ADVANCES IN GROUPER AQUACULTURE

Editors: **M.A. Rimmer, S. McBride and K.C. Williams**

Australian Centre for International Agricultural Research
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Abbreviations and acronyms

4-NPC	4-nitrophenyl caproate
AB-PAS	alcian blue–periodic acid Schiff
ACIAR	Australian Centre for International Agricultural Research
ACP	acid phosphatase
AD	apparent digestibility
ADMD	apparent dry matter digestibility
ALP	alkaline phosphatase
AMP	amino peptidase
ANOVA	analysis of variance
AOAC	Association of Official Analytical Chemists
A-P	Asia-Pacific
APD	apparent protein digestibility
APEC	Asia-Pacific Economic Cooperation
APMFAN	Asia-Pacific Marine Finfish Aquaculture Network
APNa	L-ascorbyl-2-monophosphate-Na-Ca
ARA	arachidonic acid
BF	body fat
bsd	bile salt dependent
BW	body weight
CM	circular muscle layer
CMC	carboxymethylcellulose
CP	crude protein
CSIRO	Commonwealth Scientific and Industrial Research Organisation
DHA	docosahexaenoic acid
DM	dry matter
DO	dissolved oxygen
DOM	dissolved organic matter
DPH	days post hatch
DPI&F	Department of Primary Industries and Fisheries (Queensland, Australia)
DW	dry weight
E	energy
EA	environmental assessment
EAA	essential amino acid
EPA	eicosapentaenoic acid
FCR	food conversion ratio
GBRMPA	Great Barrier Reef Marine Park Authority
GC	gas chromatograph
GE	gross energy
GIS	Geographic Information System
GMO	genetically modified organism
HACCP	Hazard Analysis and Critical Control Point

HPLC	high pressure liquid chromatography
HSI	hepatosomatic index
HUFA	highly unsaturated fatty acid
LCFA	long chain fatty acids (C18+)
LIP	lipase
LM	longitudinal muscle layer
LP	lamina propria
lx	lux
MAL	maltase
MCFA	medium chain fatty acids (C10–C14)
n-	naupliar stage (copepods)
N	nitrogen
NACA	Network of Aquaculture Centres in Asia-Pacific
NEAA	non-essential amino acid
NFE	nitrogen free extract
NGO	non-governmental organisation
NHL	newly-hatched larvae
NL	neutral lipid
NSC	no significant change
NSE	non-specific esterase
PER	protein efficiency ratio
PL	polar lipid
POM	particulate organic matter
ppt	parts per thousand
PUFA	polyunsaturated fatty acid
PVC	polyvinylchloride
R&D	research and development
RDE	retention of digestible energy
RDN	retention of digestible nitrogen
RT-PCR	reverse transcriptase-polymerase chain reaction
S	serosa layer
S-	small (strain rotifers — *Brachionus rotundiformis*)
SD	standard deviation
SE	standard error
SEAFDEC AQD	Southeast Asian Fisheries Development Centre Aquaculture Department
SGR	specific growth rate
SM	submucosa
SPF	specific pathogen free
SS-	super small (strain rotifers — *Brachionus rotundiformis*)
STREAM	Support to Regional Aquatic Resources Management
SV	supranuclear vacuoles
TL	total length/total lipid
TRP	trypsin
UNSRAT	Sam Ratulangi University (Manado, Northern Sulawesi, Indonesia)
USA	United States of America
UV	ultra-violet
VNN	viral nervous necrosis

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Foreword

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 in Hong Kong and southern China has increased pressure on wild stock resources.

During the late 1990s several regional workshops were held to look at the fisheries and aquaculture of grouper and reef fish. These workshops concluded that aquaculture of reef fish species will contribute to regional economies by providing product for domestic and export markets. However, the technologies for production of grouper were not yet commercially viable and a range of research issues were identified as high priority for the development of aquaculture production technology for groupers. It was also apparent that there were limited opportunities for interaction amongst many grouper researchers in the region and that improved communication and collaboration would reduce duplication and increase resource utilisation.

The ACIAR grouper project was designed to address some of the recommendations from these regional workshops by undertaking research in several critical areas of grouper aquaculture technology and by developing a collaborative network of grouper aquaculture researchers in the Asia-Pacific region.

The project had three major components and these are covered in this book; larval rearing to improve growth and survival of groupers during the hatchery phase; diet development to produce feeds with low environmental impact; and support for the NACA Grouper Aquaculture Research and Development Network.

The information gained during the project and reported here will be useful for further development and optimisation of grouper aquaculture.

Peter Core
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Australian Centre for International Agricultural Research

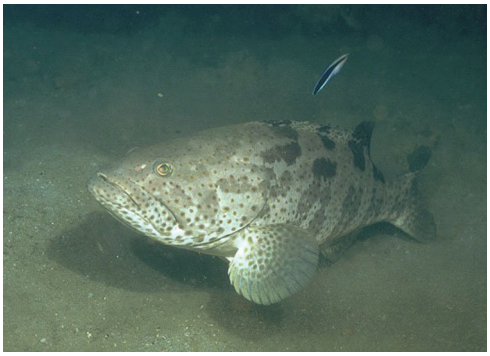
SECTION 1
INTRODUCTION

M.A. Rimmer

Aquaculture of high-value marine finfish species continues to develop rapidly in Southeast Asia. Many groupers (members of the Family Serranidae, Subfamily Epinephelinae) bring high prices (up to US$70/kg wholesale) in the live markets of Hong Kong and southern China (McGilvray and Chan 2001). Increasing market demand and the real or perceived profitability of the live reef food fish trade has led to many Southeast Asian and Pacific countries focussing on supplying this apparently lucrative trade through wild capture fisheries and aquaculture (Sadovy et al. 2003).

Worldwide, most grouper aquaculture production is from Southeast Asia. Based on FAO data, Taiwan and Indonesia are the major producers of farmed grouper, followed by Thailand and Malaysia (Table 1). However, unreported production may be substantial. Mainland China produced an estimated 8256 t of groupers in 1997 according to unofficial reports (NACA/TNC 1998), and production is likely to have increased substantially since then. Vietnam produced an estimated 2600 t of marine fish in 2001, of which a high proportion was cultured groupers (Le 2002). Based on these estimates, the regional total production of groupers through aquaculture in 2001 may have been more than 23,000 t and valued at around US$160 million.

Despite the continuing expansion of grouper aquaculture in the Asia-Pacific region, there remain several important constraints to the sustainable development of this industry sector. Foremost amongst these is the limited availability of fingerlings. Grouper aquaculture remains heavily dependent on the capture and grow-out of wild-caught juvenile fish; around 70–85% of cultured groupers are grown out from wild-caught fry (Sadovy et al. 2003). In addition, there is a recognised need to replace the widespread use of 'trash' fish as a feed source for groupers with compounded diets, as has been done for other marine finfish species such as barramundi/seabass (*Lates calcarifer*) and milkfish (*Chanos chanos*).



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

Photo: David Cook

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Table 1. Aquaculture production (tonnes [t]) reported to FAO by country and total value (US$ million) of groupers, 1990–2001.

Country	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001
Brazil	—	—	—	—	—	—	—	—	1	2	3	~4
Hong Kong SAR	365	265	55	632	627	620	1110	1036	312	280	523	910
Indonesia	—	—	—	—	—	—	—	—	—	1759	1159	3818
Korea, Republic of	—	—	—	—	—	—	9	—	—	5	6	20
Kuwait	—	—	—	—	—	—	—	—	—	5	6	3
Malaysia	144	153	288	1006	931	834	857	799	465	948	1217	1101
Philippines	2363	6765	349	772	2129	715	595	654	135	151	167	136
Saudi Arabia	—	—	—	—	—	—	—	—	1	—	—	—
Singapore	185	198	233	147	133	101	93	82	97	94	111	157
Taiwan	2206	1229	1125	3942	1841	2104	1883	2525	3471	4122	5053	5386
Thailand	415	355	965	755	1078	674	74	795	1390	1143	1332	1442
Tunisia	—	—	2	~1	~1	<0.5	<0.5	<0.5	—	—	—	—
United Arab Emirates	—	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
Total production (t)	5678	8965	3017	7255	6740	5048	5321	5891	5872	8509	9577	12,977
Total value (US$m)	28.5	64.4	29.4	59.5	81.1	67.5	65.5	73.0	45.7	63.9	67.2	89.9

Marine finfish aquaculture is an important contributor to the economies of coastal communities in the Asia-Pacific region, and aquaculture of high-value species such as groupers provides greater economic benefits to farmers than aquaculture of lower-value species such as milkfish (Yap 2002). Consequently, the development of sustainable grouper aquaculture can potentially provide widespread benefits to coastal communities in the Asia-Pacific region. The results described in this publication are the outcomes of a four-year research and development project funded by the Australian Centre for International Agricultural Research (ACIAR), involving collaborative research by institutions in Australia, the Philippines, Indonesia and the Asia-Pacific region. The project targeted two of the major production technology constraints for grouper aquaculture: increasing fingerling supply by improving hatchery production technology; and determining the requirements of juvenile groupers for critically-important nutrients, which is a prerequisite for the development of practical compounded diets to replace the feeding of 'trash' fish. In addition, the project provided a regional structure to coordinate and promote collaboration in grouper aquaculture research, development and extension activities to better utilise the existing institutional efforts in the Asia-Pacific region.

Larval rearing

As noted earlier, wild-caught groupers still make up the bulk of the seedstock supply in many parts of Southeast Asia, including Vietnam, Thailand and the Philippines. The trade in wild fry is associated with a number of resource management issues including overfishing, use of unsustainable harvesting techniques (including cyanide), high levels of mortality, and inadequate supply to support the demand of a developing aquaculture industry (Sadovy 2000). To meet aquaculture's demand for seedstock and to reduce pressure on wild fisheries, there is a recognised need to develop commercial marine finfish hatcheries throughout the Asia-Pacific region to supply hatchery-reared seedstock.

At the beginning of this project, despite at least a decade of research, hatchery production of groupers remained a limiting factor in grouper aquaculture. Average survival to fingerling stage (about 25 mm in length) was low, generally in the range 0–10% (Rimmer 1997, 1998, Rimmer et al. 2000). In addition, survival was highly irregular — one or two larval rearing 'runs' often resulted in reasonable (up to 10%) survival, while others had zero survival. Both of these factors (low average survival and unreliable hatchery production) were major constraints to the commercialisation of grouper larviculture technology.

In 1997 Taiwan was the only commercial producer of grouper fingerlings in the Asia-Pacific region (Rimmer 1998; Rimmer et al. 2000; Liao et al. 2001). Production of grouper fingerlings in Taiwan is largely limited to *Epinephelus coioides* and *E. malabaricus*, although there has been experimental production of several other

species, including *E. lanceolatus*, *Plectropomus* spp. and *Cromileptes altivelis* (Rimmer 1998; Liao et al. 2001). Larval rearing of groupers in Taiwan remains problematic, and many (wild caught) fingerlings are imported from other parts of Asia, particularly Thailand (Ruangpanit 1993; Sadovy 2000). Overall survival estimates are not available, but in 1997 Taiwanese farmers regarded the equivalent of 7% survival to 2.5–3 cm in length as excellent (Rimmer 1998).

The larval rearing component of the ACIAR project concentrated on a relatively broad range of research topics to assess which areas were likely to be limiting factors in successful grouper larviculture. These were:

- Environmental requirements to optimise survival in pre–feeding larvae.
- Nutritional (particularly fatty acid) requirements of larval groupers.
- Isolating and culturing SS–strain rotifers for use in grouper larviculture.
- Assessing the capacity of larval groupers to digest live and artificial prey, including documenting the development of the digestive tract and the ontogeny of digestive enzymes.
- Defining hatchery management techniques for larval rearing of groupers.

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

Photo: GBRMPA

Research results were continually integrated into hatchery verification trials to assess the practical outcomes of the research. The results of the larval rearing component are detailed in Section 2 of this publication.

Development of grow–out diets

The need for compounded (pellet) feeds to replace the feeding of 'trash' fish is widespread throughout the Asia–Pacific region. Issues regarding the use of trash fish have been identified in detail in several publications (for example New 1996) and include: competition for fishery products with human nutritional requirements and with other agricultural sectors; relatively low efficiency of utilisation of 'trash' fish (dry matter FCRs typically range from 5:1 to 10:1, compared 1:1 with 2:1 for pellet diets); and localised pollution due to losses of feed material during feeding (Phillips 1998). Because using 'trash' fish for feed is not economic in Australia, marine finfish aquaculture relies on the development of suitable cost-effective pelleted feeds. In addition, Australia's strict environmental regulation of aquaculture requires feeds that minimise nutrient release to the environment.

The grow-out diet component of the project was 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. Major research areas within this overall structure were:

- Inventory and categorise the composition of feed ingredients in Indonesia.
- Determine the apparent digestibility of a range of feed ingredients available in Indonesia, the Philippines and Australia.
- Determine the requirements of groupers for critical nutrients, with emphasis on protein and energy requirements.
- Assess the capacity to replace fishmeal in grow-out diets for groupers with terrestrial protein sources.

Research results from the grow-out nutrition component were validated through trials using experimental pellet diets in comparison with 'trash' fish, and later through trials using

commercially produced diets. Results of this component of the project are detailed in Section 3 of this publication.

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

Research coordination and information dissemination

Although there has been considerable research and development effort extended on developing sustainable grouper aquaculture in the Asia-Pacific, at the start of the project it was apparent that this effort had been relatively fragmented and uncoordinated. Discussion with other grouper aquaculture researchers indicated that most felt that they were working in isolation. This lack of communication and coordination between research and development institutions in the region caused overlap, and in some cases outright duplication, of research effort, which diluted the overall research progress in this field.

The third component of the ACIAR project targeted this issue by improving communication between grouper aquaculture researchers worldwide, but with particular emphasis on the Asia-Pacific region. The primary mechanism for this component of the project was the development and maintenance of the Asia-Pacific Grouper Network, coordinated through the Network of Aquaculture Centres in Asia-Pacific (NACA). Participants at the Grouper Aquaculture Workshop held in Bangkok in April 1998 (Rimmer et al. 2000) committed to reducing duplication and overlap of research effort by participating in a Grouper Aquaculture Research and Development Program to be coordinated by NACA. This component of the project was augmented with an APEC Fisheries Working Group project *Collaborative APEC Research and Development Grouper Network*.

Section 4 of this publication outlines the activities of the Asia-Pacific Grouper Network and its successor, the Asia-Pacific Marine Finfish Aquaculture Network, and provides examples of how a networking approach can more widely spread research and development benefits in the region.

Further Information

This publication summarises the results of ACIAR project FIS/07/73 *Improved hatchery and grow-out technology for grouper aquaculture in the Asia-Pacific region*. Additional details of the research work carried out under this project can be found at www.enaca.org/aciar/. A list of project and Asia-Pacific Marine Finfish Network publications is provided in Appendix 2. Many of these publications are available for download from www.enaca.org/grouper/.

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SECTION 2
LARVAL REARING

Improvements in larval rearing techniques for groupers increased larval survival from around 3% to 20–40% for *E. coioides* and from <10% to 30–50% for *C. altivelis*. This has transformed grouper hatchery technology to commercial viability, as evidenced by the dramatic increase in grouper fingerling production from Indonesian hatcheries. Grouper hatchery technology has been disseminated more widely than the project participants through the activities of the Asia-Pacific Marine Finfish Aquaculture Network.

Summary

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

An essential pre-requisite for successful marine finfish larviculture is to maximise survival and condition of the larvae prior to the commencement of exogenous feeding. Optimal environmental parameters during egg incubation and rearing of pre-feeding larvae were established for *E. coioides* and *C. altivelis* (Table 1).

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

A better understanding of the nutritional physiology of grouper larvae was established through this project. The histological study of the digestive tract in *E. coioides* and descriptions of the ontogeny and of digestive enzymes in *E. coioides* and *C. altivelis* provide a basis for assessing the digestive capacities at different developmental stages. The low activities of digestive enzymes and the rudimentary structure of the digestive tract indicated that grouper larvae have a low digestive capacity prior to 10 days post hatch. As the structural complexity of the

Juvenile tiger and humpback grouper in an Indonesian hatchery. The outcomes of ACIAR-supported research have contributed substantially to overcoming the bottleneck of fingerling supply of high-value species such as these.

Table 1. Environmental conditions for incubation of eggs and early larvae of two grouper species for optimal survival.

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

[1] determined in a 100L tank.
[2] determined in a 4L tank.

stomach progressed between 10 and 16 days post hatch, fluctuations in trypsin and total protease activities were observed suggesting a change in digestive physiology. The appearance of gastric glands coincided with a general increase in enzyme activities indicating an increase in the digestive capacity of the larvae preceding metamorphosis. It is also apparent that there are differences between grouper species in the emergence of digestive enzymes implying there may be different capabilities between genera for digesting the major macronutrients.

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

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

With improvements in larval nutrition and husbandry techniques, survival has now increased from around 3% to 20–40% for *E. coioides* and from <10% to 30–50% for *C. altivelis*. This has moved larval rearing technology for groupers into the realm of commercial viability. That this technology is commercially viable has been well demonstrated in Indonesia where, in 2003, there were an estimated 67 hatcheries (52 'backyard', six medium and nine large hatcheries) producing grouper fingerlings for the food fish and ornamental markets. Estimated production of grouper fingerlings from Indonesian hatcheries in 2002 was 3,350,200 fish (5–10 cm total length) comprising 2,656,200 *E. fuscoguttatus*, 697,800 *C. altivelis*, and 2200 *E. coioides* (Sugama 2003).

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

Marine finfish hatcheries provide important socio-economic contributions to coastal communities. The socio-economic assessment of 'backyard' hatcheries in Bali, Indonesia, showed that hatcheries are important sources of employment and economic benefit in northern Bali. Economic features of these hatcheries include: high profitability ($6300–$100,000 per annum), high internal rates of return (>12%), positive benefit-cost ratios (1.3–3.1), and rapid payback of capital cost (often with one year). These hatcheries are important sources of employment for local people, including women, either directly or in associated industries such as fish brokerage. A feature of these hatcheries is that they may switch between different species as commodity prices fluctuate; thus, the industry as a whole is relatively robust to market fluctuations.

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

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Environmental Factors Affecting Embryonic Development, Hatching and Survival of Early Stage Larvae of the Grouper (*Epinephelus coioides*)

J.D. Toledo, N.B. Caberoy and G.F. Quinitio

Introduction

The orange spotted grouper, *Epinephelus coioides*, is known to spawn readily in tanks and floating net cages (Toledo et al. 1993). Despite recent developments in seed production techniques (Duray et al. 1997; Toledo et al. 1999), survival of early-stage larvae is still low. Low survival during the early larval stage is likely due to the effects of poor egg quality, the size and nutritional value of prey at the onset of feeding, and the environmental conditions during the incubation of fertilised eggs and early stage larvae. In this study, a series of experiments was conducted to determine the effects of density, salinity, aeration and light intensity on the survival of fertilised eggs and early stage larvae of *E. coioides.*

Methods

The effects of density, aeration, and salinity on embryonic development, hatching, and survival of the early larval stages of grouper were determined. Spontaneously spawned grouper eggs were collected 15–20 minutes (min) after spawning. Eggs at early cleavage (8–32 celled stage) were stocked in 4 L plastic containers at 200, 400, 800 or 1600 eggs/L. Fertilised grouper eggs at early cleavage stage were stocked in 4 L containers at a density of 400 eggs/L. Four levels of aeration were tested (0 ml/min, 100 ml/min, 500 ml/min, 1000 ml/min) to determine the effect of aeration intensity on egg and larval survival. To examine the sensitivity of grouper eggs to various salinities, eggs at early cleavage or 'eyed stage' were abruptly transferred to salinities of 8, 16, 24, 32 and 40 ppt. Survival rate was determined by the percentage of eggs that reached the eyed stage. There were three replicates for each treatment.

Newly hatched larvae (day of hatching = day-0) were randomly distributed in 15 units of 40 L aquaria containing 35 ppt seawater at a density of 1,500 larvae/aquarium. Five aeration levels were tested: 0 (no aeration); 0.62 ml/min/L; 1.25 ml/min/L; 2.50 ml/min/L and 3.75 ml/min/L. A single air-stone was positioned at the centre of the floor of each aquarium. Five salinity levels were tested: 8, 16, 24, 32 and 40 ppt. Five light intensities (0, 120, 230, 500 and 700 lx) were randomly assigned in five enclosed chambers. Three 40 L aquaria containing 22 ppt seawater were placed in each chamber. The desired light intensities were obtained by adjusting the number of 21 to 100 watt fluorescent bulbs in each chamber. Except for the dark chamber (0 lx), the photoperiod was maintained at a 12-hour light and 12-hour dark cycle. All aquaria were provided with aeration at 0.62 ml/min/L.

Twenty larvae in each aquarium were sampled daily at 1100 hrs. Total length (TL) and the diameter of the oil globule of each larva were measured using an Image Analysis System equipped with Image-Pro Plus Imaging Software for Windows. Larvae were then preserved in 5% formalin-seawater for gut content analysis.

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Results and Discussion

Embryonic survival, hatching rates and percentage of normal larvae were greatly affected by stocking density, intensity of aeration and salinity during egg incubation. The highest viability, hatching rates and percentage of normal larvae were observed at a stocking density of 400 eggs/L, aeration level of 100 ml/min and salinity of 32–42 ppt (Figs. 1–3). Low hatching rates were noted at salinities below 32 ppt. Almost all of the eggs exposed to a salinity of 8 ppt failed to hatch. Eggs at the eyed stage were more sensitive to salinity change than those at early cleavage. The most remarkable abnormalities were the presence of curvature in the body and some exceptionally small (shrunken) larvae.

Salinity, aeration and light intensity greatly influenced oil globule absorption, feeding incidence, growth and survival of *E. coioides* early stage larvae (Figs. 4–6). Under static conditions, early stage grouper larvae survived much better and with a bigger oil globule under gentle aeration of 0.62 and 1.25 ml/min/L than those under stronger aeration of 2.50–3.75 ml/min/L

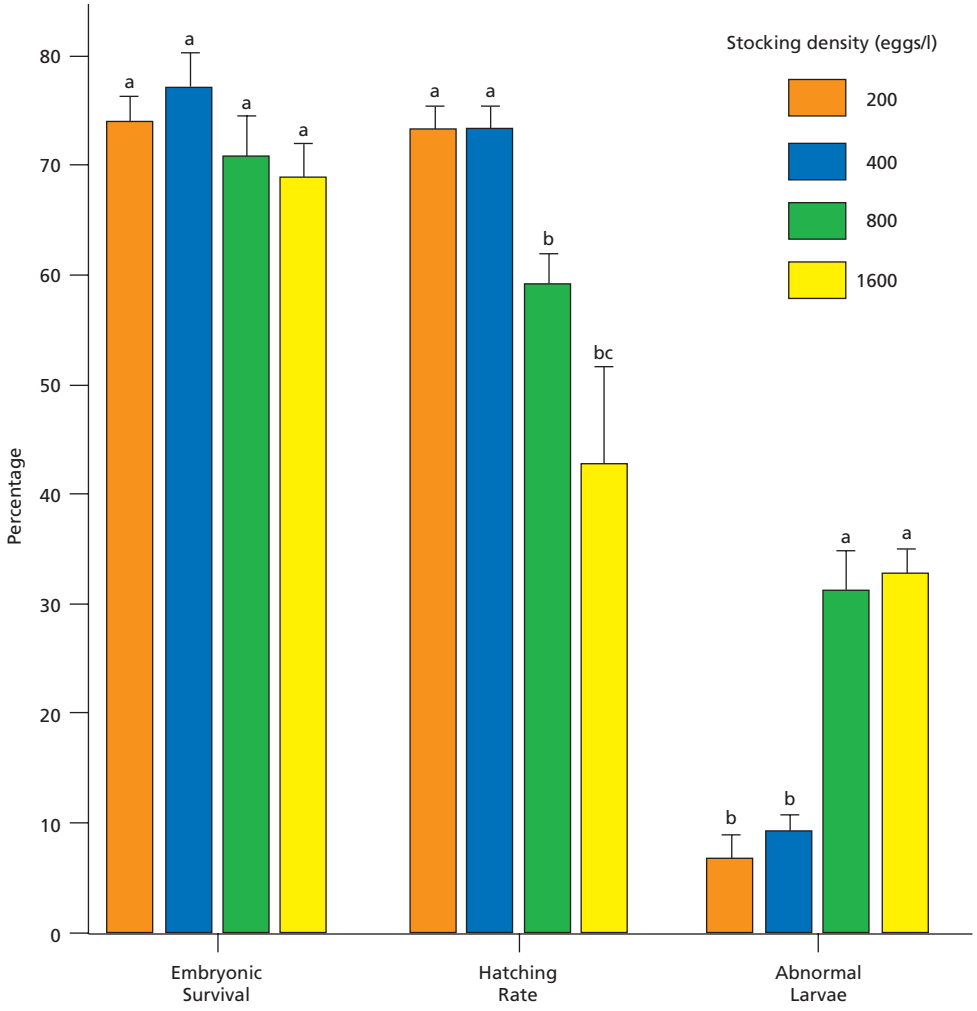

Figure 1. Effect of stocking density on embryonic survival, hatching and occurrence of abnormal larvae in the grouper *E. coioides*. Bars with the same letters are not significantly different (P > 0.05).

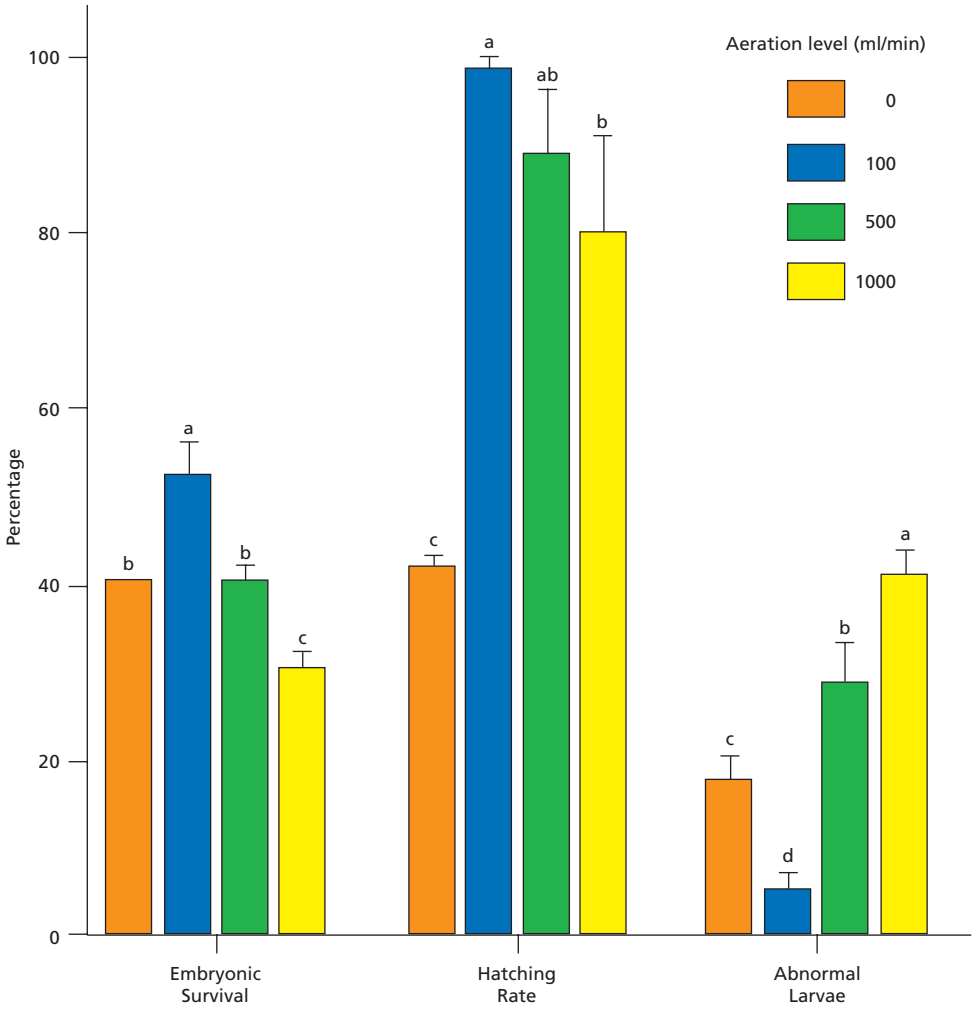

Figure 2. Effect of the intensity of aeration on embryonic survival, hatching and occurrence of abnormal larvae in the grouper *E. coioides*. Bars with the same letters are not significantly different (P > 0.05).

and under static conditions (Fig. 4). Larvae exposed to higher aeration levels most likely required more energy to maintain their swimming position. Low feeding incidence of larvae at higher aeration on day-5 also suggests that larvae experienced difficulty in catching prey.

Newly hatched larvae in this study survived an abrupt transfer from a spawning and hatching salinity of 35 ppt to salinity in the range of 8 to 40 ppt. This indicates that *E. coioides* larvae have a strong tolerance to salinity fluctuations. On day-1, larvae at salinities of 16 and 24 ppt swam to the middle or upper-portion of the water column while larvae at 8 ppt remained clustered at the bottom of the aquarium. They did not die and float as described by Yamaoka et al. (2000) for *E. akaara*.

Larval survival was significantly higher at the light intensities of 500 and 700 lx than the lower intensities tested. The lowest level of growth and biggest volume of remnant oil globules observed at 0 lx could have been caused by the lower activity of the larvae in total darkness.

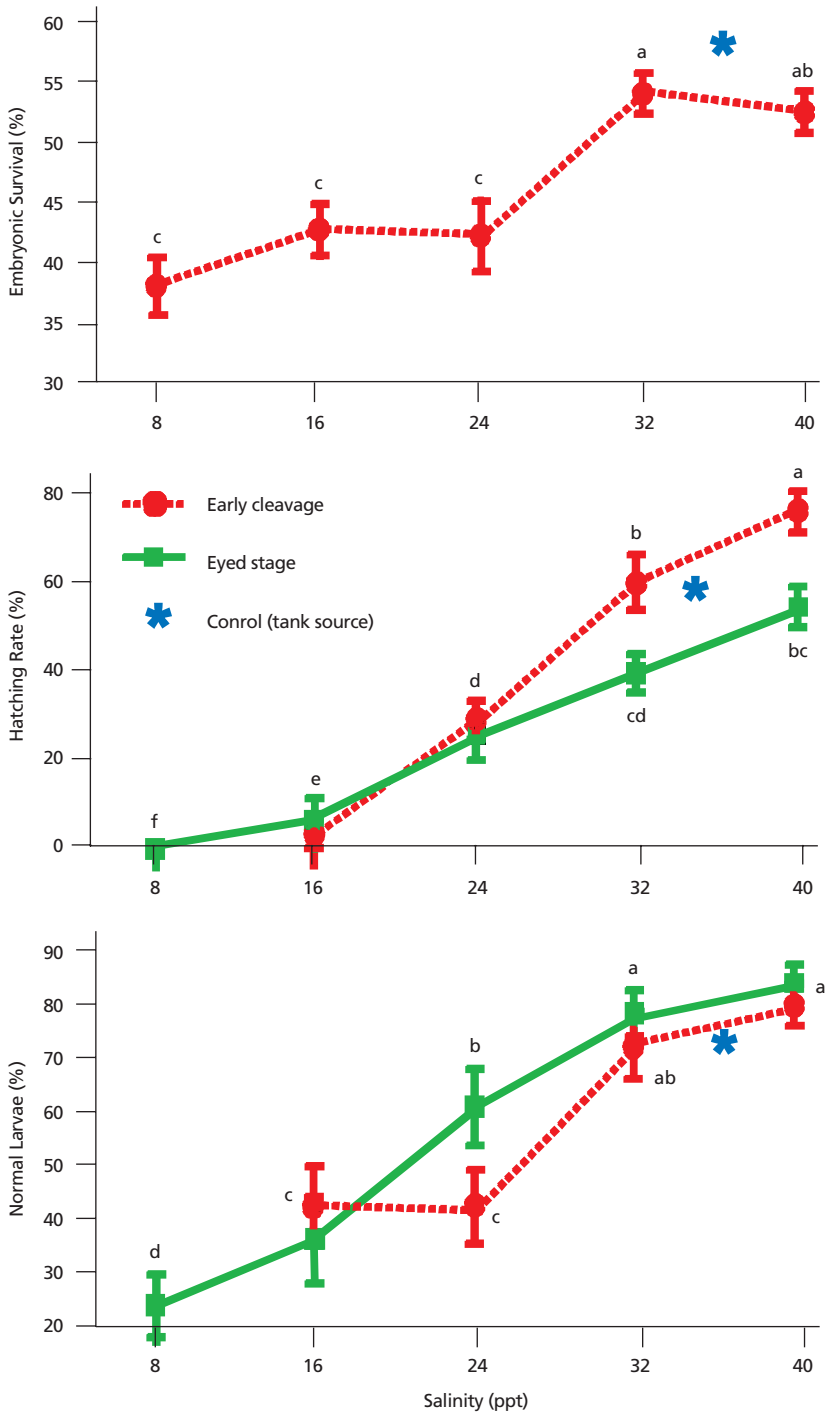

Figure 3. Embryonic survival, hatching and percentage of normal larvae of *E. coioides* abruptly transferred to different salinities at early cleavage (8–32 celled) and eyed stages. Means with the same superscripts are not significantly different ($P > 0.05$).

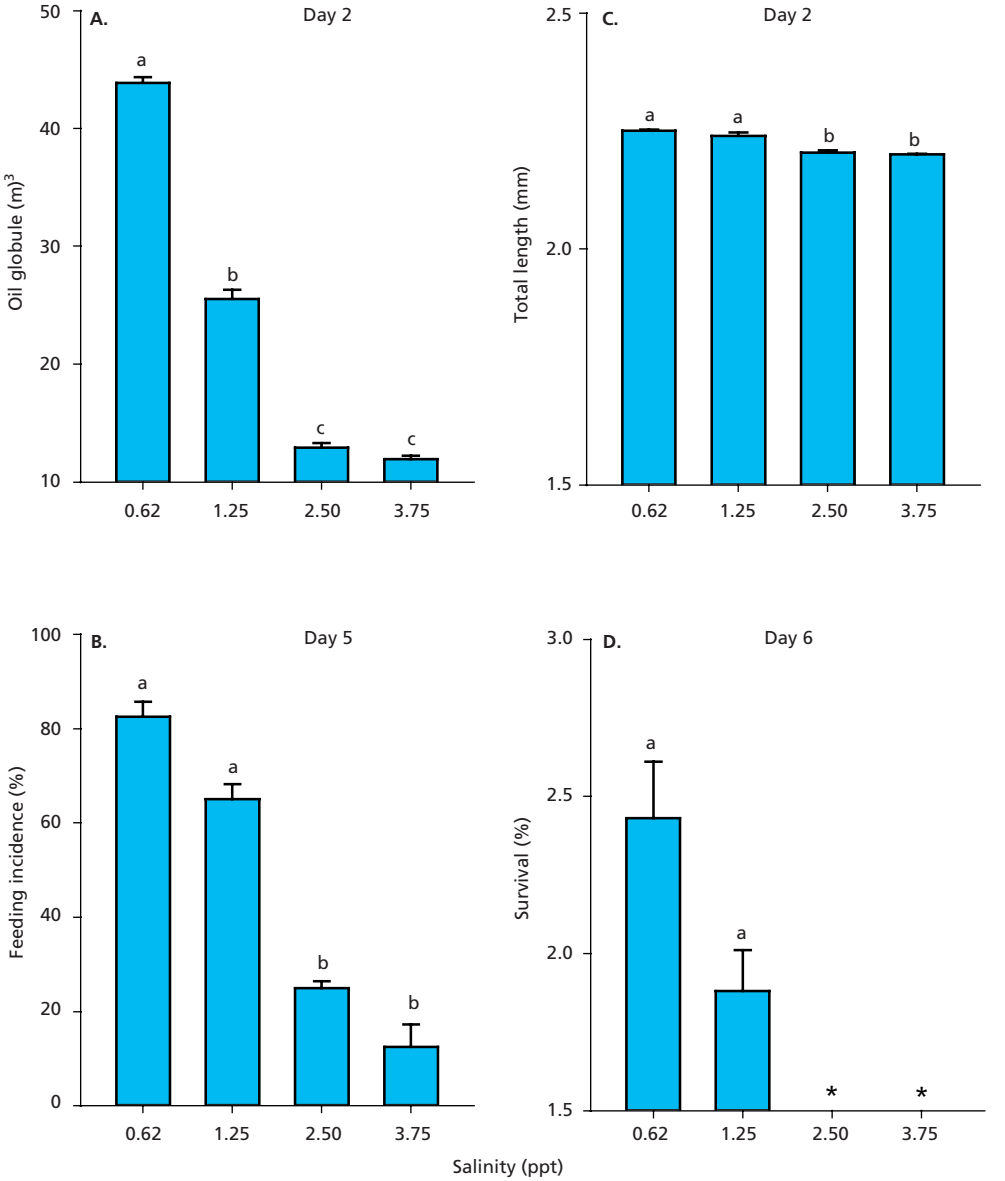

Figure 4. Effects of various aeration levels on A) oil globule absorption, B) feeding incidence, C) total length and D) survival of *E. coioides* larvae. Each bar represents mean ± SE of 3 replicates (n = 20). Bars with different letters are significantly different (P > 0.05). *All larvae died on Day 6.

No survival of larvae at light intensities 0 1x and 120 lx, and very low survival at 230 lx, suggests that the light intensity appropriate for first-feeding *E. coioides* larvae would be higher than 500 lx. Yamaoka et al. (2000) suggested that light intensity for seed production of *E. akaara* must be lower than 1000 lx at the water surface level of rearing tanks until 10 days after hatching.

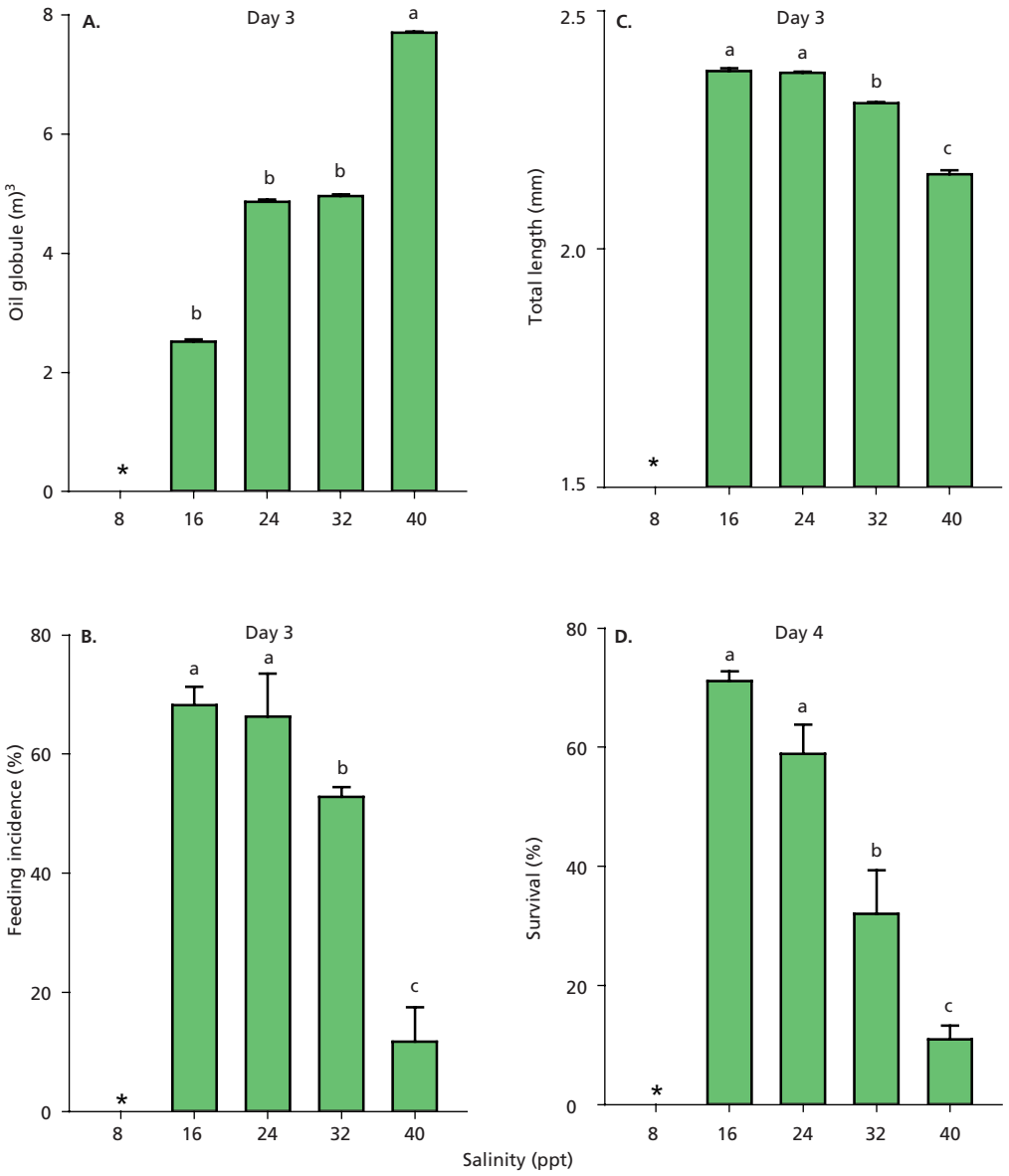

Figure 5. Effects of various salinity levels on A) oil globule absorption, B) feeding incidence, C) total length and D) survival of *E. coioides* larvae. Each bar represents mean ± SE of 3 replicates (n = 20). Bars with different letters are significantly different (P > 0.05). *All larvae died on Day 3.

