries and Rural Communities, Mr Henry Palaszczuk, and provided an opportunity for the Australian aquaculture industry to hear about the results of DPI's Reef Fish Aquaculture Project as well as the ACIAR Grouper Project. The symposium was well attended by over 60 industry, research and government representatives from throughout Australia. Details of the symposium are given in section 4.3 of this report.

Figure 1 ACIAR Project Meeting participants during the field trip to inspect Barramundi Waters barramundi farm, Innisfail, Queensland.

The next project meeting will be held at SEAFDEC AQD, Tigbauan, Iloilo, Philippines in July 2001.

1.2 Training

Mr Ketut Suwirya (RSCF, Gondol) spent 3 weeks (June 2000) at CSIRO Marine Research Cleveland laboratory for training in lipid class analysis. The training was provided by Ms Margaret Barclay, analytical chemist at CSIRO Cleveland laboratory. Eighteen samples of *Artemia* from an enrichment experiment at NFC Cairns were analysed for total lipid (chloroform-methanol extraction) and fatty acids following methyl esterification and quantification using HPLC procedures. Training was also provided in phospholipid analysis using HPLC procedures. Fatty acid analysis was also carried out on lipid extracts of grouper feeds brought from Gondol by Ketut Suwirya.

Mrs Reni Yulianingsih undertook training in HPLC chemical analysis techniques at the Bogor Agricultural Institute, Bogor, Indonesia.

Additional training is planned for 2001–2002, as listed in the table below:

Laboratory	Aspects	Candidate	Time	Place
SEAFDEC AQD	Enzyme biochemistry – fluorometric techniques	Gerry Quinitio	late 2001	DPI–NFC
RIM Gondol	Nutritional analysis – GC	Ketut Suwirya	(completed)	CSIRO Cleveland
RICF Maros	Nutrition research methodology	Asda Laining	September 2001	CSIRO Cleveland
	Grow-out nutrition / chemical analysis	Neltje Palinggi	September 2001	(TBA)
	Chemical analysis – HPLC	Reni Yulianingsih	(completed)	Bogor Ag. Institute

1.3 Calibration exercise

The calibration exercise was completed in the first year of the project. Details are given in the first annual project report (July 1999 – June 2000).

1.4 End-of-project workshop

The end-of-project workshop is tentatively planned for Singapore in September 2002, in conjunction with a major cage aquaculture workshop to be held by the Singapore Primary Production Department. Singapore PPD is yet to agree to the joint meeting, and finalisation of the proposed arrangement is to be negotiated by NACA.

2 Larval rearing

2.1 Pre-feeding larvae / environmental factors

This component of the research has focused on determining optimal conditions for grouper larvae during the egg and early larval stages. This is to ensure that newly-hatched larvae are provided with optimal environmental conditions prior to the commencement of first feeding, to improve survival during the early larval rearing stages.

Research at SEAFDEC has shown that the optimal conditions for *E. coioides* larvae are:

	Eggs	Early stage larvae
Density	400 eggs/litre	–
Aeration	100 ml/min	25–50 ml/min
Salinity	>32 ppt	16–24 ppt
Light level	–	500–700 lux

Similar experiments carried out with *Cromileptes altivelis* at RIM Gondol have shown that egg density, water exchange rate and aeration rate all affected time to hatching, hatching rate and survival of newly hatched larvae. The best hatching rate was observed at an egg density of 500 eggs / litre (77%), water exchange rate of 200% per day (71.6%), and an aeration rate of 600 ml/min (78.7%). An aeration rate of 600 ml/min also resulted in the best survival rate (62.3%) for D3 larvae.

A separate experiment compared the effects of temperature (25, 28, 31°C, plus control [ambient temperature]) on growth, feeding activity and survival of early-stage *C. altivelis* larvae. The best growth was achieved at a temperature of 31°C. Survival of larvae ranged from 4.77 to 48.11% and the highest survival was exhibited by larvae reared at 28°C. The highest feeding rate was exhibited by larvae reared at 28 and 31°C. Based on these results, the optimal temperature for the early larval rearing of *C. altivelis* is 28°C. Details of these experiments are provided in Appendix 1.

These experiments have been carried out in static hatching tanks. Similar experiments will be repeated in flow-through tanks at NFC later in the project using *E. fuscoguttatus* eggs and larvae.

Research into larval rearing techniques at NFC has been constrained by the poor spawning performance of broodstock of both *E. fuscoguttatus* and *C. altivelis*. The *E. fuscoguttatus*, which have demonstrated a very short spawning season (2–4 weeks

per year) are now in photothermally controlled tanks where they will be cycled through a 120-day photothermal cycle. Hopefully, this will allow for multiple spawnings per year even with the short spawning season exhibited by this species. Male *C. altivelis* held at NFC have demonstrated poor gonadal development and many have reverted to female. This issue is now being addressed through a DPI-funded study into sex change and gonadal maturation in groupers.

2.2 Larval nutrition

2.2.1 Nutritional composition of live feeds

This component of the research aims to improve larval survival by providing live prey of better nutritional value for larval rearing. In particular, fatty acid and vitamin composition of live prey organisms will be examined.

Research at SEAFDEC has investigated the fatty acid and lipid class composition of:

1. Phytoplankton and yeast: *Chlorella vulgaris*, *Isochrysis galbana*, *Nannochloropsis oculata, Tetraselmis tetrahele*, *Chaetoceros calcitrans*, *Thalassiosira pseudonana*, *Chlorella*-like (Oton, Iloilo), and yeast (Bactoagar–DIFCO) was used.

2. Rotifers: starved or enriched with phytoplankon or with various n-3 HUFA enrichment products.

3. *Artemia* nauplii: unenriched newly hatched *Artemia* nauplii and *Artemia* nauplii starved or enriched with various n-3 HUFA boosters.

4. Copepods and brackishwater cladocerans: *Pseudodiaptomus annandalei* fed with *Tetraselmis chuii*, Isocrysis, *Chlorella vulgaris* and *Chaetoceros calcitrans*. *Pseudodiaphanosoma celebensis* cultured on *Tetraselmis chuii*, cow dung and rice bran.

Data obtained to date are presented in Appendix 1. Work to assess the nutritional effect of n-3 HUFA enrichment products is on-going and these products will probably be necessary to get the high DHA levels and DHA:EPA ratios that grouper larvae are likely to require.

2.2.2 Nutritional requirements of grouper larvae

To determine the patterns of conservation and loss of neutral and polar lipid class composition and fatty acid in grouper larvae, samples of eggs and larvae at different developmental stages have been collected and analysed for lipid class and fatty acid composition.

1. Egg to larva

Results showed that neutral lipids (NL) are the major energy sources in egg and newly hatched larvae. Unfed larvae at day 2.5 and 4 conserved polar lipid (PL) fatty acids and primarily spent NL for energy. In neurula egg, DHA:EPA: ARA ratios of 2.6: 1.4: 1 were found in PL while 2.6: 0.7: 1 ratios were found in NL. NL EPA was depleted at day 4 while DHA was highly conserved in PL.

2. Fed and starved grouper larvae

With continuous feeding, both NL and PL increased with time. Fed larvae consistently contained higher NL than PL whereas starved larvae retained more PL than NL. Starvation for three days resulted to very low larval NL and PL contents. ARA, EPA and DHA were conserved more in PL than in NL.

3. Wild grouper 'tinies' (wild-caught grouper juveniles)
PLs were consistently higher than NLs during the whole starvation period. NL was primarily used for energy. NL and PL DHA was lost after a week of starvation (Table 4 and 5). Except for DHA, PL fatty acids were highly conserved.

Details of the results are given in Appendix 1. These results will be integrated with those obtained from activity 2.2.1 (nutritional composition of live feeds) to develop nutritional profiles that match as closely as possible the nutritional requirements of the larvae.

2.2.3 Natural and artificial diets

This work is ongoing, and is integrated with the larval rearing research. RIM Gondol in particular has had good success in rearing larvae of *C. altivelis* using commercial larval artificial diets in conjunction with live prey. See Appendix 1 for details.

2.3 Development of the digestive tract and enzymes

This component of the research aims to add substantially to our knowledge of the ability of fish larvae to utilise various prey types. It complements earlier work on the physical constraints (in particular, small mouth size) of grouper larvae at first feed which limit their ability to ingest many prey types.

2.3.1 Histology

The larval development of *E. coioides* has been documented at SEAFDEC using histological samples photographed using an image analyser (see Appendix 1 for further details). Histological samples of *C. altivelis* from RIM Gondol are waiting analysis at NFC.

2.3.2 Digestive enzymes - qualitative

This work has been delayed because of technical problems with chemical supply to the Philippines and problems in getting new blades for the SEAFDEC cryotome. The work will focus on localising the activity of various enzymes in larval groupers, principally *E. coioides*.