Conclusions

- Under static incubation conditions, the highest egg viability, hatching rate and percentage of normal larvae were obtained at a stocking density of 400 eggs/L with moderate aeration (100 ml/min), and salinity of 32–42 ppt.
- Gentle aeration at 0.62 to 1.25 ml/min/L, rearing water of 16 to 24 ppt and a light intensity of 500 to 700 lx maximised the survival of early stage *E. coioides* larvae in the hatchery.

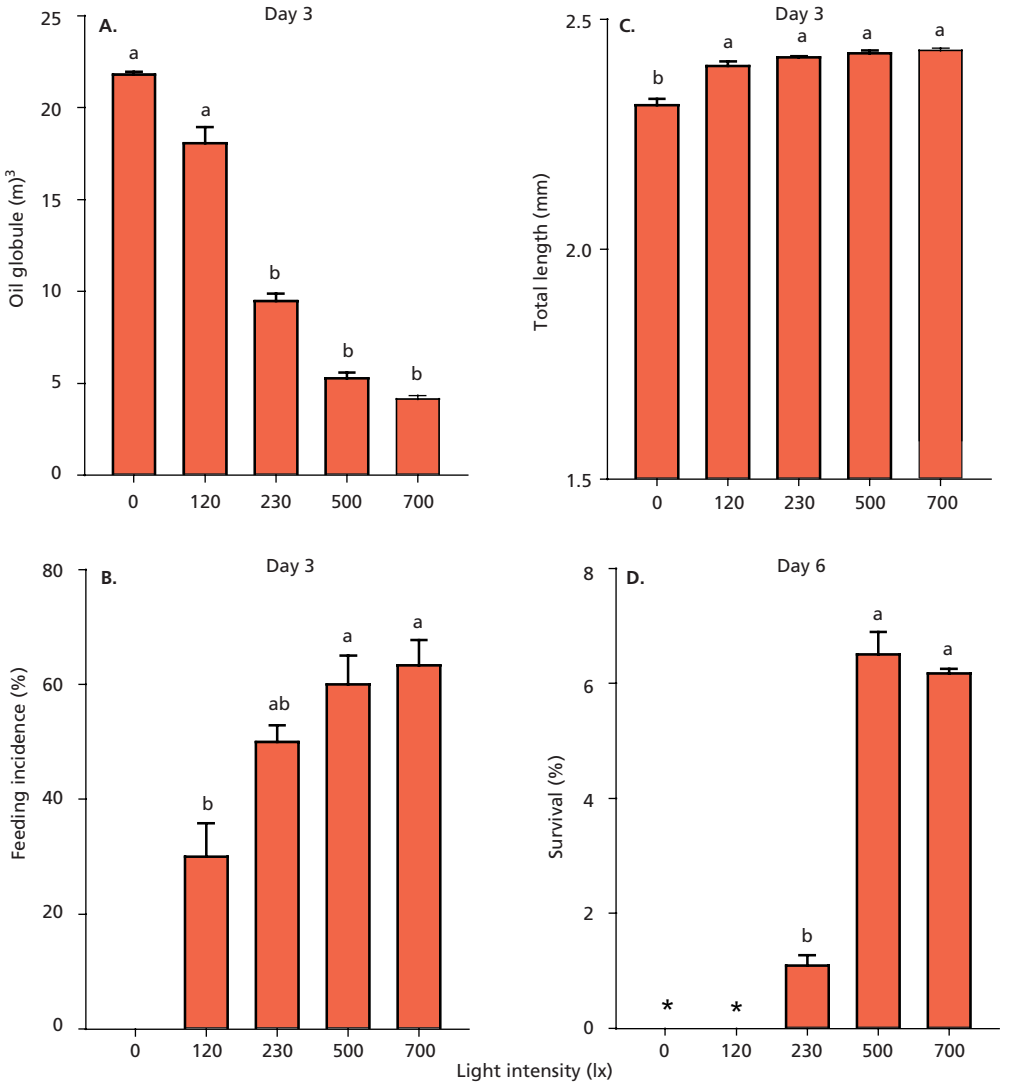

Figure 6. Effects of various light intensities on A) oil globule absorption, B) feeding incidence, C) total length and D) survival of *E. coioides* larvae. Each bar represents mean ± SE of 3 replicates (n = 20). Bars with different letters are significantly different (P > 0.05). *All larvae died on Days 4 and 6, respectively.

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Environmental Factors Affecting Embryonic Development and Hatching of Humpback Grouper (*Cromileptes altivelis*) Larvae

K. Sugama, Trijoko, S. Ismi and K. Maha Setiawati

Introduction

Larvae of humpback grouper show drastic changes in morphology as they develop from hatched larvae to juvenile stage (Mishima and Gonzares 1994). Until larvae complete metamorphosis to juvenile stage, they are very sensitive to environmental conditions and are prone to stress and high mortality rates.

Egg handling should be done very carefully to prevent mechanical shocks. Sensitivity of humpback grouper eggs may be different at each stage of egg development. Eggs are very sensitive before the embryonic stage and just before hatching. Salinity, temperature and egg density are important factors during the incubation of marine fish eggs (Holliday 1988).

The purpose of the present studies was to determine the effect of egg density, water exchange and aeration rate on embryonic development and hatching rate of humpback grouper, *Cromileptes altivelis,* eggs.

Material and Methods

Spawners and Eggs

Grouper spawners weighing 1.06–3.16 kg each, total length 41.5–57.0 cm were held in round concrete tanks (75 m^3) with a density of 24 fish (16 female and 8 male). Broodfish were fed trash fish and squid that were supplemented with a vitamin mix and vitamin C. Water exchange in the broodstock tanks was 300–500% per day using flow through system.

Humpback grouper spawn at night, mostly around 22:00–24:00 hours. Since fertilised grouper eggs float, spawned eggs flowed directly into the egg collector (300–400 μm mesh size) that was connected to the tank overflow pipe. Collected eggs were then harvested on the following morning and transferred into a transparent 30-litre polycarbonate tank filled with filtered sea water. Only floating eggs were transferred into the polycarbonate tank for the experiment.

Experiment 1: Effect of Egg Density

Six treatments of egg density were studied in this experiment: 500, 1000, 1500, 2000, 2500 and 3000 eggs/litre. The experiment was conducted using three litre transparent bottles each filled with two litres of sea water. The experiment was a completely randomised design with three replicates for each treatment. Samples of eggs were collected every hour after stocking for observation of embryonic development.

Experiment 2: Effect of Water Exchange

This experiment used 12 transparent tanks, 100 litres in volume and filled with sea water (34 ppt). In each tank were stocked newly spawned eggs at a density of 500 eggs/litre. Treatments for this experiment were: without water exchange; 100% water exchange; and 200% water exchange per day. The experiment was a completely randomised design with three

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treatments and three replicates per treatment. Samples were taken every hour after stocking for embryonic development and hatching observation.

Experiment 3: Effect of Aeration Rate

This experiment used 12 transparent bottles, three litres in volume filled with sea water (34 ppt). In each bottle was stocked newly spawned eggs at a density of 500 eggs/litre. The treatments in this experiment were: (A) aeration rate of 0 ml/min, (B) 200 ml/min, (C) 400 ml/min, and (D) 600 ml/min. The experiment was a completely randomised design with four treatments and three replicates per treatment. Samples were taken every hour after stocking for observation of embryonic development and hatching.

Embryonic Development

Time from spawning to various embryonic stages was recorded for the different treatments. Ten to 30 eggs were analysed each hour and their development was examined by microscope.

Result

Experiment 1

Egg development from spawning to various embryonic stages at different egg density is summarised in Table 1. Total time required from spawning to hatching ranged from 18 hours and 41 minutes to 20 hours and 29 minutes. The hatching rate was significantly higher for eggs incubated at lower density (Table 2). However, eggs incubated at the density of 2000, 2500 and 3000 eggs per litre resulted in the same hatching rate.

Table 2. Average value of hatching time and hatching rate of humpback grouper eggs incubated at different densities.

Treatment (eggs per litre)	Hatching time (hrs)	Hatching rate (%)
A (500)	18.68 ± 0.55^{a}	77.0 ± 12.3^{a}
B (1000)	18.76 ± 0.47^{ab}	71.3 ± 6.6^{b}
C (1500)	18.85 ± 0.38^{ab}	65.0 ± 0.2^{c}
D (2000)	19.18 ± 0.02^{ab}	59.7 ± 5.2^{d}
E (2500)	19.43 ± 0.02^{b}	56.7 ± 8.2^{d}
F (3000)	20.48 ± 1.28^{c}	59.0 ± 5.8^{d}

Values followed by the same letter within a column are not significantly different (P > 0.05).

Experiment 2

Table 3 shows that embryonic development from many cells to hatching was faster at higher water exchange. Almost 70% of eggs had hatched at 17:00 for eggs incubated at a water exchange of 200%/day. On the other hand, eggs incubated at 100%/day water exchange and without water exchange had a hatching rate of

Table 1. Time observation on the development of humpback grouper eggs from spawning to hatching during the incubation at different egg densities.

Developmental stages	Egg density (per litre)					
	A (500)	B (1000)	C (1500)	D (2000)	E (2500)	F (3000)
Spawning	23:19	23:19	23:19	23:19	23:19	23:19
1-cell	23:30	23:35	23:37	23:37	23:40	23:40
2-cells	23:45	23:50	23:50	23:50	23:55	23:55
4-cells	23:46	23:46	23:55	23:55	23:55	23:57
16-cells	23:54	00:00	00:00	00:00	23:05	23:05
64-cells	00:00	00:10	00:10	00:15	00:17	00:17
Many cells	00:10	00:15	00:20	00:20	00:22	00:22
Morula	02:15	02:20	02:25	02:25	02:25	02:25
Blastula	02:46	02:50	02:50	02:55	02:57	02:57
Gastrula	03:00	03:02	03:02	03:02	03:10	03:10
Head and tail bud	06:56	06:58	06:58	06:58	06:58	06:58
Optic bud	07:00	07:05	07:05	07:10	07:15	07:15
Digestive system	08:40	08:50	08:50	08:55	09:00	09:00
Form fin	11:05	11:15	11:20	11:25	11:30	11:30
Heart beating	11:10	11:25	11:25	11:30	11:35	11:35
Pre-hatching	14:20	14:30	14:30	14:35	14:45	14:45
Hatching	18:00	18:05	18:10	18:30	18:45	19:10

Table 3. Observations of embryonic development of humpback grouper eggs at different water exchange-rates during incubation.

Embryonic development	Water exchange (% per day)					
	200		100		0	
	Time	%	Time	%	Time	%
1-4 cell	21:10	80	21:15	80	21:17	80
Many cells	22:30	80	22:35	80	22:37	80
Morola stage	23:00	—	—	—	—	—
Blastula stage	02:10	70	02:15	65	02:20	50
Gastrula stage	03:37	—	—	—	—	—
Neorula stage	05:29	70	05:35	62	05:40	50
Digestive system	09:55	—	08:30	60	—	—
Hatching	17:00	70	17:30	60	18:00	50

only 60% and 50% at 17:30 and 18:00, respectively. The hatching rate of eggs incubated at 200%/day (71.6%) and 100%/day (57.7%) water exchange was not significantly different, but significantly higher compared with no water exchange (48.3%).

Experiment 3

Hatching time of eggs incubated at different aeration rates are shown in Table 4. Hatching time is significantly different ($P < 0.05$). All eggs hatched at around 18:00 in the afternoon. Dissolved oxygen increased and ammonia concentration decreased in incubation tanks with increasing aeration rate (Table 4). Hatching rate and survival of 3-day old larvae increased significantly ($P < 0.05$) with increasing aeration rate (Table 5).

Table 4. Hatching time of humpback grouper eggs incubated at different aeration rates, dissolved oxygen (DO) and ammonia concentration in the incubation water.

Aeration rate (ml/min)	Hatching time (hour)	Dissolved Oxygen (ppm)	Ammonia (ppm)
A (0)	18.63 ± 0.39^{a}	2.50–3.40	0.445–0.892
B (200)	18.20 ± 0.03^{ab}	4.50–5.00	0.380–0.646
C (400)	18.13 ± 0.03^{ab}	4.80–5.40	0.241–0.553
D (600)	18.00 ± 0.23^{b}	4.90–5.50	0.221–0.443

Values in column followed by same letter are not significantly different ($P > 0.05$).

Table 5. Hatching rate of humpback grouper eggs and survival of 3-day old larvae after the eggs were incubated at different aeration rates.

Aeration rate (ml/min)	Hatching rate (%)	Survival (%)
A (0)	21.0 ± 3.6^{a}	31.7 ± 2.9^{a}
B (200)	38.3 ± 2.9^{b}	40.3 ± 2.5^{b}
C (400)	69.3 ± 1.2^{c}	52.3 ± 2.5^{c}
D (600)	78.7 ± 3.2^{d}	62.3 ± 2.5^{d}

Values within a column followed by the same letter are not significantly different ($P > 0.01$).

Discussion

After fertilisation, humpback grouper eggs hatched within 18.41–19.51 hours at an egg density of 500–3000/litre; 18–18.38 hours at aeration rate of 0–600 ml/min; 19.50–20.50 hours at water exchange of 0–200%/day at 29–32°C water temperature and salinity 34 ppt. Hussain et al. (1975) (cited in Kawahara et al. 1997) reported that grouper *Epinephelus tauvina* eggs hatched within 26–35 hours after fertilisation at 27–30°C, much longer than the present observation. Hatching time of *E. fuscoguttatus* eggs was 18–19 hours at 28–30°C water temperature (Chao et al. 1993 cited in Kawahara et al. 1997). The hatching time of artificially-fertilised egg of *E. striatus* was 21.1–22.3 hours for the first-hatching and 23–25.5 hours for the complete hatching at 28°C (Watanabe et al. 1995). However, other environmental conditions, such as

light intensity, aeration and salinity could influence the incubation time of grouper eggs.

The average hatching rate success of *Cromileptes altivelis* eggs was 77%, with an egg density of 500/litre, 71.6% with a water exchange 200%/day, and 78.67% with an aeration rate of 600 ml/minute. Watanabe et al. (1995) reported that hatching success of *E. striatus* eggs was as high as 82.5% at the water temperature of 26–30°C, but the mortality of yolksac larvae was accelerated at higher temperatures. The result of survival rate of pre-feeding stage of *C. altivelis* larvae (day-3) in present study was 62.3% with an aeration rate of 600ml/min. Biochemical quality of eggs that related to the condition of the broodstock can influence larval survival (Watanabe et al. 1984). Other environmental factors such as aeration (water circulation) and light intensity may also influence metabolism and survival of yolksac larvae.

Conclusion

- For optimum hatching rates of *C. altivelis* eggs, an egg density of 500 eggs/litre, water exchange of 200%/day and an aeration rate of 600ml/minute are recommended.

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SS-strain Rotifer Culture for Finfish Larvae with Small Mouth Gape

Richard M. Knuckey, Inneke Rumengan and Stenly Wullur

Introduction

In the aquaculture of temperate marine finfish, the rotifer *Brachionus plicatilis* (L-strain) has been the primary live food for early larval stages. As aquaculture has diversified to include tropical marine species the small strain rotifer, *B. rotundiformis,* is now routinely cultured. Many marine finfish have larvae with a small mouth gape that has required the selection of a super small (SS) strain of *B. rotundiformis.* However, even this SS-strain of rotifer is not an optimal size and a smaller size would be ideal for first-feeding finfish larvae.

The objective of this work was to develop culture techniques that would select for smaller sized rotifers and reduce the average size of rotifers in a population. A typical population of rotifers contains reproductive females of varying sizes with the distribution skewed toward the smaller animals. The aim was to apply selection pressures that would exaggerate this skewed distribution and result in a higher proportion of smaller rotifers.

Methods

Small rotifer body size, as a heritable trait related to the size of amictic and resting eggs, was determined by: measuring amictic eggs attached to rotifers; measuring harvested eggs (amictic and resting); isolating individual eggs to form clonal colonies; and measuring adult rotifers of resulting populations. Initial rotifer populations and clonal lines were maintained at 28°C, 30 ppt salinity and fed *Nannochloropsis oculata* at 3×10^6 cell/ml.

Effects of the environmental factors salinity and diet on rotifer body-size were determined. To examine the effect of salinity on the development rate and final size of rotifers, 30 eggs from a clonal culture were placed in each well of a 24-well plate at three salinities (5, 20 and 30 ppt). Over a 24-hour period following hatching, rotifers were removed hourly from a well (n = 30), measured and the time of appearance of reproductive females was noted.

The effect of diet on rotifer body size was determined by feeding rotifers an equal ash-free, dry-weight ration (equivalent to 3×10^6 cells/ml of *N. oculata*) of algae of different cell mass. Algal mass ranged from 1 pg/cell for *Stichococcus* to 10 pg/cell for *N. oculata,* 170 pg/cell for *Tetraselmis* and 572 pg/cell for *Heterocapsa niei*.

In the first experiment, first laid eggs from a clonal culture were distributed among wells of 24-well plates containing each of the algal diets. Three-day old, F1 rotifers were collected from the resulting populations and measured. In the second experiment, 16×1 L rotifer cultures (20 rotifers/ml) were fed with four different algae (4 replicates/algal diet).

Replicates were fed daily and the population adjusted to 20 rotifers/ml. A sample (~40) of harvested, egg-bearing rotifers was measured every second day. After 14 days, the size distribution of the replicate populations was compared to one fed the control species, *N. oculata*.

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Results and Discussion

The SS-strain rotifer was isolated from Centenary Lakes, Cairns. At the start of the program, reproductive females had an average lorica length of 151 ± 15 µm and width of 111 ± 10 µm. The distribution was skewed toward smaller sizes with the length of the smallest reproductive female measured being 96 µm (Fig. 1). A poor relationship was found between the length of the parent and the length of its egg (r^2 = 0.09); and between the area of the parent rotifer and the area of its egg (r^2 = 0.24). The maximum width of amictic eggs averaged 96 ± 11 µm of which 14% were smaller than 85 µm (average –1 SD). Offspring hatched from this sub-group of eggs had an average size of 147 µm and a distribution similar to the initial population. The optimal salinity for hatching and resting eggs was 5 ppt seawater. Resting eggs, collected and sorted into small resting eggs (77 ± 6 µm), hatched to produce females with an average length of 135 ± 9 µm at commencement of egg production. However, selection and culture of the two smallest females (100–120 µm) produced populations with an average body length of 140 and 148 µm (Fig. 2).

Salinity affected the rate of rotifer development. Development was fastest at the lowest tested salinity of 5 ppt and slowed as salinity rose to 20 ppt and 30 ppt (Fig. 3). Rotifers also became reproductive earlier at lower salinity and egg-bearing rotifers appeared before maximal size was attained.

Diet also affected the development of rotifers. After feeding on four equal ration diets of varying particle size (1 pg/cell to 572 pg/cell) for 14 days, the average length and width of the rotifer populations was not significantly different (Fig. 4). However, the distribution of sizes within the populations was different. Rotifers raised on the control diet of *N. oculata* had an average body dimension of 179 µm in length and 140 µm in width. Fifty-six per cent of the population had a body length less than the average and 46% had a body width greater than the average. Rotifers fed *Stichococcus*-like algae, (the smallest diet at 1 pg/cell) had 72% of the population with a body length less than the average length of the control rotifers fed *N. oculata*. Rotifers fed *Tetraselmis* had a larger proportion of wide rotifers with 64% being larger than the average width of those fed the

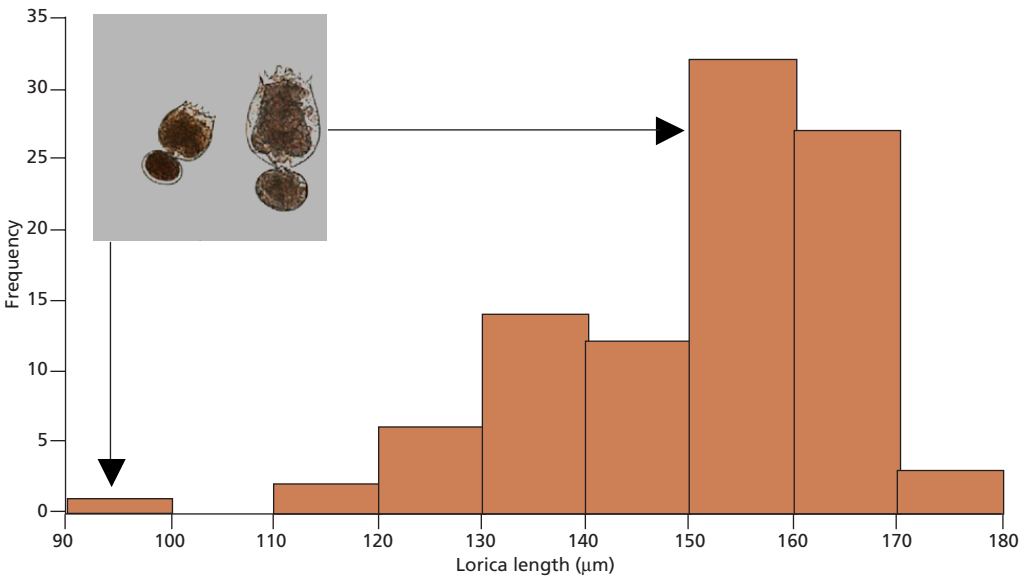

Figure 1. Size distribution of the Cairns isolate of the SS-strain rotifer. Average lorica length = 151 ± 15 µm.

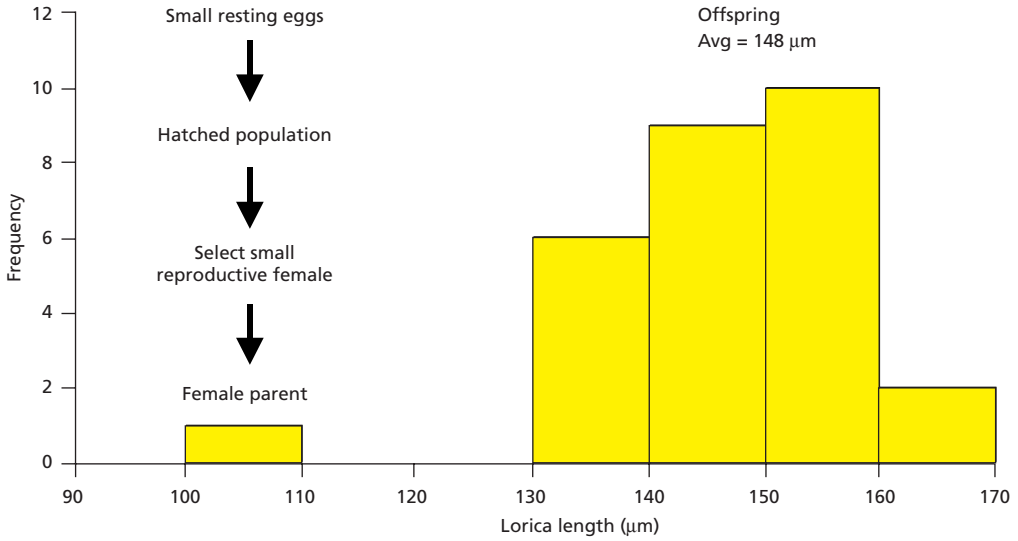

Figure 2. Example of mean size (lorica length) of resulting population of rotifers bred from a single, small parent isolated from a population hatched from smallest resting eggs.

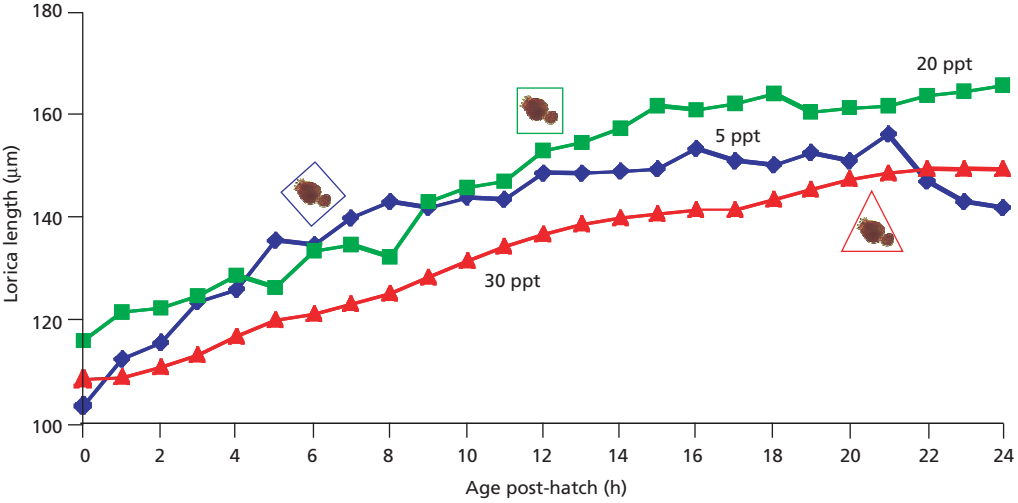

Figure 3. Effect of salinity on the rate of rotifer development (increase in lorica length). Rotifer symbols indicate the time of the first appearance of egg production. All sample points n = 30 rotifers.

control diet *N. oculata*. Rotifers fed the largest size alga, *H. niei,* were similar to those fed the control. This indicates that this alga could be too large for rotifers to ingest so they are feeding on algal cell debris and bacteria.

The results confirm the plasticity of the rotifer lorica and the polymorphism that occurs in populations. Rotifer size may vary by more than 100% between habitats (Ruttner-Kolisko 1977). Increase in rotifer lorica size when a fed diet of large-celled algae (*Tetraselmis*) has been reported (Rumengan et al. 1998). However, Reitan et al. (1997) found differences in lorica length due to different diets that were not large enough to affect their availability to fish larvae. We found diet had no significant effect on the

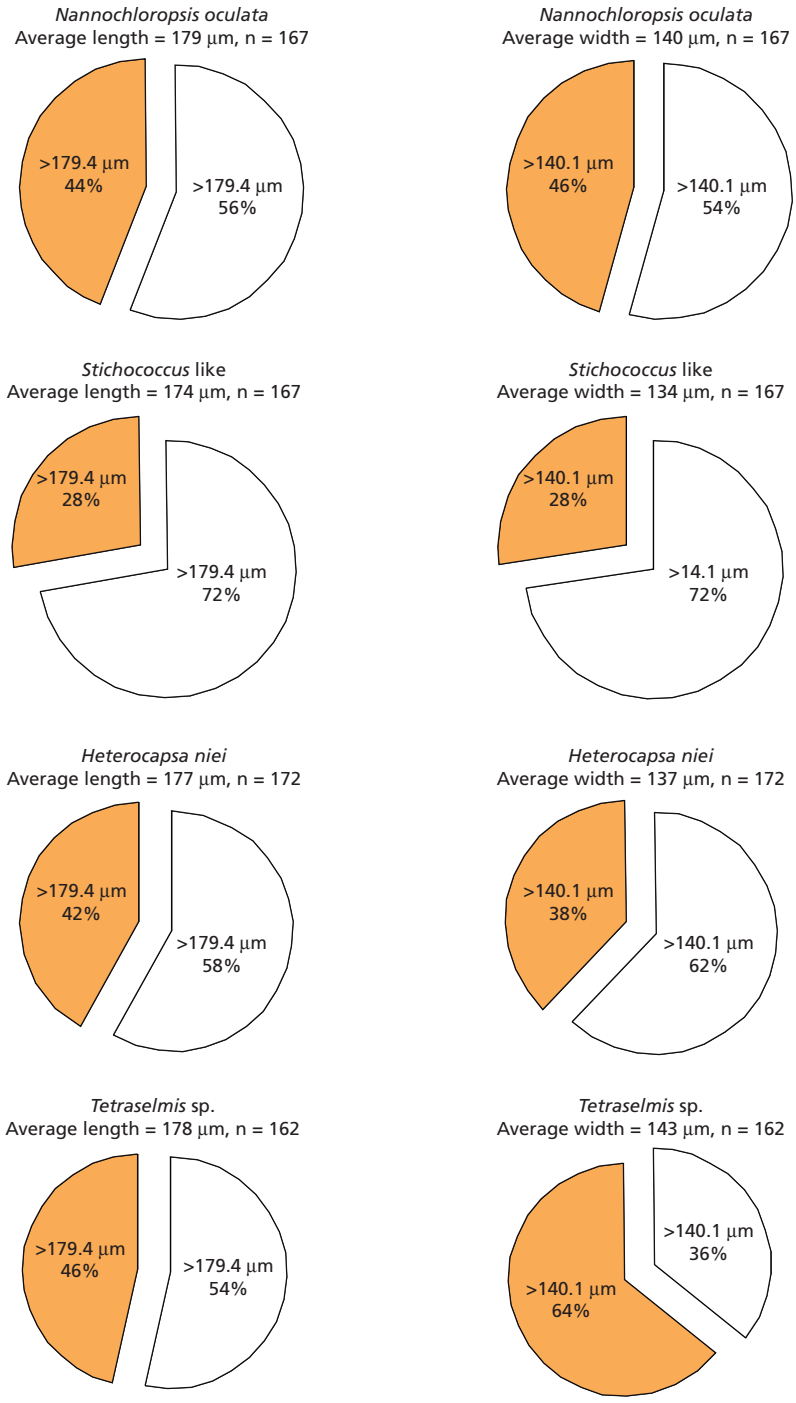

Figure 4. The average size (left: body length; right: body width) and size distribution of populations of rotifers raised for 14 days on equal ration diets of four microalgae of varying particle size (cell mass). Algal cell mass (ash-free, dry-weight/cell) for the control diet *N. oculata* = 10 pg/cell. For the test diets, *Stichococcus*-like = 1 pg/cell, *Tetraselmis* sp. 170 pg/cell and *H. niei* = 572 pg/cell. Size distributions are in relation to the average size (length 179.4 µm and width 140.1 µm) of the control rotifers fed *N. oculata*.

average size of rotifers but feeding with very small algae did increase the percentage of smaller rotifers within a population. This is beneficial when rotifers are used to feed fish larvae with a small mouth gape.

Conclusions

- Higher percentages of smaller rotifers suitable for first feeding larvae are obtained when rotifers are raised on ultra-small algae such as *Stichococcus*.
- Selection of small resting eggs is more successful than amictic eggs in producing a rotifer population of reduced size.
- Much of the skewness toward smaller sized reproductive rotifers in a population is a result of rotifers reproducing before achieving maximal size. This reduces the chance of successfully reducing the size of rotifers by selecting for small reproductive females since most of these rotifers will continue to increase in size as they mature. Synchronous rotifer cultures are required for this purpose, in which all rotifers have reached maximal size, before the smallest individuals are selected.

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DPI&F researcher Dr Richard Knuckey with UNSRAT staff and students.

Changes in the Gastrointestinal Tract and Associated Organs During Early Development of the Grouper (*Epinephelus coioides*)

G.F. Quinitio, A.C. Sa-an, J.D. Toledo and J.D. Tan-Fermin

Introduction

Production of grouper, *Epinephelus coioides,* juveniles for stocking in grow-out ponds and floating net cages is hindered by problems encountered during larval rearing. Although some advances in grouper larval rearing have been made using live food organisms (Duray et al. 1997; Toledo et al. 1999), there is still a need to further improve survival. One approach is to understand the nutritional physiology of the larvae. The development of the gastrointestinal tract during growth may be related to habitat, body form, and diet of the fish (Ferraris et al. 1987). Knowledge of the changes directly associated with the process of food assimilation is important for understanding the nutritional physiology of the larvae (Segner et al. 1993) and thereby improve survival under laboratory rearing conditions. This paper describes the morpho-histological changes in the gastrointestinal tract of *E. coioides* and associated organs during its early development.

Methods

Larvae of *E. coioides* were reared in five-tonne rectangular concrete tanks using the semi-intensive culture system described by Toledo et al. (1999). Copepods were propagated by adding nauplii, adults, and copepodids of *Acartia* and *Pseudodiaptomus* to the larval rearing tank 2–3 days before stocking with newly hatched larvae. *Brachionus rotundiformis* were added to the tank at increasing density (2–10 individual/ml) from day 2 to day 18. *Artemia* nauplii and metanauplii were given to satiation starting on day 16 until they could feed on 'trash fish' or pellets. Weaning to trash fish or an artificial diet started at day 30 to day 35. Larval samples were collected at day 0 (newly hatched), day 2, day 4, day 6, day 8, day 10, day 12, day 14, day 16, day 20, day 25, day 30, day 35, day 40, day 45, and day 60. Total length (TL) of about 10–20 larvae per sampling was measured. Water temperature and salinity during the rearing period were 26–28°C and 20–30 ppt, respectively.

Larvae were preserved in Bouin's solution, dehydrated through a graded alcohol series, and embedded in paraffin. Prior to embedding in paraffin, a portion of the head and tail were cut-off in large larvae to reduce the size of the sample for sectioning. About 6–8 µm longitudinal sections were stained with Harris' hematoxylin and eosin and alcian blue-periodic acid-Schiff (AB-PAS) (Cook 1990). At least three samples were examined from each stage for longitudinal sections using light microscopy. Depending on the number of samples available (1–3 larvae), 6–8 µm cross-sections were also processed and examined to counter-check the observations made in the longitudinal sections.

Results and Discussion

Newly hatched (day 0) grouper larvae (TL: 1.42–1.80 mm, n = 20) have a large yolk that contains a single oil globule. The primordial digestive tract was a straight tube located above the yolk sac and below the notochord. At day 2 (TL: 2.00–2.70 mm, n = 20), the mouth and anus opened. The intestine and rectum were already distinct from each other due to the presence of the intestinal-rectal valve between them. Moreover, the liver was observed with the gall bladder next to it while the pancreas was located at the dorsal side of the mid-portion of the intestine and posterior to the swim bladder.

At day 4 (TL: 2.37–2.66 mm, n = 20), the yolk sac and oil globule were resorbed. The oesophagus was differentiated and the anterior part of the intestine had clearly formed into the primordial stomach. Coiling of the intestine was observed in day 6 larvae (TL: 2.50–3.21 mm, n = 10) and the spleen was located posterior to the swim bladder. At day 10 (TL: 2.33–3.60 mm, n = 20), the primordial stomach had broadened into a voluminous pouch and increased in size by day 12 (TL: 2.55–4.23 mm, n = 20) and transformed into a stomach.

At day 30 (TL: 11.38–22.6 mm, n = 20), the pancreas continued to enlarge and extend posteriorly along the intestine. The pyloric caeca was already prominent at this age. A prominent blind sac was observed at day 35. From day 40 (TL: 14.20–22.4 mm, n = 20) to day 60 (TL: 31.61–66.95 mm, n = 20), the pancreas and liver continued to enlarge. These changes are very similar to the description by Trijuno (2001) of coral trout, another grouper species.

The histological changes in the digestive tract of *E. coioides* from day 0 to day 60 are summarised in Table 1. The straight, undifferentiated digestive tract of day 0 larvae was composed of simple cuboidal cells. In day 2 larvae, the pharynx, oesophagus, primordial stomach, and intestine showed cellular differentiation. Mucosal folds with villi at the apical region were already apparent at day 4. Moreover, supranuclear vacuoles with eosinophilic granules were seen in the rectum, which increased in number as the larvae grew. These vacuoles have also been observed in many marine fish larvae (Tanaka 1971, 1972 a, b; Walford and Lam 1993; Kaji et al. 1996, 1999; Trijuno 2001). The function of these supranuclear vacuoles is for protein absorption before differentiation of the digestive glands before the stomach is fully functional. These vacuoles gradually disappeared as the stomach became fully functional.

Mucus cells were observed in the pharynx and oesophagus at day 8 and were AB-PAS positive. In the intestine, goblet cells were seen from day 12 and were also AB-PAS positive. At day 20, gastric glands were first observed in the stomach and few goblet cells were observed in the pyloric caeca. Tanaka (1973) described these occurrences as an indication of transformation from larva to juvenile.

Proliferation of gastric glands was observed at day 30 which may be an indication that the larvae were developing the ability to feed on fish as reported for Pacific bluefin tuna (Kaji et al. 1996) and yellowfin tuna (Kaji et al. 1999). At day 35, the lamina propria and submucosa in the stomach were very distinct while the blind sac had become prominent. The tissue layers in the intestine had also become well developed. Therefore, feeding minced fish to *E. coioides* larvae at day 35, when reared using the semi-intensive system as developed by Toledo et al. (1999), appears to be appropriate. There is minimal morpho-histological change from day 40 to day 60.

Conclusion

- The digestive tract of day 0 larvae was a straight, undifferentiated tube composed of simple cuboidal cells.
- At day 2, cellular differentiation was observed in the pharynx, oesophagus, primordial stomach, and intestine.
- The primordial stomach broadened into a voluminous pouch at day 10.
- The gastric gland was observed in the stomach from day 20.
- Day 35 seems to be the proper time to feed fish larvae fish flesh when using the semi-intensive rearing system.
- There were no significant morpho-histological changes in the metamorphosing grouper larvae from day 40 to day 60.

Table 1. Summary of histological changes in the digestive tract of the grouper, *E. coioides,* larvae.

Age (post-hatching)	Region of Digestive Tract				
	Pharynx	Esophagus	Stomach	Pyloric Caeca	Intestine
0	Straight, undifferentiated tube made up of simple cuboidal cells				
2	Stratified squamous cells	Stratified squamous cells, +CM	Cuboidal cells, +CM	Absent	Tall columnar cells, +CM
4	Sac-like widening, muscle layer	Mucosal folds	Tall columnar cells, mucosal folds	Absent	Mucosal folds, wider lumen, apical region with villi, SV with eosinophilic granules in rectum
6	+LP and +SM but not distinct from each other	+LP and +SM but not distinct from each other	NSC	Absent	Increased no. of SV in rectum
8	Pharyngeal teeth, mucus cells, taste buds	Mucus cells, developing LM, thicker CM than stomach	NSC	Absent	NSC
10	NSC	NSC	NSC	Absent	NSC
12	NSC	LP/SM, CM and +S distinct from each other	Distinct CM, hollow space below mucosal epithelium	Absent	+Goblet cells, hollow space below mucosal epithelium
14	NSC	Increased no. of mucus cells, CM continued thickening	Developed mucosal folds, +LP and +SM not distinct, +S	Absent	Few goblet cells, thin strand along hollow space, decreased no. of SV in rectum
16	NSC	NSC	NSC	Absent	+S
20	Moderate no. of mucus cells	+LM below LP and SM which are now distinct from each other, numerous mucus cells	2 mucosal cell layers (outermost mucosal epithelium and below are gastric glands), thicker CM in pyloric region than cardiac region	Present with goblet cells	Moderate no. of goblet cells, taller mucosal folds, LP and SM not distinct, no SV in rectum
25	NSC	Reduced SM by the invasion of LM	NSC	NSC	NSC, abundant SV with eosinophilic granules in rectum
30	Abundant mucus cells, developed LP and SM	Developed 4 tissue layers (taller mucosal folds with numerous mucus cells, SM with LM, thicker CM and S)	Gastric glands proliferate, taller mucosal folds and deeper pits	Present with abundant goblet cells	Abundant goblet cells, distinct LP, mucosal folds branching, no SV in rectum
35	LP and SM very distinct	LP and SM very distinct	LP and SM very distinct, blind sac distinct	Prominent	Well-developed tissue layers, abundant SV in rectum
40	NSC	Well-developed LP	NSC	Branching	Further height increase of mucosal folds and more branching, 1 fish with SV in rectum
45	NSC	Well-developed tissue layers	Well-developed tissue layers	Pronounced branching	Distinct SM
60	NSC	NSC	NSC	NSC	NSC

CM = circular muscle layer; NSC = no significant change; LM = longitudinal muscle layer; LP = lamina propria; S = serosa layer; SM = submucosa; SV = supranuclear vacuoles; + = present.

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Localisation of Enzymes in the Digestive System During Early Development of the Grouper (*Epinephelus coioides*)

G.F. Quinitio, A.C. Sa-an, J.D. Toledo and J.D. Tan-Fermin

Introduction

With growth, the morphological structure of the digestive tract of fish larvae becomes more complex and is accompanied by periods of sharp increases in enzyme activity (Timeyko and Novokov 1987). Understanding this process would be helpful in improving growth and survival of fish larvae. Several studies have been done on the localisation of digestive enzymes in larval fishes, including those by Ferraris et al. (1987), Minjoyo (1990), Sarasquete et al. (1995), Gawlicka et al. (1995), and Trijuno (2001).

This paper describes the occurrence of some digestive enzymes in the gastrointestinal tract during early development. The work was conducted to provide information on formulating an appropriate feeding scheme and an artificial diet for the early development of the grouper *Epinephelus coioides*.

Methods

Larvae of *E. coioides* were reared in five-tonne rectangular concrete tanks using the semi-intensive culture system described by Toledo et al. (1999). Copepods were propagated by adding nauplii, adults and copepodids of *Acartia* and *Pseudodiaptomus* to the larval rearing tank two to three days before stocking with newly hatched larvae. *Brachionus rotundiformis* were added to the tank at increasing density (2–10 individual/ml) from day 2 to day 18. *Artemia* nauplii and metanauplii were given to satiation starting on day 16 until they began to feed on 'trash fish' or pellets. Weaning to trash fish or an artificial diet started at day 30 to day 35.

Larval samples were collected at day 0 (newly-hatched), day 2, day 4, day 6, day 8, day 10, day 12, day 14, day 16, day 20, day 25, day 30, day 35, day 40, day 45, and day 60.

Water temperature and salinity during the rearing period were 26-28°C and 20-30 ppt respectively.

Larvae were collected and fixed in formal calcium at 4°C for 18 hours, washed in tap water, blotted dry, placed in gum sucrose at 4°C for 18 hours, blotted dry again and then embedded in an embedding medium for frozen specimens (Bancroft 1990 — with slight modification). Samples were kept in a freezer at –80°C until cryostat sectioning. Longitudinal sections (8–10 µm) were cut using a Minotome cryostat at about –12°C. Sections were collected on a glass slide. The digestive enzymes localised were acid phosphatase (ACP), alkaline phosphatase (ALP), non-specific esterase (NSE), aminopeptidase (AMP), trypsin, (TRP), maltase (MAL), and lipase (LIP).

Techniques used were based on those of Cousin et al. (1987), Bancroft (1990), Cook (1990), Gawlicka et al. (1995), and Goodsell et al. (1995). About three to four larvae were used per enzyme.

Results and Discussion

The distributions of enzyme activity in the digestive system of grouper larvae at different ages are summarised in Tables 1 and 2. Most of the digestive enzymes were not observed at day 0 in *E. coioides* larvae, except for weak activity of the ACP and MAL enzymes in the yolk sac and weak staining of NSE in the oil globule.

Enzymes were localised in the pharynx, esophagus, intestine, and liver of day 2 larvae. NSE was found in all of these organs at this age with the intestine having the most enzymes. Among the enzymes observed in the intestine at day 2 (ACP, ALP, NSE, TRP, and MAL), ALP and NSE were stained intensely whereas ACP and MAL were moderate.

In general, weak activity of the enzymes occurs during the yolk sac stage because the larvae are still dependent on endogenous nutrition for metabolism as observed in other fishes (Buddington and Christofferson 1985; Ferraris et al. 1987; Minjoyo 1990). However we observed strong staining of ALP and NSE in the intestine from day 2 in the grouper larvae. Stroband et al. (1979) suggested that the presence of ALP during the early larval stage is necessary for nutrient transport in the intestine when the larvae start exogenous feeding. High levels of ALP are usually associated with absorptive cells (Troyer 1980).

NSE was already strong in the intestine from day 2 while AMP was weak at day 2 but its activity slowly became strong as the larvae grew. Ferraris et al. (1987) also made this observation in milkfish and suggested that esterases may be more essential than aminopeptidases since grouper and milkfish larvae feed on rotifers and copepods as their initial food. The late occurrence of high levels of AMP in grouper larvae (day 14) happened several days prior to feeding on brine shrimp (Toledo et al. 1999). Minjoyo (1990) correlated a high level of AMP in day 20 sea bass larvae, *Lates calcarifer,* to its carnivorous habit.

TRP in the intestine of *E. coioides* larvae exhibited weak staining during initial feeding, then stronger staining during active feeding, weak staining again at the start of brine shrimp feeding and strong again thereafter. Such fluctuations were also seen in the grouper *Plectropomus leopardus* (Trijuno 2001) and sea bass *Dicentrachus labrax* (Cahu and Zambonino-Infante 1994). It is also interesting to note that this enzyme was very weak in all the digestive organs at day 16, but was again strong in most organs at day 20 in *E. coioides.* The latter coincided with the appearance of the gastric glands. In the sea bass, Walford and Lam (1993) observed there is sharp decline in TRP activity after the stomach has become functional.

MAL was also always present in the intestine from day 2 and was strong from day 14 onwards indicating that grouper larvae have the capacity to digest carbohydrates. A similar trend was seen in flatfishes by Martinez et al. (1999). Several other carnivorous larvae have also been shown to have this capacity, particularly during the first half of larval development (Oozeki and Bailey 1995; Moyano et al. 1996; Kim, 2001; Divakaran et al. 1999).

LIP activity in the intestine was weak from day 14, moderate at day 20, and stronger from day 25. The increase in enzyme activity coincided with the occurrence of gastric glands at day 20 and increased in number thereafter.

Conclusions

- Weak enzyme activity occurs during the yolk sac stage when the grouper larvae are still dependent on endogenous nutrition for metabolism.
- High AMP activity started at day 14 prior to *Artemia* feeding at day 16.
- Fluctuations in TRP activity may be related to stomach formation.
- Occurrence of MAL during early development demonstrates a capacity to digest carbohydrates.
- An increase in LIP activity coincides with the occurrence of gastric glands.
- No significant changes in digestive enzymes were observed in the metamorphosing grouper larvae from day 40 to day 60.

Table 1. Distribution of enzyme activity in the digestive system of day 0 to day 14 grouper, *E. coioides,* larvae.

Age of Larvae (post-hatch)	Digestive Organs								
	Pharynx	Esophagus	Stomach		Pyloric caeca	Intestine	Liver	Pancreas	Spleen
			Columnar cells	Gastric glands					
0						++ALP ++NSE			
2	+ACP +NSE +MAL	+ACP +NSE				++ACP +++ALP +++NSE +TRP ++MAL	+ALP ++NSE +MAL		
4	+ACP ALP* ++NSE +TRP +MAL	+ACP ALP* ++NSE +TRP +MAL	+ACP +MAL			+++ACP +++ALP +++NSE +AMP +TRP ++MAL	+++ACP ++ALP +++NSE +MAL	ACP* ALP* TRP* +MAL	
6	+ACP ALP* ++NSE +TRP +MAL	+ACP ALP* ++NSE +TRP +MAL	+ACP ++NSE +MAL			+++ACP +++ALP +++NSE +AMP +TRP ++MAL	+++ACP ++ALP +++NSE +TRP +MAL	ACP* ALP* NSE* TRP* +MAL	++ACP ALP* +NSE TRP* MAL*
8	+ACP ++ALP ++NSE +TRP +MAL	+ACP +ALP ++NSE +TRP +MAL	+ACP ++NSE +TRP +MAL			+++ACP +++ALP +++NSE +AMP +++TRP ++MAL	+++ACP ++ALP +++NSE ++TRP +MAL	ACP* ALP* +TRP +MAL	++ACP ALP* +NSE +TRP +MAL
10	+ACP ++ALP ++NSE ++TRP +MAL	+ACP ++ALP ++NSE ++TRP +MAL	+ACP ++NSE +++TRP +MAL			+++ACP +++ALP +++NSE +AMP +++TRP ++MAL	+++ACP ++ALP +++NSE ++TRP +MAL	+ACP ALP* ++TRP +MAL	++ACP ALP* +NSE ++TRP +MAL
12	+ACP ++ALP ++NSE ++TRP +MAL	+ACP ++ALP ++NSE ++TRP +MAL	+ACP ++NSE +++TRP +MAL			+++ACP +++ALP +++NSE ++AMP +++TRP ++MAL	+++ACP ++ALP +++NSE ++TRP +MAL	+ACP +ALP ++TRP +MAL	++ACP ALP* +NSE ++TRP +MAL
14	++ACP ++ALP ++NSE ++TRP +MAL	++ACP ++ALP ++NSE ++TRP +MAL	+ACP ++NSE ++TRP +MAL			+++ACP +++ALP +++NSE +++AMP +++TRP +++MAL +LIP	+++ACP ++ALP +++NSE ++TRP +MAL	+ACP ++ALP +++TRP +MAL	+++ACP ALP* +NSE +++TRP +MAL

– = negative; + = weak.; ++ = moderate; +++ = intense; ACP = acid phosphatase; ALP = alkaline phosphatase; NSE = non-specific esterase; AMP = aminopeptidase; TRP = trypsin; MAL = maltase; LIP = lipase. * = organ not observed in samples.

Table 2. Distribution of enzyme activity in the digestive system of day 16 to day 60 grouper, *E. coioides,* larvae.

Age of Larvae (post-hatch)	Digestive Organs								
	Pharynx	Esophagus	Stomach		Pyloric caeca	Intestine	Liver	Pancreas	Spleen
			Columnar cells	Gastric glands					
16	++ACP ++ALP ++NSE +TRP +MAL	++ACP ++ALP ++NSE +TRP +MAL	+ACP ++NSE +TRP +MAL			+++ACP +++ALP +++NSE +++AMP +TRP +++MAL +LIP	+++ACP ++ALP +++NSE +TRP +MAL	+ACP ++ALP +TRP +MAL	+++ACP +ALP +NSE +TRP +MAL
20	++ACP ++ALP ++NSE +AMP ++TRP +MAL	++ACP ++ALP ++NSE +AMP ++TRP +MAL	+ACP ++NSE ++TRP +MAL	+ACP		+++ACP +++ALP +++NSE +++AMP +++TRP +++MAL ++LIP	+++ACP ++ALP +++NSE ++TRP +MAL	+ACP ++ALP +++TRP +MAL	+++ACP +ALP ++NSE +++TRP +MAL
25	++ACP ++ALP ++NSE +AMP +TRP	++ACP ++ALP ++NSE +AMP +TRP	++ACP ++NSE +TRP	++ACP ++NSE	+++ACP +++ALP +++NSE +++AMP TRP* +++MAL +++LIP	+++ACP +++ALP +++NSE +++AMP +TRP +++MAL +++LIP	+++ACP ++ALP +++NSE +TRP	++ACP ++ALP +NSE +TRP	+++ACP +ALP +NSE +TRP
30	++ACP ++ALP ++NSE +AMP +TRP +LIP	++ACP ++ALP ++NSE +AMP +TRP +LIP	++ACP ++NSE +TRP +LIP	++ACP ++NSE	+++ACP +++ALP +++NSE +++AMP +++TRP +++MAL ++LIP	+++ACP +++ALP +++NSE +++AMP +++TRP +++MAL +++LIP	+++ACP ++ALP +++NSE +TRP +LIP	++ACP ++ALP +NSE +++TRP 	+++ACP +ALP +NSE +TRP
35	++ACP ++ALP ++NSE +AMP +TRP +LIP	++ACP ++ALP ++NSE +AMP +TRP +LIP	++ACP ++NSE +TRP +LIP	++ACP ++NSE	+++ACP +++ALP +++NSE +++AMP ++TRP +++MAL +++LIP	+++ACP +++ALP +++NSE +++AMP ++TRP +++MAL +++LIP	+++ACP ++ALP +++NSE –TRP +LIP	++ACP ++ALP +NSE ++TRP 	+++ACP +ALP +NSE –TRP
40	++ACP ++ALP ++NSE +AMP +TRP LIP*	++ACP ++ALP ++NSE +AMP +TRP LIP*	++ACP ++NSE +++TRP +LIP	++ACP ++NSE	+++ACP +++ALP +++NSE +++AMP +++TRP +++MAL +++LIP	+++ACP +++ALP +++NSE +++AMP +++TRP +++MAL +++LIP	+++ACP ++ALP +++NSE ++TRP +LIP	++ACP ++ALP +NSE ++TRP 	+++ACP +ALP +NSE ++TRP
45	++ACP ++ALP ++NSE +AMP –TRP +LIP	++ACP ++ALP ++NSE +AMP –TRP +LIP	++ACP ++NSE +++TRP ++LIP	++ACP ++NSE	+++ACP +++ALP +++NSE +++AMP +++TRP +++MAL +++LIP	+++ACP +++ALP +++NSE +++AMP +++TRP +++MAL +++LIP	+++ACP ++ALP +++NSE –TRP +LIP	+++ACP ++ALP +NSE ++TRP 	+++ACP +ALP +NSE +TRP
60	++ACP ++ALP ++NSE +AMP +TRP +LIP	++ACP ++ALP ++NSE +AMP +TRP +LIP	++ACP ++NSE +AMP +++TRP ++LIP	++ACP ++NSE +AMP	+++ACP +++ALP +++NSE AMP* +++TRP +++MAL +++LIP	+++ACP +++ALP +++NSE +++AMP +++TRP +++MAL +++LIP	+++ACP ++ALP +++NSE AMP* ++TRP ++LIP	+++ACP ++ALP ++NSE AMP* ++TRP ++LIP	+++ACP ALP* NSE* AMP* TRP*

– = negative; + = weak.; ++ = moderate; +++ = intense; ACP = acid phosphatase; ALP = alkaline phosphatase; NSE = non-specific esterase; AMP = aminopeptidase; TRP = trypsin; MAL = maltase; LIP = lipase. * = organ not observed in samples.

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Digestive Enzyme Activity in Developing Grouper (*Epinephelus coioides*) Larvae

P.S. Eusebio, J.D. Toledo, R.E.P. Mamauag and M.J.G. Bernas

Introduction

Different fish species have their own unique digestion and food assimilation properties due to differences in the structure of their digestive tracts and in their way of feeding. Knowledge of the functional changes that are taking place in the digestive tract during food ingestion, digestion and assimilation is necessary to determine the ability of fish larvae to utilise a given diet (Segner et al. 1994). Timeyko and Novokov (1987) found that the complexity of the morphological structure of the digestive tract is accompanied by periods of sharp increases in enzyme activity. The variations in digestive enzyme activity during larval development are indicative of the type and level of macronutrients that should be included in artificial feeds (Cahu and Zambonino-Infante 1995). This study was undertaken to determine the activity of alkaline and acid type proteases, α-amylase, lipase, trypsin, chymotrypsin, leucine aminopeptidase, and alkaline and acid phosphatases during larval development of the grouper *Epinephelus coioides*.

Methods

Samples for measuring digestive enzyme activity were collected (sampling time: 8:00–11:00 hours) at different larval stages: day 0, day 2, day 4, day 8, day 12, day 16, day 20, day 25, day 30, day 35, day 40, day 45, day 50, day 55 and day 60. Whole larvae were used in the preparation of a crude enzyme extract from day 0 to day 30 and the mid-portion of the larvae was used from day 35 to day 60. Samples were freeze-dried, weighed and stored in a bio-freezer at–68°C prior to the preparation of crude enzyme extracts. Freeze-dried larvae (70 mg/3.5 ml) were homogenised in 50 mM Tris-HCl buffer, pH 7.5, centrifuged (12,500 × G, 30 min at 4°C), filtered through a Sephadex G-25 M column (1 × 10 cm.), centrifuged (2000 × G, 5 min at 4°C) and then decanted. The supernatant (crude enzyme extract) was used for total protein and different enzyme assays.

Total protein was determined using the method of Lowry et al. (1951). Alkaline type protease activity was measured using 1% casein as substrate; and one unit of enzyme activity was defined as the amount of enzyme catalysing the formation of 1 μg of tyrosine per minute (modified method of Walter (1984)). Acid type protease (pepsin) activity was determined using haemoglobin as a substrate; and one unit of pepsin activity expressed in tyrosine was equal to 0.001 of TCA soluble hydrolysis products per minute under standard conditions (Worthington Biochemical Corporation 1993). α-Amylase activity was quantified using soluble starch as a substrate; and one unit was defined as the amount of enzyme able to produce one micromole of reducing groups (calculated as maltose) per minute at 25°C (Worthington Biochemical Corporation 1993).

Lipase activity was measured as the rate of hydrolysis of an olive oil emulsion that was determined by titration using a pH meter (Worthington Biochemical Corporation 1993). One unit of activity was equal to one micromole

of acid produced per minute at 25°C under specified conditions. The activity of trypsin, chymotrypsin and leucine aminopeptidase was quantified according to methods described by Worthington Biochemical Corporation (1993). Trypsin activity was equivalent to one micromole of Nα-p-Tosyl-L-arginine Methyl Ester (TAME) that was hydrolysed per minute (25°C; pH 8.1), chymotrypsin activity was equivalent to one micromole of N-Benzoyl-2-monophosphate-Na-Ca (BTEE) that was hydrolysed per minute (25°C; pH 7.8), and the activity of leucine aminopeptidase was equal to one micromole of leucinamide hydrolysed per minute (25°C; pH 8.5). The activities of acid and alkaline phosphatases were determined at pH 4.8 and pH 9.8 respectively with nitrophenyl phosphate as the substrate (Bergmeyer 1974). The amount of 4-nitrophenol liberated per unit time in acidic solution was a measure of acid phosphatase activity, while the amount of 4-nitrophenol liberated per unit time in alkaline solution was a measure of alkaline phosphatase activity.

Results and Discussion

The total protein concentration of the newly hatched larvae (day 0) to day 2 was negligible (0.04 μg/larva). The concentration gradually increased with age of the larvae from 0.3 μg/larva at day 12 to 84.8 μg/larva at day 60 (Fig. 1). Both alkaline and acid type protease activities (Fig. 2) were detected at early stages of development in grouper larvae. Alkaline type protease activity was identified in the newly hatched larvae (0.01 mU/larva) and gradually increased to a peak at day 50 (7334.9 mU/larva). In contrast, acid type protease (pepsin) activity was not detected in the newly hatched larvae, but was detected at day 2 (2.2 U/larva). Its activity started to progress from day 12 (53.2 U/larva), which can be associated with the formation of the stomach. A two-fold increase in pepsin activity was observed from day 16 (53.2 U/larva) and every five days thereafter until day 40 (2706.7 U/larva). The decrease in the activity of alkaline type protease from day 50 to day 60 can be linked to metamorphosis. The relationship between a marked decrease in the specific activity of alkaline type protease and metamorphosis was reported by Tanaka et al. (1996) in Japanese flounder, *Paralichthys olivaceus,* and also by Alliot et al. (1980) in Senegal sole.

Figure 3 shows that α-amylase activity was detected in day 2 larvae (0.03 U/larvae) and a progressive increase was observed from day 16 (0.7 U/larva) until day 60 (141.9 U/larva). Early detection of α-amylase activity has also been reported for other marine fish larvae and in all cases, the activity increased with age (Munilla-Moran et al. 1990). Moyano et al. (1996) observed that a marked increase in the activity of α-amylase in seabream was closely related to

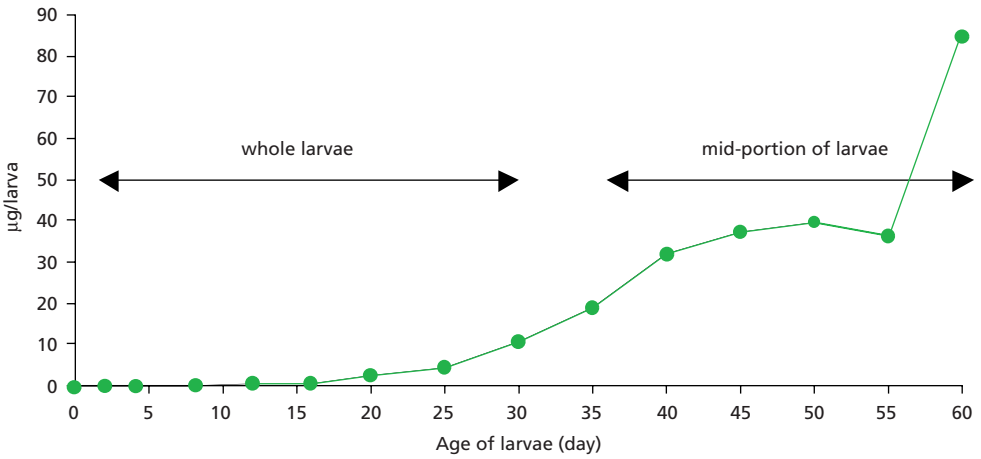

Figure 1. Total protein concentration in crude enzyme extracts from grouper larvae.

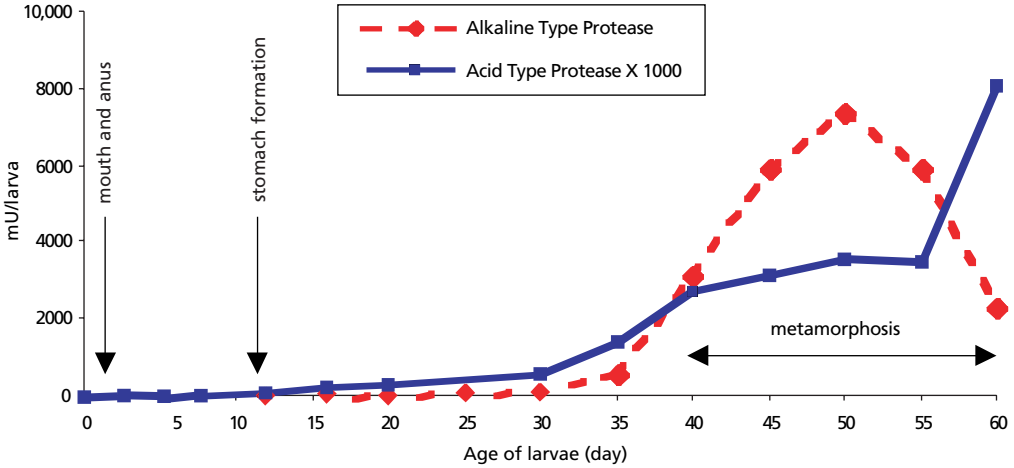

Figure 2. Protease activity in crude enzyme extracts from grouper larvae.

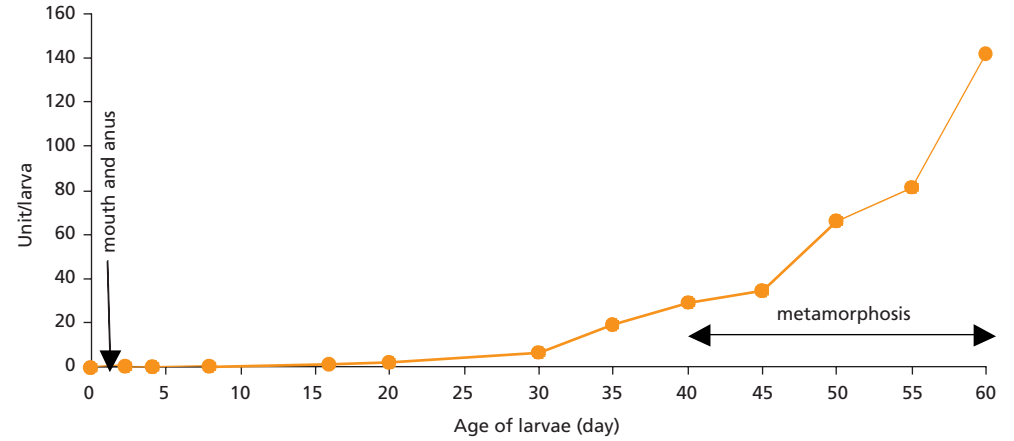

Figure 3. α-amylase activity in crude enzyme extracts from grouper larvae.

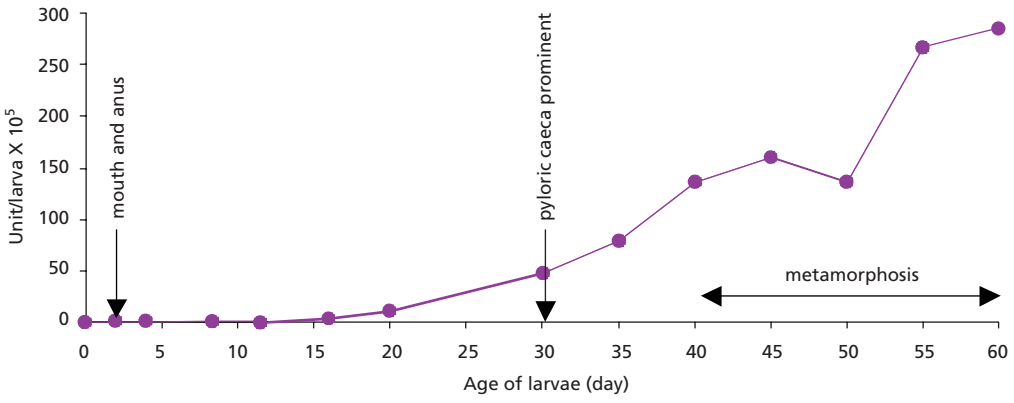

Figure 4. Lipase activity in crude enzyme extracts from grouper larvae.

its feeding habits. In this study, α-amylase activity in grouper larvae increased with age and they may be capable of digesting carbohydrates at day 16. Also, the activity of lipase increased with age of grouper larvae (Fig. 4). The gradual increase in lipase activity (0.04–285 × 10^{-5} U/larva) can be related to the development of the pyloric caeca and intestine, which were fully developed at day 30.

Leucine aminopeptidase activity started to increase to an appreciable amount from day 16 (5.4 U/larva) until day 60 (447.3 U/larva) but was highest at day 40 (601.5 U/larva), which was the onset of metamorphosis in the grouper larvae (Fig. 5). The trypsin and chymotrypsin activity patterns are shown in Figure 6. Trypsin activity (9.0–16,407.5 mU/larva) seemed to be higher than chymotrypsin activity (1.3–10,368.7 mU/larva) from day 8 until day 60. However, there was a change in the pattern when chymotrypsin activity increased from 1964.6 mU/larva at day 40 to 2932.3 mU/larva at day 45 while trypsin activity decreased from 3130.6 mU/larva at day 40 to 709.5 mU/larva at day 50. As shown in Figure 7, the activity of acid phosphatase was increasing, which started from day 12 until day 60 (0.1–46.5 mU/larva), whereas alkaline phosphatase activity started from newly hatched

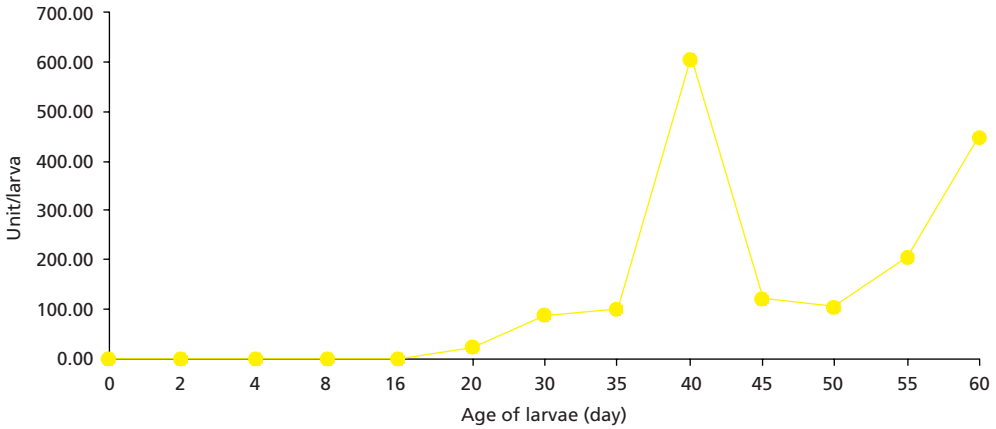

Figure 5. Leucine aminopeptidase activity in crude enzyme extracts from grouper larvae.

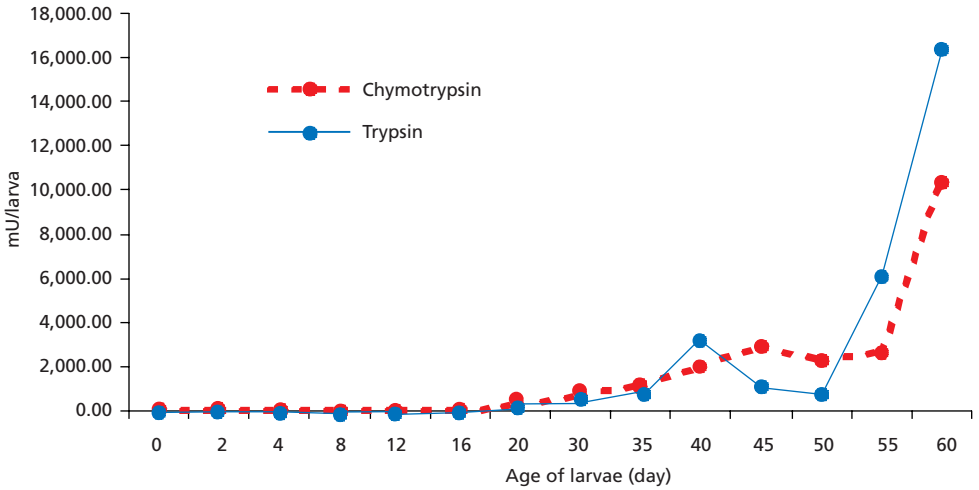

Figure 6. Trypsin and chymotrypsin activity in crude enzyme extracts from grouper larvae.

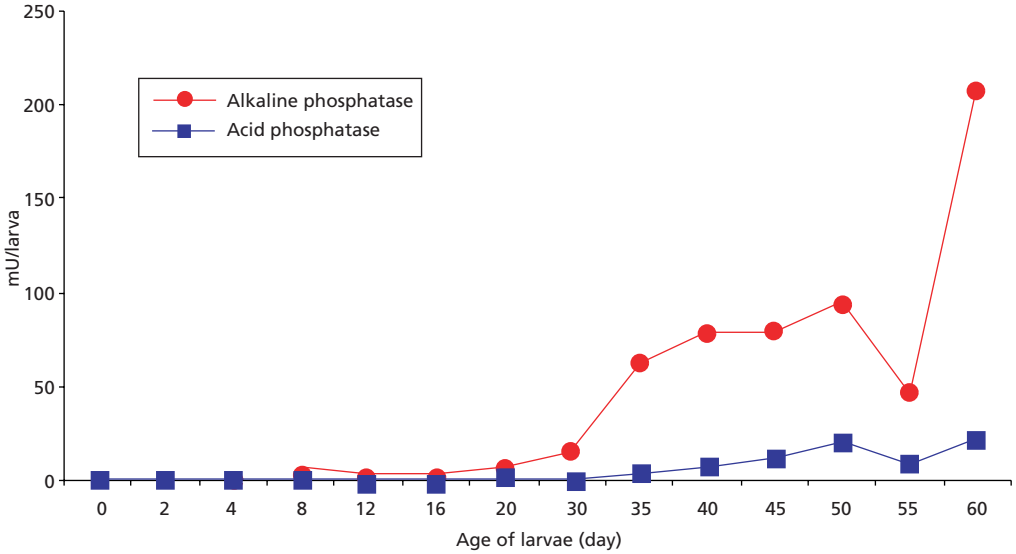

Figure 7. Acid and alkaline phosphatase activity in crude enzyme extracts from grouper larvae.

larvae (day 0) to day 60 (0.03–207.2 mU/larva). Both enzymes showed similar profiles, with higher values for alkaline phosphatase activity during metamorphosis. Moyano et al. (1996) also found that the activity of alkaline phosphatase was higher than that of acid phosphatase in gilthead seabream larvae.

Conclusion

- The maximum variation in specific activities of alkaline and acid type proteases, α-amylase, lipase, trypsin, chymotrypsin, leucine aminopeptidase, and acid and alkaline phosphatases in the digestive tract of grouper larvae are mostly related to the onset or the end of metamorphosis during larval development.

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The Activity of Digestive Enzymes in Larval Grouper and Live Feed

S. McBride

Introduction

Low and inconsistent survival in the larval rearing of groupers is a major production bottleneck (Hussain and Higuchi 1980; Kohno et al. 1997; Toledo et al. 1999). The digestive tract of first feeding grouper larvae is rudimentary (G. Quinitio unpublished data; McBride unpublished data) and there is a short window of opportunity for the successful transition from endogenous to exogenous nutrition in comparison with many other marine species (Kohno et al. 1990; Ordonio-Aguilar et al. 1995). A better understanding of the digestive physiology of grouper larvae could assist in improving the quality and accessibility of nutrients from the diet. The aim of these studies was to investigate the ontogeny of digestive enzymes in larval groupers and assess the suitability of different live feeds.

Methods

Larvae of *Epinephelus coioides* were reared in a green-water semi-intensive system in five-tonne tanks at the Southeast Asian Fisheries Development Center (SEAFDEC) facility in Tigbauan (Iloilo, Philippines) as described by Toledo et al. (1999). Larvae of *Cromileptes altivelis* were reared at the Gondol Research Institute for Mariculture (Bali, Indonesia) in five-tonne concrete tanks in a green-water culture system as described by Sugama et al. (2001). Larvae of *E. fuscoguttatus* were reared in 300 L tanks in a clear-water recirculation system at the Northern Fisheries Centre (Cairns, Australia). Samples of live prey organisms (SS-strain rotifers *Brachionus rotundiformis* and the copepod *Acartia sinjiensis*) were collected from standard cultures at the Northern Fisheries Centre.

Three to five replicates of pooled larvae (5–30 depending on age) were collected each sampling day except for *C. altivelis* where one or two replicates were collected. Samples of *E. fuscoguttatus* were only collected from three to six days post hatch (DPH) after which there was total mortality. A known number of live prey organisms were collected in triplicate. Samples were homogenised in a 10 mM Tris-HCl (pH 7.5) buffer, and centrifuged before the supernatants were collected for enzyme and protein analysis.

Concentration of soluble protein was determined using BioRad Protein Assay (Bradford) reagents (USA). Total protease and α-amylase activity were measured by sensitive fluorescent assays using casein and starch substrates respectively (Molecular Probes, USA). The activity of bile salt-dependent (bsd) lipase was estimated by an absorbance assay using the substrate 4-nitrophenyl caproate (4-NPC) (Gjellesvik et al. 1992). All enzyme assays were performed at 30°C. One unit of total protease activity was defined as the percentage change in fluorescence units from a negative control per hour. One unit of amylase activity was defined as the amount of enzyme required to liberate one milligram of maltose from starch in three minutes. One unit of bsd lipase was defined as nmoles 4-NPC hydrolysed per hour.

Differences in the emergence of digestive enzyme activity between *E. coioides* and *C. altivelis* were investigated using non-linear regression. A generalised logistic model was found the most appropriate with the enzyme activity modelled against age and grouped into species.

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Results and Discussion

Generally the emergence of digestive enzyme activity in grouper larvae was characterised by three phases.

1. Low activities were detected in the three grouper species prior to nine DPH. An exception was bsd lipase activity, which was not detected in *E. coioides* or *E. fuscoguttatus* over this period.
2. The second phase occurred between 10 and 18 DPH in *E. coioides* and *C. altivelis*. Modulations in digestive enzyme activity were observed and corresponded with key developmental changes of the gastro-intestinal tract in *E. coioides* (G. Quinitio unpublished data) and *C. altivelis* (McBride, unpublished data).
3. From 20 DPH, enzyme activity generally increased with age in both *E. coioides* and *C. altivelis* (Figs. 1 and 2).

The emergence of total protease and amylase activity with age in *E. coioides* was significantly different to the activities in *C. altivelis* ($P < 0.001$; adjusted $R^2 = 0.892$ and 0.960 respectively). The emergence of bsd lipase activity with age was similar between the two species ($P = 0.238$). These findings suggest the two species may have different abilities to digest proteins and carbohydrates at the larval stage and this is likely to have implications for the development of artificial diets for larvae and juveniles.

Total protease activity in early feeding *E. coioides* larvae increased in response to initial feeding incidence (Fig. 3). In contrast, amylase activity was not correlated with feeding incidence (Fig. 3). Live food organisms may stimulate enzyme activity in the gut of early stage larvae either by their physical presence (Hjelmeland et al. 1988; Pedersen et al. 1987), the release of hormonal factors (Hjelmeland et al. 1988; Kamisaka et al. 2001; Srivastava et al. 2002) or by contributing an exogenous source of digestive enzymes (Dabrowski and Glogowski 1977; Lauff and Hofer 1984; Munilla-Moran et al. 1990; Oozeki and Bailey 1995; Pedersen et al. 1987).

Significant differences in digestive enzyme activities were observed between the live feed organisms (Fig. 4). The potential contribution from the live feed to the enzyme activity measured in a larva was estimated by multiplying the activity per individual prey item by the total number of prey items observed in the

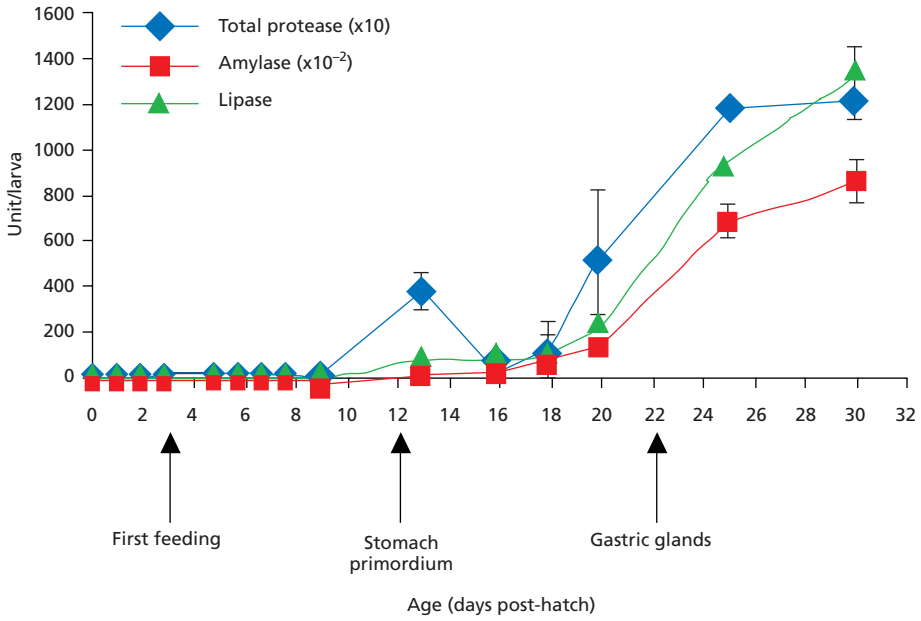

Figure 1. Emergence of digestive enzyme activities in *E. coioides* larvae with age. Arrows indicate major morphological changes in the gut development of larval *E. coioides*.