2.3.3 Digestive enzymes - quantitative

Work to date in this component of the research has focussed on technique development. Research at SEAFDEC and at NFC is proceeding along parallel lines, using slightly different analysis techniques. While SEAFDEC researchers are using established photometric procedures, NFC researchers are developing fluorimetric analysis techniques to measure digestive enzyme levels in fish larvae. The advantage of the latter approach is that only small samples (2–20 larvae) are needed, rather than the larger samples (thousands of larvae) required for photometric techniques.

As summarised below, most of the biochemistry for assaying digestive enzyme activity is now resolved. The major limitation is still access to suitable fluorescent probes and their cost.

Enzyme	Status	Comments
Total Protease	Completed	Comparison to a control and expressed as a % change in fluorescent units.
Trypsin	Completed	Using a trypsin inhibitor (TLCK) in the total protease assay. Also photometric assay.
α–amylase	Completed	Method based on standard curve.
Lipase (bile-salt-dependent)	Completed	Photometric assay completed. Standard curve not available; will have to use extinction coefficients. Substrate available for fluorescence but is very expensive ie. $500/mg
Aminopeptidase (L-leucine)	Not started	Substrate available. $300/mg

Details of these assays are provided in Appendix 1.

To date, much of the technique development work has been done with barramundi (*Lates calcarifer*). The enzyme activities in larval barramundi reared in ponds at OVL were found to be higher than their tank-reared counterparts. However, the development of the pond reared larvae was also much more advanced at the same age. It would be expected that digestive enzyme capacity would be greater in larger larvae. The investigation of diurnal and post-prandial changes in digestive enzyme activities in barramundi larvae is continuing.

Initial analyses of total protease activity in rotifers (*Branchionus rotundiformis*) and copepods (*Acartia* sp.) have been completed. These results indicate that the early feeding stages of the nauplii (n3–n4) have the highest activity (6.7 mU trypsin/min/nauplii). The early non-feeding stages had negligible activity. The total protease activity in the rotifers appears to be much lower (0.03mU trypsin/min/rotifer) in comparison to the n3-n4 copepod nauplii. The low level of protease activity in live prey organisms contradicts suggestions that marine fish larvae obtain a major proportion of their digestive enzymes from exogenous sources, particularly the live prey that they consume. This work will be repeated, with particular attention on the methodology and to confirm the negligible activity found in the rotifers and non-feeding copepod nauplii stages.

Shannon McBride's visit in April to the collaborating laboratories (RIM Gondol and SEAFDEC) was very productive. Techniques developed at NFC for collecting larvae and processing for enzyme analyses were demonstrated to staff at both centres. Mr Ketut Suwirya was keenly interested in learning the techniques. There is already a 96-well plate reader at RIM Gondol capable of reading both absorbance and fluorescence. With the purchase of appropriate filters, the staff at the centre would be able to perform a number of different assays. Ms Perla Eusebio has already established the techniques for her work at SEAFDEC. However, there was an opportunity to demonstrate the use of a 96-well plate reader for use in her assays, particularly for protein determination. The use of the plate reader will reduce the amount of chemicals needed to undertake these analyses.

Another purpose of the visit to both RIM Gondol and SEAFDEC was to sample *C. altivelis* and *E. coioides* larvae respectively. Ms Eusebio (SEAFDEC) had already collected a number of samples to be used as a comparison between the two laboratories. Further samples of *E. coioides* from age D1 to D10, including a diurnal series, were collected. These samples should provide a good picture of the ontogeny of the digestive enzymes in this species from first feeding, transition to *Artemia* and possibly through to weaning. Older *C. altivelis* larvae (D10–D18) were sampled at Gondol, and the samples returned to Australia for subsequent analysis.

Another purpose of the visit to both RICF Gondol and SEAFDEC was to sample *Cromileptes altivelis* and *Epinephelus coioides* larvae respectively. Initial results from day 12 post hatch *E. coioides* larvae demonstrated a diurnal fluctuation of enzyme activity (Fig. 2). The percentage feeding frequency also altered diurnally. As would be expected, there was little to no feeding at night and maximum feeding frequencies occurred by late afternoon. Maximum rotifer intake by *E. suillus* larvae aged day 14, has been reported to occur between 2 – 3 pm (Duray 1994).

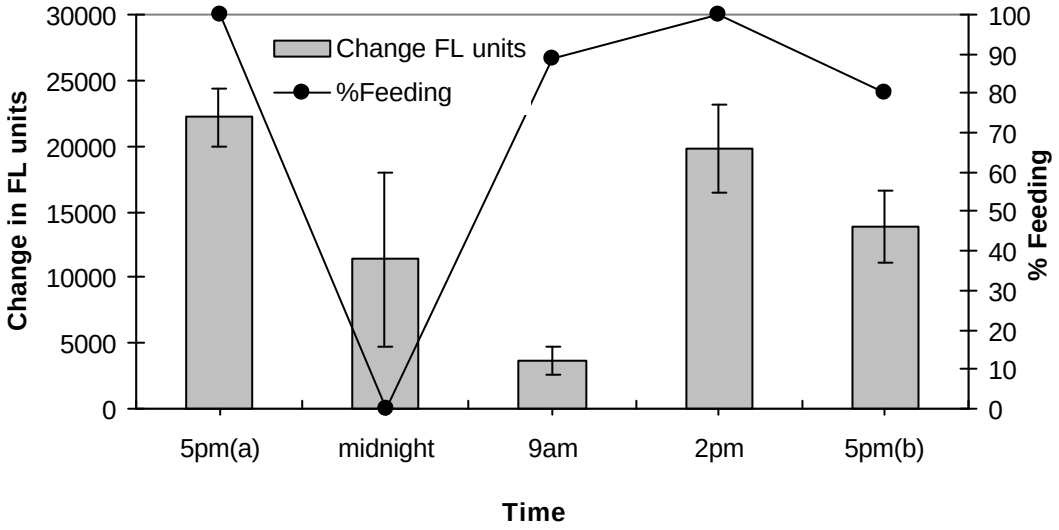


Figure 1 Total protease activity (measured as change in FL units) and feeding incidence, in day 12 post-hatch E. coioides larvae over 24 hours.

Initial results from *E. fuscoguttatus* larvae have indicated that the highest activities for total protease occur in the late afternoon in comparison to early morning.

2.4 Verification – larval rearing

2.4.1 Intensive larval rearing

Details of intensive larval rearing procedures were provided in the 1999–2000 annual report and these have not changed substantially since then.

2.4.2 Semi-intensive larval rearing

The objectives of this component are to:
1. Improve the present protocol for semi-intensive seed production of grouper in tanks by verification of research results.
2. Examine the economic viability of semi-intensive seed production of grouper in tanks and earthen ponds.

Following the best fertilization scheme, determined last year, ponds will be prepared for zooplankton production. About a week after filling up the ponds, one- or two-day old grouper larvae will be stocked at 0.25, 0.50 or 1.0 million larvae/ha. To sustain copepod nauplii production in semi-intensive larval tanks, adults and copepodids of *Acartia* and/or *Pseudodiaptomus* will be added 3 days before stocking of larvae and every week thereafter until Day 17. Copepods mass-produced from ponds or tanks will be added into the larval tanks daily from Day 25 until harvest to minimize the use of *Artemia*. Food abundance, larval growth, and gut content of the larvae will be monitored every 3 days until harvest (completion of metamorphosis). Fry to fingerling production in concrete tanks or in net cages set in ponds will be developed using either fish bycatch or SEAFDEC formulated diet for carnivorous fish. Economic analysis to estimate production cost for copepods, grouper fry and fingerlings will be done.

Results to date have been positive, with the exception of the occurrence of VNN in several batches of larvae. Newly-hatched grouper larvae were stocked in 2 to 6 units 5-ton tanks at 50,000 larvae/tank. Larvae were fed copepod nauplii and enriched rotifer from 3 to 15 days post hatching. From Day 15 onwards, separate tanks were fed either *Artemia* alone or a mixture of *Artemia* and pond-grown copepods. Several larval rearing runs indicate comparable survival rates after Day 25 (more than 20%). However, massive mortality always occurred from Day 22 onwards. Histological and tissue culture analysis indicates the presence of VNN in moribund samples examined.

Separate larval rearing runs were conducted to provide larval samples for studies on the development of digestive tract and digestive enzymes as well as for experiments on larval nutrition.

2.5 Selective breeding of SS-strain rotifers

In March 2001 ACIAR approved an extension proposal entitled 'Development of super-small strain rotifers for finfish aquaculture in the Asia-Pacific region'. The proposal is incorporated in the ACIAR grouper project and will operate until its conclusion in December 2002. The rotifer proposal developed following an APEC funded visit to NFC in September 2000 by Dr Inneke Rumengan, Sam Ratulangi University, Manado, Sulawesi. During this visit Dr Rumengan worked with NFC live-feeds staff to investigate methods to reduce the average size of the NFC SS-strain rotifer.