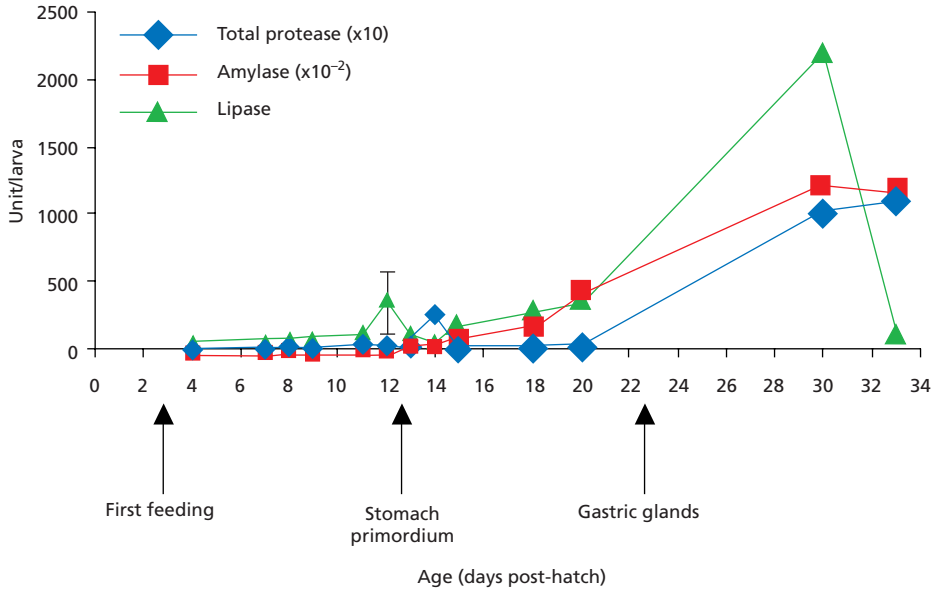

Figure 2. Emergence of digestive enzyme activities in *C. altivelis* larvae with age. Arrows indicate major morphological changes in the gut development of larval *C. altivelis*.

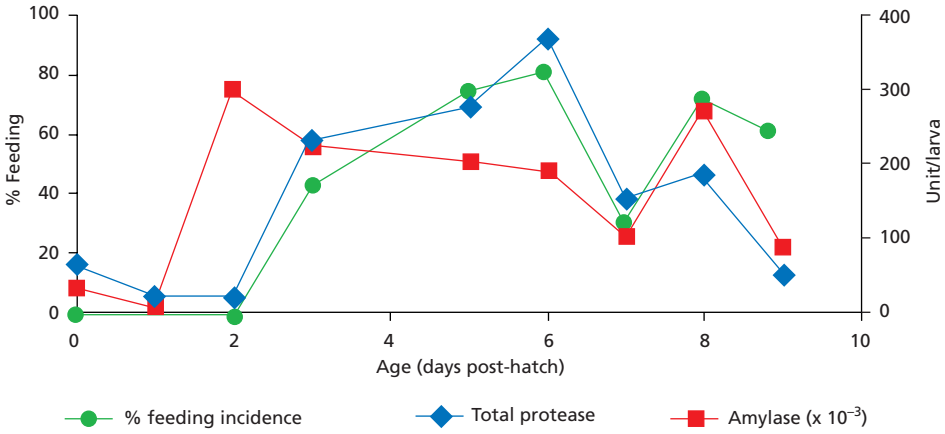

Figure 3. Correlation between feeding incidence in *E. coioides* larvae and the activity of total protease (r = 0.791, P = 0.011) and amylase (r = 0.468, P = 0.204).

gut for each age reported by Toledo et al. (1999). Rotifers contributed only 0.7% of total protease so it is unlikely that they make a significant contribution to larval digestion by providing exogenous protease enzymes. Non-feeding naupliar stages of *Arcartia* (n1–n2) contributed less than 2.5% of total protease activity and the feeding stages (n3–n4) contributed up to 35.6% of total protease activity. These results indicate that n3–n4 copepod nauplii are potentially a significant source of exogenous proteases for the larvae. Surprisingly, the potential contribution of amylase from rotifers and copepod nauplii was relatively high (Fig. 5).

Copepod nauplii contained approximately twice the amount of soluble protein than

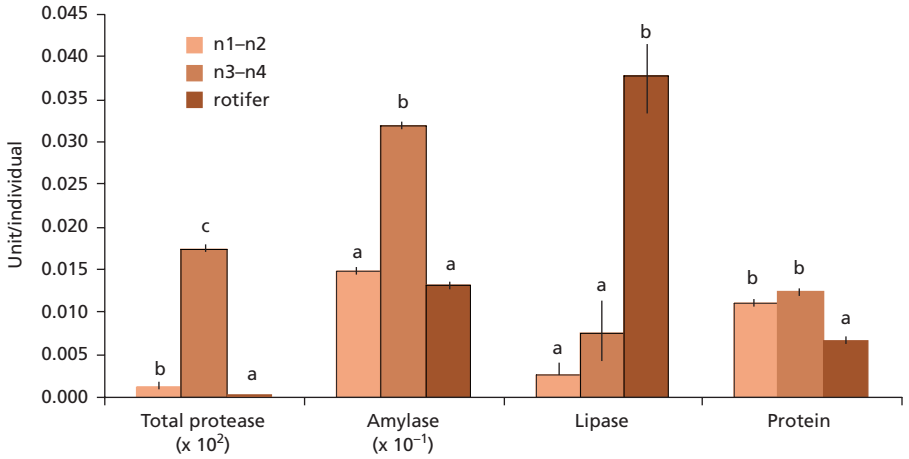

Figure 4. The digestive enzyme activities and protein content in rotifers, and n1–n2 and n3–n4 copepod nauplii. A unit/individual is a unit of enzyme activity/individual (total protease, amylase and lipase) or one µg of protein/individual (protein). Means within a category that are not significantly different share common superscripts (ANOVA; $P > 0.01$).

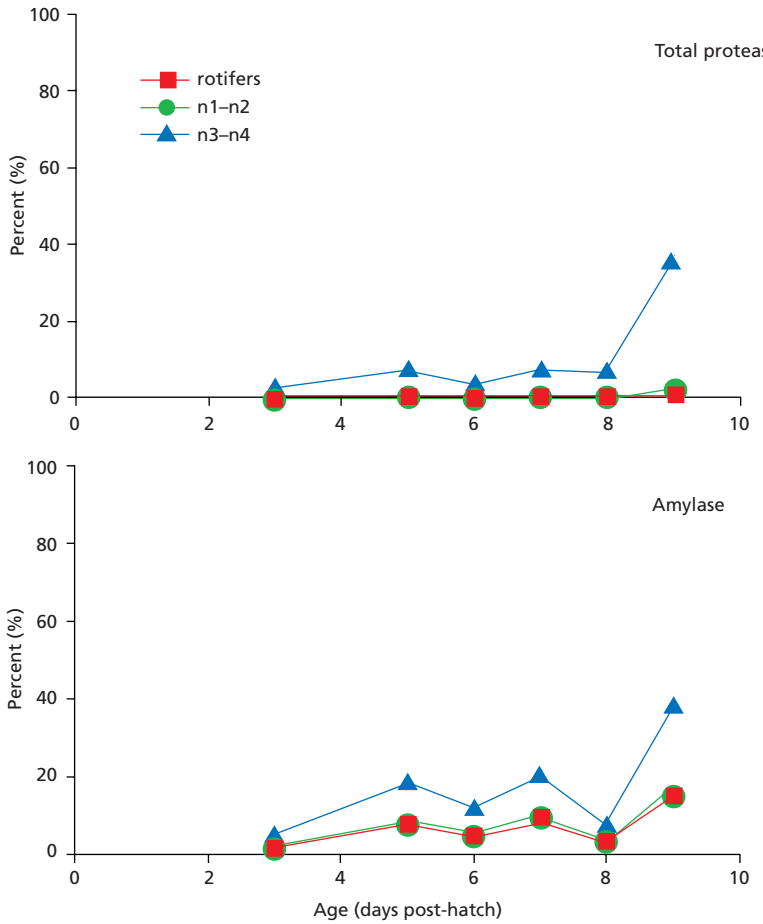

Figure 5. The estimated percent contribution of total protease and amylase activity from live feed to the respective activities measured in *E. coioides* larvae.

rotifers. The greater amount of soluble protein and protease enzymes in copepod nauplii indicates that they may provide a greater opportunity for access to protein than rotifers. This may have implications for the successful transition to exogenous feeding in grouper larvae (Ordonio-Aguilar et al. 1995). Improving nutrition during the initial feeding stages (3 to 9 DPH) may be a key to improving the quality of larvae, which are then able to undergo the major morphological changes between 10 to 20 DPH, faster and more successfully.

Conclusions

- Generally, digestive enzyme activities in larval *E. coioides* and *C. altivelis* were low prior to 18 DPH and then increased with age.
- Changes in the activity of digestive enzymes were associated with the morphological development of the digestive system.
- Total protease activity increased with feeding incidence in early feeding (3 to 9 DPH) *E. coioides* larvae.
- The emergence of total protease and amylase activity was different between *E. coioides* and *C. altivelis* larvae.
- n3–n4 copepod nauplii contained high total protease and amylase activities in comparison to n1–n2 nauplii and rotifers.

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Lipid Nutrition Studies on Grouper (*Epinephelus coioides*) Larvae

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Introduction

In marine fish, n-3 highly unsaturated fatty acids (HUFA), such as eicosapentaenoic acid (20:5n-3, EPA) and docosahexaenoic acid (22:6n-3, DHA), are important constituents of cell membranes, especially in the brain and retina; and are needed during early life stages to assure normal visual and neural development. Since arachidonic acid (20:4n-6, ARA) has an important function in producing eicosanoids, it is also an essential fatty acid (EFA) for marine fish larvae.

There were three main objectives of this project. The first was to study the lipid chain transfer from the egg stage through hatching and the patterns of lipid conservation or loss during starvation and feeding of larvae in order to elucidate the lipid metabolism of grouper (Phase 1).

The second objective was to determine the fatty acid composition of HUFA boosters and enriched live food organisms to make it possible to choose food organisms that provide various dietary levels and ratios of DHA: EPA: ARA (Phase 2).

The third objective was to determine the effect of *Brachionus* and *Artemia,* containing different levels and ratios of DHA: EPA: ARA, on the growth and survival of grouper larvae (Phase 3).

Methods

Total lipids (TL) of samples were extracted (Folch et al. 1957), separated into neutral (NL) and polar lipids (PL) in silica cartridges (Juaneda and Rocquelin 1985), the fatty acids methyl esters were prepared (AOAC 1996) and analysed on a gas chromatograph (Shimadzu GC-17A). Three to six replicate samples and analyses were done. Results were compared using ANOVA and Duncan's multiple range test at $P < 0.05$.

In Phase 1, the samples collected were: floating neurula eggs, newly hatched (NHL) and unfed 4-day old larvae (Table 1); larvae fed with live food organisms for 25 and 35 days or starved for three days (day 28 and 38, Table 2); and wild-sourced larvae starved for a week (Table 3).

In Phase 2, the samples collected were: phytoplankton, *Brachionus* cultured in phytoplankton for four days; the cladoceran *Diaphanosoma celebensis*; and the copepod *Pseudodiaptomus annandalei* (Table 4 A, B, C and D). Other samples collected in this phase were: enrichment products and *Brachionus* enriched with products (HUFA boosters) at 320 mg/million for 14 hours (Table 5A and B); *Brachionus* starved for 3, 6 and 12 hours, or one hour enriched with an emulsion (Table 6); and *Artemia* nauplii and pre-adults enriched with different HUFA boosters at 200 mg/L of seawater/100,000 nauplii for 14 hours (Table 7A and B).

In Phase 3, early feeding larvae (day 2) stocked at 20/L were fed *Brachionus* (Table 5B) until day 14 in 300 L of water with a salinity of 22–24 ppt, while at early metamorphic stage (day 25) larvae were stocked at 1/L and fed *Artemia* (Table 7B) for 10 days in 200 L of seawater. A completely randomised design with three replicates per treatment was followed and results were compared using ANOVA and Duncan's multiple range test at $P < 0.05$.

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Table 1. Neutral (NL) and polar (PL) fatty acids in *E. coioides* eggs, newly hatched larvae (NHL), and unfed day 4 larvae (μg ind^{-1} DW)[1,2].

	NL			PL			NL % loss[3]		PL % loss[3]	
	Egg	NHL	Day 4	Egg	NHL	Day 4	NHL	Day 4	NHL	Day 4
NL or PL	5.15^a	3.04^b	0.61^c	1.77^a	1.49^b	1.25^c	40.9	88.16	15.8	29.37
Fatty acids:										
Saturates	1.75^a	1.10^b	0.16^c	0.84^a	0.65^b	0.60^c	37.1	90.86	22.6	28.57
Monoenes	1.52^a	0.96^b	0.15^c	0.27^a	0.24^b	0.19^c	36.8	90.13	11.1	29.63
PUFA	1.88^a	0.99^b	0.29^c	0.66^a	0.60^b	0.47^c	47.3	84.57	9.1	28.79
20:4n-6	0.16^a	0.11^b	0.06^c	0.10^a	0.10^a	0.05^b	31.2	62.50	0.0	50.00
20:5n-3	0.22^a	0.12^b	0.00	0.07^a	0.04^b	0.02^c	45.4	100.00	42.7	71.43
22:6n-3	0.58^a	0.21^b	0.06^c	0.18^a	0.16^b	0.14^c	63.7	89.66	11.1	22.22
Ratio:			—							
DHA:EPA	2.63^a	1.75^b	1.00^c	2.57^a	4.00^b	7.00^c				
DHA:ARA	3.63^a	1.91^b	—	1.80^a	1.60^b	2.80^c				
EPA:ARA	1.37^a	1.09^b	0.61^c	0.70^a	0.40^b	0.40^b				

[1] Egg and larval dry weight (μg ind^{-1} DW): neurula eggs, 26.82; NHL, 24.04; and unfed day 4 larvae, 7.36.
[2] Treatment means in rows under NL or PL with the same superscripts are not significantly different ($P > 0.05$).
[3] Percent loss from egg stage.

Table 2. Larval dry weight (DW), total (TL), polar (PL), and neutral (NL) lipids and fatty acids of *E. coioides* larvae fed live food organisms for 25 and 35 days then starved for three days.

	Fed (day)		3 day starving		% gain	% loss	
	25	35	28	38	25–35	25–28	35–38
Larval DW (mg ind^{-1})	3.2^a	51.0^b	1.7^a	44.8^b	1622.0	46.2	12.2
TL (μg ind^{-1})	172.7^a	2664.3^b	76.0^a	1905.4^b	1542.2	56.0	28.5
PL (μg ind^{-1})	37.2^a	867.0^b	21.3^a	608.4^b	1325.7	42.6	27.8
NL (μg ind^{-1})	135.6^a	1797.3^b	54.7^a	1297.0^b	2331.8	59.7	29.8
NL FA (μg ind^{-1}):							
Saturates	47.7^a	530.3^b	18.3^a	404.3^b	1111.8	61.6	23.8
Monoenes	39.2^a	605.3^b	15.8^a	495.2^b	1542.5	59.8	18.2
PUFAs	48.6^a	661.8^b	20.6^a	397.5^b	1360.5	57.7	39.9
20:4n-6	4.9^a	67.6^b	2.3^a	42.4^b	1369.9	52.7	37.3
20:5n-3	7.4^a	114.4^b	1.6^a	75.6^b	1538.6	78.3	33.9
22:6n-3	18.3^a	173.1^b	4.0^a	131.7^b	947.7	77.9	23.9
Ratio:							
DHA:EPA	2.5^a	1.5^b	2.5^a	1.7^b			
DHA:ARA	3.7^a	2.6^b	1.7^a	3.1^b			
EPA:ARA	1.5^a	1.7^b	0.7^a	1.8^b			

[1] Treatment means in rows under 'fed' or '3 day starving' with the same superscripts are not significantly different ($P > 0.05$).

Table 3. Neutral (NL) and polar (PL) lipids and fatty acids in wild-sourced starved *E. coioides* larvae.

	NL		PL		% loss	
	Initial	day 7	Initial	day 7	NL	PL
NL or PL (mg ind^{-1})	1.3^a	0.5^b	1.5^a	1.3^b	64.8	19.4
FA (μg ind^{-1}):						
Saturates	572.4^a	100.8^b	560.6^a	152.6^b	82.4	72.8
Monoenes	318.9^a	103.5^b	368.8^a	324.1^b	67.5	12.1
n-3 FA	337.1^a	134.7^b	300.3^a	489.4^b	60.0	
n-3 HUFA	304.9^a	131.9^b	268.7^a	466.4^b	56.7	
20:4n6		34. 9		39.2		
20:5n3	56.6^a	27.1^b	57.2^a	134.4^b	52.2	
22:6n3	170.0^a	0.0	211.5^a	15.0^b	100.0	92.9
Ratio:						
DHA:EPA	3.0		3.7^a	0.1^b		
DHA:ARA	—		—	0.4		
EPA:ARA	—	0.8	—	3.4		

[1] Dry weight (mg ind^{-1}) of initial larvae and starved for seven days were 116.1 ± 3.6 and 96.5 ± 2.3.
[2] Treatment means in rows under NL or PL with the same superscripts are not significantly different ($P > 0.05$).

Table 4. Total lipids (TL), HUFA levels and ratios in phytoplankton, *Brachionus*, *Diaphanosoma*, and *Pseudodiaptomus* cultured in various feeds.

	TL % DM	% of total fatty acids			Ratio		
		20:4n-6	20:5n-3	22:6n-3	DHA:EPA	DHA:ARA	EPA:ARA
A. *Phytoplankton*:							
Chlorella vulgaris	0.3[e]	—	—	2.9[b]	—	—	—
Isochrysis galbana	3.8[a]	—	—	12.1[a]	—	—	—
Nannochloropsis oculata	3.3[b]	—	—	—	—	—	—
Tetraselmis tetrahele	2.2[c]	—	3.5[c]	—	—	—	—
Chaetoceros calcitrans	1.8[d]	—	9.4[b]	—	—	—	—
Thalassiosira pseudonana	0.1[f]	—	16.2[a]	1.8[c]	0.1	—	—
B. *Brachionus* cultured in phytoplankton:							
Chlorella vulgaris	13.6[a]	5.0[a]	13.5[a]	8.4[a]	0.6[a]	1.7	2.7[b]
Tetraselmis tetrahele	9.7[c]	1.2[c]	1.3[d]	—	—	—	1.1[c]
Chaetoceros calcitrans	12.1[b]	2.8[b]	10.0[b]	—	—	—	3.6[a]
Isochrysis galbana	8.2[d]	—	—	1.3[b]	—	—	
Nannochloropsis oculata	6.1[e]	1.2[c]	2.5[c]	—	—	—	2.1[b]
Thalassiosira pseudonana		—	9.7[b]	1.7[b]	0.2[b]	—	—
C. *Diaphanosoma celebensis* cultured in:							
Rice bran	15.3[a]	0.1	—	—	—	—	—
Cow dung	8.7[c]	—	—	—	—	—	—
Tetraselmis tetrahele	10.1[b]	—	0.4	0.1	0.2	—	—
D. *Pseudodiaptomus annandalei* cultured in:							
Chlorella vulgaris		3.9[b]	7.6[a]	28.6[c]	3.8[b]	7.3[c]	1.9[b]
Chaetoceros calcitrans		3.8[b]	7.3[a]	30.4[a]	4.2[a]	8.0[b]	1.9[b]
Isochrysis galbana		3.3[c]	7.5[a]	29.2[b]	3.9[b]	8.7[a]	2.3[a]
Tetraselmis		5.6[a]	6.5$_b$	10.0[d]	1.5[c]	1.8[d]	1.2[c]

[1]Treatment means in columns under each subheading with the same superscripts are not significantly different ($P > 0.05$).

Table 5. Total lipids (TL), HUFA levels and ratios in HUFA boosters; and in *Brachionus* fed these HUFA boosters.

	TL % DM	% of total fatty acids			Ratio		
		20:4n–6	20:5n–3	22:6n-3	DHA:EPA	DHA:ARA	EPA:ARA
A. HUFA boosters:							
Algamac 2000 (Alg2000)	27.0[e]	—	7.0[c]	20.0[g]	2.9[b]	—	—
Algamac 3050 (Alg3050)	34.8[d]	—	—	38.2[d]	—	—	—
Aquagrow Advantage (AgAdv)	10.8[h]	—	—	58.6[a]	—	—	—
Aquagrow *Chlorella* (AqChl)	20.5[g]	—	—	45.6[c]	—	—	—
Aquagrow Feed 15 (AqF15)	15.7[g]	—	—	54.1[b]	—	—	—
Aquagrow AA (AqAA)	25.2[f]	45.4[a]	0.4[e]	0.3[j]	1.0[d]	0.01[e]	0.01[e]
HUFA Enrich	64.3[a]	0.8[c]	14.3[b]	13.3[i]	0.9[d]	16.2[d]	17.4[b]
Ratio HUFA	61.4[b]	0.5[d]	5.7[d]	22.7[f]	4.0[a]	49.2[a]	12.3[d]
Super HUFA	60.8[c]	1.2[b]	23.8[a]	28.5[e]	1.2[c]	24.4[c]	20.3[a]
DHA Protein Selco	27.3[e]	0.5[d]	7.0[c]	17.0[h]	2.4[b]	37.7[b]	15.5[c]
B. *Brachionus* fed HUFA boosters:							
Initial (*Chlorella*-fed)	10.1[f]	0.8[c]	9.4[c]	1.5[g]	0.2[f]	1.8[h]	11.4[a]
Alg2000	16.0[d]	1.3[b]	3.9[f]	18.2[c]	4.6[c]	14.5[d]	3.1[d]
Alg3050	15.8[d]	1.1[b]	3.4[f]	29.0[a]	8.6[b]	26.4[c]	3.1[d]
AqAdv	17.2[c]	0.7[c]	3.1[f]	28.6[a]	9.3[a]	39.2[a]	4.2[c]
AqChl	12.5[e]	1.2[b]	5.8[d]	11.7[d]	2.0[d]	9.8[e]	4.8[c]
AqF15	13.0[e]	0.6[c]	4.8[e]	21.2[b]	4.4[c]	34.3[b]	7.7[b]
HUFA Enrich	20.4[b]	1.5[b]	10.9[b]	4.8[e]	0.4[f]	3.2[f]	7.3[b]
Ratio HUFA	21.8[a]	1.1[b]	12.7[a]	1.6[g]	0.1[f]	1.5[h]	11.5[a]
Super HUFA	21.6[a]	1.1[b]	3.6[f]	2.5[f]	0.7[e]	2.3[g]	3.4[d]
DHA Protein Selco	8.1[a]	3.2[a]	4.4[e]	2.8[f]	0.6[e]	0.9[i]	1.4[e]

[1]Treatment means in columns under each subheading with the same superscripts are not significantly different ($P > 0.05$).

Table 6. Total (TL), neutral (NL), and polar (PL) fatty acids of starved and emulsion-enriched rotifers (% of dry weight)[1,2].

	NL									
	Initial	Starved			Enriched[1]	Inital	Starved (hrs)			Enriched[1]
		3	6	12			3	6	12	
NL or PL, % DW	4.5[a]	4.3[b]	3.9[c]	3.6[d]	7.1	3.7[a]	3.5[b]	2.6[c]	2.3[d]	4.3
Fatty acids (% DW):										
Saturates	1.0[a]	0.8[b]	1.0[a]	0.7[c]	1.8	0.1[a]	0.5[b]	1.1[c]	1.1[c]	2.3
Monoenes	2.0[a]	1.8[b]	1.6[c]	0.8[d]	2.6	2.2[a]	1.7[b]	0.6[c]	0.9[d]	1.3
n-3 HUFAs	1.2[a]	1.2[a]	1.2[a]	0.6b	1.7	0.3[a]	0.4[b]	0.6[b]	0.5[d]	0.4
20:4n6	0.1[a]	0.1[a]	—	—	0.3	0.1[a]	0.1[a]	0.1[a]	0.1[a]	0.1
20:5n3	0.8[a]	0.7[b]	0.7[b]	0.3[c]	1.0	0.3[a]	0.3[a]	0.3[a]	0.2[b]	0.3
22:6n3	0.1[a]	0.1[a]	—	—	0.2	0.1[a]	0.1[a]	0.1[a]	—	0.1
Ratio:				—					—	
DHA:EPA	0.1[a]	0.1[a]	—	—	0.2	0.2[a]	0.2[a]	0.2[a]	—	0.2
DHA:ARA	1.4[a]	1.4[a]	—	—	0.6	0.6[a]	0.5[b]	2.2[c]	0.5	
EPA:ARA	10.7[a]	14.0[b]	—	—	3.6	4.0[a]	3.8[b]	3.1[c]	—	2.9

[1] Dripping emulsion of cod liver oil, egg yolk, vitamins, and water for one hour.

[2] Treatment means in rows under NL or PL with the same superscripts are not significantly different ($P > 0.05$).

Table 7. Total lipids (TL), HUFA levels and ratios in *Artemia* nauplii and pre-adults enriched with HUFA, and grouper larvae after feeding with pre-adult *Artemia.*

	TL % DM	% of total fatty acids			Ratio		
		20:4n-6	20:5n-3	22:6n-3	DHA:EPA	DHA:ARA	EPA:ARA
A. *Artemia* nauplii fed HUFA boosters:							
Initial	12.6	2.4	—	—	—	—	—
AqAdv	17.0[d]	2.0[a]	0.3[c]	13.6[a]	50.4[a]	6.7[a]	0.1[c]
AqChl	16.2[e]	2.4[a]	0.2[c]	1.3[f]	6.1[c]	0.6[d]	0.1[c]
AqF15	18.1[c]	2.4[a]	0.3[c]	6.5[b]	20.9[b]	2.7[c]	0.1[c]
Alg2000	17.0[d]	2.4[a]	0.3[c]	1.7[f]	5.8[c]	0.7[d]	0.1[c]
Alg3050	15.6[e]	2.7[a]	1.5[a]	5.7[c]	3.8[d]	2.2[c]	0.6[b]
HUFA Enrich	21.9[a]	0.8[b]	0.8[b]	2.8[e]	3.5[d]	3.5[b]	1.0[a]
Ratio HUFA	22.0[a]	0.8[b]	0.8[b]	2.9[e]	3.4[d]	3.8[b]	1.1[a]
Super HUFA	20.8[b]	0.8[b]	0.8[b]	3.1[d]	3.8[d]	3.9[b]	1.0[a]
B. Pre-adult *Artemia* fed HUFA boosters:							
Initial	13.0	2.7	3.5	—	—	—	1.3[e]
Rice bran extract (RB, control)	11.2[f]	1.3[d]	1.7[e]	—	—	—	1.3[e]
Ratio HUFA	24.3[a]	2.1[b]	5.3[c]	7.4[b]	1.4[a]	0.5[a]	2.6[c]
HUFA Enrich	23.3[b]	1.7[c]	7.7[b]	3.6[e]	0.5[b]	2.1[c]	44.5[a]
Super HUFA	18.9[e]	2.0[b]	8.1[a]	4.1[d]	0.5[b]	2.1[c]	4.1[b]
Alg2000	18.7[e]	2.3[b]	4.3[d]	6.4[c]	1.5[a]	2.8[b]	1.9[d]
Alg3050	21.2[c]	2.9[a]	4.8[d]	8.6[a]	1.8[a]	2.9[b]	1.8[d]
Mixed[2]	20.8[d]	3.0[a]	5.1[c]	7.6[b]	1.5[a]	2.5[b]	1.7[d]
C. Grouper larvae fed pre-adult *Artemia* fed HUFA boosters:							
Rice bran extract (RB, control)	5.1[e]	1.6[c]	1.7[f]	1.0[f]	0.6[d]	0.6[d]	1.1[d]
Ratio HUFA	14.8[b]	2.2[b]	3.9[d]	6.8[c]	1.8[b]	3.1[a]	1.8[c]
HUFA Enrich	16.1[a]	1.7[c]	5.9[b]	5.2[d]	0.9[c]	3.0[a]	3.5[a]
Super HUFA	13.6[c]	2.3[b]	6.5[a]	3.2[e]	0.5[d]	1.4[c]	2.8[b]
Alg2000	12.3[d]	3.1[a]	3.0[e]	7.8[b]	2.6[a]	2.5[b]	1.0[e]
Alg3050	14.0[c]	3.7[a]	3.7[d]	9.8[a]	2.6[a]	2.7[b]	1.0[d]
Mixed[2]	15.1b	3.8[a]	4.4[c]	8.0[b]	1.8[b]	2.1[b]	1.2[d]

[1] Treatment means in columns under each subheading with the same superscripts are not significantly different ($P > 0.05$).

[2] Mixed: AqAA, Alg3010, HUFA Enrich at 1:1:2 ratio.

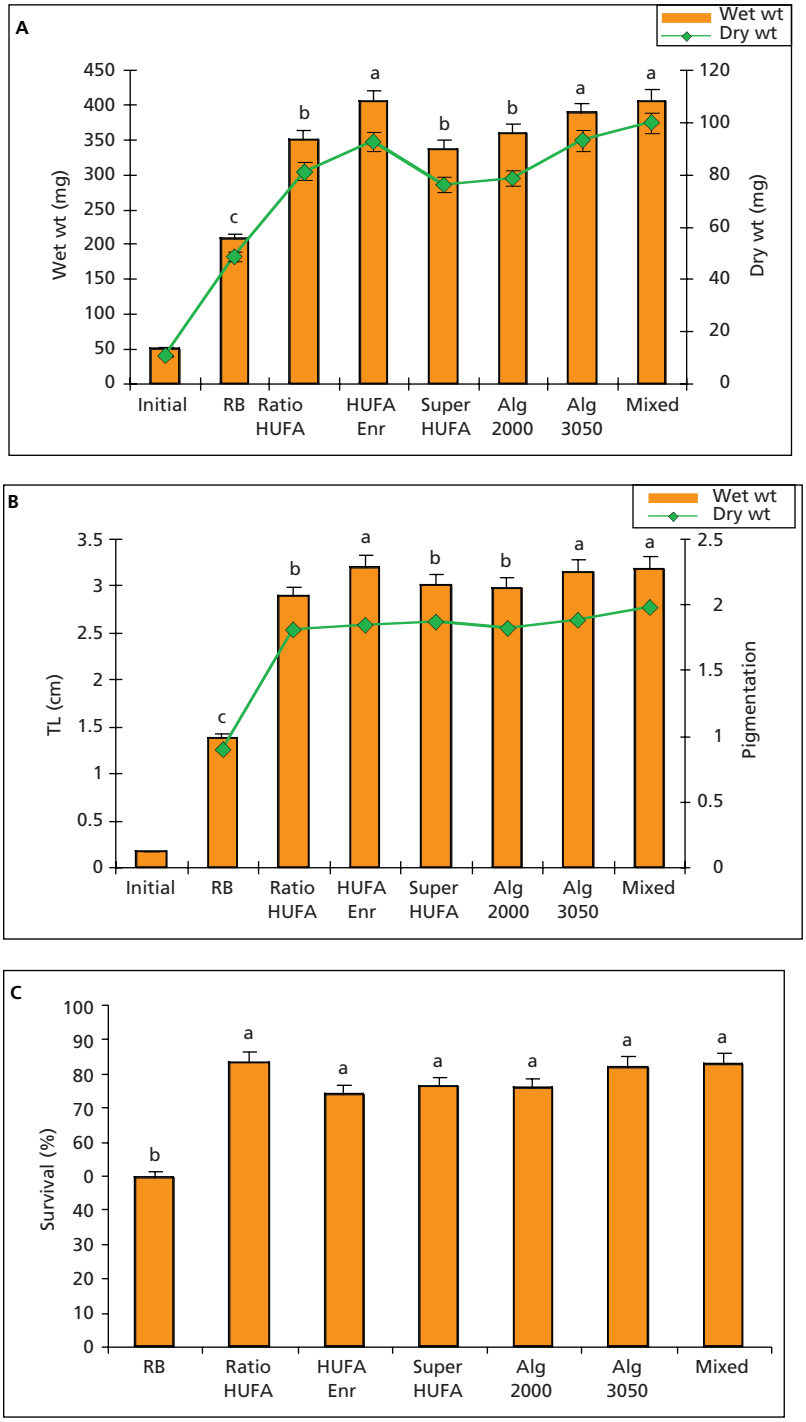

Figure 1. Wet and dry weight (A), total length and pigmentation (B), and survival (C) of grouper larvae (day 25 to –35) fed unenriched or enriched *Artemia*. Initial weight and TL = 51.1 ± 3.1 mg, 16.2 ± 0.3 mm. RB = rice bran extract; Ratio HUFA; HUFA Enrich; Super HUFA; Algamac 2000 (Alg 2000); Algamac 3050 (Alg 3050) and Mixed: Aquagrow AA, Algamac 3050 and HUFA Enrich at 1:1:2 ratio. Bars with the same letters are not significantly different (P > 0.05).

Table 8. Growth and survival of grouper larvae fed unenriched and HUFA-enriched rotifers (day 2–14).

Treatment	Weight mg ind^{-1}	Standard length mm	Total length mm	Survival %
Control	0.2 ± 0.2^{d}	1.5 ± 0.1^{c}	1.8 ± 0.1^{c}	1.3 ± 0.7^{b}
Alg 2000	0.8 ± 0.1^{b}	3.3 ± 0.2^{b}	3.6 ± 0.3^{b}	3.1 ± 0.2^{a}
Alg 3050	1.0 ± 0.3^{a}	3.5 ± 0.3^{a}	3.9 ± 0.4^{a}	3.2 ± 0.1^{a}
AqAdv	1.1 ± 0.2^{a}	3.6 ± 0.1^{a}	3.9 ± 0.1^{a}	3.1 ± 0.1^{a}
AqChl	0.9 ± 0.1^{b}	3.3 ± 0.1^{b}	3.6 ± 0.2^{b}	2.9 ± 0.3^{a}
AqF15	0.8 ± 0.1^{b}	3.4 ± 0.1^{b}	3.7 ± 0.2^{b}	2.8 ± 0.7^{a}

[1] Treatment means in columns with the same superscripts are not significantly different (P > 0.05).

Results and Discussion

Polar lipids (PL) were generally conserved while NL was primarily spent as energy in eggs, newly hatched larvae and unfed day-4 larvae (Table 1). In eggs, neutral and polar DHA:EPA ratios were similar, whereas neutral DHA:ARA and EPA:ARA ratios were twice those of PL. In day 4 unfed larvae, neutral and polar DHA and ARA were retained but EPA was low in PL and depleted in NL. Hatchery-bred larvae contained higher NL than PL; their EFA increased with feeding but three days of starvation decreased these (Table 2). Wild larvae had higher levels of PL than NL. One week of starvation totally spent the neutral DHA while some polar DHA was retained (Table 3).

DHA was present only in *Chlorella*, *Isochrysis* and *Thalassiosira* (Table 4A) and in *Brachionus* cultured in phytoplankton (Table 4B). *Diaphanosoma* grown in *Tetraselmis* contained only a little DHA and EPA indicating that HUFA enrichment is necessary to improve its nutritional value (Table 4C). *Pseudodiaptomus* reared in *Chlorella, Chaetoceros,* or *Isochrysis* had better HUFA ratios than it did cultured in *Tetraselmis* (Table 4D). Except for AqAA that contained high ARA, all boosters provided DHA, particularly high in AqAdv, AqChl, AqF15, and Alg3050 (Table 5A). *Brachionus* enriched with AqAdv and Alg3050 contained the highest DHA (Table 5B).

In starved *Brachionus*, lipids declined with time (Table 6) and to ensure optimal essential fatty acids content, *Brachionus* should be fed to larvae right after harvest or within the next three hours. Supplements of AqAdv, AqF15, and Alg3050 improved the DHA in *Artemia* nauplii (Table 7A), while in pre-adult *Artemia*, Alg3050, mixed HUFA, Ratio-HUFA and Alg2000 enhanced DHA levels and HUFA-Enrich and Super-HUFA increased EPA levels (Table 7B).

HUFA-enriched *Brachionus* and *Artemia* enhanced better growth, survival or pigmentation in early feeding (Table 8) and metamorphic larvae (Fig. 1) than un-enriched live food. Dietary HUFAs were reflected in the larvae (Table 7C).

Conclusions

- *Epinephelus coioides* eggs contained high DHA, EPA and ARA demonstrating their importance in larval development; larvae primarily spent NL as energy while PL was generally conserved.
- Wild grouper larvae had higher levels of PL than NL, whereas hatchery-sourced eggs and larvae contained higher levels of NL than PL. Based on the lipid content of wild larvae, high phospholipid diets are essential for larval survival and normal development.
- A variety of enrichment products were effective in enriching the HUFA content (particularly dietary levels and ratios of DHA, EPA and ARA) of live food organisms.
- HUFA-enriched live food organisms enhanced growth, survival and pigmentation in grouper larvae.

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Amino and Fatty Acid Profiles of Wild-Sourced Grouper (*Epinephelus coioides*) Broodstock and Larvae

V.R. Alava, F.M.P. Priolo, M. Arnaiz and J.D. Toledo

Introduction

There is a lack of information on the amino acid content of grouper broodstock tissues, eggs and newly hatched larvae. This study was carried out to provide information on levels of amino acids in muscle, liver, and gonad of wild-sourced broodstock and larvae, as well as on neurula eggs and day 35 larvae from a hatchery. The fatty acid compositions of grouper broodstock tissues were also determined. This information can be used as to guide the development of a high quality diet for grouper broodstock and larvae since their dietary nutrient requirements will be closely related to their nutrient profiles.

Methods

Samples analysed for crude protein and amino acids were: (1) abdominal muscle, liver and gonad tissues dissected out from a broodfish collected from a trap at Tigbauan Bay, Panay Gulf, Philippines; (2) wild-sourced larvae from Samar, Leyte, Philippines; (3) neurula eggs obtained from broodstock groupers in a tank fed raw fish; and (4) larvae reared in a hatchery and fed live food organisms for 35 days.

The broodfish tissues were also analysed for total lipids and fatty acid content. Crude protein (CP) was analysed using the micro-kjeldahl method (AOAC 1980). Samples were hydrolysed with trichloroacetic acid and analysed for amino acid contents using the HPLC (Shimadzu LC-10AT). Total lipids (TL) were extracted based on the method of Folch et al. (1957), fatty acid methyl esters were prepared by trans-esterification with BF_3 methanol (AOAC 1996) and analysed using a gas chromatograph (Shimadzu GC-17A). Three replicate analyses were done per sample.

Results

Table 1 shows the CP and amino acid contents of early maturing grouper broodstock tissues, eggs and larvae. Muscle contained higher levels of crude protein (dry matter basis) and amino acids than ovary and liver. CP and amino acid contents in wild-sourced larvae were higher than in the hatchery-sourced eggs and larvae. Among the ten essential amino acids (EAA), leucine and lysine were dominant in all samples analysed.

Of the non-essential amino acids (NEAA), glutamine and asparagine were the highest. TL and fatty acid content in grouper broodstock is given in Table 2. Total lipids content was highest in liver, followed by ovary then muscle. The levels of highly unsaturated fatty acids (HUFA) in these three tissues were: 22:6n3 (DHA) > 20:4n-6 (ARA) > 20:5n-3 (EPA). In the ovary, a DHA:EPA ratio of 6.8 and a DHA:ARA ratio of 2.5 was obtained.

Conclusions

- At the early maturing stage, the grouper ovarian protein was 73.3% and lipid was 19.3% indicating high dietary requirements of these nutrients for ovarian development.
- Crude protein and amino acids in wild-sourced larvae were higher than the eggs and larvae sourced from a hatchery.

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Table 1. Crude protein and polymerised amino acids (% of protein, DW) of wild *E. coioides* broodstock tissues and larvae, eggs and day 35 larvae.