In May 2001 Dr Richard Knuckey (NFC) visited Dr Rumengan to discuss the rotifer proposal and the work already carried out on the NFC SS-strain rotifer by her students (Appendix 5). We were fortunate to have Mrs Tida Pechmanee, National Institute of Coastal Aquaculture, Songkhla, Thailand attend the Manado meetings. Mrs Pechmanee has many years of experience in all aspects of live prey production. During these meetings, factors that influence rotifer size were identified and a

sequence of experiments planned to measure the potential of each factor to reduce the average body size of a rotifer population. An ACIAR-funded research assistant, Mr Erly Kaligis has been appointed at Sam Ratulangi University. He is currently running experiments to fully characterise the NFC SS-strain rotifer. During these experiments, optimal feed density, salinity and development rate will be determined.

Laboratory space at Sam Ratulangi University is very limited making large scale culture of rotifers impossible. Dr Rumengan attended the annual ACIAR meeting at SEAFDEC where RIM Gondol kindly agreed to make their facilities available for large-scale experimental work. In October 2001 Dr Rumengan, Dr Knuckey and Mrs Semmens (NFC) will travel to RIM to undertake a set of experiments looking at the effect of diet particle size of the growth rate and final body size of a population of rotifers.

3 Grow-out nutrition

3.1 Inventory of feed ingredients

This activity was completed in the first year of the project and full details were provided in the 1999–2000 Annual Report.

3.2 Nutritional composition

3.2.1 Chemical analyses of feed ingredients in South Sulawesi

This activity was completed in the first year of the project and full details were provided in the 1999–2000 Annual Report.

3.2.2 Digestibility of key ingredients

At SEAFDEC, protein digestibility studies have been carried out with *E. coioides*. ADMD of gluten meal was highest among the feed ingredients tested followed by tuna fish meal and imported meat and bone meal. However, ADMD and APD of blood meal from Australia were lowest among the feed ingredients tested. Further, the APD value for the imported meat and bone meal was comparable with that of gluten meal but significantly higher than that of tuna fish meal. The growth performance of fish fed locally available meat and bone meal, HP 300, meat and bone meal from Australia, and locally available gluten meal and tuna fish meal were comparable with that of the control. Fish fed blood meal and Protamino Aqua-based diets had the poorest growth performance based on specific growth rate (SGR). No significant effect on survival was observed among fish fed diets containing the test ingredients. The present findings suggest that ADMD and APD can be used as indicators to determine the nutritional value of feed ingredients tested. Also, all feed ingredients tested except blood meal and Protamino Aqua can be used as protein sources to replace 16-31% of grouper diet without affecting their growth. Further details are provided in Appendix 1.

At RICF Maros, digestibility studies are being carried out with *C. altivelis* which are sourced from RIM Gondol, using locally available feed ingredients. Apparent digestibility data for the test ingredients are presented in Appendix 1. Digestibility rate of dry matter ranged from 22.7– 86.4% and relatively lower for plant ingredients compared to animal ingredients. The highest digestibility observed was for fishmeal (sardines: 86.4%) and the lowest in rice bran (22.7%). It appears from these results

that humpback grouper can more effectively digest the dry matter from animal than from plant ingredients.

Digestibility coefficient of protein is relatively high for all ingredients except blood meal (only 55.2%) and rice bran (59.5%). Fortunately, the digestibility rate of blood meal could be increased up to 87.5% through fermentation. The digestibility rate of energy varied in all ingredients, ranging from 40.4–85.2% with the highest rate for sardine fish meal and the lowest for palm oil cake. The digestibility rate for 3 other types of blood meal could not be computed due to insufficient sample mass.

3.3 Nutritional requirements

3.3.1 Protein; P:E

At RIM Gondol, nutrition work has focussed on the protein and lipid requirements of *C. altivelis*, using experimental diets containing three protein levels (44, 50 or 56%) and three lipid levels (6, 9 or 12%) each. Results of the experiment showed that the dietary protein level significant affected final weight, percent weight gain, total length, feed efficiency, and lipid retention. However, the effect of lipid level was significant only for lipid retention. Interaction between these two factors was significant only for final weight and weight gain. At the dietary lipid level of 9%, increasing level of dietary protein significantly increased the weight gain of the fish, and the highest weight gain was found at 56 % dietary protein. Increasing the level of dietary protein at the lipid levels of 6 and 12 % did not improved fish growth. Regardless of dietary lipid levels, increasing level of dietary protein also increased feed efficiency. These results indicate that the best performing diet for juvenile humpback grouper was ttha containing 56% protein, 9% lipid, energy content of 4.77 kcal/g diet, with a protein/energy ratio of 118 mg/kcal.

Complementary grow-out nutrition work with juvenile *C. altivelis* at CSIRO Cleveland examined the effects of diets containing five levels of crude protein (41–62%, DM basis) and energy (two levels of added oil to provide total dietary DM lipid content of 15 or 24%) during an eight-week comparative slaughter growth assay and in-experiment digestibility experiment. The results showed that:

- The apparent digestibility of starch and of a 3:1 blend of fish and soybean oil appears to be poor while that of casein is high in *C. altivelis*.
- *C. altivelis* will preferentially use protein over that of lipid or carbohydrate as a source of metabolic energy. Thus, growth rates (and FCR) will increase in proportion to the amount of protein in the diet (as such, designation of an optimum dietary protein requirement is spurious).
- Increasing the energy concentration of the diet through the addition of unsaturated oil may give rise to a small net gain in digestible energy intake but has no productivity value to the fish other than to predispose towards a greater deposition of body fat.

Further details of these experiments are provided in Appendix 1. As discussed later in this report (Future Research Plans, p.23), these results contrast with the results obtained for barramundi using high energy (high protein, high lipid) diets, which has important implications for the development of diets for groupers, or at least for *C. altivelis*.

Researchers at RIM Gondol have also examined the nutritional requirements of juvenile *C. altivelis* for dietary choline and lecithin, using test diets containing 0% or 0.9% choline chloride and 0% or 8% lecithin. The results showed a requirement for both supplementary dietary choline and lecithin, as evidenced by improved growth (192.5 – 240.5%) and feed efficiency (46.9–73.3%). Details of this experiment are provided in Appendix 1.

3.3.2 Fatty acids

Experimental work at Gondol has investigated the n-3 HUFA requirements of *C. altivelis*, with the objective of identifying the minimum dietary requirement to prevent n-3 HUFA deficiency. The results indicated that growth of *C. altivelis* was significantly affected by the level of n-3 HUFA in diets. Fish fed diet without n-3 HUFA supplementation had significantly lower growth than those fish fed diets with n-3 HUFA level of 1.0% – 3.0%. Growth of fish that were fed diets with levels of n-3 HUFA 1.0%, 1,5%, 2.0% and 3.0% were not significantly different ($P>0.05$). This experiment shows that the minimum dietary n-3 HUFA requirement for growth of humpback grouper juveniles is 1.0%. Details of this experimental work are appended (Appendix 1).

3.3.3 Phospholipids

This component has not yet commenced. Based on the results of the inter-laboratory calibration exercise, which demonstrated substantial differences between laboratories for phospholipid analyses, some additional cross-checking of phospholipid analyses will be necessary.

3.4 Fishmeal replacement

At SEAFDEC a feeding experiment in tanks was conducted to determine the efficacy of low fish-meal based diets for juvenile grouper. Processed meat meal and blood meal at 4:1 combination were used to replace Chilean fish meal at 0, 10, 20, 30, 40, 50, 60, 80, 100% in an isonitrogenous diet. Trash fish feeding was used as control.

The results showed best weight gain and SGR in fish fed the diet with 20% fish meal replacement. There were no significant differences in growth performance among fish fed diet with 0-80% fish meal replacement compared with those fed trash fish. However, fish fed the 20% fish meal diet had significantly higher ($P<0.05$) growth than those fed the diet with 100% fish meal replacement. Survival among fish fed the experimental diets did not significantly differ (96–100%) but was significantly higher ($P<0.05$) than survival of fish fed trash fish (90%). These results show that up to 80% of fish meal protein can be replaced by processed meat meal and blood meal coming from terrestrial animals with no adverse effects on growth survival, and feed conversion efficiency of *E. coioides* juveniles.

Fishmeal replacement research at RICF Maros has been aimed at obtaining the optimal percentage substitution of fish meal with shrimp head meal and blood meal for barramundi cod grow-out feed. Based on digestibility assessment, particularly for the apparent digestibility of protein, all ingredients except blood meal and rice bran appear to be promising as a partial or even complete replacement of fish meal in humpback grouper diets. Blood meal should be fermented prior to be used as fish meal replacement.

The substitution of fish meal by shrimp head meal up to 10% does not reduce the quality of feed as indicated by DGR, FCR,FI, FE and PER and reduce the cost of feed production. Substitution of fish meal by blood meal (10–40%) is not applicable.

Further details of these trials are given in Appendix 1.

3.5 Diet validation

A field trial was conducted at SEAFDEC's Igang Substation to confirm the results of tank studies, under on-farm conditions. *E. coioides* juveniles were stocked in net cages, 2m wide x 2 m length x 2m deep at 50 fish per cage and reared on three experimental diets from September 2000 to January 2001: (A) all fish meal-based diet, (B) 80% of fish meal replaced by 4:1 meat meal and blood meal and (C) trash fish as feed. Weight gain and SGR were highest in the trash fish diet and lowest in the 80% fish meal replacement diet while best survival and FCR were obtained in the 80% replacement diet and poorest in the all fish-meal based diet.

3.6 Economic evaluation

The three economic models (hatchery, nursery, grow-out) developed by Bill Johnston (DPI) were presented at the second annual project meeting held in Cairns in July 2000. Based on feedback from this meeting, Bill has revised some aspects of the models. The models are written in Excel 97® and provide a detailed assessment of the capital and operating costs for each phase of grouper aquaculture. Outputs include a breakdown of annual costs and a profitability analysis (using a discounted cash flow model). The revised models will be distributed in mid-2001.

Work has commenced to obtain commercially valid input data to develop a 'model hatchery / nursery / farm' for each country. The models will be used to investigate aspects of the profitability of the various components of grouper aquaculture, and to evaluate the economic impacts of the outcomes of the ACIAR grouper project.

4 Communication and coordination

The communication and coordination component of the project was developed from a recommendation of the Grouper Aquaculture Research Workshop held in Bangkok in 1998 that communication and coordination between grouper aquaculture researchers in the Asia-Pacific region needed to be improved in order to increase the efficiency of the existing research effort in this field by reducing overlap and outright duplication of research effort. Because the communication and coordination functions fall within NACA's mandate, NACA is the central point for these activities.

4.1 Research program development

The overall research program for the Asia-Pacific Grouper Network was presented for discussion at the APEC–NACA Collaborative Grouper R&D Workshop held in Medan, Indonesia, on 17–20 April 2000. The research program was accepted, with some modifications, by the workshop participants. The research program outline is now:

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

1.5 Post-harvest
2 Environment
3 Marketing
4 Food supply, certification
5 Socio-economics, livelihoods
6 Fish health
7 Training and extension

4.2 Research coordination

Coordination of the above program is being undertaken by NACA, in conjunction with ACIAR (Mr Barney Smith), AFFA (Mr Matthew Dadswell), SEAFDEC (Dr Clarissa Marte), DPI (Dr Mike Rimmer) and CSIRO (Dr Kevin Williams).

Additional funding to support the APGN has been provided by APEC through the Fisheries Working Group, and this supports annual workshops and staff exchanges. These mechanisms are being used to 'value-add' the ACIAR Grouper Project, and to expand the networking component of the project.

4.3 Dissemination of results

Results are disseminated through five mechanisms:

1. The Grouper Electronic Newsletter, compiled and sent by Sih-Yang Sim (NACA). Since the establishment of the Grouper Electronic Newsletter in 1999, the number of subscribers has continued to expand and now numbers about 230. The newsletter serves as means of exchange of research results from the ACIAR project, and from other projects and researchers active throughout the grouper network. Subscribers to the E-newsletter include those from Asia and the Pacific, the Americas, and Europe. There have been 12 issues of the newsletter so far, and the 2000–2001 newsletters are appended (Appendix 2). All newsletters are available on the new website at http://www.enaca.org/E-Newsletter/ so that new subscribers have access to previous issues.

2. The 'Grouper News' segment in regional magazines and newsletters, particularly 'Aquaculture Asia' and 'The Live Reef Fish Bulletin'. This has appeared regularly since July 1998.

3. The newly constructed and upgraded ACIAR Grouper Project website is (http://www.enaca.org/aciar/) has been available from August 2001. The newly completed website is divided into the following sections: Background; Project Objectives; Reports Section; Results Summary; Meetings, News, etc; Collaborating Institutes & Contacts, and direct link to APGN websites. The site has a complete listing of ACIAR project reports and allows researchers to access all relevant project material in one location. The web site is kept updated on a regular basis by Sih Yang Sim (NACA).

   The new Asia-Pacific Grouper Network (APGN) website titled "Asia-Pacific Regional Cooperation in Aquaculture of Groupers and Coral Reef Species" is now available from the internet address http://www.enaca.org/grouper/. This new web site has been moved to a faster server, making it easier and quicker to access. The new design includes several sections: Background; E-Newsletter; Projects Section; Meetings & Workshops; Publications & Articles; Institute Profiles;

Database; and Linkages. Further work is being undertaken to expand the scope of the website with a discussion forum and updated library/reference database. The process is time consuming but the completed version is expected to be ready by the end of October 2001.

4. Project publications are listed in detail in Appendix 3. In addition to magazine and conference proceeding papers, several publications have been submitted (and one accepted) to international scientific journals including *Aquaculture* and *Fisheries Science*. A number of other journal papers are currently in development.

5. Presentations at regional conferences, workshops and meetings. These are covered in more detail in the report section *Publications and other communication activities* (p. 31) and a full list of conferences, workshops and meeting attended is appended (Appendix 4).

Although the grouper network was initially targeted on the Asia-Pacific region, the network has also attracted attention from the African region (Tanzania, Mozambique), the Americas, and Europe.

Further activities associated with the Asia-Pacific Grouper Network in 2000–2001 included:

- A survey of institutions involved in research on grouper aquaculture, as part of a process of formalisation of the grouper network. Plans are being made to formalise the network at the 13th meeting of the NACA Governing Council that will be held in January 2002 in Malaysia.
- Preparation for publication of two reports from APEC/NACA grouper workshops, held in 1999 and 2000. The reports, to be published during 2001, contain material presented from the ACIAR grouper research project.
- Preliminary work has been undertaken to promote more effective cooperation and exchange of information with NGO's actively involved with promoting aquaculture as an alternative to destructive fishing practices.

Importance of results

Future research plans

Larviculture

Results to date have suggested no major change to the proposed research program. Future research will continue to build on the work done to date, investigating the nutritional requirements of grouper larvae, and validating experimental outcomes through verification trials.

Grow-out nutrition

The unexpected findings in the earlier protein to energy study with polka dot grouper need to be confirmed with other species of grouper. More estuarine species such as *E. coioides*, *E. malabaricus* etc may have a metabolism more adapted to using lipid as a source of energy. If other species can be sourced in Australia (either reared at Northern Fisheries Center or imported from partner laboratories) further protein to energy experiments will be carried out to test their capacity to utilize dietary lipid.

The apparent limited capacity of polka dot grouper to utilize the potential energy of high lipid diets was very surprising. Other carnivorous fish such as salmonids and Asian seabass are able to metabolise high lipid diets, sparing dietary protein, and growing faster and converting food into growth more efficiently. The earlier study showed that polka dot grouper grew better on high protein diets (growth continuing to improve up to the maximum examined dietary protein concentration of 63% DM). Such growth enhancement was due to a large proportion of the digested amino acids being deaminated and oxidized to yield metabolic energy (as evidenced by N retention in the fish being reduced as dietary protein increased). Although the better fish growth obtained by increasing the protein concentration of the diet may be justified on economic grounds, and particularly if less expensive protein feed ingredients could be used instead of fish meal, such diets will result in more of the dietary N being excreted as ammonia into the surrounding water to the detriment of the environment.

Two lines of future investigation are suggested from the above study with polka dot grouper. One aspect would be to examine the extent to which groupers can utilize cheaper terrestrial animal or plant protein meals as a potential source of metabolic energy. This direction is being pursued by collaborators at SEAFDEC and Maros laboratories. However, this ignores the N excretion problem that may well be exacerbated by using poorly balanced protein in the diet. An alternative aspect for investigation is to understand why polka dot grouper has only a low capacity to utilize dietary lipid as a source of metabolic energy. That is, is it possible to develop high lipid diets for polka dot grouper that have a sparing effect on protein oxidation?

The type of the lipid in the diet could have a marked bearing on the manner in which the constituent fatty acids are subsequently metabolized. Short and medium chain fatty acids (i.e. with a chain length of 14 or fewer carbon atoms) are more likely to be oxidized than longer chain fatty acids (i.e. with a chain length of 18 or more carbon atoms). This is because the absorbed larger long chain fatty acids can only be transported in the lymph and then only after being re-esterified into triglycerides and complexed with protein to form chylomicrons. These triglycerides by-pass the liver

and are distributed to peripheral adipose and muscle tissues where they are stored *in situ* or upon enzymic (lipase) hydrolysis, constituent fatty acids are carried across the cell membrane into the mitochondria. Once in the mitochondria, the fatty acids can either be chain elongated to longer chain fatty acids or alternatively be β-oxidised, ultimately yielding energy. In contrast, absorbed short and medium chain fatty acids are bound to albumin and transported in the blood to the liver and thence to all other tissues. Because of their small size, these fatty acids can pass easily across the cell wall membrane and into mitochondria. As these fatty acids are invariably fully saturated, they are more likely to be β-oxidised rather than being elongated. If this reasoning is correct, feeding fish with diets rich in short or medium chain fatty acid lipids may induce greater rates of β-oxidation and spare protein from being preferentially oxidized as a source of metabolic energy. Studies to test this hypothesis are in progress with polka dot grouper.