	Wild grouper			Wild	Hatchery	
	Ovary[1]	Liver[1]	Muscle	larvae	Eggs	larvae
Crude protein, % DW	73.33	26.62	94.34	72.22	69.14	69.53
Larval DW, mg ind^{-1}				116.21	0.03	51.04
EAA[2]:						
Arg	3.66	1.41	4.20	4.67	4.31	4.23
His	1.80	0.78	2.46	1.62	1.83	1.62
Ile	3.54	1.31	4.91	3.47	3.35	3.58
Leu	6.15	2.45	9.34	6.16	6.19	5.88
Lys	5.88	2.21	8.01	6.49	4.35	6.40
Met	2.34	0.77	3.14	2.11	1.34	2.09
Phe	3.03	1.42	4.27	3.19	3.50	3.27
Thr	3.66	1.40	4.71	3.55	3.83	3.44
Val	4.43	1.55	4.96	3.83	4.99	3.88
Sum EAA	34.49	13.29	46.04	35.11	33.70	34.39
NEAA:						
Asp	9.30	2.94	8.81	7.85	5.75	7.41
Ser	3.24	1.28	4.71	3.17	3.01	2.95
Glu	14.02	3.78	14.31	11.75	10.24	11.10
Pro	1.98	1.15	5.05	3.02	5.07	2.97
Gly	2.93	1.41	3.83	3.95	2.80	3.85
Ala	4.68	1.87	6.94	4.54	4.66	4.30
Tyr	2.62	0.88	4.62	2.80	3.90	2.56
Sum NEAA	38.76	13.30	48.28	37.07	35.44	35.14

[1] The wild broodstock (2.90 kg) had a gonadosomatic index (GSI) of 0.73 and hepatosomatic index (HSI) of 1.24.
[2] Tryptophan was undetected and might have been destroyed during sample hydrolysis.

Table 2. Total lipids and fatty acids in wild *E. coioides* tissues.

	Ovary[1]	Liver[1]	Muscle
Total lipids (% DM)	19.28	40.13	4.73
Fatty acids (% of TL, DM):			
14:0	0.64	0.82	0.11
16:0	4.46	13.31	1.51
16:1n-7	2.32	7.62	0.02
18:0	1.10	1.43	0.35
18:1n-9	4.31	9.40	0.98
18:2n-6	0.09	0.05	0.03
20:1n-9	0.19	0.88	0.05
20:4n-6	0.99	1.25	0.36
20:5n-3	0.37	0.63	0.13
20:4n-3	0.16	0.04	0.03
22:4n-6	0.46	0.03	0.12
22:4n-3	0.30	0.23	0.09
22:5n-3	0.58	0.51	0.09
22:6n-3	2.53	1.85	0.59
Total:			
Saturates	6.31	15.75	2.01
Monoenes	6.89	17.90	1.08
n-3 FA	4.09	3.72	1.02
n-6 FA	1.53	1.24	0.50
n-3 HUFA	3.92	3.22	0.93
Ratio:			
n-3: n-6	2.67	3.00	2.04
DHA: EPA	6.84	2.94	4.54
DHA: ARA	2.55	1.48	1.64
EPA: ARA	0.37	0.50	0.36

[1] See Table 1.

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Studies on Semi-Intensive Seed Production of Grouper (*Epinephelus coioides*)

J.D. Toledo, D. Chavez and J. Rodriguez Jr.

Introduction

Previous studies have demonstrated that the use of copepod nauplii, alone or in combination with rotifers, increase the growth and survival of early larval stages of the grouper *Epinephelus coioides.* An average survival rate of 3% was obtained after metamorphosis in a pilot-scale production using 10-tonne tanks (Toledo et al. 1999). Other studies in this project, performed at a laboratory-scale, reported higher survival rates when early larval stages were reared under appropriate salinity, aeration and light intensities. Higher growth and survival were also observed when live prey organisms were enriched with highly unsaturated fatty acids (HUFA). The main objective of this study was to improve hatchery survival by verifying and incorporating laboratory-scale experimental results to a hatchery-scale operation.

Materials and Methods

Nine units of 200–metre square ponds were used to verify mass culture techniques for zooplankton. Three fertilisation schemes were tested with three replicate ponds per fertilisation treatment. Incoming water was screened with a 0.8–1.0 mm mesh net to minimise entry of predators but allow entry of natural populations of copepods. The initial water depth was one metre. The culture period was 45 days. The initial quantity and quality of copepods and other zooplankton in each pond were monitored a day after filling, then every three to four days thereafter. Zooplankton samples were collected, preserved and identified as described by Ohno et al. (1996).

To test copepod production in tanks, adults and copepodids were transferred into six one-tonne oval fibreglass production tanks at a starting density of 60 individuals/L. Zooplankton in three of the tanks were fed daily with a mixture of *Nannochloropsis* sp., *Tetraselmis* sp., and *Chaetoceros* sp. at a final density of 300,000 cells/ml. Zooplankton in the remaining three tanks were fed daily with half the amount of a mixture of the same algae (150,000 cells/ml) and bread yeast (0.5 gm/100,000 individuals). A moderate airlift system kept the algae and bread yeast suspended in the water column.

Larval rearing runs using five-tonne tanks were performed from 2000 to 2002 to verify earlier experimental results. The protocol of Toledo et al. (1999) was tested in 2000. To propagate copepod naulpii in larval tanks, *Acartia* copepodids and adults were inoculated in four 10-tonne larval rearing tanks at 60–80 individuals/L, two to three days before stocking of grouper eggs or newly hatched larvae. *Brachionus* were added daily from day 2 to day 18 at increasing densities of 2–10 individuals/ml. *Artemia* nauplii and metanauplii were fed to satiation from day 15 until metamorphosis. Pond-grown zooplankton was added in separate tanks from day 15 onwards as a supplement to *Artemia.* In 2001, environmental conditions (20–25 ppt, moderate aeration, ca. 700 lux light) found appropriate for early larval stages were tested. To further improve larval survival, live prey organisms were 'enriched' in 2002 with

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commercial enrichment products or homemade oil emulsions made of fish oil, bread yeast, egg yolk and vitamin mix.

Results and Discussion

The population of copepods in ponds fertilised with various combinations of organic and inorganic fertilisers increased a week after flooding of ponds and fertilisation (Figs. 1–3). In all treatments, the density of copepods rapidly increased from 86–148 individuals/L a day after flooding to 1524-3186 individuals/L 9–12 days thereafter. Zooplankton compositions were: *Apocyclops* and *Oithona* sp. in Treatment I; *Apocyclops*, *Brachionus rotundiformis*, *Oithona,* and *Penilia* in Treatment II; and *Apocyclops*, *Pseudodiaptomus* sp. and *Penilia* in Treatment III. Salinity, temperature and water transparency during the experiment varied from 26–31 ppt, 29–31°C and 36–80 cm, respectively.

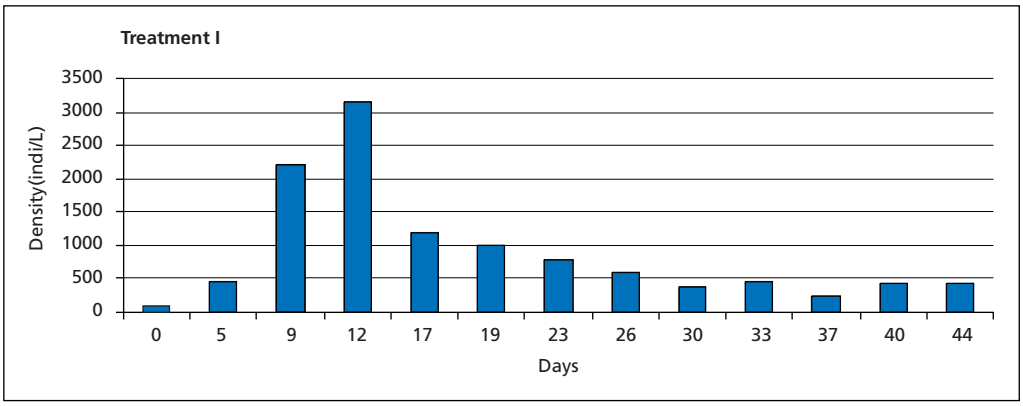

Figure 1. Density of zooplankton in earthen ponds fertilised following the methods of Ohno and Okamoto (1988). Chicken manure was applied evenly as a basal fertiliser at 500 kg/ha. After filling the pond with water, urea, ammonium sulfate and ammonium phosphate were added and then every three days thereafter at a rate of 2.7, 4 and 6.0 kg/ha, respectively.

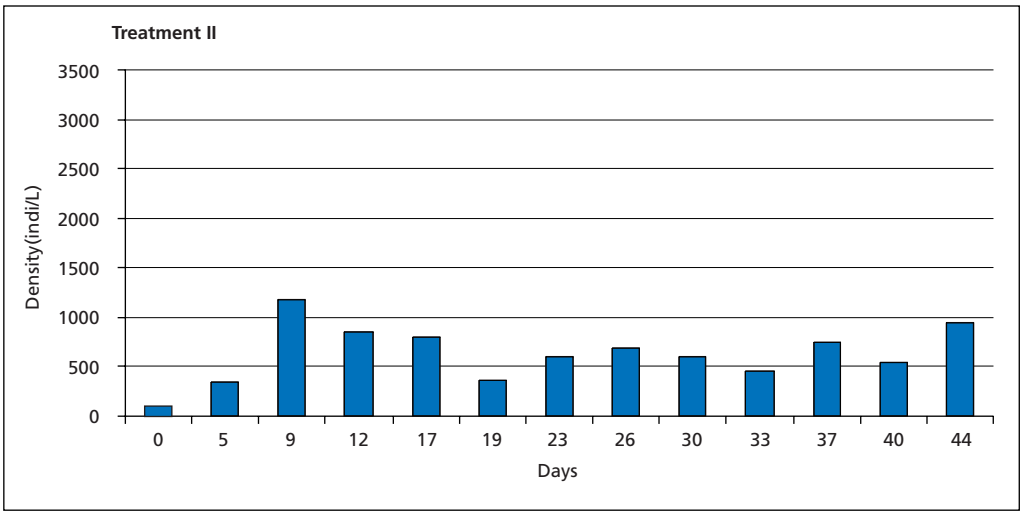

Figure 2. Density of zooplankton cultured in ponds fertilised following SEAFDEC AQD. Basal fertiliser composed of two-tonnes of chicken manure, 25 kg urea, and 50 kg ammonium phosphate per hectare.

Copepodids and adults of *Acartia tsuensis* fed a mixture of algae alone, or in combination with bread yeast, seemed to propagate well in tank conditions (Fig. 4). Density of *Acartia* rapidly increased from 60 individuals/L to about 900 individuals/L (including various naupliar stages) three days after stocking. A decrease in density was observed 5–6 days after stocking, probably due to cannibalism and contamination of rotifers. The present results suggest that bread yeast could be used in combination with algae for nauplii production of *Acartia* in tanks.

Verification runs indicate that pond-grown copepods (*Oithona, Pseudodiaptomus* and *Acartia*) can be used as a supplement to *Artemia.* Larvae fed copepods and *Artemia* starting at day 15 showed similar survival rates to those fed *Artemia* only (Fig. 5). Larval survival from days 5–15 was higher in larvae reared in 20–25 ppt (36.6–73%) to those reared in normal seawater (21.8–41.7%) (Fig. 6). However, survival at harvest appeared similar (4.9–6.4%) (Figs. 5–6). From day 20 onwards, moribund larvae swimming listlessly near the water surface with abrupt swirling movements were commonly observed.

Thirty-nine out of 71 tanks were discarded in 2000, while three of the 12 and eight out of 26 production runs were aborted in 2001 and 2002 (Table 1), respectively. Mean survival at harvest

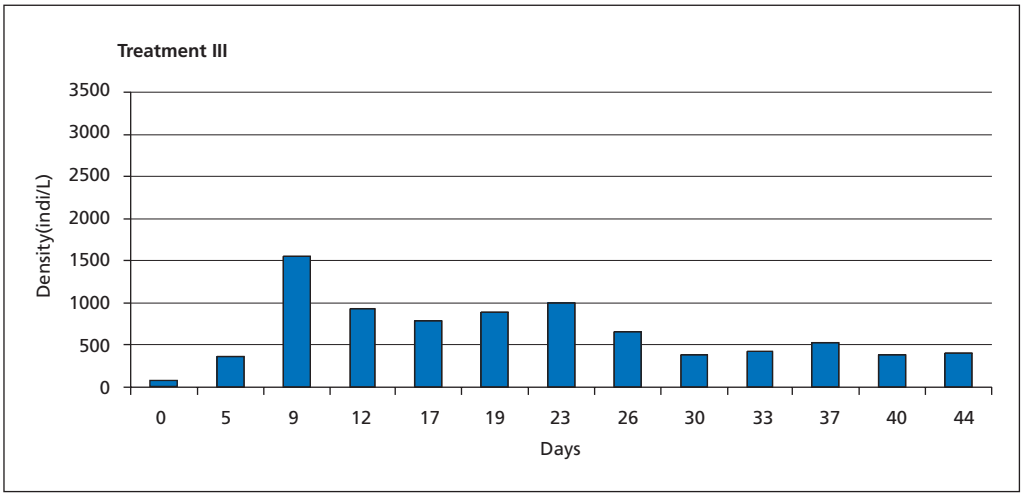

Figure 3. Density of zooplankton cultured in ponds (modifying Geiger et al. (1983)). Rice bran and liquid inorganic fertiliser were added weekly, at a rate of 300 kg and 50 L per hectare, respectively.

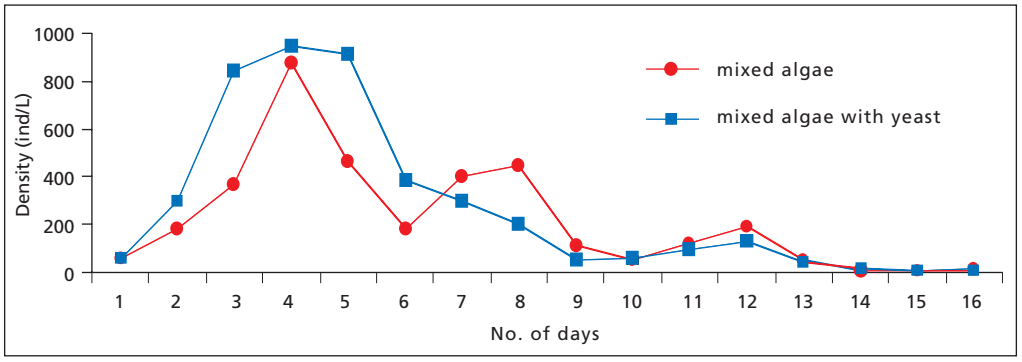

Figure 4. Density of *Acartia tsuensis* cultured in a one-tonne tank fed with mixed algae alone or in combination with bread yeast.

(day 35) increased from 3.06% (0.62–10.2%) in 2000 to 5.33% (1.2–12.1%) in 2001 and 10.39% (1.1–49.4%) in 2002. Larvae in discarded or aborted tanks had high cumulative mortalities with clinical signs of viral nervous necrosis (VNN). Mortalities were associated with VNN (Maeno et al. 2002). Fertilised eggs as well as larvae at various ages were shown to be positive for VNN by cell culture and RT-PCR. Histopathological observations revealed vacuoles in the retina and brain of moribund larvae.

Table 1. Summary of larval rearing runs in five-tonne tanks from 2000–2002.

	2000	2001	2002
Total number of tanks	71	12	20
Aborted/discarded	39	3	8
Mean survival (%)	3.1	5.3	10.4
Range (%)	0.6–10.2	1.2–12.1	1.1–49.4

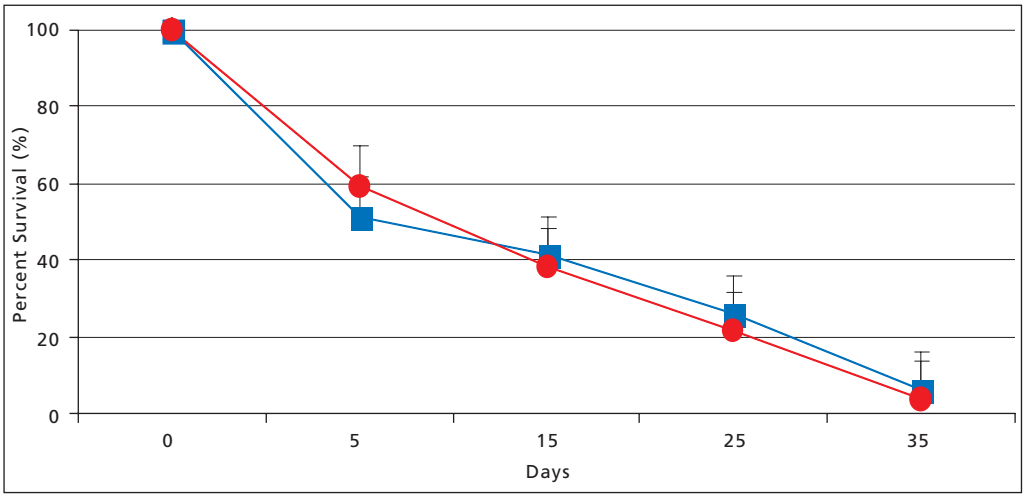

Figure 5. Percentage survival of grouper larvae fed with *Artemia* alone (circles) or in combination with copepods (squares). Figures are mean ± SEM of four replicates.

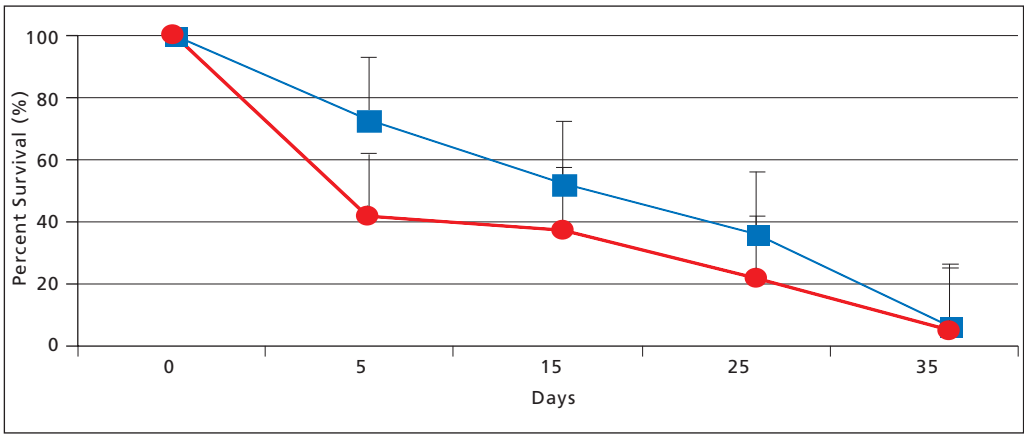

Figure 6. Percentage survival of grouper larvae reared in salinities of 20–25 ppt (squares) or 34–35 ppt (circles). Figures are means ± SEM of six replicates.

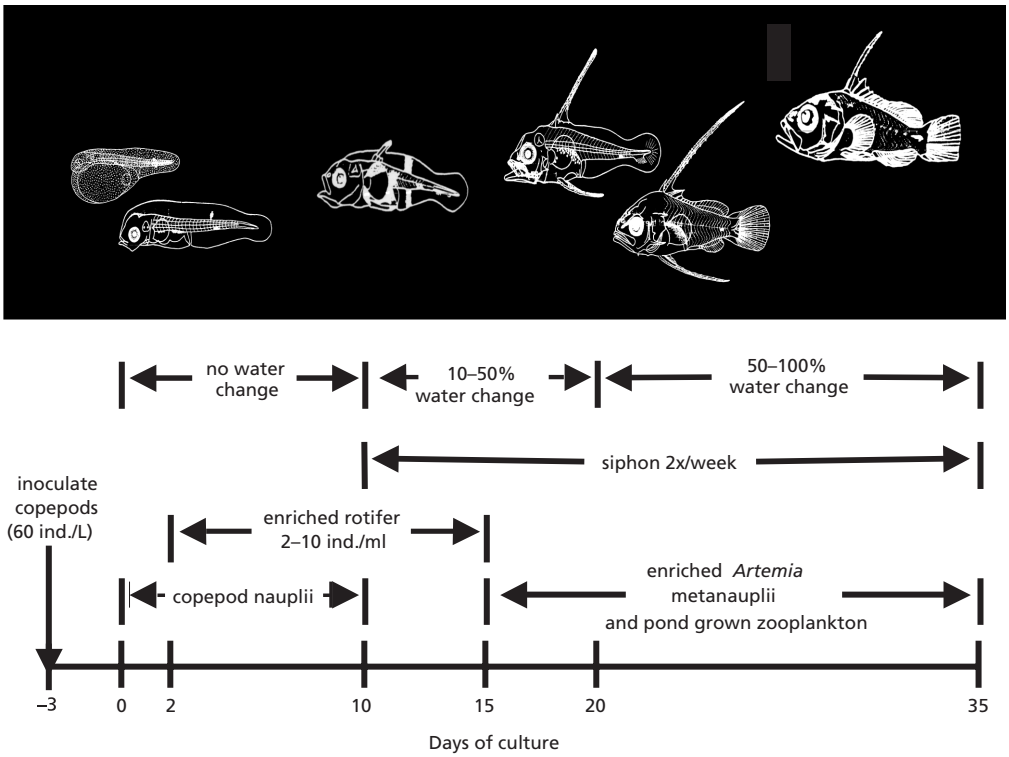

Figure 7. Feeding and water management scheme for semi-intensive rearing of grouper larvae.

Sampling larval *Epinephelus coioides* from larval rearing tanks at the Southeast Asian Fisheries Development Centre Aquaculture Department, Iloilo, Philippines.

Conclusions

- Brackish water (20–25 ppt) could increase survival at early larval stages.
- Pond-grown copepods can be used as a supplement to *Artemia.*
- Up to 40% survival at harvest may be obtained following the protocol shown in Figure 7.
- Larval survival in the hatchery was highly affected by VNN infection.

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Effect of Water Temperature on Growth, Survival and Feeding Rate of Humpback Grouper (*Cromileptes altivelis*) Larvae

K. Sugama, Trijoko, S. Ismi and K. Maha Setiawati

Introduction

The primary problem in grouper propagation is high mortality at early larval stage. Some experiments have been carried out concerning this aspect for some species of groupers, such as *Epinephelus akaara* (Tseng and Ho 1979), *Epinephelus salmoides* (Hamanto et al. 1986; Huang et al. 1986; Lin et al. 1986), *Epinephelus tauvina*[1] (Husain et al. 1975; Chen et al. 1977; Husein and Higuchi 1980), *Epinephelus fuscoguttatus* (Supriatna and Kohno 1990), and *Cromileptes altivelis* (Aslianti 1996; Slamet et al. 1996; Kumagai et al. 1998; Sugama et al. 1998). Results of these experiments showed that high mortality was observed during the initial stage up to day-9 old larvae.

In general, the main factors that determine larvae mortality are biotic factors (for example food, disease, parasitism and predation) and abiotic factors (for example oxygen, pH, salinity, toxic substances and temperature) (Kamler 1992). Temperature has a major effect on the development of marine fish. Temperature also influences efficiency of yolk utilisation, growth, feeding rate, time to metamorphosis, behaviour and swimming speed, digestion and gut evacuation rate, and metabolic demand (Blaxter 1988). Kamler (1992) reported that the main factors contributing to the variability in developmental rate during early exogenous feeding of larvae are temperature and food. Therefore information on the effect of temperature on larval developmental is important for marine fish.

The aim of the present experiment was to identify the optimum water temperature for the growth, survival and feeding rate of early stage humpback grouper larvae.

Materials and Methods

Twelve transparent polycarbonate tanks (30 litres volume) were used in this experiment. Rearing tanks were placed in a water bath system with a temperature controller and maintained at 25°, 28°, 31°C and without a temperature controller (control). Eggs were collected from naturally-spawning broodstock and then incubated in a 200 litre transparent tank. One day old larvae (D-1) with a hatching rate of 96% were stocked in each rearing tank at a density of 10 larvae/litre. Light intensity was adjusted to 750–850 lux according to larval stage using TL lamp.

Two types of rotifer, i.e. SS-type and S-type were used as food during larval rearing and fed to the larvae once per day at 8:00 in the morning. Rotifers were enriched with *Nannochloropsis* and a commercial enrichment product (Selco) before feeding to the larvae. SS-type rotifers were fed at day 3–5 at a density of 5 individuals/litre. S-type rotifers were fed at day 6–10 at a density of 10 individuals/litre.

[1] *E. salmoides* is a synonym of *E. coioides*; *E. tauvina* is likely a misidentification of *E. coioides*.

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Feeding rate was determined directly by calculating the number of rotifers in the gut of each larva. Ten samples of larvae were collected from each tank everyday. To calculate the growth of larvae, ten larvae were collected and measured for total length (TL) at the beginning and at the end of the experiment.

Prototype 'backyard' hatchery for grouper, Gondol Research Institute for Mariculture, Indonesia. The prototype hatchery is used to train farmers and extension officers in grouper hatchery technology.

Commerical marine finfish hatchery, Gondol, Bali, Indonesia. The blue-coloured tanks are used for larval rearing of groupers.

Results

Results of the experiment showed that water temperature influenced growth, development of dorsal and pectoral spines, survival, and feeding rate of the larvae.

The effect of temperature on growth of humpback grouper larvae is shown in Table 1. Growth of the larvae reared at 31°C was the best and significantly higher ($P < 0.05$) than other treatments. Growth of larvae reared at 28°C was significantly higher than larvae reared at 25°C and control larvae ($P < 0.05$). These results indicate that the growth rate of the larvae increases with increasing water temperature.

Table 1. Growth (TL) of humpback grouper larvae reared at different temperatures.

Water temperature (°C)	Initial TL (mm)	Final TL (mm)	Growth (mm d^{-1})
Control	2.517 ± 0.082	3.403 ± 0.115^a	0.099 ± 0.013^a
25	2.517 ± 0.082	3.385 ± 0.200^a	0.096 ± 0.022^a
28	2.517 ± 0.082	3.815 ± 0.074^b	0.144 ± 0.008^b
31	2.517 ± 0.082	4.173 ± 0.094^c	0.184 ± 0.010^c

Means with the same superscript in the same column are not significantly different ($P > 0.05$).

The effect of temperature on dorsal spine and pectoral spine development is shown in Table 2. Except for the larvae reared at 25°C, the dorsal spine started to develop in most of the larvae on day-7 i.e. 0.001 mm, 0.019 mm, and 0.246 mm for the larvae reared at control, 28°C, and 31°C, respectively. The pectoral spine also started to develop on day-7, but only for the larvae reared at 31°C (0.484 mm). On day-8, development of the dorsal spine and pectoral spine were more significant, except for the larvae reared at 25°C. Dorsal spine and pectoral spine for the larvae reared at 25°C started to develop on day-9, with the length of 0.019 mm and 0.094 mm for dorsal spine and pectoral spine, respectively. These results indicate that development of dorsal spine and pectoral spine of the larvae is more rapid as water temperature increases.

The effect of temperature on feeding rate of humpback grouper larvae is shown in Figure 1. Feeding rate of larvae reared at 31°C was significantly higher ($P < 0.05$) than those of larvae reared at 25°C and control, but was not significantly different ($P > 0.05$) compared with the larvae reared at 28°C. Feeding rate of larvae

reared at 28°C was higher than larvae reared at 25°C and control. These data indicate that feeding activity of humpback grouper larvae reared at 28°C and 31°C was high.

Table 2. The length of the dorsal spine and pectoral spine of 10 days old humpback grouper larvae.

Water temperature (°C)	Dorsal spine (mm)	Pectoral spine (mm)
Control	0.396 ± 0.131	0.530 ± 0.172
25	0.143 ± 0.033	0.299 ± 0.155
28	0.986 ± 0.108	1.399 ± 0.100
31	1.849 ± 0.210	2.033 ± 0.250

Survival of humpback grouper larvae reared at different temperatures are shown in Figure 2. Survival of larvae reared at 28°C was higher than the other treatments. Survival of larvae reared at 25°C was higher than larvae reared at control and at 31°C ($P < 0.05$).

Counting and grading *Cromileptes altivelis* juveniles in a 'backyard' hatchery in Bali, Indonesia. Hatcheries are an important source of employment for local people in northern Bali.

Discussion

The result of the present experiment showed that growth of humpback grouper larvae increased with rising water temperature. This result agrees with the result reported by Akatsu et al. (1983) for brown spotted grouper *Epinephelus tauvina* (= *E. coioides*). In their experiment, larvae of brown spotted grouper were reared for 12 days at different water temperatures. Total length of larvae reared at high temperature (32°C) was the highest (6.5 mm) compared with larvae reared at 23°C (4.1 mm).

The development of dorsal spine and pectoral spine of humpback grouper larvae increased with rising water temperature. Blaxter (1988) reported that temperature is known to influence growth and time required to metamorphosis of fish larvae. In this experiment, development of pectoral spine was faster than that of dorsal spine, although dorsal spine started to develop earlier than pectoral spine. Slamet et al. (1996) also reported that the development of the pectoral spine was faster than the dorsal spine. Aslianti (1996) found that development of dorsal spine of humpback grouper *Cromileptes altivelis* larvae was started on day-7, the same as the result of the present experiment. Development of dorsal spine of grouper *Plectropomus maculatus* larvae also started on day-7 (Diani et al. 1991).

Feeding rate of humpback grouper larvae increased with increasing water temperature. This data indicates that feeding activity of humpback grouper larvae increases with increasing water temperature. Similar results have been reported by Jobling (1994), who found that feed intake of Baltic salmon increased approximately threefold as temperature increased from 2° to 6°C. In another experiment, Koskela et al. (1997) reported that the feeding rate of juvenile Baltic salmon reared at 6°C was approximately two times higher than that reared at 4°C. Elliott (1991) and Jobling (1994) stated that the metabolic processes of fish are sensitive to the changes of environmental temperature and a decrease in water temperature to the below optimum level results in reduced feed intake and growth. Fish eventually lose their appetite when maintained below the temperature tolerance range.

The highest survival (48.11%) was found for the larvae reared at 28°C that was much higher compared with the survival of larvae reared at 31°C, which was only 4.77%. This data indicates that optimal water temperature for rearing of humpback grouper larvae is 28°C, even though

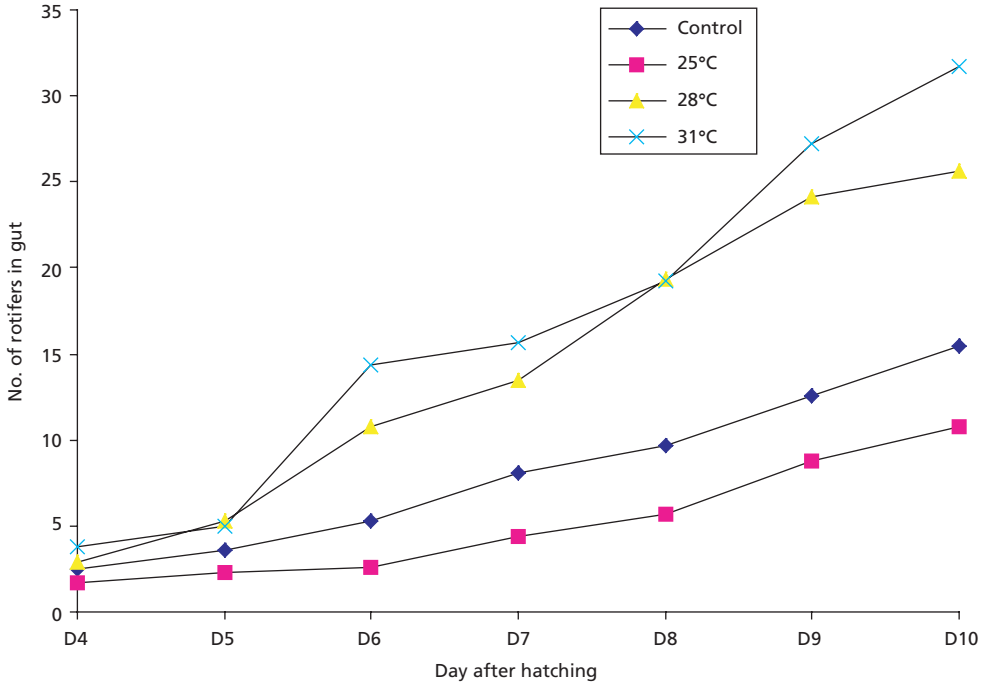


Figure 1. Feeding rate of humpback grouper larvae reared at different temperatures.

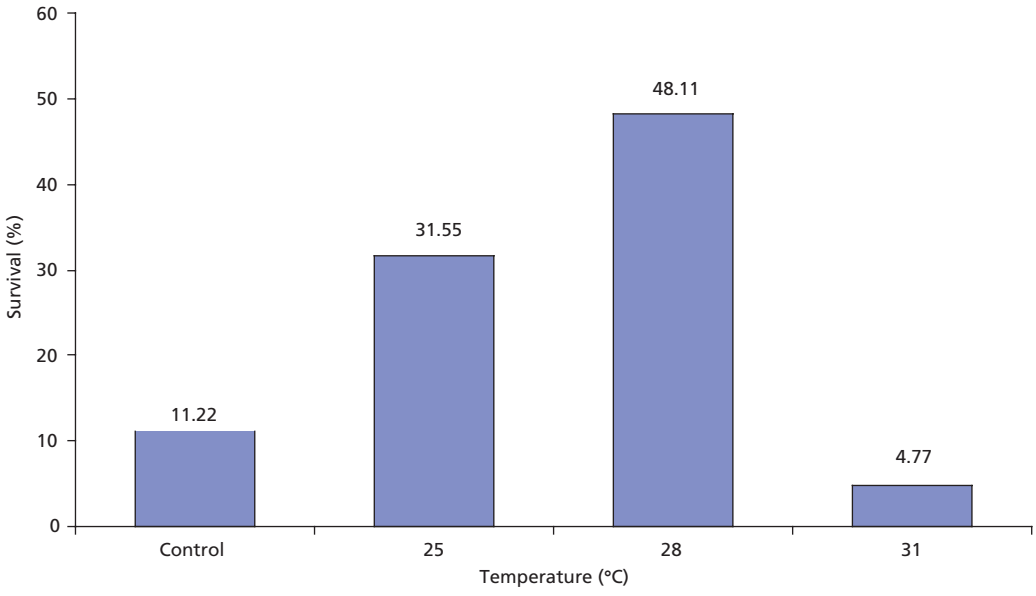
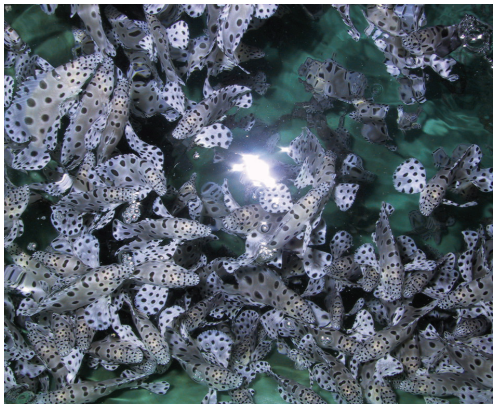


Figure 2. Survival of humpback grouper larvae reared at different temperatures.

the best growth rate of larvae was at the water temperature of 31°C. This is similar to the findings reported by Akatsu et al. (1998), who found that survival of grouper *E. tauvina* (= *E. coioides*) larvae reared for 12 days at different temperatures, i.e. 18°, 23°, 29° and 32°C were 0%, 0.29%, 9.8% and 0.6%, respectively. The highest survival was at the water temperature of 29°C.



Juvenile humpback grouper/barramundi cod (*Cromileptes altivelis*) reared at Gondol Research Institute for Mariculture, Bali, Indonesia.

Conclusions

- Growth and feeding rate of humpback grouper (*Cromileptes altivelis*) larvae increase with increasing water temperature.
- The optimum temperature for rearing of early stage humpback grouper larvae is 28°C.

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Larval Rearing Tank Management to Improve Survival of Early Stage Humpback Grouper (*Gromileptes altivelis*) Larvae

K. Sugama, Trijoko, S. Ismi and K. Maha Setiawati

Introduction

High mortality during the early larval stages of humpback grouper *Cromileptes altivelis* is one factor hindering the development of mass production of this species (Slamet et al. 1996, Aslianti, 1996). Difficulties in rearing grouper larvae also have been reported by Kohno et al. (1994; 1997). Kawahara et al. (2000) reported that larvae at 0–5 days after hatching are easily trapped at the water surface by surface tension. Those larvae once trapped at the water surface cannot escape from the surface and eventually die. High mortality also frequently occurs at 10–25 days after hatching because of larval entanglement with the spines.

The purpose of the present study was to determine if survival was improved by the addition of an oil film on the water surface and by increasing the percentage water exchange.

Methods

Experiment 1: Effect of oil addition on water surface of larval rearing tank for humpback grouper larvae.

The experiment was conducted using transparent tanks, 200 litres in volume and filled with sea water (34 ± 1 ppt). Eggs of humpback grouper were stocked into each tank at the density of 10 eggs/litre. Starting at day 1 after hatching, squid oil at different concentrations (0, 0.1, 0.2 and 0.3, ml/m2 for Study–1; 0.3, 0.4, and 0.5 ml/m2 for Study–2) was added to each tank. The experiment was a completely randomised design with three replicates per treatment. Survival and growth rate were measured at the end of the experiment (day 6 from hatching). Study–3 was conducted to determine the optimal time for the oil to be added to the rearing water. For this study, oil was added to the larval rearing tanks during the rearing period of day 1–3, day 1–6, day 1–9, day 1–12; the control group had no oil added to the tanks during the rearing period. The experiment was terminated at day 15 and survival of larvae was calculated.

Experiment 2: Effect of different water exchange on initial feeding incidence of humpback grouper larvae.

This experiment was conducted using nine transparent tanks, 200 litres in volume filled with sea water (salinity of 34 ± 1 ppt.). Eggs of humpback grouper were stocked into each tank at a density of 10 eggs/litre. During the experiment, larvae were reared without water exchange (treatment A), 100% water exchange per day (treatment B), and 200% water exchange per day (treatment C). Water exchange was started on day 3 by flow-through system. The experiment was designed in a completely randomised design with three treatments and three replicates for each treatment for 10 days. Samples of larvae were taken every day for observation of larval growth and

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stomach contents. Survival rate was measured at the end of the experiment.

Results and Discussion

Experiment 1

The addition of an oil film on the water surface during larval rearing, influenced survival rate of the larvae. Survival rate of larvae without oil addition was significantly lower ($P < 0.05$) than the treatments with the addition of oil. Addition of oil at the concentration of 0.3–0.4 ml/m^2 resulted in the highest survival (Tables 1 and 2). The highest survival rate occurred with the addition of 0.3 ml/m^2 of oil to the water surface. The total length of larvae in all treatments was the same. Larvae at 0–5 days after hatching were still very weak and slow moving. At this stage, larvae are easily trapped at the water surface by surface tension. Once trapped at the water surface, the larvae cannot escape and eventually die. The larvae trapped at the surface secrete a sticky mucus and this appears to contribute to additional larvae being trapped at the water surface. Finally, a significant number of larvae die in a short time (Kawahara et al. 2000). The addition of an oil film to the water surface reduces the surface tension and therefore the number of larval mortalities was reduced. However, if the amount of oil added is too low (i.e. 0.2 mL/m^2 or lower) the surface tension is still strong enough to trap the larvae leading to surface deaths.

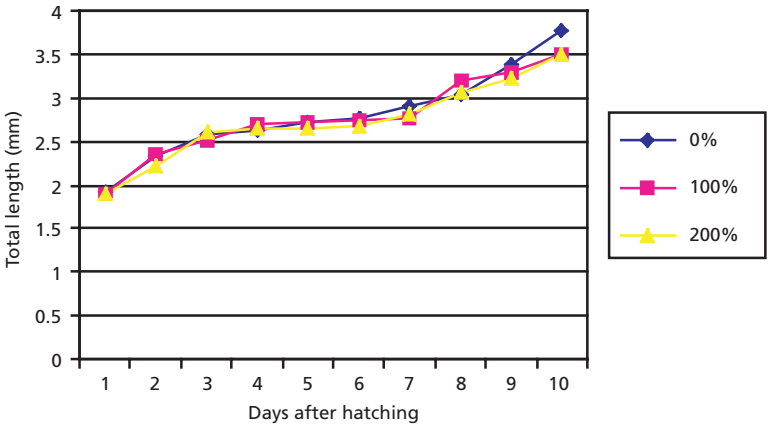

Figure 1. Total length (mm) of humpback grouper larvae reared at different water exchange rates (%/day).