Future project budget

The research activities of the project are currently being managed within budget. A variation to the project budget was accepted in early 2001, to incorporate an additional research component on selective breeding of rotifers, and to assist with attendance at the end-of-project workshop.

Conduct of other research projects

The ACIAR work is strongly linked with other projects in place at all the participating laboratories. A major closely-linked project is the APEC Collaborative Grouper R&D Network Project (FWG 01/99), which is administered by AFFA and coordinated by NACA. The objectives of the APEC project are to:

1. Through the development of a regional research network develop the capacity to establish a sustainable grouper aquaculture industry which will benefit all collaborating economies.
2. Provide an alternative source of income / employment to people currently engaging in dangerous and illegal fishing practices.
3. Protect endangered reefs and reef fish from the pressures of illegal and dangerous fishing practices.
4. Develop a new aquaculture industry with significant export potential and economic benefit to a diversity of stakeholders.
5. Reduce substantially the current reliance on wild-caught fingerlings for aquaculture purposes because capture of wild juveniles is probably unsustainable, and is sometimes carries out using destructive fishing techniques which can have significant impact on the long-term status of reef fish stocks.

Through APEC involvement, the expansion of an existing South-East Asian initiative on collaborative research into grouper culture can be extended to more economies in the Asia-Pacific region. The role of APEC will be to enhance the extension of grouper research and facilitate the development of a network throughout the APEC region and beyond, to ensure that all economies in the region can benefit from the development of improved technology in live reef fish culture techniques.

The APEC Collaborative Grouper R&D Network Project has held two regional workshops to date :

1. Hat Yai, Thailand, 7–9 April 1999

This workshop was attended by 43 delegates from 14 APEC and NACA member economies. Economies represented included Australia; Brunei-Darussalam; China; Chinese Taipei; Hong Kong, China; Indonesia; Japan; Korea; Malaysia; New Caledonia; Peru; Philippines; and Thailand.

The major outcomes from the workshop were:

- Agreement on the need to expand and strengthen the grouper aquaculture research and development network, particularly through technical exchanges.
- Development of a strategic research plan for to support grouper aquaculture development; improve survival and food safety of live fish during handling and transport; and address destructive fishing practices.
- Preparation of three projects for consideration by the APEC FWG.
- Submission of a proposal for APEC to work with other regional bodies to develop a cooperative and equitable means of addressing the issue of cyanide fishing.

2. Medan, Sumatra, Indonesia, 18–20 April 2000.

This meeting was held in conjunction with the Regional Seafarming Workshop and was hosted by the Government of Indonesia in cooperation with the Bay of Bengal Programme (BOBP/FAO), and NACA. The workshop involved 53 participants from APEC economies from throughout the Asia-Pacific, including Australia; Hong Kong, China; Indonesia; Japan; Korea; Malaysia; Philippines; Singapore; Thailand; and Vietnam. The meeting was attended by representatives from NACA, the Secretariat for the Pacific Community (SPC), the Solomon Islands, Myanmar, INFOFISH, and a non-governmental organisation, The Nature Conservancy (TNC).

The meeting was very successful, with a number of key recommendations being made in support of APEC FWG and NACA objectives for grouper aquaculture. Specifically, the Workshop recommended further expansion of activities to cover coastal livelihoods, improved environmental management of cage aquaculture, and most importantly the formalisation of the participation of the centres/institutions involved in the network.

In addition to the Grouper Collaborative R&D Network project, APEC is supporting a number of associated projects:

- Regional survey of grouper fry collection methods (Hong Kong University).
- Production of a 'farmer-friendly' Grouper Health and Husbandry Manual (SEAFDEC AQD).
- Development of a regional disease research project, concentrating on viral diseases in groupers (AAHRI).

Additional projects are likely to be developed as a result of the Medan workshop. All these projects are being coordinated through NACA as part of the coordinated R&D program of the Asia-Pacific Grouper Network.

Related research grants received or applied for

John Allwright Fellowship for Agricultural Research

Ms Josette Bangcaya (SEAFDEC AQD) was awarded a John Allwright Fellowship for Agricultural Research to undertake an MSc degree at the Queensland University of Technology, Brisbane. Josette's research topic will investigate biotechnology applications for evaluation of egg quality in marine finfish, including groupers. Her research will be undertaken in collaboration with the marine finfish programs based at DPI's Bribie Island Aquaculture Research Centre and Northern Fisheries Centre, Cairns.

PhD scholarship – Japan Society for the Promotion of Science

Joebert Toledo (SEAFDEC AQD) has been awarded a PhD scholarship from the Japan Society for the Promotion of Science at the Faculty of Applied Biological Science, Hiroshima University. Joebert's dissertation will be titled: 'Studies on the seed production of grouper *Epinephelus coioides*' and will cover a range of research activities at SEAFDEC, including some ACIAR-funded activities. The scholarship is for 4 years, from 2000 to 2004. Joebert is required to attend Hiroshima University for at least 30 days each year to undertake some laboratory work and to consult with his supervisor, but most of the research will be undertaken at SEAFDEC.

Capacity-Building for Agriculture and Rural Development (CARD) Program – CSIRO / NTUF Feeds Development Project

Under a newly established bilateral arrangement between Australia and Viet Nam, AusAID is supporting a project to build *Aquafeeds R&D capacity for intensive aquaculture in Viet Nam*. Participants in the Project are CSIRO Marine Research (Kevin Williams) and the University of Fisheries (UoF), Nha Trang, Viet Nam (Le Anh Tuan). The overall objective of the project is to increase food security and income of rural Vietnamese in coastal communities by improving the profitability, and environmental sustainability, of intensive aquaculture in Viet Nam. This objective will be achieved by:

- Developing a collaborative aquafeed research project between CSIRO and UoF to facilitate the transfer of aquafeeds technology between Australia and Viet Nam.
- Instituting a training program to increase the aquaculture skill base at UoF and to provide Vietnamese post-graduates with opportunities to study aquaculture nutrition.
- Disseminating and show-casing project achievements at scientific forums, by the holding of a technical workshop in Viet Nam and extension of research findings to the aquaculture industry.

In the CARD project, grouper and rock lobsters are priority species for which feeds and feeding management research will be carried out. There is an obvious advantage for the CARD project to be closely linked with the ACIAR Grouper project and this will benefit both projects. Wherever possible, key Vietnamese staff engaged in the CARD project will be invited to attend Project meetings of the ACIAR Grouper project.

AusAID GSLP funding for Komodo Mariculture Project

AusAID funding (under the Government Sector Linkages Program) has been provided to AFFA and DPI has been subcontracted to provide training and technical support by Australian and Indonesian experts in support of GOI and TNC management of Komodo National Park (KNP) in the Nusa Tenggara Timur region of Indonesia. The expected outcomes from the project are to:

- provide an alternative source of high-value marine finfish (particularly groupers) which are currently captured using unsustainable fishing practices such as cyanide;
- provide alternative livelihoods for local coastal communities in the Komodo region.

The GSLP project will provide training in:

- broodstock collection, quarantine, prophylaxis and management techniques;
- broodstock nutrition, vitamin application, feeding techniques;
- disease identification, diagnosis and treatment techniques;
- captive spawning, egg collection and incubation;
- polymerase chain reaction (PCR) viral diagnostic techniques.

APEC Collaborative Grouper R&D Network Staff Exchange

A collaborative project staff exchange was funded for Dr Inneke Rumengan (Laboratory of Marine Biotechnology, Faculty of Fisheries and Marine Science, Sam Ratulangi University) to visit Northern Fisheries Centre, Cairns, to undertake collaborative research to selectively breed SS-strain rotifer (*B. rotundiformis*) for application in marine finfish hatcheries, particularly grouper hatcheries. Dr Rumengan undertook this exchange in late 2000.

The research initiated under this exchange has been incorporated in the ACIAR grouper project. The rotifer selective breeding component will investigate the development of selective breeding procedures for *B. rotundiformis* to determine whether it is practical to select for size as a genetic trait. If rotifers can be selectively bred for size, then a population of the desired size (say, <90µm width) could be established for routine use in hatcheries. This would preclude the need to sieve rotifers, reducing wastage of rotifer production and increasing hatchery productivity by making more rotifers available for larviculture using the same rotifer culture facilities.

This activity was subsequently included in the ACIAR Grouper Project (FIS/97/73) as a separate component of the larviculture work to be undertaken during the last 18 months of the project.

CARD Program – DPI / RIA1 / RIA2 Developing marine finfish hatchery technology for mariculture in Vietnam

A CARD application was submitted to promote the development of sustainable aquaculture for marine finfish by providing training for Vietnamese personnel in barramundi / seabass (*Lates calcarifer*) hatchery technology, and in copepod culture technology. This proposal sought to address the current major constraint to the development of sustainable finfish mariculture in Vietnam, which is the availability of seedstock (fish fingerlings).

This application was rejected on administrative grounds by the consultants assigned by AusAID to process all CARD applications. Apparently several pages of the application were misplaced prior to it being sent by DPI to ITC.

Development of linkages with collaborating-country organisations

DPI and RIM Gondol have developed strong linkages with The Nature Conservancy, a US-based NGO, which is providing natural resource management services to the Government of Indonesia in Komodo National Park. Although most KNP inhabitants mainly derive their income from a pelagic lift net fishery targeting squid and small pelagic fish, several surrounding communities, are involved in fishing with cyanide and hook-and-line for valuable fish species (groupers and Napoleon wrasse) to supply foreign markets (mainly Hong Kong) with live food fish. The extremely high exploitation pressure on the grouper stocks, and the poisoning of the coral reefs through the use of cyanide severely threaten the marine biodiversity in and around KNP.

To abate these threats, TNC's Indonesia Coastal and Marine Program together with the Indonesian Park authority implemented an extensive marine conservation program for Komodo National Park. Development of a fish culture enterprise that involves local communities was identified as a possibility to steer fishermen away from destructive and unsustainable fishing methods. Also, the development of a fish culture enterprise would serve as a model for other rural areas in Indonesia, thereby contributing to the market transformation of the life reef fish trade from unsustainable, capture-based to sustainable, culture-based.

Construction of the Labaun Bajo hatchery has commenced and TNC have recruited an Australian consultant, Dr Phillip Arumagam, to oversee the project. Training and assessment of the disease status of the broodstock is being supported by an AusAID GSLP project, as noted above. The GSLP project will further enhance linkages between TNC, CRIA, DPI and other institutions in the Asia-Pacific region.

Student Contributions to RICF Activities

RICF Maros has developed enhanced linkages with Indonesian universities by involving undergraduate and postgraduate students in the research:

1. Andiu Roni, undergraduate of Sekolah Tinggi Perikanan, (School of Fisheries) Jakarta - Effect of feeding frequency on growth of barramundi cod in floating net cages. The activity finished in June 2001.

2. Rusli, undergraduate of University of Moeslim Indonesia, Makassar - The substitution of soybean meal with soybean cake as protein sources in milkfish diet.

3. Andi Tenriawaruwaty, undergraduate of University of Moeslim Indonesia, Makassar; The effect of palm oil cake for growth and survival rate of milkfish.

4. Rosmawati Anwar, Master degree of Hasanuddin University, Makassar. Bioprocessing of soybean cake using *Aspergillus niger* for milkfish diet (preliminary study for grouper diet experiment).

5. Ibrahim Sidiq and Indah Sriwana; undergraduate of Haluoleo University, Kendari Southeast Sulawesi have a field work on grouper grow-out for 1 month and is still ongoing.

Optimal methods / channels of extension / outreach of results to end-users

All the participating organisations in FIS/97/73 have well developed extension facilities to assist with the application of research results.

DPI has a range of effective extension activities in the field of aquaculture. Regular (currently six-monthly) workshops / conferences are held in association with the Australian Barramundi Farming Association which represents the tropical marine finfish farming sector in Australia. Technical outputs are provided to industry at these workshops / conferences. In addition, dedicated technical workshops on specific aspects of marine finfish culture are held on an 'as needed' basis. Other extension activities provided by QDPI are:

- publication three times per year of 'Queensland Aquaculture' Magazine, allowing widespread dissemination of research outcomes to the Queensland aquaculture industry;
- publication of technical material in a range of formats, such as the 'Going Farming' series and the Information Series publications.

DPI held a dedicated Reef Fish Aquaculture Symposium in conjunction with the ACIAR FIS/97/73 Project Meeting held in Cairns in late July 2000. The Reef Fish Aquaculture Symposium provided intending farmers and aquaculture investors with a 'snapshot' of the current status of reef fish / grouper aquaculture.

CRIA utilises the services of the Assessment Institute for Agricultural Technology, which has an office in each province in Indonesia. AIAT is administered by the Agency for Agricultural Research and Development, which also administers CRIA. CRIA research progress and outcomes are reported to AIAT at seminars and by direct contact. AIAT then extends research results to farmers through meetings and workshops. The development of small scale backyard fish hatcheries for milkfish and seabass has provided RIM Gondol with experience in extending research results to industry. About 2000 units of backyard hatcheries now operate in Bali, with many in the Gondol-Singaraja area. About 10 backyard hatcheries have adopted the production technology for groupers developmed by RIM Gondol and are producing fingerlings of *C. altivelis* and *E. fuscoguttatus*.

SEAFDEC operates a Technology Verification Program (TVP), which is effectively a farm extension service. There are five SEAFDEC staff assigned to TVP. Most emphasis at the moment is on providing alternative technologies for the shrimp farms in The Philippines that have gone out of operation because of disease problems. One alternative to shrimp farming that is being actively promoted by TVP is grouper aquaculture. SEAFDEC held a 'Grouper Festival' on the island of Negros in March 1998, to promote grouper aquaculture specifically. According to SEAFDEC TVP staff, there is huge interest in

grouper aquaculture in The Philippines and the development of additional grouper farms is constrained only by the poor availability of fingerlings.

A major component of this project is dedicated to ensuring that there is greater cooperation and exchange of information amongst grouper researchers and industry throughout the Asia-Pacific region. Research outcomes from the ACIAR project, as well as results from other research projects, are publicised in the dedicated 'Grouper News' section of 'Aquaculture Asia' (published by NACA) and the 'Live Reef Fish Bulletin' (published by the Secretariat for the Pacific Community). The widespread readership of these established and respected publications will ensure widespread dissemination of the results of the ACIAR project. This information is also provided on the NACA Grouper web site (http://www.enaca.org/grouper/) and outcomes of the ACIAR project are provided on the ACIAR project web site (http://www.enaca.org/aciar/). These mechanisms will ensure the widest possible extension of the outcomes of this project, and will 'value add' the ACIAR project by accessing the outcomes of other regional research projects to broaden the knowledge base relating to grouper aquaculture technology.

In addition, transfer of results to farmers and coastal communities is being actively supported under the APEC grouper research and development project.

Environmental impacts

The proposed research will potentially provide positive environmental impacts with regard to alleviating pressure on wild stocks that currently supply the bulk of the live reef fish markets. Currently, the demand for live groupers for the high value live fish markets of Hong Kong and southern China is being met largely by the capture fishery, and this fishery has been associated with unsustainable fishing practices, particularly the use of sodium cyanide as a capture technique.

The development of sustainable grouper aquaculture will contribute to the alleviation of adverse environmental impacts associated with unsustainable fishing practices by providing an alternative source of supply which will assist in meeting demand for grouper product. A specific strategy to develop grouper mariculture to reduce pressures on wild stocks is being developed in collaboration with TNC in the Komodo region of Nusa Tenggara Timur, Indonesia.

Increased cage and pond culture of groupers is likely to have some localised adverse environmental impacts, particularly related to water quality degradation associated with uneaten fish feed and fish wastes. Although published studies indicate that the overall contributions of nutrients and organic matter from coastal cage and pond culture of finfish are small compared with other coastal discharges, localised water quality changes and sediment accumulation may occur [Phillips, 1998 #544]. Such impacts tend to be greater from cage farms than from ponds because wastes can be assimilated to some extent within the pond environment, while wastes from cage farms are discharged directly into the local environment [Phillips, 1998 #544]. However, such impacts are highly localised and the overall impact of marine cage culture in coastal environments is minor [Phillips, 1998 #544; Wu, 1995 #545].

This project will assist in reducing adverse environmental impacts by developing a dry diet for grouper culture to replace the now commonly used

trash fish diet. Losses associated with feeding trash fish are around 20–38% [Wu, 1995 #545] compared with around 10% for pelleted feeds [Wu, 1995 #545; Beveridge, 1996 #551]. Furthermore, this issue will be addressed directly through the development of guidelines on environmental management of grouper cage aquaculture planned for 2000–2001 under the APEC grouper research and development project.

Gender impacts

The impacts of this project will be gender neutral. Both men and women are employed in finfish aquaculture in the affected countries. The outcomes of the project will result in increased development, and improved sustainability, of grouper aquaculture. These outcomes will not impact on one gender exclusively, but will promote the economic development of the community in general.