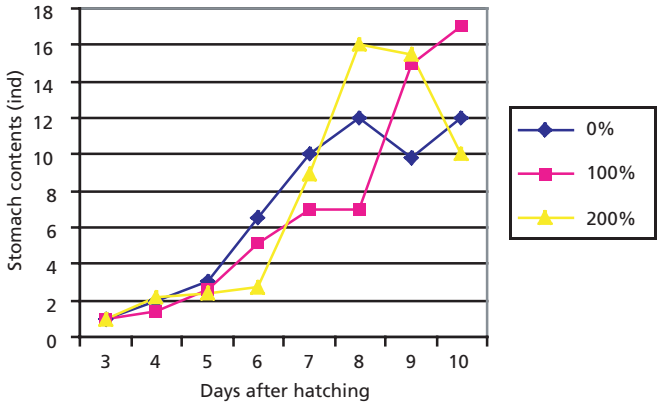

Figure 2. Stomach contents of humpback grouper larvae reared at different water exchange rates (%/day).

Study 3 demonstrated that the addition of oil up to day-9 post hatch resulted in the best larval survival (Table 3). This result correlates with the morphological development of the larvae. Long spines of dorsal and pelvic fin start to develop in day-9 old larvae. At this time larvae become more active and floating death decreases.

Table 1. Survival and total length of humpback grouper larvae with different concentration of oil addition during the 6 days larval rearing.

Oil addition (ml/m^2)	Total length (mm)	Survival rate (%)
0	2.80 ± 0.078[a]	0.30[a]
0.1	2.90 ± 0.073[a]	14.85[b]
0.2	2.99 ± 0.091[a]	42.30[c]
0.3	2.80 ± 0.078[a]	56.25[d]

Values with the same letter within a column are not significantly different (P > 0.05).

Table 2. Survival and total length of humpback grouper larvae with different concentration of oil addition during the 6 days larval rearing.

Oil addition (ml/m^2)	Total length (mm)	Survival rate (%)
0	2.93 ± 0.067[a]	7.4[a]
0.3	3.03 ± 0.089[a]	52.0[b]
0.4	3.09 ± 0.091[a]	50.0[bc]
0.5	2.97 ± 0.034[a]	48.9[bc]

Values with the same letter within a column are not significantly different (P > 0.05).

Table 3. Survival and total length of humpback grouper larvae after addition of oil at different larval rearing period.

Oil addition (day)	Total length (mm)	Survival rate (%)
1–3	5.10 ± 0.045[a]	4.8[a]
1–6	5.54 ± 0.056[a]	4.0[a]
1–9	5.23 ± 0.089[a]	7.9[b]
1–12	4.86 ± 0.023[a]	5.8[a]
No oil	4.35 ± 0.076[a]	0.8[c]

Values with the same letter within a column are not significantly different (P > 0.05).

Experiment 2

Results of this experiment showed that the highest survival rate was found for the treatment without water exchange during the first 10 days of larval rearing (Table 4). This result suggests that early stage larvae (days 0–10) are sensitive to fluctuations in environmental factors due to water exchange. However, there was no difference in growth (Figure 1) or feeding activity (Figure 2) at different water exchange rates.

Table 4. Survival and total length of humpback grouper larvae reared with different water exchange rates from day 0 to day 10 post hatch.

Water exchange (%/day)	Total length (mm)	Survival rate (%)
No exchange	3.61[a]	6.48[a]
100	3.54[a]	4.12[b]
200	3.50[a]	4.67[b]

Values with the same letter within a column are not significantly different (P > 0.05).

Conclusions

- Addition of an oil film to the water surface improved survival in day 0–day 9 humpback grouper *Cromileptes altivelis* larvae.
- Zero water exchange resulted in the highest survival of humpback grouper *Cromileptes altivelis* for the first 10 days of larval rearing.

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SECTION 3
GROW-OUT DIET DEVELOPMENT

Marine finfish cage farm in HaLong Bay, Vietnam. Fish farms in Southeast Asia are often family-run operations.

Nutritional research identified the major dietary requirements of groupers and enabled the development of appropriately formulated pelleted dry diets to replace fresh fishery bycatch ('trash' fish). Fish fed dry diets performed as well as, or better than, fish fed fishery bycatch. A high proportion of fishmeal (up to 80%) could be substituted by high quality meat meals, providing an option to decrease reliance on fishmeal. The use of dry diets rather than fishery bycatch provides a more efficient use of fishmeal resources, enables farmers to provide a nutritionally optimised and consistent feed source, and reduces pollution associated with feeding 'trash' fish.

Summary

The overall goal of the project's grow-out diet development research was to develop compounded pelleted grouper feeds as a more sustainable, lower-polluting and cost-effective alternative to the feeding of fresh fishery bycatch (or 'trash' fish) (Tacon and Forster 2003). The grouper species studied were humpback or polka dot grouper, *Cromileptes altivelis*, tiger or flowery grouper, *Epinephelus fuscoguttatus* and the gold spot or estuary cod, *Epinephelus coioides*. The research approach was to define the requirements of groupers for the key nutrients that largely determine the rate at which fish grow, determine the nutritive value of locally available marine and terrestrial feed ingredients and examine the extent to which high cost marine protein feed ingredients could be replaced using cheaper and more renewable terrestrial protein feed ingredients.

The crude protein (CP) requirement of humpback grouper and tiger grouper was met with diets that contained not less than 44% dry matter (DM) digestible CP (about 50% on an as-fed CP basis). Increasing the lipid content of the diet beyond about 9–10% did not improve fish growth rates but instead reduced the fish's appetite and resulted in higher rates of body fat deposition (Giri et al. 1999; Williams et al. 2004). Adding dietary lipid in the form of coconut oil as a rich source of medium chain fatty acids (C 10–14) resulted in an accelerated rate of lipid oxidation in humpback grouper compared with diets in which the lipid was provided as long chain (C 18+) fatty acids. However, this led to a profound depression of the fish's appetite and a profound decline in the fish's growth rate. Growth rate and survival of sea-caged humpback groupers were improved when diets were supplemented with up to 150 mg/kg of vitamin C as the heat-stable form of L-ascorbyl-2-monophosphate-Na-Ca (Laining et al. 2002). This benefit of vitamin C supplementation was most apparent following heavy flood rains, which caused a marked deterioration in water quality (increased turbidity and reduced dissolved oxygen content) around the cages. The dietary requirement for the essential omega-3 highly unsaturated fatty acids (n-3 HUFA) was examined for humpback grouper and tiger grouper. Increasing the supplementation rate up to 1–1.5% of the diet resulted in improved fish growth rates and better survival. In studies examining the capacity of humpback grouper to utilise different types of carbohydrate as energy sources, the best results were achieved using glucose, while starch and sucrose were the least effective (Usman 2002).

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

The apparent digestibility of a comprehensive range of ingredients available in the Philippines and Indonesia was determined for gold spot grouper and humpback grouper respectively. The CP in both marine and terrestrial animal meals was well digested (above 76%) by both grouper species with the exception of oven-dried blood meal, which was poorly digested (55%) (Laining et al. 2003). The protein digestibility of plant products was more variable (from 43% to 100%) with high fibre meals such as rice bran and lucaena (ipil-ipil) meal being poorly digested. The DM digestibility of the meals was adversely affected by the amounts of ash and fibre they contained. A collation of the DM and CP apparent digestibility values of the tested ingredients is presented in Table 1.

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

Table 1. The dry matter (DM) and crude protein (CP) apparent digestibility (AD) of selected feed ingredients determined for gold spot grouper in the Philippines and for humpback grouper in Indonesia.

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

[1] Mean ± SD.

was used at inclusion rates above 10% as a replacement for fishmeal protein.

In laboratory and field cage studies, a practical low-cost dry pelleted diet was formulated on a digestible nutrient basis to meet the requirements of juvenile gold spot grouper and compared with feeding either a commercial pellet diet or fresh fishery bycatch (Millamena 2002). In both studies, fish fed the project formulation diet survived and grew as well as those fed the fresh bycatch. In the laboratory study, fish fed the commercial pellet diet grew significantly slower and converted feed less efficiently than those fed either the project diet or fresh by-catch. The analysis of the commercial pellet diet showed a sub-optimal specification. When the commercial mill adjusted the formulation to meet these specifications, fish fed that diet in the field study performed as well as those fed either the project diet or fresh by-catch.

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

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Apparent Digestibility of Selected Feed Ingredients in Diets for Grouper (*Epinephelus coioides*) Juveniles

P.S. Eusebio, R.M. Coloso and R.E.P. Mamauag

Introduction

Cultured grouper are commonly fed trash fish. Because of the insufficient supply, high cost and variable quality of trash fish, there is a need to develop cost-effective and environment-friendly formulated diets (Tokwinas 1989). Inexpensive feed formulation can be achieved with incorporation of a variety of feed ingredients (Boonyaratpalin et al. 1998). Effective incorporation of an ingredient however, requires information on its digestibility for the target species. This study was conducted to determine the quality of selected feed ingredients as protein sources in grouper diets based on their nutrient composition and apparent digestibility coefficients for dry matter (ADMD) and crude protein (APD).

Methods

Grouper juveniles were used in a series of feeding experiments. A total of 56 juveniles (mean body weight ± standard deviation (s.d.) = 85.4 ± 5 g) were used for the first batch of test ingredients (Chilean fish meal, white fish meal, shrimp meal, defatted soybean meal, white cowpea meal and ipil-ipil leaf meal). 54 juveniles (mean body weight ± s.d. = 125.8 ± 5 g) were used for the second batch (squid meal, local meat and bone meal, meat solubles, soy protein concentrates, and rice bran), 72 juveniles (mean body weight ± s.d. = 198.2 ± 8 g) for the third batch (tuna fish meal, imported meat and bone meal, blood meal, corn gluten meal and wheat flour) and 48 juveniles (mean body weight ± s.d. = 211.4 ± 6 g) for the fourth batch (poultry feather meal, lupin seed meal and corn germ meal). White cowpea (*Vigna unguiculata*) and lupin (*Lupinus albus*) seeds were used in their raw dried state. Ipil-ipil (*Leucaena leucocephala*) leaves were soaked in tap water for 24 h, drained, rinsed with water and air-dried. All feed ingredients were oven-dried for 12 h at 60°C, ground and sieved using a No. 60 mesh size. Samples were taken for proximate analysis using standard methods (AOAC 1990).

Apparent digestibility coefficients were measured *in vivo* by using a flow through modified Guelph faecal collection system with filtered aerated sea water (flow rate = 800–1000 ml/min for 45–65 days. The method by Cho et al. (1982) was adapted using a ratio of 70:30 (reference diet to test ingredient) in each test diet (Table 1). A reference diet was formulated to meet the known nutrient requirement of grouper (45% protein, 10% fat and 375 kcal/kg metabolizable energy). All experimental diets contained 1% Cr_2O_3 as an external indicator and 1% carboxymethylcellulose (CMC) as binder. The fish were acclimated with reference diet (without Cr_2O_3) for 5 days before feeding them experimental diets twice daily (08:30 h and 14:30 h) at a rate of 5–8% of body weight. The seawater temperature and salinity ranged from 27°C to 28°C and 30 ppt. to 31 ppt., respectively. Each tank was provided with a faecal decantation column. Water from the 60L tank (first two batches) or 250 L tank (3rd and 4th batch) flowed through the decantation column into an attached clear plastic bottle where the faecal material (voided from 17:30 h to 07:30 h) settled and remained until collected.

The tanks and the faecal collection apparatus were cleaned twice daily; 2 h after feeding in the

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afternoon and before feeding in the morning. Faecal collection was started when the colour of the faeces became greenish, and were collected every morning (08:00 h) from the plastic bottle, washed three times with distilled water, freeze-dried and prepared for crude protein and Cr_2O_3 analyses. Apparent protein digestibility (APD) of the feed ingredients was computed using the formula described by Spyridakis et al. (1989) and Forster (1999). Apparent dry matter digestibility (ADMD) of feed ingredients was computed following the formula of Spyridakis et al. (1989) and Cho et al. (1982).

All data were analyzed using ANOVA for a completely randomized design. Treatment means were compared by Duncan's New Multiple Range Test. Differences were considered significant at $P<0.05$.

Results and Discussion

The proximate composition (g/100g dry weight) of various feed ingredients is shown in Table 2. Protein levels in fish meals and other feedstuffs of animal origin were generally high (47–87%). The protein content of feed ingredients from plant ranged only from 11% to 61%. Likewise, the levels of ash in fish and shrimp meals were higher (15–16%) compared with those in feed ingredients of plant origin (7–12 %). The levels of fiber in rice bran and ipil-ipil leaf meal (10–16%) were higher compared with those of other feed ingredients (<7%). Rice bran had the highest fat content (11%).

Table 3 shows the apparent digestibility coefficients for dry matter (ADMD) and crude protein. No significant difference was observed among the ADMD or APD of the reference diets in all batches of experiments, which indicated that variations in time, body weight of experimental fish and size of tanks have not affected the apparent digestibility measurements. ADMD and APD values for a reference diet in each batch were the constants used in the computation of ADMD and APD of the respective feed ingredients.

Table 1. Proximate composition of various feed ingredients for *in vivo* digestibility experiment (g/100 g dry weight)[1].

Test ingredients	%H_2O	Protein	Fat	Fibre	NFE[2]	Ash
Animal by-product						
Blood meal[3]	3.27	87.33	4.36	0.04	6.02	2.05
Fish meal, Chilean	10.03	73.57	7.99	0.08	2.09	16.27
Fish meal, tuna[4]	9.56	56.76	10.94	0.88	11.66	19.76
Fish meal, white[5]	7.64	74.63	7.59	0.00	1.72	16.06
Meat and bone meal[3]	10.40	49.05	9.02	1.18	9.41	31.34
Meat and bone meal[4]	4.95	46.96	10.49	0.78	4.75	37.02
Meat solubles (Protamino Aqua)	4.20	76.52	1.16	0.20	10.34	11.78
Poultry feather meal[4]	4.82	70.88	17.68	0.62	8.32	2.50
Shrimp meal, *Acetes* sp.	7.40	72.39	2.89	2.80	6.82	15.10
Squid meal	6.70	76.50	4.00	0.60	11.00	7.90
Plant by-product						
Corn germ meal[4]	4.51	8.54	47.35	6.38	36.91	0.82
Cowpea meal, white[6]	7.06	25.62	0.54	6.23	63.19	4.42
Corn Gluten meal[4]	7.96	60.62	6.96	3.36	27.84	1.22
Soy protein concentrates (HP 300)	5.73	56.87	1.03	5.09	28.71	8.30
Ipil-ipil leaf meal[7]	9.40	21.37	7.34	15.50	46.59	9.20
Lupin (*Lupinus albus*) seed meal[4]	7.62	28.54	5.52	14.24	48.72	2.98
Rice bran	8.78	12.26	10.46	10.32	55.24	11.72
Soybean meal, defatted	7.82	54.82	0.92	5.62	31.76	6.88
Wheat Flour	13.18	10.93	1.09	0.58	86.90	0.50

[1] Analysis done in Centralized Analytical Laboratory at SEAFDEC/AQD.
[2] Nitrogen free extract = 100 – [% crude protein + % ash + % crude fiber + %crude fat].
[3] Australia.
[4] Philippines.
[5] Alaskan white fish meal, USA.
[6] *Vigna unguiculata*, whole seeds in their raw state.
[7] Native ipil-ipil (*Leucaena leucocephala*) leaves soaked in water for 24 hours.

Table 2. The composition of reference and test diets for *in vivo* digestibility experiment of various feed ingredients (g/100 g feed).

Feed Ingredient	Reference Diet	Test Diet
Fish meal, Chilean	37.00	25.90
Squid meal	5.00	3.50
Shrimp meal, *Acetes* sp.	10.00	7.00
Soybean meal, defatted	13.00	9.10
Wheat flour	7.80	5.46
Rice bran	13.94	9.14
Cod liver oil	2.50	1.75
Soybean oil	2.50	1.75
Vitamin mix[1]	4.20	2.94
Mineral mix[1]	2.00	1.40
Ethoxyquin	0.05	0.05
Phosphitan C[2]	0.01	0.01
Chromic oxide (Cr_2O_3)	1.00	1.00
Carboxymethylcellulose,CMC	1.00	1.00
Test ingredient	—	30.00

[1] Biomin, commercially available vitamin and mineral mixture for shrimps, Overseas Feeds Corporation, Cebu City, Philippines.
[2] Ascorbic acid monophosphate, feed grade, Showa Denko K.K. Japan.

The apparent digestibility coefficients for ADMD ranged from 37% to 99% where squid meal and meat solubles had the highest and blood meal the lowest coefficients. Dry matter digestibility of reference diets were comparable with those of Chilean fish meal, white fish meal, tuna fish meal, poultry feather meal, *Acetes sp.,* local meat and bone meal, soy protein concentrates, defatted soybean meal, white cowpea meal, corn gluten meal and corn germ meal (74–94%). Low ADMD values seem to indicate that the overall efficiency of grouper to utilise the nutrients decreased as dietary fiber in feedstuff increased (de Silva et al., 1990). Reduced ADMD can also be associated with an increase in the nitrogen free extract of the respective feed ingredients, which also suggests that grouper had limited ability to digest starch and other carbohydrate components of the feed ingredients.

The apparent protein digestibility (APD) of all feed ingredients tested were relatively high (79–99%), except rice bran (43%) and blood meal (15%). APD values for the reference diets were comparable with those of white fish meal, Chilean fish meal, *Acetes* sp., squid meal, meat

Table 3. Apparent digestibility coefficients for dry matter and crude protein of various feed ingredients (%).

Batch No.	Diet/Feed Ingredient	ADMD[1]	APD[2]
1	Reference diet[3]	85.37 ± 0.29^{bc}	97.16 ± 0.10^{a}
	Fish meal, Chilean	83.56 ± 3.09^{bc}	98.03 ± 0.07^{a}
	Shrimp meal[4]	75.98 ± 4.00^{cd}	95.01 ± 0.72^{a}
	Soybean meal, defatted	75.68 ± 1.98^{cd}	96.03 ± 0.13^{a}
	Fish meal, white	89.22 ± 1.69^{b}	98.57 ± 0.31^{a}
	Cowpea meal, white	74.17 ± 3.14^{cd}	93.53 ± 1.22^{a}
	Ipil-ipil leaf meal	55.97 ± 0.04^{e}	78.83 ± 2.64^{b}
	Squid meal	99.37 ± 0.95^{a}	94.21 ± 0.21^{a}
	Meat and bone meal[5]	77.73 ± 0.09^{c}	83.79 ± 1.66^{b}
	Soy protein	76.32 ± 4.88^{cd}	85.50 ± 0.40^{b}
	Meat solubles	99.33 ± 0.45^{a}	97.59 ± 0.08^{a}
	Rice bran	68.50 ± 7.02^{d}	42.70 ± 5.38^{c}
3	Reference diet[3]	84.14 ± 1.14^{bc}	93.16 ± 0.41^{a}
	Meat and bone meal[6]	60.80 ± 0.80^{de}	98.91 ± 1.32^{a}
	Blood meal[6]	36.85 ± 0.98^{f}	15.45 ± 2.01^{d}
	Corn Gluten meal	93.98 ± 2.03^{ab}	99.52 ± 0.65^{a}
	Fish meal, tuna	75.39 ± 3.61^{cd}	76.24 ± 1.92^{b}
	Wheat flour	72.75 ± 0.85^{d}	82.86 ± 1.26^{b}
4	Reference diet[3]	84.35 ± 1.06^{bc}	94.58 ± 0.30^{a}
	Corn germ meal	85.15 ± 2.81^{bc}	82.86 ± 4.71^{b}
	Lupin seed meal	54.11 ± 1.24^{e}	97.48 ± 3.65^{a}
	Poultry feather meal	74.32 ± 3.06^{cd}	81.83 ± 2.58^{b}

[1] ADMD = Apparent Dry Matter Digestibility, Spyridakis et al., 1989; Cho et al., 1982.
[2] APD = Apparent Protein Digestibility, Spyridakis et al., 1989; Forster, 1999.
[3] ADMD and APD = Spyridakis et al., 1989.
[4] *Acetes* sp.
[5] Philippines.
[6] Australia.

solubles, imported meat and bone meal, defatted soybean meal, white cowpea meal, corn gluten meal, and lupin seed meal (93–99%). These were significantly higher than those of tuna fish meal, local meat and bone meal, poultry feather meal, soy protein concentrates, wheat flour, ipil-ipil leaf meal and corn germ meal (76–85%). Low APD values for rice bran and ipil-ipil leaf meal may be due to their low protein or high fiber contents (McGoogan and Reigh 1996). High APD values were obtained for some feed ingredients with high protein content. Increases in protease enzyme activity were observed when dietary protein increased suggesting that enzyme activity was related to the amount of protein in the gut (Eusebio and Coloso 2002). However, blood meal with high protein content had low APD value, which can be attributed to the processing method used in its preparation. This method can damage the amino acids, thus contributing to low nitrogen digestibility.

Conclusions

- ADMD values vary with the levels of fiber and other carbohydrate substances in feed ingredients.
- Grouper can utilise dietary protein efficiently regardless of whether it is of animal or plant origin.
- High APD values are generally obtained in feed ingredients with high protein content.
- Low digestibility coefficients for feed ingredients can also be attributed to the processing methods used in their preparation.

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Evaluation of some Terrestrial Proteins in Complete Diets for Grouper (*Epinephelus coioides*) Juveniles

P.S. Eusebio, R.M. Coloso and R.E.P. Mamauag

Introduction

Various protein sources have been evaluated with the objective of partially or completely replacing fish meal in fish diets. Some plant protein sources have promoted reasonable growth even at high (≥25%) dietary inclusion levels (Jackson et al. 1982; Elangovan and Shim 2000; Gouveia and Davie, 2000). Also, other plant and animal protein sources were used in the diets for carnivorous fish without negative effects on growth (Gomez et al. 1995; Carter and Hauler 2000; Eusebio and Coloso 2000). This study was undertaken to determine the nutritive value of some of the more widely available protein sources in the diets for grouper juveniles based on apparent digestibility coefficients for dry matter (ADMD) and crude protein (APD), food conversion ratio (FCR), specific growth rate (SGR) and survival.

Methods

A series of feeding experiments were conducted to determine the growth performance of grouper juveniles (initial body weights = 2–5 g). The test diets were formulated for growth (4 replications/treatment) and digestibility experiments (3 replications/treatment). Each diet contained a test ingredient: experiment 1 (white fish meal, white cowpea meal and ipil-ipil leaf meal), experiment 2 (local meat and bone meal, soy protein concentrates and meat solubles) and experiment 3 (imported meat and bone meal, blood meal and corn gluten meal). The APD values for feed ingredients obtained from the previous experiments[1] were considered in the formulation. The composition and chemical analyses (AOAC 1990) of the formulated diets is shown in Tables 1–3.

A feeding trial for each experiment was conducted for 85 days in a flow-through system with filtered and aerated seawater. Ten and twenty juveniles were stocked in each of 60 L and 250 L oval fiberglass tanks, respectively. The fish were acclimated for five days prior to feeding them test diets. Feed was given twice daily (8:00 h and 16:00 h) at 15% of body weight at the start and computed thereafter to 8% towards the end of the feeding period. Water temperature and salinity ranged from 27–28°C and 29–30 ppt., respectively. Other water quality parameters were within the ranges appropriate for growth. The fish were weighed as a group every 20 days for 80 days. The growth performance of grouper juveniles was evaluated based on SGR (% day^{-1}), FCR and survival. Apparent digestibility coefficients (ADMD and APD) of formulated diets were also measured using the modified Guelph Faecal Collection System with Cr_2O_3 as an external indicator (Eusebio and Coloso 2000). The feeds and faeces were analyzed for moisture and protein using standard methods (AOAC 1990), and Cr_2O_3 (Carter et al. 1960). ADMD and APD were

[1] Eusebio, P.S., Coloso, R.M. and Mamauag, R.E.P. Apparent digestibility of selected feed ingredients in diets for grouper (*Epinephelus coioides*) juveniles. This volume, pp. 75–78.

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Table 1. Composition of the control and test diets (Experiment 1)[1].

Feed Ingredient	Control	Diet 1	Diet 2	Diet 3
Fish meal, Chilean	39.00	—	38.00	35.00
Fish meal, white	—	30.00	—	—
Cowpea meal, white	—	—	20.50	—
Ipil-ipil leaf meal	—	—	—	15.10
Squid meal	5.00	5.00	5.00	5.00
Shrimp meal, *Acetes* sp.	10.00	10.00	10.00	10.00
Soybean meal, defatted	17.00	24.40	12.96	13.21
Wheat flour	9.80	6.20	—	7.00
Rice bran	7.74	12.84	1.28	2.63
Cod liver oil	2.10	2.15	2.50	2.40
Soybean oil	2.10	2.15	2.50	2.40
Vitamin mix[2]	4.20	4.20	4.20	4.20
Mineral mix[2]	2.00	2.00	2.00	2.00
Ethoxyquin	0.05	0.05	0.05	0.05
Phosphitan C	0.01	0.01	0.01	0.01
Carboxymethylcellulose	1.00	1.00	1.00	1.00
Proximate Composition[3]				
Crude protein (N × 6.25)	48.6	48.3	47.5	46.5
Crude fat	9.3	9.2	9.2	9.8
Nitrogen-free extract[4]	23.4	24.7	23.4	25.6
Crude fiber	2.9	3.5	3.5	2.9
Metabolisable energy (kcal/100g)[5]	370	372	365	372
Calculated digestible protein	42.6	42.6	43.0	42.0

[1] As fed basis (g/100g feed).
[2] Biomin, commercially available vitamin and mineral mixtures, Overseas Feed Corporation, Cebu City, Philippines.
[3] Dry weight basis (g/100g feed).
[4] NFE = 100 – (% crude protein + % crude fat + % crude fiber + % crude ash).
[5] Metabolizable energy was calculated based on the standard physiological values of 4.5kcal/g protein, 3.3kcal/g carbohydrate and 8.0kcal/g fat (Brett and Groves, 1979).

Table 2. Composition of the control and test diets (Experiment 2)[1].

Feed Ingredient	Control	Diet 1	Diet 2	Diet 3
Fish meal, Chilean	39.00	30.00	14.00	—
Meat and bone meal[2]	—	16.00	—	—
Soy protein concentrates	—	—	35.00	—
Meat solubles	—	—	—	30.00
Squid meal	5.00	5.00	5.00	5.00
Shrimp meal, *Acetes* sp.	10.00	10.00	10.00	10.00
Soybean meal, defatted	17.00	14.50	8.30	21.50
Wheat flour	9.80	10.40	—	4.70
Rice bran	7.74	3.34	14.74	14.04
Cod liver oil	2.10	2.00	2.60	3.25
Soybean oil	2.10	2.00	2.60	3.25
Vitamin mix[3]	4.20	4.20	4.20	4.20
Mineral mix[3]	2.00	1.50	2.50	3.00
Ethoxyquin	0.05	0.05	0.05	0.05
Phosphitan C	0.01	0.01	0.01	0.01
Carboxymethylcellulose	1.00	1.00	1.00	1.00
Proximate Composition[4]				
Crude protein (N × 6.25)	48.6	47.7	43.9	46.9
Crude fat	9.3	9.3	9.2	9.9
Nitrogen-free extract[5]	23.4	24.8	31.1	27.9
Crude fiber	2.9	2.8	3.9	3.1
Metabolisable energy (kcal/100g)[6]	370	371	374	381
Calculated digestible protein	42.6	42.2	40.1	42.4

[1] As fed basis (g/100g feed).
[2] Locally available.
[3] Biomin, commercially available vitamin and mineral mixtures, Overseas feed Corporation, Cebu City, Philippines.
[4] Dry weight basis (g/100g feed).
[5] NFE = 100 – (% crude protein + % crude fat + % crude fiber + % crude ash).
[6] Metabolizable energy was calculated based on the standard physiological values of 4.5 kcal/g protein, 3.3 kcal/g carbohydrate and 8.0 kcal/g fat (Brett and Groves, 1979).

Table 3. Composition of the control and test diets (Experiment 3)[1].

Feed Ingredient	Control	Diet 1	Diet 2	Diet 3	Diet 4
Fish meal, Chilean	36.00	25.00	—	12.00	18.00
Meat and bone meal[2]	—	19.00	—	—	—
Blood meal[2]	—	—	27.00	—	—
Corn gluten meal	—	—	—	31.00	—
Fish meal, tuna	—	—	—	—	22.00
Squid meal	5.00	5.00	5.00	5.00	5.00
Shrimp meal, *Acetes* sp.	10.00	10.00	10.00	10.00	10.00
Soybean meal, defatted	16.50	14.00	20.00	13.00	18.50
Wheat flour	9.80	12.00	7.60	3.60	8.80
Rice bran	10.96	3.74	14.24	12.54	6.14
Cod liver oil	2.24	2.25	3.20	2.30	2.15
Soybean oil	2.24	2.25	3.20	2.30	2.15
Vitamin mix[3]	4.20	4.20	4.20	4.20	4.20
Mineral mix[3]	2.00	1.50	4.50	3.00	2.00
Ethoxyquin	0.05	0.05	0.05	0.05	0.05
Phosphitan C	0.01	0.01	0.01	0.01	0.01
Carboxymethylcellulose	1.00	1.00	1.00	1.00	1.00
Proximate Composition[4]					
Crude protein (N × 6.25)	47.5	47.2	48.1	47.6	47.7
Crude fat	11.4	11.4	10.4	13.8	12.0
Nitrogen-free extract[5]	23.7	23.00	28.4	25.5	24.5
Crude fiber	3.4	2.2	2.2	2.6	1.4
Metabolisable energy (kcal/100g)[6]	384	379	394	409	391
Calculated digestible protein	43.0	43.5	28.3	43.3	40.6

[1] As fed basis (g/100g feed).
[2] Australia.
[3] Biomin, commercially available vitamin and mineral mixtures for shrimps, Overseas Feed Corporation, Cebu City, Philippines.
[4] Dry weight basis (g/100g feed).
[5] NFE = 100 – (% crude protein + % crude fat + % crude fiber + % crude ash).
[6] Metabolizable energy was calculated based on the standard physiological values of 4.5 kcal/g protein, 3.3 kcal/g carbohydrate and 8.0 kcal/g fat (Brett and Groves, 1979).

computed using the formula of Spyridakis et al. (1989).

The data were analyzed using ANOVA for a completely randomized design. Treatment means were compared by the use of Duncan's Multiple Range Test (SAS Institute Inc. 1988). Differences were considered significant at $P < 0.05$.

Results and Discussion

Experiment 1

Table 4 shows the growth performance of grouper and digestibility of the diets used in the growth experiment. Based on SGR, the growth performance of grouper juveniles (initial body weight ± standard error (s.e.) = 3.7 ± 0.6g) fed control diet, white fish meal and white cowpea meal-based diets was comparable (3.2–3.3% day^{-1}). Fish fed ipil-ipil leaf meal-based diet had the poorest growth performance (2.7% day^{-1}). No significant difference was observed between the FCR of white fish meal and white cowpea meal-based diets, and the control diet (1.3–1.4). Ipil-ipil leaf meal-based diet with FCR value of 1.6 was less efficient than the other three diets. Survival was 100% in all treatments. ADMD values for the control diet, white cowpea meal and ipil-ipil leaf meal-based diets were not significantly different (65–72%) but lower than that of the white fish meal-based diet (81%). The APD value for white fish meal-based diet was the highest (95%), followed by the control diet (91%) and white cowpea meal-based diet (88%). Ipil-ipil leaf meal-based diet had the lowest APD value of 79%. The poor growth performance of grouper fed ipil-ipil leaf meal-based diet can be associated with the low APD value of the respective diet. Jackson et al. (1982) found that ipil-ipil leaf meal (25% replacement of fish meal) in a diet for tilapia resulted in poor growth, which can be attributed to the toxic effect of mimosine present in ipil-ipil leaves.

Experiment 2

The growth performance of grouper juveniles (mean initial weight ± s.e. = 2.5 ± 0.1g) and digestibility of the diets used in the growth experiment are shown in Table 5. The SGR of fish fed the control diet was comparable with that of fish fed local meat and bone meal-based diet (3.6% day^{-1}). The growth of fish (SGR = 3.4% day^{-1}) fed soy protein concentrates-based diet was not as excellent as that of the control fish, which can be associated with the lower values for ADMD (58%) and APD (93%) of their diet compared with the control diet (ADMD = 73%; APD = 95%). Fish fed meat solubles-based diet (FCR = 2.7) had the poorest growth performance (SGR = 2.4% day^{-1}) because the diet that was given to them was not as efficient as the other diets (FCR = 1.4–1.5). However, no significant difference was observed on the survival of fish fed the four diets (80–93%). Also, the poor growth performance of fish given soy protein concentrates and meat solubles-based diets can be due to the processing methods used in the preparation of the respective feed ingredients. Heat can damage the amino acid components of the feed ingredients thus making them unavailable to the fish (Opstevedt et al. 1984).

Experiment 3

The SGR of fish (mean initial weight ± s.e. = 3.9 ± 0.4g) fed the control diet and imported meat and bone meal, corn gluten meal and tuna fish meal-based diets were comparable (3% day–1), but significantly higher than that of fish given blood meal-based diet (2% day–1) (Table 6). FCR values for the control diet, meat and bone meal

Table 4. Growth performance of grouper, *Epinephelus coioides* juveniles and apparent digestibility coefficients (%) for dry matter and protein of the diets (Experiment 1)[1].

Dietary treatment	Initial weight (g)	Specific growth rate (% day^{-1})[2]	FCR[3]	ADMD[4]	APD[5]
Control diet	3.71 ± 0.24^{a}	3.22 ± 0.03^{a}	1.43 ± 0.02^{a}	71.90 ± 2.21^{b}	90.65 ± 0.43^{b}
Fish meal, white	3.7 ± 0.35^{a}	3.34 ± 0.05^{a}	1.28 ± 0.07^{a}	80.75 ± 1.79^{a}	94.99 ± 0.33^{a}
Cowpea meal, white	3.67 ± 0.37^{a}	3.24 ± 0.05^{a}	1.38 ± 0.10^{a}	64.72 ± 1.80^{b}	87.84 ± 0.41^{b}
Ipil-ipil leaf meal	3.68 ± 0.38^{a}	2.67 ± 0.10^{b}	1.64 ± 0.05^{b}	72.45 ± 0.50^{b}	78.74 ± 0.14^{c}

[1] Treatment means in columns followed by different superscripts are significantly different ($P < 0.05$; mean value ± standard error of the mean; n = 4 and 3 for growth and digestibility experiments, respectively); survival was 100% in all treatments.
[2] Specific growth rate = 100 × (ln W_{final} – ln $W_{initial}$/time (days).
[3] Feed conversion ratio = dry weight feed (g)/wet weight gain (g).
[4] ADMD = 100 – [(% Cr_2O_{3diet}/% $Cr_2O_{3faeces}$ × % DM_{faeces}/% DM_{diet} × 100)].
[5] APD = 100 – [(% Cr_2O_{3diet}/% $Cr_2O_{3faeces}$ × % $protein_{faeces}$/% $protein_{diet}$ × 100)].

Table 5. Growth performance of grouper, *Epinephelus coioides* juveniles and apparent digestibility coefficients (%) for dry matter and protein of the diets (Experiment 2)[1].

Dietary treatment	Initial weight (g)	Specific growth rate (% day^{-1})[2]	FCR[3]	Survival rate (%)	ADMD[4]	APD[5]
Control	2.54 ± 0.06^{a}	3.63 ± 0.02^{a}	1.41 ± 0.08^{a}	80.00 ± 5.47^{a}	73.26 ± 1.08^{a}	94.59 ± 0.71^{a}
Meat and bone meal (Philippines)	2.49 ± 0.09^{a}	3.60 ± 0.05^{a}	1.38 ± 0.04^{a}	91.67 ± 4.01^{a}	64.14 ± 2.67^{b}	91.87 ± 0.59^{c}
Soy protein concentrates (HP 300)	2.49 ± 0.08^{a}	3.38 ± 0.05^{b}	1.53 ± 0.10^{a}	81.67 ± 6.01^{a}	58.45 ± 1.99^{c}	92.55 ± 0.37^{bc}
Meat solubles (Protamino Aqua)	2.48 ± 0.08^{a}	2.43 ± 0.04^{c}	2.72 ± 0.09^{b}	93.33 ± 3.33^{a}	70.97 ± 0.39^{a}	93.74 ± 0.18^{ab}

[1] Treatment means in columns followed by different superscripts are significantly different ($P < 0.05$; mean value ± standard error of the mean; n = 4 and 3 for growth and digestibility experiments, respectively).
[2] Specific growth rate = 100 × (ln W_{final} – ln $W_{initial}$/time (days).
[3] Feed conversion ratio = dry weight feed (g)/wet weight gain (g).
[4] ADMD = 100 – [(% Cr_2O_{3diet}/% $Cr_2O_{3faeces}$ × % DM_{faeces}/% DM_{diet} × 100)].
[5] APD = 100 – [(% Cr_2O_{3diet}/% $Cr_2O_{3faeces}$ × % $protein_{faeces}$/% $protein_{diet}$ × 100)].

Table 6. Growth performance of grouper, *Epinephelus coioides* juveniles and apparent digestibility coefficients (%) for dry matter and protein of the diets (Experiment 3)[1].

Dietary treatment	Initial weight (g)	Specific growth rate (% day^{-1})[2]	FCR[3]	Survival rate (%)	ADMD[4]	APD[5]
Control	3.92 ± 0.55^{a}	3.10 ± 0.10^{a}	1.08 ± 0.05^{a}	97.5 ± 0.01^{a}	79.7 ± 1.3^{a}	94.6 ± 0.3^{a}
Meat and bone meal (Australia)	3.93 ± 0.48^{a}	3.00 ± 0.01^{a}	1.09 ± 0.09^{a}	97.5 ± 0.01^{a}	60.4 ± 3.6^{b}	87.6 ± 1.1^{b}
Blood meal (Australia)	3.92 ± 0.43^{a}	2.44 ± 0.13^{b}	1.44 ± 0.02^{c}	95.0 ± 0.02^{a}	82.9 ± 0.9^{a}	89.4 ± 0.5^{b}
Corn gluten meal	3.93 ± 0.36^{a}	2.82 ± 0.13^{a}	1.24 ± 0.02^{b}	90.0 ± 0.05^{a}	73.8 ± 1.5^{a}	93.3 ± 0.6^{a}
Fish meal, tuna	3.91 ± 0.31^{a}	3.05 ± 0.08^{a}	1.10 ± 0.04^{a}	97.5 ± 0.01^{a}	78.6 ± 2.0^{a}	93.1 ± 0.6^{a}

[1] Treatment means in columns followed by different superscripts are significantly different ($P < 0.05$; mean value ± standard error of the mean; n = 4 and 3 for growth and digestibility experiments, respectively).
[2] Specific growth rate = $100 \times (\ln W_{final} - \ln W_{initial}/\text{time (days)}$.
[3] Feed conversion ratio = dry weight feed (g)/wet weight gain (g).
[4] ADMD = $100 - [(\% Cr_2O_{3diet}/\% Cr_2O_{3faeces} \times \% DM_{faeces}/\% DM_{diet} \times 100)]$.
[5] APD = $100 - [(\% Cr_2O_{3diet}/\% Cr_2O_{3faeces} \times \% \text{protein}_{faeces}/\% \text{protein}_{diet} \times 100)]$.

and tuna fish meal-based diets were comparable (1.1) and better than that of corn gluten meal-based diet (1.2). Blood meal-based diet was the least efficient with FCR value of 1.4. The poor growth performance of fish fed blood meal-based diet can be associated with the poor efficiency and low APD of the diet. Also, Allan et al. (2000) observed that the poor availability of isoleucine in blood meal for rainbow trout was associated with its low isoleucine and high leucine contents. ADMD values for the blood meal, tuna fish meal and corn gluten meal-based diets, and the control diet were comparable (74–83%). The imported meat and bone meal-based diet had the lowest ADMD value (60%). On the other hand, APD coefficients for the control diet, and corn gluten meal and tuna fish meal-based diets were comparable (93–95%), but were higher than those of the imported meat and bone meal-based diets (88%) and blood meal-based diets (89%). Furthermore, no significant difference was observed on the survival of fish fed the five diets (90–98%).

Conclusions

- White cowpea meal (20.5% incorporation), local (16% incorporation) and imported (19% incorporation) meat and bone meals can partially replace fish meal in the diets for grouper juveniles without affecting their growth.
- Low ADMD and APD values for the processed feed ingredients (meat and bone meal, soy protein concentrates and blood meal-based diets) can be associated with the processing methods used in the preparation of the respective feed ingredients, which can damage the amino acids and contribute to low nitrogen digestibility.
- Apparent digestibility coefficients (ADMD and APD) and growth can be used as indicators to determine the nutritional value of feed ingredients. However, the availability and optimal balance of amino acids must also be considered.

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Apparent Digestibility of Selected Local Feed Ingredients for Humpback Grouper (*Cromileptes altivelis*)

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Introduction

The main objective of this study was to determine the apparent digestibility of several feed ingredients locally available in South Sulawesi for juvenile humpback grouper. Feedstuff digestibility assessment in fish is essential for determining nutrient requirements, screening the potential nutritive value of alternative feed ingredients and in the development of nutritionally adequate diets at least cost (Hajen et al. 1993). At present, digestibility coefficients of common feed ingredients have been reported for only a few species of warmwater marine carnivorous fishes (NRC 1983) and none for humpback grouper.

Methods

A reference diet and nine test diets, namely shrimp head meal, three types of blood meal (dried blood meal, formic acid-preserved blood meal, propionic acid-preserved blood meal), two types of fish meal (sardine meal, local-mixed fish meal), soybean meal, palm oil cake meal and rice bran were tested (Table 1). Chromic oxide was added to the diets at an inclusion rate of 1% as the digestibility marker.

Three experiments were carried out sequentially. Each experiment constituted a 4×4 latin square design in which four diets (one reference diet and three test ingredient diets) were examined over four collection periods. In each experiment, 20 fish of 20g initial weight from each cage were transferred to a 200L cylindro-conical faecal collection tank that was fitted with a faeces collection chamber (Allan et al. 1999). Following a five-day acclimatisation period, during which the fish were fed their prescribed diet twice daily to satiety, faeces were collected. The collected faeces were oven-dried (40°C) and stored in a sealed bottle at –40°C until analysed. Faecal collection continued for five to seven days when it was judged that a sufficient sample had been collected for chemical analysis. After each faecal collection period, diets were reallocated to the collection tanks in accordance with the latin square design and faecal collection recommenced after a further five-day acclimatisation period. This process was repeated for each of the four collection periods and sequentially for each of the three experiments.

The nutrient content of feed and faecal samples was analysed by AOAC (1990) procedures. After acid digestion, chromium concentration was determined by a spectrophotometric method (Furukawa and Tsukahara 1966). The apparent digestibility coefficient (ADC) of a nutrient in an ingredient was calculated according to procedures described by Foster (1999).

Results and Discussion

Humpback grouper generally showed a high capacity to digest protein. The apparent digestibility of plant protein (67.2% to 80.5%) was almost as good as that of animal protein (78.0% to 92.5%) except for the poorly digested oven-dried blood meal and rice bran meal. However, the apparent digestibility of dry matter (DM) was generally poor and especially for plant feed ingredients where values ranged from 22.2% for rice bran meal to 54.8% for soybean meal. By comparison, the DM apparent digestibility of

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Table 1. Formulation (g/kg, air dry) of experimental diets.

Ingredient and international feed number (IFN)		Diet		
Ingredient	**IFN**	**Reference**	**Test diet 1**	**Test diet 2**
Fish meal	5-01-985	570	342	399
Soybean meal; roasted; full-fat	5-14-005	80	48	56
Wheat gluten	—	100	60	70
Wheat flour	4-05-199	60	36	42
Rice bran	4-03-928	80	48	56
Fish oil	7-08-049	40	24	28
Squid oil	—	30	18	21
Vitamin mix[1]	—	30	18	21
Mineral mix [2]	—	10	6	7
Test ingredient 1 (animal origin)[3]	—	0	400	0
Test ingredient 2 (plant origin)[4]	—	0	0	300
Chromic oxide	—	10	10	10

[1] At 30 g/kg inclusion level, provided in 1 kg of final diet: retinol, 540 mg; cholecalciferol, 9.125 mg; α-tocopherol, 212.4 mg; menadione, 375 mg; thiamin, 300 mg; riboflavin, 750 mg; pyridoxine, 300 mg; cyanocobalamin, 3.5 mg; ascorbic acid, 4500 mg; folic acid, 150 mg; nicotinic acid, 1800 mg; d-pantothenic acid, 1500 mg; biotin, 3.75 mg; and d/l methionine, 1500 mg.

[2] At 10 g/kg inclusion level, provided in 1 kg of final diet: Ca, 3.25 g; P, 1.0 g; Fe, 60 mg; Mn, 40 mg; I, 0.75 mg; Cu, 3 mg; and Zn, 37.5 mg.

[3] Animal origin:

Shrimp head meal (5-04-226) and Local mixed fish meal (5-01-974): Manufactured by TAS Coy, Makassar, South Sulawesi.

Sardine meal(5-02-015): Dried sardine from fish landing site and extruded at RICF feed mill.

Blood meal (5-00-380): Oven-dried (60°C) and ground fresh bovine blood from local abattoir, Makassar, South Sulawesi.

[4] Plant origin:

Soybean meal (5-14-005): Whole soybean seed supplied by PT Inti Tani and heat-extruded at RICF feed mill.

Palm oil cake (5-04-487): Manufactured by PT. Pertani, Luwu, South Sulawesi.

Rice bran (4-03-928): Supplied by PT. Pertani, Sidrap, South Sulawesi.

Table 2. The dry matter (DM), crude protein (CP) and gross energy (GE) apparent digestibility coefficients (%) of diets and of substituted test feed ingredients examined in three experiments. Each coefficient is the mean of four replicates.

Diet and ingredient designation	Dry matter	Crude protein	Gross energy
Shrimp head meal	58.5 ± 3.33^{a}	78.0 ± 1.32^{b}	63.6 ± 0.89^{a}
Soybean meal (full-fat)	54.8 ± 2.72^{b}	67.2 ± 1.29^{c}	51.1 ± 0.89^{b}
Palm oil cake meal	45.3 ± 2.37^{c}	80.5 ± 1.30^{a}	40.4 ± 3.74^{c}
Dried blood meal	48.1 ± 0.85^{c}	55.2 ± 1.35^{c}	nd
Formic blood meal	67.9 ± 1.63^{a}	87.5 ± 0.55^{a}	nd
Propionic blood meal	61.7 ± 2.60^{b}	84.2 ± 0.69^{b}	nd
Local sardine meal	87.2 ± 2.53^{a}	92.5 ± 1.40^{a}	85.2 ± 0.90^{a}
Local mixed-fish meal	59.1 ± 1.23^{b}	82.4 ± 1.99^{b}	77.2 ± 1.91^{b}
Rice bran meal	22.2 ± 1.52^{c}	59.5 ± 1.41^{c}	44.3 ± 0.97^{c}

[a,b,c,d] Treatment means within each column with the same superscript letters are not significantly different ($P > 0.05$).
nd Not determined as insufficient faecal sample for energy analysis.

animal feed ingredients was higher, especially for the local sardine meal (87.2%). The local mixed-fish meal and the shrimp head meal were far less digestible with a DM apparent digestibility of only 59.1% and 58.5%, respectively, and lower than either of the two fermented blood meals (61.7% and 67.9%). The apparent digestibility of gross energy (GE) was comparatively high for the two fish meals, with the local sardine meal being significantly more digestible than the local mixed-fish meal (85.2% compared with 77.7%, respectively). Although, the apparent energy digestibility of the shrimp head meal was low (63.6%), it appeared to be higher than for each

of the three plant feed ingredients; full-fat soybean meal (51.1%), palm oil cake meal (40.4%) and rice bran (44.3%).

The low energy digestibility of the plant feed ingredients can be attributed to their high carbohydrate content and poor digestibility by carnivorous fish (Lupatsch et al. 1997). Other warmwater carnivorous marine fish, such as Asian sea bass and red drum, appear to have a higher capacity to digest plant ingredients than humpback grouper (Gaylord and Gatlin 1996).

Generally, freshwater and warmwater fish appear to digest carbohydrates more effectively than marine fish and coldwater fish (Wilson 1994). This study has shown that humpback grouper are able to efficiently digest the protein of both plant and animal feed ingredients. However, they have a very limited capacity to digest carbohydrate-rich products such as many plant feedstuffs and shrimp head meal that additionally has a high ash content (25.1%). Blood meal was digested better than plant meals and thus has greater potential to be used as a dietary replacement of fish meal in humpback grouper diets, particularly if the nutritive value of blood meal is enhanced through preservation. The two types of local sources of fish meal were digested well by humpback grouper and thus have good potential to substitute for imported fish meal. However, use of these products as aquaculture ingredients would directly compete with their traditional use for human consumption.

Conclusions

- Humpback grouper are able to digest the protein of both plant and animal feed ingredients.
- Humpback grouper have a very limited capacity to digest carbohydrate-rich products found in many plant feedstuffs, and shrimp head meal which additionally has a high ash content.
- Several feed ingredients could be used as protein sources as fish meal replacement.

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The Optimal Dietary Protein and Lipid Specification for Rearing Humpback Grouper (*Cromileptes altivelis*) Fingerlings

K.C. Williams, D.M. Smith, I.H. Williams, S. Irvin, M. Barclay and M. Jones

Introduction

A major objective of this project was to develop compounded grouper grow-out feeds that are more cost-effective and less environmentally damaging than the alternative of feeding fresh fishery by-catch. Information on the nutritional requirements of epinepheline groupers is limited (Boonyaratpalin 1997; Chen 2001) and non-existent for humpback grouper. Moreover, it is not known whether or not groupers can effectively utilise dietary lipid as an energy source in order to spare protein and so reduce nitrogen (N) discharge to the environment. As a first step in the development of formulated grow-out feeds for humpback grouper fingerlings, a series of growth, digestibility and metabolic assays were carried out to determine the optimum dietary protein and lipid specification for maximising productivity of humpback fingerlings.

CSIRO researcher Dr Kevin Williams assisting RICA staff to design digestibility experiments.

Methods

The optimum dietary protein to lipid ratio for humpback grouper was determined by feeding fingerlings one of 10 pelleted diets in which the dry matter (DM) crude protein (CP) concentration varied from 41% to 63% at 5.5% increments and in combination with either 15% or 24% DM lipid (a 3:1 mixture of fish oil and soybean oil). These diets were fed to four replicate tanks of fingerlings in an 8-week comparative slaughter growth and nutrient digestibility assay.

A second comparative slaughter growth and digestibility assay, employing the same culture conditions as before, was carried out to see if supplying dietary lipid at moderate (15% added oil) or high (30% added oil) concentrations and in the form of either long-chain fatty acids (LCFA, C18+, as olive oil) or medium-chain fatty acids (MCFA, C12–C16, as coconut oil) affected the way the fish used the lipid as an energy source. Five diets, a low-lipid (7% DM), high-protein (82% CP DM) control diet and four 'lipid' diets that together comprised a 2×2 factorial of the two types and two concentrations of lipid, were fed to six replicate tanks of fingerlings for eight weeks. The formulation of the 'lipid' diets was identical to the control except that the required amount of lipid was included at the expense of defatted fish meal with a concomitant lowering of the dietary CP from 82% to 69% and 57% DM for the 15% and 30% lipid treatments, respectively. These same 'lipid' diets were radioactively labelled with ^{14}C-octanoic

acid (as a marker of MCFA) and ^{14}C-oleic acid and ^{14}C-palmitic acid (as markers of LCFA) were fed to fish and the fate of the labelled ^{14}C was determined using metabolism chambers. For these studies, seven to nine replicates of each treatment were used and the presence of the ^{14}C in the fish, in the chamber water and in the respired CO_2, was quantitatively determined for the ensuring 22 hr post-feeding.

Results and Discussion

Fish productivity and CP digestibility improved linearly with increasing dietary CP; energy digestibility was lower for the high lipid diets and fish on these diets were fatter, but did not grow faster, than those fed low lipid diets (Table 1). Asian seabass, *Lates calcarifer*, showed a similar improvement in growth rate with

Table 1. Apparent digestibility (AD) of crude protein (CP) and energy (E) of diets and specific growth rate (SGR), dry matter (DM) food conversion ratio (FCR), DM body fat (BF) and retention of digestible N (RDN) and digestible E (RDE) of fish.

Response	CP (%)					Fat (%)	
	41.0	46.5	52.0	57.5	63.0	15	24
ADCP (%)	46.8^{C}	55.3^{BC}	58.5^{A}	69.7^{A}	74.0^{A}	59.8	61.9
ADE (%)	59.9^{A}	58.4^{B}	51.3^{C}	61.3^{B}	68.1^{A}	62.2^{X}	57.5^{Y}
SGR (%/d)	1.12^{C}	1.11^{C}	1.26^{B}	1.42^{A}	1.52^{A}	1.31	1.26
FCR (g:g)	1.58^{C}	1.49^{C}	1.24^{B}	1.08^{A}	1.00^{A}	1.28	1.27
BF (%)	23.5	23.2	23.7	23.1	23.5	21.7^{X}	25.1^{Y}
RDN (%)	58.6^{A}	48.8^{B}	50.3^{B}	42.3^{C}	38.8^{C}	48.9	46.7
RDE (%)	35.0^{C}	38.6^{C}	52.3^{A}	47.5^{B}	44.2^{B}	40.7^{Y}	46.3^{X}

$^{A,B,C; X,Y}$ Within comparisons, means without a common letter differ ($P < 0.05$).

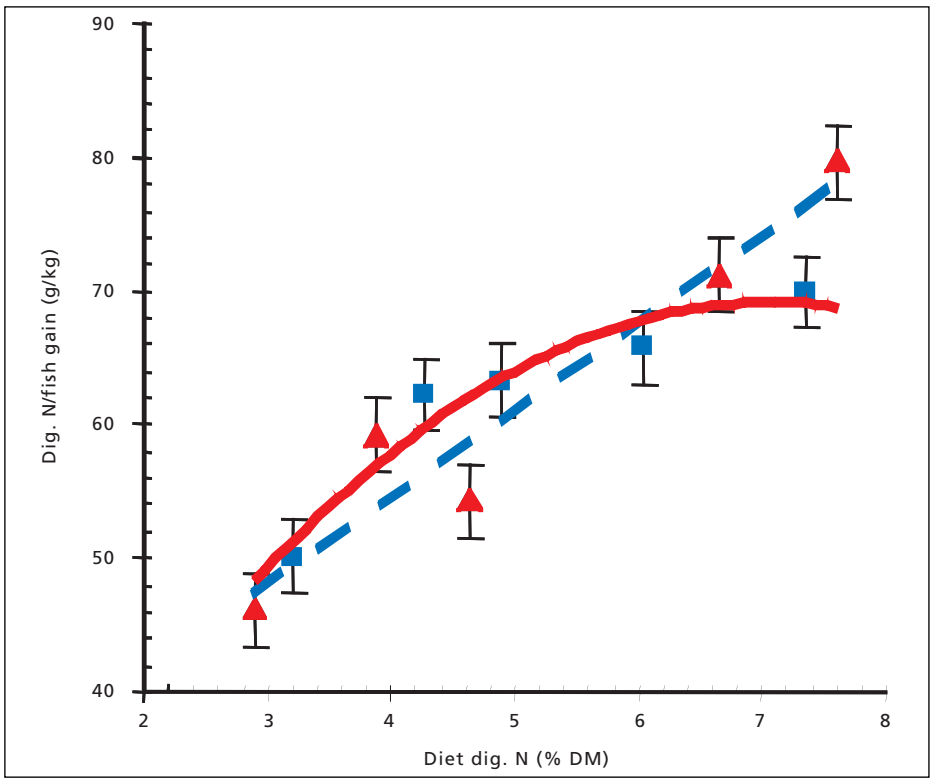

Figure 1. Relationship between the digestible N content of the diet and the amount of digestible N required per kg weight gain of fish-fed diets containing either 15 (▲; $R^2 = 0.93$) or 24 (■; $R^2 = 0.94$)) % fat.

increasing dietary CP content, but differed in that growth rate and FCR improved incrementally with increasing dietary lipid content (Williams et al. 2003). Humpback grouper required significantly more digestible N per unit weight gain with increasing dietary CP content and this relationship was unaffected by the amount of lipid in the diet (Fig. 1).

Increasing the amount of lipid in the control diet by adding 15% of olive oil (LCFA) at the expense of fish meal resulted in a 14% to 20% improvement in growth rate and food conversion, a doubling of the body fat content of the fish (from 15% to 29% DM) and the retention of dietary protein was increased by 28% (from 25% to 32%). A higher addition of olive oil (30%) reduced voluntary food intake by 40%, and consequently depressed growth rate by 32% while protein retention and body fat content were unchanged. Adding coconut oil (MCFA) instead of olive oil depressed food intake by 59%, with a similar reduction in growth rate and no increase in protein retention. The amount of dietary lipid retained as body fat in the fish relative to that oxidised for energy decreased with increasing dietary lipid and was less for MCFA than for LCFA lipids (Fig. 2).

The percentage distribution of radioactivity following ingestion of ^{14}C-labelled diets containing either olive oil or coconut oil at inclusion rates of 15% or 30% showed by humpback grouper oxidised MCFA far more rapidly than LCFA (Table 2). The respiration rate of fish fed diets containing MFA was significantly higher than those fed LCFA (Fig. 3).

Table 2. Percentage distribution of radioactivity following ingestion of ^{14}C-labelled diets containing varying inclusion rates of either coconut oil (MCFA) or olive oil (LCFA).

Diet lipid	Distribution of radioactivity (%)			
	Fish	Respired CO_2	DOM	POM
15% LCFA	70^{B}	15^{B}	11^{B}	3.9^{B}
30% LCFA	67^{B}	11^{B}	11^{B}	11.5^{C}
15% MCFA	23^{A}	51^{A}	26^{A}	0.6^{A}
30% MCFA	17^{A}	49^{A}	34^{A}	0.6^{A}

DOM = Dissolved organic matter in metabolic chamber water.

POM = Particulate organic matter in metabolic chamber water.

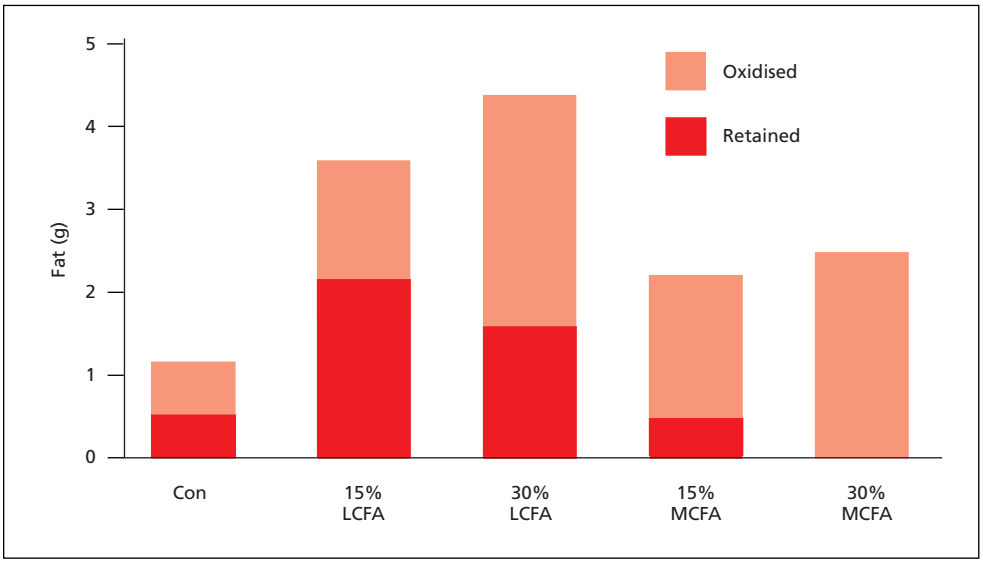

Figure 2. The amount of consumed dietary lipid retained as body fat or oxidised by fish fed either a low lipid (7% DM) control (Con) diet or diets with either 15% or 30% added olive oil (LCFA) or coconut oil (MCFA).

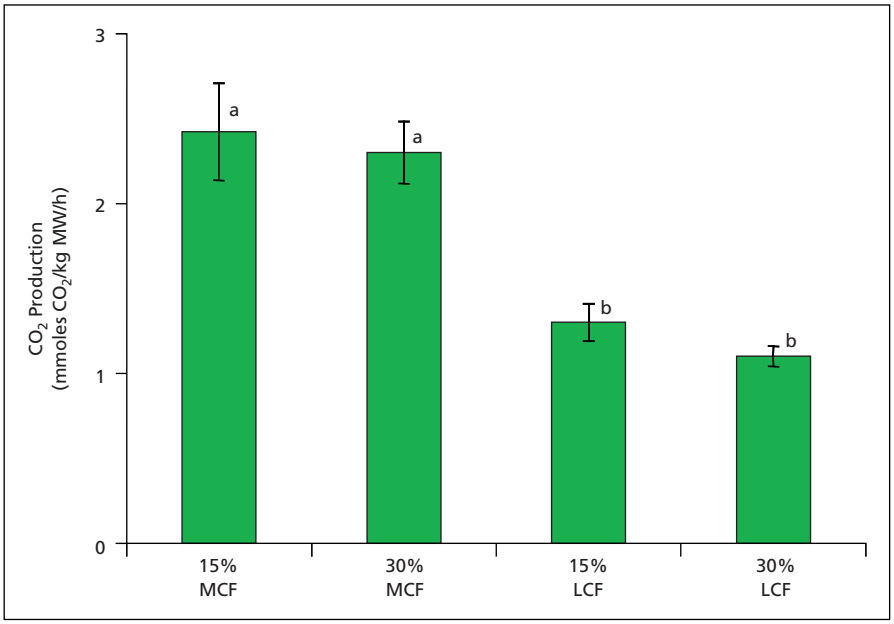

Figure 3. Respiration rate as measured by CO_2 production of fish following ingestion of diets containing 15% or 30% of either coconut oil (MCFA) or olive oil (LCFA).

Conclusions

- Diets for fingerling humpback grouper should contain not less than 44% DM digestible protein (about 60% CP).
- Increasing the lipid content of the diet above about 15% did not promote greater oxidation of the fat but rather led to increased body fat deposition, a reduction in food intake and a slowing of growth rate.
- Replacement of LCFA lipids (such as fish or long-chain vegetable oils) with MCFA lipids (such as coconut oil) did increase the rate of fat oxidation but had a detrimental effect on food intake, and consequently also on growth rate.

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Optimum Level of Dietary Protein and Lipid for Rearing Juvenile Tiger Grouper (*Epinephelus fuscoguttatus*)

N.A. Giri, K. Suwirya and M. Marzuqi

Introduction

Live tiger grouper, *Epinephelus fuscoguttatus*, is a commercially high-value fish. Recently, success in propagation of this species has been reported (Sudjiarno et al. 2001) following success in the development of hatchery technology for humpback grouper, *Cromileptes altivelis* (Sugama et al. 2001). Generally, cultured groupers are primarily fed trash fish. Feed represents a large part of production costs during intensive aquaculture. As protein represents the most expensive component in fish diet, it is important to determine the optimal level of dietary protein for growth of fish. Some researchers have reported that the dietary protein requirement of groupers ranges from 47.8–60%, depending on species, but there is no information on dietary protein requirement for tiger grouper. Dietary lipid is a source of energy and essential fatty acids for fish. Some researchers have reported that the optimum dietary lipid requirement for groupers varies between 9–13.5% depending on species (Boonyaratpalin 1997, Lin and Shiau 2003, Williams et al. 2004). There is no information on lipid requirement for *E. fuscoguttatus*. The balance of dietary protein and lipid is an important aspect in diet formulation to minimise utilisation of protein for energy source and also to produce a cost-effective diet.

Methods

Two series of experiments were conducted to determine crude dietary protein (CP) and lipid requirement for growth of juvenile tiger grouper. For the first experiment, six dry diets were prepared to contain graded levels of CP from 32% to 57% DM at 5% increments. Diets were formulated using fish meal, casein, mysid shrimp meal and squid liver meal as the protein source. The energy content of the experimental diets was adjusted by inclusion of dextrin. Hatchery produced juveniles of tiger grouper of 11.9 ± 0.02 g average body weight were stocked in 30 litre polycarbonate tanks with the density of 10 fish per tank. Each tank was equipped with flow-through seawater and aeration to maintain good water quality in the rearing tank. Fish were fed twice every day at satiation level for 42 days. The experiment was a completely randomised design with six treatments and three replicates for each treatment.

For the second experiment, six experimental dry diets were prepared to contain graded levels of lipid from 0 to 50% DM at 3% increments. Diets were formulated using casein and chloroform-methanol extracted fish meal, squid liver meal and mysid meal as the main protein source. The gross energy content of the experimental diets was adjusted by adding dextrin and α-starch so that all diets contained 4.0–4.2 kcal/g diet. Hatchery produced juveniles of tiger grouper of 4.7 ± 0.40 g average body weight were stocked in 30 L polycarbonate tanks with a density of 12 fish per tank. Each tank was equipped with flow-through seawater and aeration to maintain good water quality in the rearing tank. Fish were fed twice daily at satiation level for 56 days. The experiment was a completely randomised design with six treatments and three replicates for each treatment.

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Results and Discussion

Dietary CP significantly affected final weight, percent weight gain and feed efficiency responses of the fish (Table 1). Fish fed 47% dietary CP showed the highest weight gain (45.9 g) and percent weight gain (287%), but not significantly better than fish fed 52% or 57% CP diets. As for growth rate, feed efficiency improved with increasing dietary CP up to 47% but worsened at higher levels of dietary CP. Fish fed the 47% CP diet showed the best protein efficiency ratio and protein retention. These results indicate that juvenile tiger grouper require a diet of 47% CP for best growth. This finding is similar to that of Chen and Tsai (1994) who reported a dietary CP requirement of 48% for the marbled grouper *E. malabaricus*. Vergara *et al*. (1996) reported that dietary protein levels above the optimum level caused growth rate and feed efficiency of juvenile gilthead sea bream, *S. aurata*, to worsen. They attributed this result to insufficient energy being consumed by the fish with a net loss of energy due to inefficient deamination of absorbed excess amino acids and excretion of nitrogenous waste products. In the present juvenile tiger grouper study, increasing dietary protein above 47%, while not adversely affecting fish growth rate, did cause a significant impairment of feed efficiency.

Fish performance was significantly influenced by the lipid content of the diet (Table 2). Fish fed the diet without lipid supplementation had the lowest survival and the worst final weight, percent weight gain and feed efficiency. These response traits improved significantly as the dietary lipid content increased up to 9% and plateaued thereafter. These results are similar to those reported by Chu *et al*. (1996), who found that the grouper, *E. areolatus*, required a dietary lipid level of 9% for good growth. Increasing the dietary lipid content to 12% or 15% did not improve growth of tiger grouper. Whole body lipid content and protein retention of fish also increased as the dietary lipid level increased up to 9% (Fig. 1). This result indicates that juvenile tiger grouper have a limited ability to metabolize dietary lipid as an energy source.

Table 1. Final weight, weight gain, feed efficiency and protein efficiency ratio of juvenile tiger grouper fed experimental diet[1].

Protein level	Final Weight (g)	Weight gain (%)	Feed Efficiency[2]	Protein efficiency ratio[3]
32	37.6 ± 0.5^{a}	217.2 ± 6.1^{a}	69 ± 0.01^{a}	2.05 ± 0.02^{a}
37	38.6 ± 0.8^{a}	225.4 ± 6.6^{a}	72 ± 0.01^{a}	1.98 ± 0.03^{a}
42	41.3 ± 0.8^{b}	248.0 ± 6.7^{b}	81 ± 0.02^{b}	1.99 ± 0.03^{a}
47	45.9 ± 0.8^{c}	286.7 ± 6.9^{c}	99 ± 0.03^{c}	2.15 ± 0.02^{b}
52	44.9 ± 0.7^{c}	279.1 ± 5.7^{c}	88 ± 0.02^{d}	1.69 ± 0.04^{c}
57	44.4 ± 0.5^{c}	274.5 ± 4.5^{c}	87 ± 0.03^{d}	1.55 ± 0.04^{d}

[1] Initial weight = 11.9 ± 0.02 g. Values within the column with a common letter are not significantly different ($P > 0.05$).
[2] Feed efficiency: 100 × Weight gain (g)/feed intake (g).
[3] Protein efficiency ratio = $\frac{\text{Body weight gain (g)}}{\text{Protein intake (g)}}$.

Table 2. Final weight, weight gain, survival, and feed efficiency of juvenile tiger grouper fed experimental diet[1].

Lipid level	Final Weight (g)	Weight gain (%)	Survival (%)	Feed Efficiency[2]
0	16.57 ± 0.72^{a}	251.4 ± 14.5^{a}	75.1 ± 0.0^{a}	73 ± 0.08^{a}
3	19.30 ± 0.95^{abc}	307.3 ± 21.1^{abc}	97.2 ± 4.8^{b}	94 ± 0.09^{ab}
6	18.73 ± 2.41^{ab}	294.3 ± 50.1^{ab}	83.3 ± 0.0^{c}	93 ± 0.17^{ab}
9	21.93 ± 0.47^{c}	360.4 ± 10.7^{c}	100.0 ± 0.0^{b}	112 ± 0.04^{b}
12	18.80 ± 0.30^{ab}	296.1 ± 6.5^{ab}	91.7 ± 8.4^{b}	97 ± 0.10^{ab}
15	19.30 ± 2.98^{bc}	314.5 ± 58.7^{bc}	100.0 ± 0.0^{b}	99 ± 0.17^{ab}

[1] Initial weight = 4.7 ± 0.4 g. Values within the column with a common letter are not significantly different ($P > 0.05$).
[2] Feed efficiency: 100 × Weight gain (g)/feed intake (g).

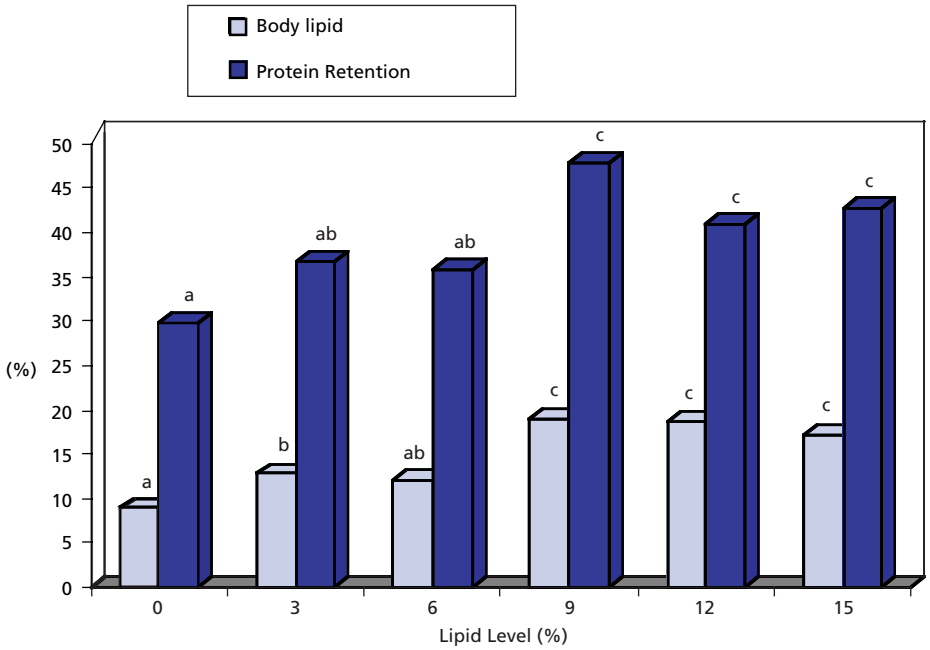

Figure 1. Whole body lipid content and protein retention of juvenile tiger grouper fed experimental diets with different lipid levels.

Conclusions

- The optimal dietary CP and lipid specifications for juvenile tiger grouper are 47% and 9% respectively.

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Dietary Optimum Protein for Tiger Grouper (*Epinephelus fuscoguttatus*) Diet Reared in Floating Net Cages

A. Laining, N. Kabangnga and Usman

Introduction

Generally, aquatic animals — particularly marine fishes — require high protein for their maximum growth. Several investigations on protein requirement of groupers have been reported such as juvenile *Ephinephelus striatus* requires more than 55% protein, (Ellis et al. 1996); *E malabaricus* 47.8% (Chen and Tsai 1994), while humpback grouper (*Cromileptes altivelis*) requires 52% (Giri et al. 1999). Moreover, it has also been reported that humpback grouper require lipid at a level of 9–11% and vitamin C in the form of L-ascorbyl-2-monophosphate-sodium-calcium at a rate of 150 ppm (Laining et al. 2002). Humpback grouper have the capability to utilise glucose as a carbohydrate source at 16% (Usman 2002). This experiment was conducted to provide preliminary information regarding the optimum level of dietary protein for a tiger grouper diet.

Methods

A 17-week experiment was carried out to determine the appropriate level of dietary protein and its effects on biological responses and apparent crude protein (CP) and dry matter (DM) digestibility of tiger grouper. The experiment was a randomised block design of five treatments and three replicates. Diets containing graded levels of protein: from 35% to 50% at 5% increments were fed to tiger grouper raised in floating sea cages. All diets were formulated to be isocaloric (4.7 kcal/g).

Tiger grouper were transferred from the Research Institute for Mariculture, Gondol, Bali and sorted into three different weight groups namely, small (53–65 g), medium (75–85 g) and large (97–105 g). Fish were stocked into fifteen $1 \times 1 \times 2.5$m cages with 12 fish per cage. The fish were fed twice daily to satiety.

The parameters measured were growth rate, feed efficiency, survival rate and the protein digestibility coefficient. Determination of the apparent digestibility coefficient was done after growth assay using chromium oxide as an inert marker.

Research Institute for Coastal Aquaculture staff feeding juvenile *Cromileptes altivelis* in experimental cages, Barru, Indonesia.

The response traits measured were growth rate, feed efficiency, survival rate and DM and CP apparent digestibility. Chromium oxide was used as the marker for determining apparent digestibility with faecal collection being carried out for this purpose upon the completion of the growth assay.

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Results and Discussion

Growth rate significantly improved as dietary protein increased, with the diet containing 50% protein resulting in the highest percent weight gain (266%), while the smallest weight gain was achieved by fish fed the 35% CP diet (77%) (Table 1). The change in average individual weight of fish over the course of the 17-week experiment is shown in Figure 1. Feed efficiency and survival rate showed a similar improvement, with increasing dietary CP. Increasing the dietary protein content from 35% to 50% resulted in almost a doubling of both the survival rate and a similar magnitude of improvement in feed efficiency. Based on broken-line analysis of

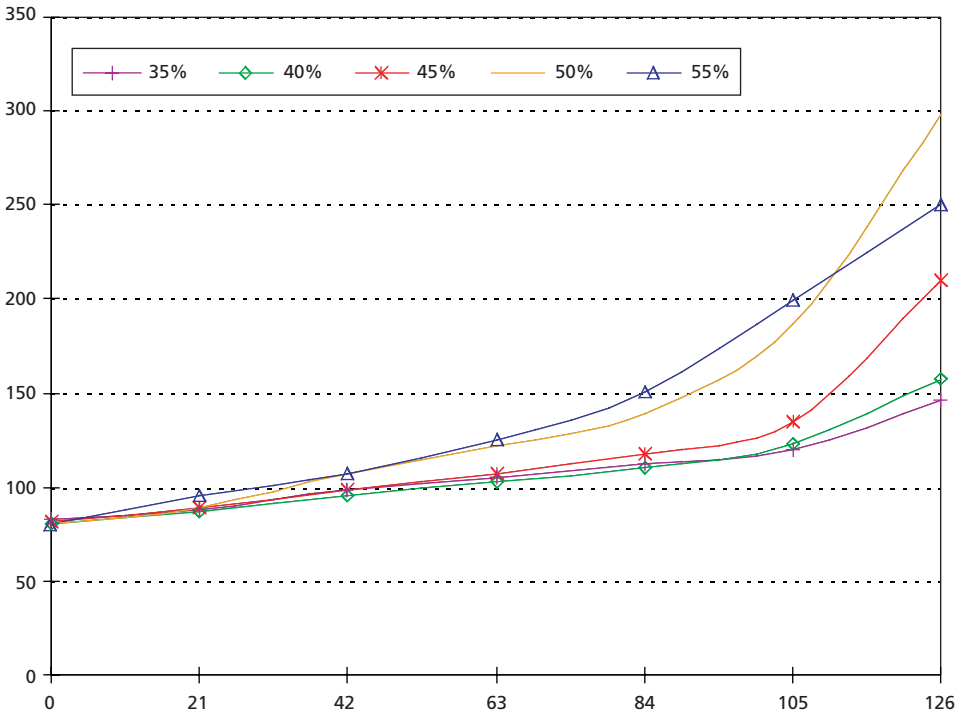

Figure 1. Average individual weight gain of tiger grouper after 126 days of culture.

Table 1. Biological performances of tiger grouper fed diets with different levels of protein.

Biological Parameter	Kadar protein/*Protein levels* (%)				
	35	40	45	50	55
Initial weight (g)	82.6	80.6	81.8	80.7	80.4
Final weight (g)	145.9	156.7	210.3	298.3	250
Weight gain (%)	77.2[a3]	94.9[a]	158.6[b]	266.2[d]	210.9[c]
Absolute growth (g/d)	0.5[a]	0.6[a]	1.0[b]	1.7[d]	1.3[c]
Survival rate (%)	41.7[a]	44.4[a]	50[ab]	72.2[c]	61.1[bc]
Feed intake (g/)[1]	168.6[a]	176.9[a]	200[ab]	274.2[c]	255.2[bc]
Feed efficiency (%)[2]	37.4[a]	48.8[a]	64.3[b]	78.6[c]	71.3[bc]
Dry matter digest.coefficient (%)	47.33	50.49	48.50	53.90	50.28
Protein digest.coefficient (%)	72.15	71.66	76.59	80.96	79.86

[1] Feed intake: Total daily feed intake/0.5 × (total fish at start + total fish at the end).
[2] Feed efficiency: Weight gain (g)/feed intake (g) × 100%.
[3] Value in rows followed by the same superscript are not significantly different (P > 0.01).

weight gain (Jobling, 1994), a dietary CP specification of 51% was determined to be optimal for tiger group over the weight range of 80 g to 300 g.

The DM apparent digestibility was not significantly affected by dietary CP (Table 1). However, CP apparent digestibility significantly increased as dietary CP increased from 35% to 50%, but with no further improvement with the 55% CP diet.

Conclusions

- Productivity responses of juvenile tiger grouper improved as the dietary CP content increased up to 50%.
- The apparent digestibility of CP, but not DM, also improved with increasing dietary CP up to 50% and protein digestibility of tiger grouper also improved.
- For tiger grouper reared from 80 g to 300 g, the optimum dietary CP specification was determined from broken-line regression analysis to be 51%.

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Effect of Dietary n-3 HUFA on Growth of Humpback Grouper (*Cromileptes altivelis*) and Tiger Grouper (*Epinephelus fuscogutatus*) Juveniles

K. Suwirya, N.A. Giri and M. Marzuqi

Research Institute for Mariculture experimental grow-out sea cages at Pegametan, Bali, Indonesia.

Introduction

Humpback and tiger grouper are two candidate species for aquaculture in Indonesia, as well as in other countries. Research is ongoing to improve the propagation techniques for these species and to increase the production of juveniles. However, little information is available on the nutritional requirements of these species and especially their requirement for the omega-3 highly unsaturated fatty acids (n-3 HUFA). Marine fish are unable to synthesise n-3 HUFA *de novo* and thus they need to be supplied in the diet for optimal health and growth (Watanabe 1993; Wanakowat et al. 1993; Lochmann and Gatlin 1993). The objective of this study was to determine the n-3 HUFA requirement of juvenile humpback and tiger grouper.