Research problems

In some cases, scientists at partner laboratories have had difficulty in accessing chemicals for their experimental work. Where possible, some chemicals have been purchased in Australia and supplied to facilitate the research at partner laboratories. This will delay the completion of at least one activity (digestive enzyme localisation) but this should not impact significantly on the project.

An on-going constraint is the limited success of larval rearing of groupers for the planned nutrition work in Australia. This has been primarily caused by the limited availability of larvae due to ongoing facility limitations and broodstock problems at NFC Cairns. Construction of a new aquaculture facility at Cairns has at last commenced, but will not be on-line until early 2002.

To facilitate the grow-out nutrition work in Australia, CSIRO has imported juvenile groupers from the overseas partner laboratories since the Cleveland laboratory is an accredited quarantine laboratory.

Publications and other communication activities

Publications

Dr Oseni Millamena (SEAFDEC AQD) has had a paper accepted for publication in *Aquaculture* on replacement of fish meal with meat meal for grouper diets. This will be the first formal scientific publication arising from the project research. A number of other publications are in preparation at the participating laboratories. A full list of project publications is appended (Appendix 3).

Project results and progress have been reported at a range of conferences, workshops and meetings throughout the region. These include:

- Joint Australia–Taiwan Aquaculture and Fisheries Resources and Management Forum III, Bribie Island, Queensland, 29–30 June 2001.
- Seminar Nasional Pengembangan Budi Daya Laut Menuju Terciptanya *Sea Farming* (National Seminar on Development of Sustainable Sea Farming), Jakarta, Indonesia, 7–8 March 2001; organised by centre of Research and

Development of Marine Exploration and Fisheries (CRIFI) in cooperation with Japan International Cooperation Agency (JICA).

- Reef Fish Aquaculture Symposium, Cairns, Queensland, Australia, 27 July 2000.
- DPI ACIAR Project Leaders' Workshops held in Brisbane, Queensland, 29 June 2000, and Caloundra, Queensland, 21 March 2001.
- Seminar Nasional Pengembangan Budi Daya Laut Menuju Terciptanya *Sea Farming*, Jakarta, Indonesia, 7–8 March 2001.
- Australian Barramundi Farming Symposium, Cairns, Queensland, Australia, 23 February 2001.
- Dissemination of research result of RICF Maros on 22 November 2000 in cooperation with Regional Office of the Ministry of Agriculture in South Sulawesi. The meeting was attended by official of regional fisheries offices from 23 regencies in South Sulawesi, companies and farmers.
- Dissemination of results of fisheries research, Sukamandi, West Java on 21–22 September 2000.
- Workshop on Sustainable Marine Aquaculture Development in Sabah, Malaysia, held at Kota Kinabalu, Sabah, Malaysia, 15–16 August 2000. Workshop jointly organised by the Institute for Development Studies (Sabah) and Jabatab Perikanan (Department of Fisheries) Sabah and co-sponsored by the Konrad Adenauer Foundation, Germany, and Jabatan Perikanan Sabah.

Reef Fish Aquaculture Symposium, Cairns, Queensland, Australia, 26 July 2000

In response to continuing interest in reef fish (grouper) aquaculture in Australia, DPI and ACIAR held a Reef Fish Aquaculture Symposium in Cairns, Queensland, Australia, on 26 July 2000, in conjunction with the second annual project meeting. The symposium provided an update on the status of aquaculture of reef fish in the Asia-Pacific region, and was targeted at potential farmers and those interested in investing in this aspect of aquaculture.

The symposium was opened by the Queensland Minister for Primary Industries and Rural Communities, Mr Henry Palaszczuk, and provided an opportunity for the Australian aquaculture industry to hear about the results of DPI's Reef Fish Aquaculture Project as well as the ACIAR project *Improved larviculture and grow-out technology for grouper aquaculture in the Asia-Pacific region*.

DPI's Reef Fish Aquaculture Project represents a major investment by the Queensland Government in the development of reef fish aquaculture to support Queensland's primary industries. The project grew out of the Reef Fish Aquaculture Feasibility Study, which was undertaken in 1996 in response to the expansion of the live reef food fish trade to Hong Kong to evaluate the potential for aquaculture production to provide additional product for the live reef fish market. The Reef Fish Aquaculture Feasibility Study showed that, although the costs of research and development to establish a reef fish aquaculture industry in Queensland were high, potential benefits to the state from this industry were also high. Prices for live reef fish have remained high, despite the Asian economic crisis, and high-value species such as barramundi cod, Maori wrasse and Queensland groper fetch average wholesale prices of $140–$160 per kilogram.

The ACIAR project is a major component of a large international effort to develop sustainable grouper (reef fish) aquaculture in the Asia-Pacific region. In addition to

DPI, it involves researchers from CSIRO and overseas laboratories in Indonesia and the Philippines, as well as the Network of Aquaculture Centres in Asia-Pacific (NACA). NACA, which Australia joined in 1998, is coordinating the development of grouper aquaculture in the Asia-Pacific region through several projects including the ACIAR project and several projects funded by the Asia-Pacific Economic Cooperation (APEC) and other agencies.

The symposium was well attended by over 60 industry, research and government representatives from throughout Australia. Speakers covered a wide range of topics relating to the development of reef fish aquaculture, including:

- development of improved larval rearing techniques, which will lead to more reliable fingerling supply;
- development of new live prey organisms, such as copepods, to improve growth and survival of grouper larvae in hatcheries;
- nutrition and feeds development, to develop cost-effective feeds using locally available ingredients to replace 'trash' fish which is commonly used as aquaculture feed in Asia.
- the status of reef fish / grouper aquaculture in Australia, Indonesia, the Philippines and Taiwan.

The symposium heard that, although great strides have been made in reef fish aquaculture in the last few years, Australia is still at least five years away from having a reef fish aquaculture industry. This is because of the need to develop reliable hatchery techniques to produce fingerlings. Unlike Asia, where juvenile fish are harvested from the wild, Australia's strict fisheries management regulations do not allow juvenile harvesting and any commercial aquaculture venture needs to be based on hatchery supply of seedstock. The DPI and ACIAR projects are bringing the Australian aquaculture industry closer to the goal of commercial reef fish aquaculture.

Program – Reef Fish Aquaculture Symposium

0830 – 0900	*Coffee*	
Session 1	**Chair: Dr Chris Barlow, DPI**	
0900 – 0915	Welcome, introduction	The Honourable the Minister for Primary Industries and Rural Communities, Mr Henry Palaszczuk
0915 – 0945	Potential for reef fish aquaculture in Queensland	Dr Mike Rimmer, DPI
0945 – 1000	Breeding and larviculture R&D at NFC	Ms Elizabeth Cox, DPI
1000 – 1030	Live prey R&D at NFC	Dr Richard Knuckey, DPI
1030 – 1100	*Morning tea*	
Session 2	**Chair: Dr Kevin Williams, CSIRO**	
1100 – 1130	Copepod culture development	Dr David McKinnon, AIMS
1130 – 1200	Grouper reproduction and induced spawning	Dr Abigail Elizur, DPI
1200 – 1230	ACIAR Grouper Project	Dr Mike Rimmer, DPI
1230 – 1330	*Lunch*	
Session 3	**Chair: Dr Mike Rimmer, DPI**	
1330 – 1400	Grouper research at SEAFDEC	Mr Joebert Toledo, SEAFDEC AQD
1400 – 1430	Barramundi cod hatchery R&D	Dr Ketut Sugama, CRIFI RSCF Gondol
1430 – 1500	Grouper nutrition	Dr Kevin Williams, CSIRO DMR
1500 – 1530	Grouper diet R&D at Gondol	Dr N.A. Giri, RSCF Gondol
1530 – 1600	*Afternoon tea*	
Session 4	**Chair: Ms Elizabeth Cox, DPI**	
1600 – 1630	Grouper diet R&D at Maros	Dr Taufik Ahmad, CRIFI RICF Maros
1630 – 1700	Grouper aquaculture research in the Northern Territory	Mr Jerome Bosmans, NTDPIF
1700 – 1730	Grouper and snapper aquaculture in Taiwan	Dr Mike Rimmer, DPI

Figure 2 Participants in the Reef Fish Aquaculture Symposium, Cairns, 26 July 2000. Foreground, from left to right: Dr Mike Rimmer (Principal Fisheries Biologist, DPI, and ACIAR Project Leader), Dr Chris Barlow (Aquaculture Program Leader, DPI), Dr Abigail Elizur (Principal Fisheries Biologist, DPI), The Honourable the Minister for Primary Industries and Rural Communities, Mr Henry Palaszczuk.

Other communication activities

Grouper electronic newsletter

The grouper electronic newsletter, compiled and sent by Sih-Yang Sim (NACA). Yang has sent out four editions of the newsletter to date and it currently has over 220 subscribers.

'Grouper News' in regional aquaculture magazines

The regional aquaculture magazine 'Aquaculture Asia' (published by NACA) continues to carry a 'Grouper News' section that provides updates on activities within the region on the development of sustainable grouper aquaculture. This has appeared regularly since July 1998. Many of these articles are also included in 'The Live Reef Fish Bulletin' published by the Secretariat of the Pacific Community.

Web site

The web site for the ACIAR grouper aquaculture project has been moved to a new web site in the US (http://www.enaca.org/aciar/) to overcome the access problems that previously plagued the web site. This site is linked to the NACA grouper web site (http://www.enaca.org/grouper/) and contains the outline of the project and detailed project documentation. The ACIAR grouper aquaculture project web site is currently being redesigned to make it more attractive and user-friendly, as are the other NACA web sites.

Value of the research

Social benefit

The development of sustainable grouper aquaculture technology will have impacts at all levels of the community in those countries involved in this industry. In Indonesia, many hatcheries are of the 'backyard' type – relatively small hatcheries that are basically family-run operations. There are currently around 2,000 units of backyard hatcheries in Bali (K. Sugama, pers. comm.) and they are important income generators in northern Bali. Currently, there are around 10 backyard hatcheries that have adopted the grouper larval rearing technology developed by GRIM and are producing barramundi cod and flowery cod fingerlings (K. Suwirya, pers. comm.).

In the Philippines, SEAFDECs TVP has found that there is excellent potential for the adaption of disused shrimp farms for grouper aquaculture. Currently, over 90% of Philippine shrimp farms lie idle because of shrimp disease problems. However, development of grouper farms is constrained by the limited availability of grouper fingerlings, which are available only from wild capture. Supply of hatchery-reared fingerlings would enable these disused farms to be productively employed for grouper culture. Because of the intensive nature of finfish farming (in Australia, barramundi farms employ one person for about every 8 tonnes of production, and this would likely be higher in the Philippines) the development of grouper farms from disused shrimp farms would be an important source of employment for local people.

Through the development of sustainable grouper aquaculture, employment opportunities would be provided for people with a wide range of skills. Hatcheries have a requirement for relatively skilled technicians, while farms utilise a wider range of skills, including relatively unskilled labour. Consequently, the development of improved grouper aquaculture technology will benefit a wide range of people in the community.

Economic benefit

Economic benefits resulting from this researched will be estimated during later stages of the project. As detailed above, the first two years of the project involved developing separate economic models for the three major phases of grouper aquaculture: hatchery, nursery and grow-out (ponds and cages), and adapting these models for use in Australia, Indonesia and the Philippines. Future work will concentrate on developing 'model farms' for Australia, Indonesia and the Philippines using available commercial data. The model farms will then be used to estimate the benefits of the research outcomes of the ACIAR project.

Existing information on the economic benefits of grouper aquaculture is summarised below.

Australia
DPI's Reef Fish Aquaculture Feasibility Study, carried out in 1995–96, predicted that considerable benefits would flow from the development of reef fish aquaculture in Australia. Costs to establish this industry were estimated at about $15 million over 10–13 years. The benefit/cost model showed that a reef fish aquaculture industry in Queensland has the potential to be highly profitable, generating revenue in excess of $1 billion within 30 years under favourable conditions. Using an 8% discount rate

over 40 years, the net present value of the research project could be of the order of $170 million, with a benefit/cost ratio of 17:1, and an internal rate of return of 29%.

An additional aspect of the development of a reef fish aquaculture industry in northern Australia is the additional employment that would be generated. Northern Australian fish farms employ one person for every 8–10 tonnes of production (DPI, unpublished data), so the development of a reef fish aquaculture industry is potentially a valuable source of employment for rural areas of northern Australia.

Indonesia
In Indonesia, RIM Gondol has developed the technology for small-scale backyard fish hatcheries and about 800 of these hatcheries now operate in Bali. Milkfish are the species most commonly cultured, but Asian seabass are also cultured. About 10 backyard hatcheries are now producing grouper fingerlings of several species. The success of the backyard hatcheries is based on economics. The land now used for backyard hatcheries was previously used for coconut production. However, production of marine fish fry produces incomes several orders of magnitude higher than coconut production – e.g. an income of about Rp80,000 (AUD$14) per annum with coconuts compared to Rp20,000 (AUD$3.50) every three weeks for milkfish fry.

Philippines
SEAFDEC has undertaken economic evaluations of grouper production in both ponds and cages in the Philippines. For pond culture, based on a production unit of 5,000 pieces per ha per year with 80% survival to harvest at 5–7 months, the return on investment is about 82% and the payback period is 1.22 years, with an annual net profit of PHP244,210 (AUD$10,950) [Baliao, 1998 #552]. For floating net cages, one module of six cages holding 3,000 pieces with a survival rate of 80% to 5–7 months will produce a net profit of PHP 111,230 (AUD$4,850) per annum, with a return on investment of 59% and a payback period of 1.68 years [Baliao, 2000 #553].

Travel and meetings

The following travel has been undertaken:

- Ketut Suwirya (RIM Gondol) travelled to Brisbane for training in GC analysis techniques at the CSIRO Division of Marine Research Laboratories at Cleveland from 4 to 24 June 2000.

- Mike Rimmer attended the 'ACIAR Live' (DPI ACIAR Project Leaders') Workshop, held in Brisbane, Queensland, 29 June 2000, and presented a summary of the results of ACIAR Project FIS/97/73.

- Joebert Toledo (SEAFDEC AQD), Ketut Sugama and Adiasmara Giri (RIM Gondol), and Taufik Ahmad (RICF Maros) visited Cairns, Queensland, Australia for the second annual project meeting, 24–27 July 2000. The formal project meeting was undertaken on 24–25 July, followed by the Reef Fish Aquaculture Symposium on 26 July. On 27 July participants undertook a field trip to barramundi farms in the Cairns–Innisfail region.

- Kevin Williams travelled to Thailand and Indonesia on 24 September – 4 October 2000. This trip involved meetings with NACA staff (H. Kongkeo, M. Phillips and

S-Y Sim) and with Thailand Department of Fisheries (M. Boonyaratpalin) and Asian Institute of Technology (P. Edwards) staff; as well as attendance at the 6th Roche Aquaculture Conference (Bangkok, 29 September 2000). At RIM Gondol discussions were held with project staff and the importation of hatchery-reared *Cromileptes* juveniles for grow-out nutrition research in Australia was arranged.

- Kevin Williams travelled to the Philippines, Vietnam and Indonesia on 28 November – 14 December 2000. This trip involved visits to SEAFDEC AQD and RICF Maros for discussions with grouper project staff.

- Mike Rimmer attended the DPI ACIAR Project Leaders' Workshop held in Caloundra, Queensland, 21 March 2001.

- Shannon McBride (DPI) attended the ACIAR Fisheries Program meeting held at Snobs Creek, Victoria, in April 2001. Shannon presented a summary of the results of the ACIAR grouper aquaculture project.

- Mike Rimmer travelled to RICF Maros (Sulawesi, Indonesia), and with Shannon McBride, to RIM Gondol (Bali, Indonesia) and SEAFDEC (Iloilo, Philippines) for discussions on project progress with partner laboratories in April 2001. Shannon undertook collaborative experiments on the development of the digestive system and ontogeny of digestive enzymes of grouper larvae at RIM and SEAFDEC. Grouper larvae samples obtained at these laboratories were returned to Australia for further analysis.

- Richard Knuckey (DPI) travelled to Sam Ratulangi University (Manado, Sulawesi, Indonesia) to initiate the project component on selective breeding of rotifers. Mrs Tida Petchmanee (NICA, Songkla, Thailand) also travelled to Manado to participate in this activity.

Budget discussion

The third and fourth six-monthly fund tranches were distributed in May 2000 and January 2001. A budget variation was approved by ACIAR in June 2001 to cover the following activities:

1. An additional component involving Sam Ratulangi University (Manado, Sulawesi, Indonesia) and DPI NFC in selective breeding of rotifers.
2. Additional funding for the end-of-project workshop, including funding for project participants.

Some research activities at the CRIA laboratories (RICF Maros and Gondol RIM) were delayed in early 2000 due to delays in these laboratories receiving their budget allocation from CRIA Jakarta. These delays had no significant impact on the project.

An acquittal for the second year of the project (July 2000 – June 2001) is appended (Appendix 6).

Conclusions

Despite delays in implementing the formal project arrangements, participating laboratories are to be congratulated for commencing work on the project immediately on its commencement. In several cases, this has meant that the laboratories have had to absorb the initial costs of the research while waiting for the ACIAR funds to be disbursed.

As this report demonstrates, significant progress has been made in a number of areas of the project. Overall the project is very close to on-target, although a few areas are slightly lagging – this is particularly the case with the training activities.