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Methods

Six pelleted diets were formulated in which the total dietary n-3 HUFA content was varied from 0% to 2.5% dry matter at 0.5% increments. In a separate 8-week growth assay experiment for each fish species, diets were fed to three replicate tanks of juveniles and measurements made of percentage weight gain, feed intake and feed efficiency.

Results and Discussion

Productivity responses of juvenile humpback grouper fed diets varying in n-3 HUFA content are presented in Table 1. Neither total feed intake nor survival rate was significantly ($P > 0.05$) affected by the amount of n-3 HUFA in the diet. However, weight gain increased curvilinearly with increasing dietary n-3 HUFA, with a maximum response at the 1.0% supplementation rate. This result demonstrates that the minimum dietary n-3 HUFA requirement for humpback grouper juveniles is 1.0% dry matter.

Responses of tiger grouper to the feeding of diets varying in n-3 HUFA content are presented in Table 2. Feed intake was unaffected by the amount of n-3 HUFA in the diet. Percentage weight gain and feed efficiency improved linearly and curvilinearly, respectively, as the amount of n-3 HUFA in the diet increased. This result implies a dietary requirement for n-3 HUFA of at least, and possibly more than, the maximum supplementation rate of 2.5% examined in this experiment.

Table 1. Percentage weight gain, total feed intake and survival rate of humpback grouper juveniles, fed diets varying in n-3 HUFA content.

Dietary level of n-3 HUFA (%)	Weight gain (%)[1]	Feed intake (g/ind)[2]	Survival rate (%)
0	115[a]	17.9[a]	100
0.5	136[ab]	18.1[a]	100
1.0	182[c]	19.4[a]	100
1.5	169[c]	19.1[a]	100
2.0	183[c]	19.4[a]	100
2.5	189[c]	19.7[a]	100

[1] Weight gain (%) = 100 × (average final weight – average initial weight)/average initial weight.
[2] Feed intake = 0.5 × sum of daily DM feed allocation/(total fish at start + total fish at end).
Values in columns followed by the same superscript letter are not significantly different ($P > 0.05$).

Table 2. Percentage weight gain, feed efficiency and total feed intake of juvenile tiger grouper fed diets varying in n-3 HUFA content.

Dietary n-3 HUFA (%)	Weight gain (%)[1]	Feed efficiency (%)[2]	Feed intake (g/ind)[1]
0.0	509 ± 10.3[a]	0.71[a]	15.2 ± 1.06[a]
0.5	528 ± 29.4[ab]	0.74[ab]	16.1 ± 0.32[a]
1.0	560 ± 11.9[ab]	0.79[ab]	15.7 ± 0.69[a]
1.5	605 ± 50.6[bc]	0.80[b]	15.2 ± 0.27[a]
2.0	621 ± 27.0[c]	0.86[b]	14.8 ± 2.62[a]
2.5	650 ± 13.7[c]	0.85[b]	15.0 ± 2.78[a]

[1] Weight gain (%) = (average final weight – average initial weight)/average initial weight × 100.
[2] 100 × (Total weight gain (g)/total feed intake (g))
means within a column with the same superscript are not statistically different ($P > 0.05$).

A lack of n-3 HUFA in the diet of marine fish has been reported to increase mortality, decrease growth rate and result in the development of an abnormal swim bladder (Sorgeloos, et al., 1988; Webster and Lovel, 1990; Koven, et al., 1990). The dietary requirement of n-3 HUFA varies with species and the size of fish. For example, the dietary n-3 HUFA requirement of gilthead sea bream larvae is about 2.2% (Salhi, et al., 1994), whereas only 0.5–1% is required for juvenile red drum (Lochmann and Galtin, 1993) and about 0.9% for juvenile Korean rockfish of 6 g size (Lee, et al., 1993). A similar dietary n-3 HUFA specification of 1% appears adequate for juvenile Asian sea bass (Wanakowat et al., 1993). Our findings suggest that the dietary n-3 HUFA requirement of humpback grouper fingerlings is only about 1%, whereas tiger grouper require much higher levels of at least 2.5%. Further studies are needed to confirm that these two grouper species do have such differing requirements for dietary n-3 HUFA.

Conclusions

- The dietary n-3 HUFA specification for optimal growth of juvenile humpback and tiger groupers should be not less than 1.5% and 2.5%, respectively.
- Further research is required to confirm the differences between these grouper species.

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Supplementation of Vitamin C, L-ascorbyl-2-monophosphate-sodium-calcium for Sea Cage Reared Humpback Grouper (*Cromileptes altivelis*) Diets

A. Laining, N. Palinggi, M. Atmomarsono and T. Ahmad

Introduction

Even though seed production of grouper culture in Indonesia has been successful (Sugama et al. 1998), there has been limited development of grow-out ventures. Several constraints of humpback grouper grow-out, especially in floating net cages, are still found; for example, slow growth and high mortality. Humpback grouper are very sensitive to improper handling and a sudden change of environmental conditions commonly leads to stress and mortality. Since very little information is known about the vitamin C requirement of humpback grouper, an experiment was carried out with fish reared in floating net cages to investigate the efficacy of stable vitamin C L-ascorbyl-2-monophosphate-Na-Ca (APNa).

Methods

A completely randomised design of five treatments with three replicates was applied in this experiment. The dietary treatments comprised three different levels of APNa at inclusion rates of 50, 100 or 150 mg/kg, a positive control (in which a standard commercial vitamin premix containing vitamin C as ascorbic acid was used) and a negative control (in which vitamin C was absent). All diets were formulated to be isonitrogenous and isoenergetic with specifications of 48.5% crude protein, 10.9% crude lipid and 3047 calories/g gross energy.

Maros researcher Ms Asda Laining inspecting farm-made pelleted fish feed at Iloilo, Philippines.

Hatchery-reared juvenile humpback grouper, *Cromileptes altivelis*, from the Research Institute

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for Mariculture, Gondol, Bali, Indonesia and weighing 5–7 g, were distributed to each of 15 cages (1 × 1 × 2.5 m). During the two months of culture, fish were fed to satiation a pelleted diet, twice daily at 0700 and 1600 h. Feed ingredients and manufactured pellets were analysed for moisture crude protein, crude lipid, crude fibre, ash and energy. The vitamin C content of liver samples was determined using HPLC procedures. Fish were weighed and their length measured every two weeks and food intake reconciled over the same periods for determination of growth rates, feed efficiency and survival. Environmental characteristics of water temperature, salinity, transparency and dissolved oxygen were measured periodically during the 8-week experiment.

Results and Discussion

Fish growth rate was significantly improved with increasing APNa. The weight gain of fish on the vitamin C-free negative control diet was only half that of fish fed the highest APNa supplementation (150 mg/kg) diet (110% vs. 254% respectively) and not significantly different to the positive control diet (120%). Feed efficiency also improved with increasing dietary APNa content, but feed intake did not differ significantly among dietary treatments (Table 1).

Fish survival rates improved with dietary APNa content and was best for the 150 mg/kg APNa supplemented diet (95%) and worst for the commercial premix control diet (72.5%) (Table 1). Mortalities first occurred in the third fortnight and continued during the final fortnight of the experiment. This corresponded with a decline of water quality around the sea cages during a period of heavy rainfall that washed silt and debris into the sea in the region of the fish cages (Table 2). A similar study undertaken by Subyakto (2000) and Giri et al. (1999), but under laboratory conditions, showed that including ascorbyl-2-monophosphate-Mg in the diet at a rate of 25–30 mg/kg was sufficient for rearing humpback grouper.

Vitamin C level in the liver increased with increasing dietary APNa supplementation (Table 3). The vitamin C content of the liver of fish fed the commercial premix diet was low (6.0 µg/g), similar to that of fish fed the vitamin C-free diet (4.2 µg/g) and only half that of similar fish sampled at the start of the experiment (12.3 µg/g).

Table 1. Weight gain, daily growth rate, feed intake, food conversion ratio (FCR) and survival rate of humpback grouper fed on diets containing different levels of APNa.

Variables	Commercial premix control	APNa level, mg/kg feed			
		0	50	100	150
Weight gain (%)	119.5	110	170	187	254
Daily growth rate (%/d)	1.40[a]	1.32[a]	1.78[b]	1.88[b]	2.26[c]
Feed intake (g/g)[1]	19.4[a]	19.7[a]	18.7[a]	19.4[a]	21.0[a]
FCR (g/g)[2]	2.99[a]	3.12[a]	2.00[b]	1.83[c]	1.43[d]
Survival rate	72.5	75.0[a]	85.0[b]	86.7[b]	95.0[c]

[1] Apparent average daily feed intake of all fish in the tank.
[2] FCR determined as total weight gain (g) divided by total feed (g) dispensed.
Within response traits, values followed by the same letter are not significantly different ($P > 0.05$).

Table 2. Water quality observed around the cages during the experiment.

Variables	Day			
	0–14	15–28	29–40	41–56
Temperature (°C)	29.5–30.4	29.1–30.4	25–27	27.9–28
Salinity (ppt)	34	32	25–26	27–31
Transparency (m)	4.0–5.8	3.9–4.5	1.0–1.4	3.1–3.9
Dissolved oxygen (ppm)	4.2–5.9	4.9–7.9	3.5–4.1	4.5–5.0

Table 3. Vitamin C contained in humpback grouper liver at the beginning and end of the experiment (mean ± SD).

Treatments	Vitamin C in liver (μg/g)
Initial	12.3 ± 4.03
Commercial premix control	6.1 ± 0.25
Vitamin C-free control	4.2 ± 1.25
50 mg APNa	26.1 ± 2.20
100 mg APNa	47.6 ± 2.14
150 mg APNa	94.2 ± 0.35

Conclusions

- Supplementation of dietary APNa at 150 mg/kg diet resulted in the best biological performance of humpback grouper as indicated by growth rate, feed efficiency, survival rate and liver vitamin C content.
- Humpback grouper require a high dietary vitamin C level especially if fish are likely to be subjected to stressful conditions such as poor water quality.

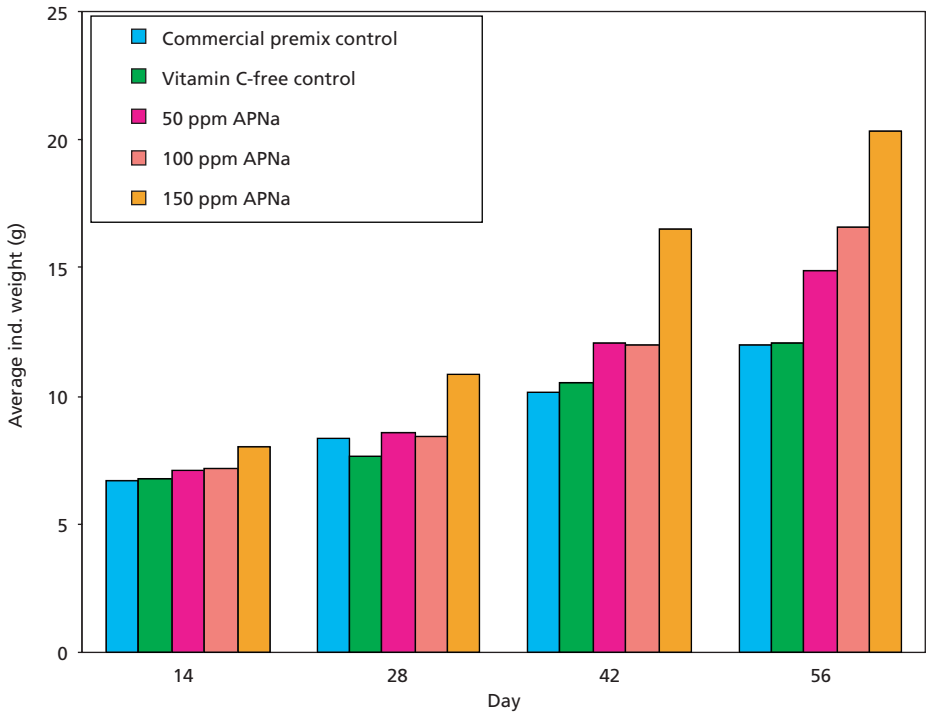

Figure 1. Average individual weight of humpback grouper fed with different levels of APNa.

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Utilisation of Different Dietary Carbohydrate Sources by Humpback Grouper (*Cromileptes altivelis*)

Usman, N. Palinggi and N.A. Giri

Introduction

Carbohydrates are an important macro-nutrient of feeds. They are a cheaper source of energy than either protein or lipid but only if they can be digested and the energy utilised by the animal. Carnivorous marine fish have little capacity to digest and utilise dietary carbohydrates as energy sources and much less than that of herbivorous or omnivorous freshwater fish (Wilson, 1994; Shiau, 1997). Utilisation of dietary carbohydrate is not only affected by species and the size of fish but also by the nature of the carbohydrate itself (Spannhof and Plantikow, 1983; Omondi and Stark, 1996; Peres and Oliva-Teles, 2002; Lee et al., 2003). As a broad generalization, freshwater fish utilise starch better than simple sugars (see Shiau 1997), whereas marine carnivorous fish appear better at utilising simple sugars than starch (Deng et al., 2001; Lee et al., 2003), but anomalies have been observed, e.g. *E. malabaricus* grouper in 23ºC water utilised starch better than glucose (Shiau and Lin 2002).

It is not known if humpback grouper can utilise carbohydrate as an energy source or whether different types of carbohydrates differ in their usefulness as dietary constituents. To better understand the capacity of juvenile humpback groupers to utilise carbohydrates, diets providing different carbohydrate types were tested.

Methods

Hatchery-reared humpback grouper juveniles of initial weight 7.8 ± 0.4 g were held in 12 black polycarbonate tanks. Each tank was filled with 80 L of filtered seawater and stocked with 15 fish. Water flow was at 45 L/h and aeration was supplied to each tank. A randomised block design (three replicates) was used to examine four pelleted dry diets that differed only in the source of carbohydrate — glucose, sucrose, dextrin or starch — each being included at 20% of the diet. The dietary crude protein, crude fat and digestible energy specifications were 54%, 11% and 3.3 kcal/g, respectively. Fish were fed twice daily to satiation for six weeks. Weight and length measurement was carried out fortnightly.

Apparent digestibility of the diet was measured using chromic oxide as the digestibility marker (Takeuchi, 1988). At the conclusion of the growth assay, blood samples were taken at 0, 3, 6, 9, 12, 18 and 24 h after feeding and plasma glucose levels determined by the procedure of Wedemeyer and Yasutake (1977).

Results and Discussion

The type of carbohydrate in the diet had a significant effect on the productivity responses of the fish (Table 1). Feeding the glucose diet resulted in the best growth rate and feed efficiency being significantly better than in all other diets, while the starch diet resulted in the worst fish performance. Fish fed either the sucrose or dextrin diets produced an intermediate performance significantly better than the starch diet but inferior to the glucose diet. Protein retention rates were highest for glucose and dextrin diets and significantly better than starch and, in turn, better than the sucrose diet. More lipid was retained by fish fed the glucose diet (68%) but differences between other carbohydrate types were not significant (range 46% to 54%). There

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Table 1. Protein retention (PR), lipid retention (LR), absolute growth rate (GR), feed consumption (FC), feed efficiency (FE), and survival rate (SR) of humpback grouper fed diets containing different types of carbohydrate.

Nutrient	Carbohydrate			
	Glucose	Sucrose	Dextrin	Starch
PR (%)	35 ± 1.4^{c}	26 ± 1.8^{a}	33 ± 1.8^{c}	30 ± 0.9^{b}
LR (%)	68 ± 5.4^{b}	46 ± 2.5^{a}	54 ± 2.8^{a}	50 ± 6.9^{a}
GR (g/d)	0.40 ± 0.03^{c}	0.27 ± 0.00^{a}	0.34 ± 0.02^{b}	0.34 ± 0.03^{b}
FC (%)	247 ± 14.5^{b}	218 ± 6.1^{a}	235 ± 6.4^{ab}	234 ± 11.6^{ab}
FE (%)	101 ± 2.3^{c}	79 ± 2.7^{a}	91 ± 4.2^{b}	90 ± 3.3^{b}
SR (%)	100^{a}	100^{a}	100^{a}	100^{a}

Means within rows with a common letter are not significantly different (P > 0.05).

Table 2. The apparent digestibility of nitrogen free extract (ADNFE), crude protein (ADCP) and lipid (ADL) of diets containing different types of carbohydrate when fed to juvenile humpback grouper.

Nutrient	Kind of carbohydrate			
	Glucose	Sucrose	Dextrin	Starch
ADNFE (%)	96.6 ± 1.42^{c}	87.7 ± .2.86^{b}	82.8 ± 2.58^{b}	96.3 ± 2.94^{a}
ADCP ADL	94.4 ± 0.28^{b}	93.4 ± 0.87^{a}	94.6 ± 0.23^{b}	94.9 ± 0.45^{b}
(%)	97.2 ± 1.11^{a}	96.2 ± 0.83^{a}	95.6 ± 0.18^{a}	95.3 ± 1.46^{a}

Means within rows with a common letter are not significantly different (P > 0.05).

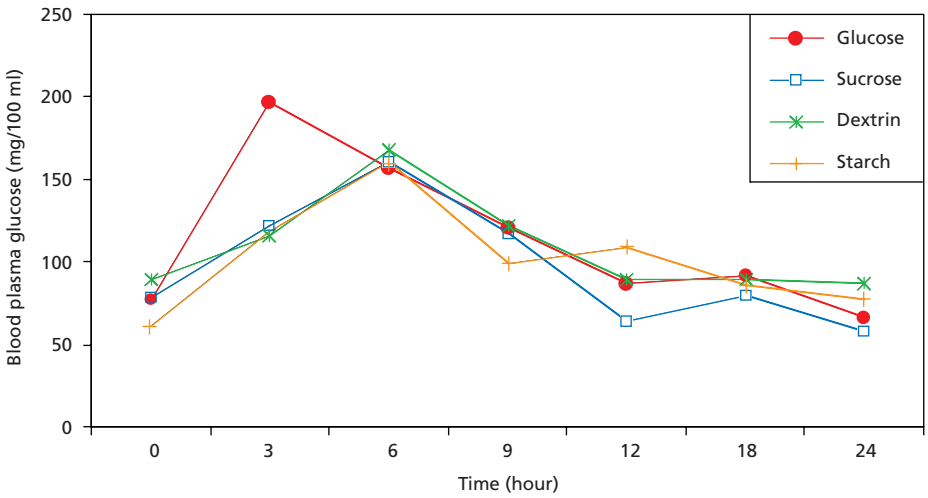

Figure 1. Change of rate and pattern of blood plasma glucose in humpback grouper (*C. altivelis*) fed different types of dietary carbohydrates.

were no fish losses on any of the treatments during the experiment. These results accord with the findings of Shiau and Lin (2002) for *E. malabaricus* grouper held in cool (23°C) water, but contrast with their earlier observations (Shiau and Lin, 2001) where starch and glucose were equally well utilised by *E. malabaricus* that were held in warm (29°C) water.

The type of carbohydrate in the diet significantly affected the apparent digestibility of nitrogen free extract (NFE) and protein, but not the digestibility of lipid (Table 2). NFE and protein digestibility was highest for the glucose diet and significantly higher than all other diets in the case of NFE, but only for the sucrose diet in the case of protein. It is difficult to understand

why the apparent digestibility of protein should have been depressed by the inclusion of sucrose and yet not with starch. However, the absence of any effect of carbohydrate type on the apparent digestibility of lipid agrees with similar findings for striped bass and sunshine bass fed diets containing glucose, maltose or dextrin (Rawles and Gatlin III, 1998).

The ability of fish to absorb and metabolize dietary carbohydrate can be gauged from the rate and pattern of change in blood plasma glucose. Humpback grouper fed the glucose diet resulted in faster glucose absorption and attained a higher plasma glucose level than fish fed diets containing other carbohydrate types (Fig. 1). Hung et al. (1989) reported that white sturgeon fish are more able to utilise dietary glucose and maltose and had higher glucose-6-phospate dehydrogenase and isocitrate dehydrogenase enzyme activities, compared to fish fed diets containing fructose, sucrose, dextrin or starch. Subsequent studies (Deng et al., 2001) confirmed the ability of white sturgeon fish to utilise glucose and maltose more efficiently than starch or dextrin. In this regard, humpback grouper appear to mimic other marine carnivorous fish in being able to utilise simple carbohydrates such as glucose better than more complex sources such as starch and dextrin.

Conclusions

- The type of carbohydrate in the diet affects the apparent digestibility of both NFE and protein, but not lipid, and consequently growth rate, feed efficiency and nutrient retention responses of humpback grouper.
- Blood plasma glucose concentration increased most rapidly and attained the highest value at 3 h post-feeding when glucose was included in the diet. Including sucrose, dextrin or starch in the diet resulted in a similar pattern of plasma glucose with peak concentrations at 6 h post-feeding.
- The best dietary carbohydrate source for juvenile humpback grouper is glucose followed by dextrin, starch and sucrose.

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Utilisation of Dietary Dextrin by Juvenile Humpback Grouper (*Cromileptes altivelis*)

K. Suwirya, N.A. Giri, M. Marzuqi and Trijoko

Introduction

As a cheaper energy source than either protein or lipid, carbohydrate should be considered when formulating cost-effective and environmentally-friendly compounded grouper grow-out feeds. Generally, carnivorous marine fish are not as good as herbivorous or omnivorous freshwater fish in utilising carbohydrate as a source of energy. For example, growth of greasy grouper *Epinephelus coioides* and Atlantic salmon *Salmo salar* was optimised when diets contained 12% and 11% of carbohydrate, respectively (Shiau and Lan, 1996; Grisdale-Helland and Helland, 1997), whereas tilapia *Oreochromis niloticus* × *O. aureus* could effectively utilise and spare for protein when included at dietary concentrations of up to 41% (Shiau and Peng, 1993).

Since information regarding the utilisation of carbohydrates in grouper diet is still very limited (Usman, 2002), an experiment was conducted to determine the optimum level of carbohydrate in diets for juvenile humpback grouper.

Methods

The experiment was a completely randomised design and comprised five treatments and three tank replicates. The dietary treatments provided graded inclusions of dextrin as the carbohydrate source from 0% to 28% at 7% increments. All diets were formulated to be isonitrogenous and isocaloric and were prepared as a dry pellet.

Hatchery-reared juvenile humpback grouper of average initial body weight 8 ± 0.3 g were stocked at a rate of 11 fish per tank into 30 L polycarbonate tanks. Each tank was supplied with a flow through water system and individual aeration to maintain good water quality during the rearing period. Fish were fed twice daily to satiation for 63 days. At the end of the experiment, two fish from each tank were taken randomly and the liver and muscle removed for glycogen analysis (Wedemeyer and Yasutaka, 1977). The hepatosomatic index (HSI) was also determined as: HSI = 100*(wet liver weight/total wet fish weight) with all weights in grams.

Results and Discussion

Percentage weight gain and feed conversion ratio (FCR) of fish improved as the amount of dextrin in the diet increased from 0 to 14% with no significant productivity change at higher dextrin levels (Table 1). Regression analysis showed that percentage weight gain and FCR of the fish

Experimental pellet diets for groupers, Pegametan, Bali, Indonesia.

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improved curvilinearly with increasing dietary dextrin with asymptotic maximum responses at 18% and 21% dextrin, respectively.

Liver glycogen and lipid concentration increased curvilinearly with increasing dietary dextrin (Table 2), with asymptotic maximum responses occurring at dietary dextrin contents of 24% and 25%, respectively. Shimeno *et al*. (1979) reported that glycogen liver content of yellowtail (*Seriola quinqueradiata*) with a weight of 144 g, increased when fed carbohydrate levels up to 14%, and then decreased at higher levels. Muscle glycogen and lipid concentration similarly increased with increasing dietary dextrin (Table 2) but the asymptote was beyond the range of dietary dextrin examined in the experiment. Liver size was also affected by the dietary carbohydrate levels as indicated by the increasing hepatosomatic index (HSI) with increasing dietary dextrin (Table 2). Regression analysis showed that the HSI attained a maximum value with a dietary dextrin content of 18%. Based on these results, it appears that humpback grouper have a reasonably good capacity to utilise carbohydrate as an energy source.

Conclusion

- Juvenile humpback grouper were able to efficiently utilise dextrin as a dietary energy source at inclusion rates of up to at least 14%.
- Further work is warranted to see if carbohydrates such as dextrin can be used by humpback grouper to spare dietary protein and so reduce the amount of nitrogen excreted by the fish.

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Table 1. Percentage weight gain, feed efficiency, and feed conversion ratio (FCR) of juvenile humpback grouper fed experimental diets.

Dietary dextrin level (%)	Weight gain (%)	Feed efficiency	FCR[1]
0	222 ± 5.1^{a}	0.77 ± 0.04^{a}	1.37 ± 0.06^{b}
7	251 ± 8.8^{b}	0.86 ± 0.03^{ab}	1.16 ± 0.04^{ab}
14	268 ± 8.2^{b}	0.92 ± 0.09^{b}	1.02 ± 0.07^{a}
21	249 ± 9.9^{b}	0.93 ± 0.05^{b}	1.03 ± 0.05^{a}
28	259 ± 14.6^{b}	0.91 ± 0.01^{b}	1.07 ± 0.03^{a}

[1]Weight of dry feed as fed (g)/fish weight gain (g).
Means in the same column with a common superscript letter are not significantly different (P > 0.05).

Table 2. Glycogen and lipid concentration of liver and muscle and the hepatosomatic index (HSI)[1] of juvenile humpback grouper fed experimental diets containing graded amounts of dextrin.

Parameter	Dietary dextrin level (%)				
	0	7	14	21	28
Liver					
Glycogen (%)	2.54 ± 0.97^{a}	5.28 ± 0.44^{b}	7.84 ± 0.56^{c}	7.96 ± 0.31^{c}	8.40 ± 0.2^{c}
Lipid (%)	17.17 ± 1.14^{a}	18.49 ± 0.94^{ab}	19.84 ± 0.55^{ab}	21.25 ± 2.16^{b}	20.52 ± 3.18^{b}
Muscle					
Glycogen (%)	0.01 ± 0.01^{a}	0.04 ± 0.02^{ab}	0.05 ± 0.02^{b}	0.07 ± 0.02^{b}	0.07 ± 0.02^{b}
Lipid (%)	16.37 ± 1.37^{a}	17.85 ± 1.65^{a}	18.92 ± 1.98^{a}	18.40 ± 0.74^{a}	19.76 ± 3.90^{a}
HSI (%)	2.07 ± 0.28^{a}	3.51 ± 0.07^{b}	3.63 ± 0.27^{b}	3.41 ± 0.44^{b}	3.46 ± 0.64^{b}

[1]HSI = 100 × (wet weight of liver (g)/total wet weight of fish (g)).
Means in the same column with a common superscript letter are not significantly different (P > 0.05).

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Replacement of Fish Meal by Animal By-product Meals in a Practical Diet for Grow-out Culture of Grouper (*Epinephelus coioides*)

O.M. Millamena

Introduction

Grouper culture has been dependent mainly on trash fish as feed (Boonyaratpalin 1993). Artificial diets have been developed for grouper (Chen and Chen 1986; Chen et al. 1987) but these diets have a high content of fish meal, the most common protein source in aquafeeds. With an increasing population and increased fishing pressure, the global production of fish meal has been in a state of decline whilst the demand for aquaculture has been steadily increasing (Tacon 1996). There is an urgent need to find suitable alternatives to fish meal. The objective of this study was to develop compounded feeds for juvenile grouper that have a low content of fish meal, and as an alternative to trash fish feeding.

Methods

Experimental diets

The basal diet contained 44.4% dietary protein supplied mainly by Chilean fish meal (40%), shrimp meal *Acetes* sp. (10%), soybean meal (6%) and squid meal (1%). The abattoir by-products, consisting of 4:1 combination of processed meat meal and blood meal, were incorporated to replace fish meal protein at increasing percentage replacements of 0% to 100% on an isonitrogenous basis in diets 1–8 (Tables 1 and 2). The 100% fish meal diet (diet 1) and trash fish as sole feed (diet 9) served as control treatments.

Culture

E. coioides juveniles, initial mean body wt (BW) = 6.0 ± 0.2g, were stocked in 36-units of 250-L fiberglass tanks at 25 fishes per tank. Tanks were supplied with sand-filtered seawater in a flow-through system provided with a standpipe at the center and cut PVC pipes to serve as shelter for the fish. Fish were initially fed on trash fish then gradually acclimatised to the diets for five days prior to start of the experimental run. Eight dietary treatments representing increasing (0%, 10%, 20%, 30%, 40%, 60%, 80%, 100%) percent replacements of fish meal protein with 4:1 combination of meat meal and blood meal were tested in quadruplicate groups of fish arranged in a completely randomised design. Fish were fed the diets twice per day at a daily feeding rate of 5–6% of BW and trash fish at 10–12% of BW for 60 days. Parameters used to determine diet efficiency were growth expressed as percent weight gain and specific growth rate (SGR), survival, food conversion rate (FCR), and body composition of grouper juveniles.

Results and Discussion

Table 3 shows the mean values of percent weight gain, SGR, survival, and FCR of *E. coioides* juveniles fed the diets. At the end of culture, grouper juveniles attained a weight gain of 448.0–570.4%. Specific growth rate ranged from 2.83% to 3.13%. Percentage weight gain and SGR tended to increase up to 20% replacement (diet 3) of fish meal with processed animal by-products, followed by a decreasing trend up to the highest level (100%, diet 8) of fish meal substitution. There were no significant differences ($P > 0.05$) in growth among fish fed diets

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Table 1. Composition of the experimental diets on a dry basis in g per 100g dry diet.

Ingredients	Diets (% Replacement)							
	1 (0%)	2 (10%)	3 (20%)	4 (30%)	5 (40%)	6 (60%)	7 (80%)	8 (100%)
Chilean fish meal	40	36	32	28	24	16	8	0
Meat meal[a]	0	4	8	12	16	24	32	40
Blood meal	0	1	2	3	4	6	8	10
Shrimp meal	10	10	10	10	10	10	10	10
Soybean meal	6	6	6	6	6	6	6	6
Squid meal	1	1	1	1	1	1	1	1
Wheat flour	15	15	15	15	15	15	15	15
Vitamin mix	4	4	4	4	4	4	4	4
Mineral mix	3	3	3	3	3	3	3	3
Cod liver oil	6	6	6	6	6	6	6	6
Rice bran	15	14	13	12	11	9	7	5

[a] Processed meat meal and blood meal produced by Consolidated Meat Group, Australia.

Table 2. Proximate composition (%) of the experimental diets on wet weight basis.

Diet	Moisture (%)	Crude protein (%)	Crude fat (%)	Crude fiber (%)	NFE* (%)	Ash (%)
1	2.4 ± 0.02	44.4 ± 0.62	12.2 ± 0.01	3.7 ± 0.03	23.0 ± 0.18	14.3 ± 0.00
2	2.8 ± 0.04	43.3 ± 0.22	11.9 ± 0.16	3.6 ± 0.11	24.2 ± 0.16	14.2 ± 0.05
3	2.8 ± 0.02	44.8 ± 0.25	11.7 ± 0.06	3.7 ± 0.08	22.2 ± 0.30	14.6 ± 0.09
4	2.8 ± 0.03	43.8 ± 0.13	12.1 ± 0.11	2.2 ± 0.14	22.9 ± 0.13	15.9 ± 0.19
5	2.7 ± 0.02	43.7 ± 0.25	11.7 ± 0.04	2.1 ± 0.13	23.8 ± 0.16	16.0 ± 0.21
6	4.0 ± 0.06	43.9 ± 0.04	11.5 ± 0.05	1.7 ± 0.16	22.6 ± 0.19	16.3 ± 0.06
7	4.0 ± 0.02	43.6 ± 0.13	11.3 ± 0.11	1.7 ± 0.63	22.8 ± 0.09	16.6 ± 0.17
8	3.8 ± 0.02	44.0 ± 0.71	11.5 ± 0.01	1.8 ± 0.12	22.2 ± 0.45	16.9 ± 0.17
9	4.2 ± 0.02	68.2 ± 0.05	5.5 ± 0.02	0.07 ± 0.01	2.0 ± 0.10	20.1 ± 0.04

*NFE; nitrogen free extract.

1–7 (0–80% fish meal replacement) including the trash fish control (diet 9). However, fish fed diet 3 had significantly higher ($P < 0.05$) growth than those fed diet 8 (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 (90%) of fish fed trash fish. Likewise, feed conversion ratios were low and ranged from 0.93 to 1.05.

This study has demonstrated that replacement of up to 80% fish meal protein with processed slaughterhouse by-products allowed growth rates similar to or better than those exhibited by the control groups (fish meal based diet and trash fish feeding). Possible reasons for the reduced growth of grouper at total replacement may be due to deficiencies in essential nutrients. Fish meal, in general, has a good amino acid and fatty acid profile for fish. On the other hand, the animal by-product meals that were used to replace fish meal were lower in essential amino acids (methionine, lysine and isoleucine) compared with those in grouper juveniles (Table 4).

Deficiencies in essential amino acids may explain the decline in growth performance of juvenile grouper particularly at full replacement levels of fish meal. Furthermore, animal meat meals are high in saturated fat and like other terrestrial proteins are characterised by high levels of n-6 polyunsaturated fatty acids but low levels of n-3 highly unsaturated fatty acids that are required by marine fish.

Another possible explanation for the reduced performance at increasing levels of fish meal substitution may be the resulting effect on diet digestibility. High ash content in meat meals may lower the digestibility of the diets and this may have caused the reduction in growth rates. In this study, the increase in ash content from 14.2% to 16.9% with increasing levels of animal by-product meals was reflected in the proximate analysis of the diets.

Table 3. Weight gain, specific growth rate (SGR), survival and food conversion ratio (FCR) of grouper fed the experimental diets for 60 days[1]. Data are presented as mean ± SE, n = 23–25 fish.

Diet/% meat replacement	% Weight gain	SGR[2]	Survival (%)	FCR[3]
1 (0)	502 ± 38.3^{ab}	2.95 ± 0.1^{a}	95 ± 0.8^{a}	1.00 ± 0.03
2 (10)	539 ± 43.7^{ab}	3.06 ± 0.1^{a}	100 ± 0.8^{a}	0.99 ± 0.02
3 (20)	570 ± 36.6^{a}	3.13 ± 0.2^{a}	99 ± 1.8^{a}	0.95 ± 0.03
4 (30)	530 ± 78.6^{ab}	3.04 ± 0.3^{a}	96 ± 1.8^{a}	0.98 ± 0.05
5 (40)	494 ± 82.8^{ab}	2.93 ± 0.3^{a}	99 ± 1.8^{a}	1.02 ± 0.06
6 (60)	501 ± 75.6^{ab}	2.95 ± 0.3^{a}	100 ± 0.0^{a}	1.05 ± 0.04
7 (80)	492 ± 85.4^{ab}	2.92 ± 0.3^{ab}	99 ± 1.8^{a}	1.04 ± 0.07
8 (100)	448 ± 87.3^{b}	2.82 ± 0.2^{b}	96 ± 3.4^{a}	0.99 ± 0.16
9 (TF)	525 ± 62.0^{ab}	3.02 ± 0.3^{a}	90 ± 6.4^{b}	0.93 ± 0.06

[1]Treatment means with different superscripts within column are significantly different (P < 0.05).

Table 4. Comparison of the amino acid content in Chilean fish meal, meat and bone meal and blood meal (4:1) mixture in experimental diets (1–8) with the EAA pattern of grouper juveniles in g per 100 g TCA precipitable protein.

Amino Acid	Grouper juvenile	Diet (% replacement)							
		1 (0%)	2 (10%)	3 (20%)	4 (30%)	5 (40%)	6 (60%)	7 (80%)	8 (100%)
Arg	2.50	3.00	3.09	3.19	3.28	3.38	3.56	3.76	3.94
His	1.20	1.73	1.79	1.84	1.90	2.01	2.07	2.18	2.29
Ile	1.66	2.48	2.33	2.19	2.04	1.88	1.60	1.30	1.01
Leu	5.04	6.31	6.46	6.60	6.75	7.00	7.19	7.48	7.77
Lys	4.60	5.22	5.11	5.00	4.88	4.82	4.55	4.32	4.10
Met	1.82	2.05	1.95	1.84	1.74	1.64	1.43	1.23	1.02
Phe	2.47	2.34	2.45	2.57	2.68	2.85	3.02	3.25	3.47
Thr	2.76	2.83	2.84	2.84	2.85	2.87	2.87	2.88	2.90
Tryp	—	—	0.04	0.09	0.13	0.18	0.27	0.36	0.45
Val	1.89	2.86	3.00	3.14	3.29	3.49	3.71	4.00	4.28

n = 4 replicate injections in the HPLC.

Conclusions

- 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.
- Use of animal by-product meals as a protein source substantially lowers the level of fish meal required in juvenile grouper diet. Furthermore, the diet can be effectively used as a substitute for trash fish feeding, thereby reducing the requirements for fishery resource.
- From an economic standpoint, replacement of fish meal with cheaper animal by-product meals in a practical diet for grouper can alleviate the problem of low fish meal availability and high cost.

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The Use of Shrimp Head Meal as a Substitute to Fish Meal in Diets for Humpback Grouper (*Cromileptes altivelis*)

Rachmansyah, A. Laining and T. Ahmad

Introduction

Fishmeal is the main source of protein in fish feed manufactured in Indonesia and most of this (about 147,000 tonnes and valued at US$123 million) is imported (Anonymous, 1998). Shrimp head meal originates from shrimp processing plant waste and contains about 50% protein. It is therefore a good potential candidate for the replacement of fishmeal. Moreover, the apparent digestibility of the protein of shrimp head meal is quite high (78.0%) and not much lower than for local fishmeal (82%) (Laining et al., 2003). The total replacement of fishmeal with a combination of meat and soybean meals in diets for juvenile barramundi, *Lates calcarifer*, resulted in equivalent fish growth (Williams et al., 2003). Grouper may also have a similar capacity to utilise protein sources other than fishmeal. The extent to which locally produced shrimp head meal can substitute for fishmeal in diets for juvenile humpback grouper was examined in this study.

Methods

The experiment was a completely randomised block design and comprised five dietary treatments and three replicates. The dietary treatments comprised graded inclusions of shrimp head meal from 0% to 40% in 10% increments, which replaced an isonitrogenous amount of fishmeal in a basal diet. Hence, all diets had a similar crude protein (CP) content of 45% and were formulated to the same gross energy specification of 4 kcal/g. The hatchery-reared fish were stocked into 15 $1 \times 1 \times 1.2$ m floating net cages set in a raft in the sea. Stocking rate was 20 fish/m^3 and the average initial individual weight of the fish was 15.9 g. Fish were fed twice daily to satiation. The experiment was carried out for 60 days and the fish were weighed and their length measured every fortnight. Apparent digestibility of the diets was determined at the conclusion of the growth assay with chromic oxide being used as a digestibility marker.

Results and Discussion

Replacing fishmeal with shrimp head meal adversely affected ($P < 0.05$) growth rate, feed conversion ratio and protein efficiency ratio responses of the fish and protein digestibility of the diet was reduced at shrimp head inclusion rates above 10% (Table 1). However, survival

Research Institute for Coastal Aquaculture experimental grow-out cages, Barru, southern Sulawesi, Indonesia.

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Table 1. Biological response of humpback grouper fed different level of shrimp head meal.

Variables	Shrimp head meal (%) in the diet				
	0	10	20	30	40
Weight gain (%)	101.5[a]	102.6[a]	76.8[b]	67.9[b]	27.8[c]
Survival rate (%)	100[a]	96.7[a]	95.0[a]	98.3[a]	96.7[a]
Daily growth rate (%/day)	1.17[a]	1.14[a]	0.85[b]	0.84[b]	0.51[c]
Feed conversion ratio	1.52[a]	1.55[a]	1.79[b]	1.78[b]	2.64[c]
Feed intake[1]	23.2[a]	23.0[a]	19.6[b]	19.0[b]	13.8[c]
Feed efficiency[2]	66[a]	67[a]	62[a]	59[b]	41[c]
Protein efficiency ratio	1.35	1.36	1.34	1.19	0.89
App. digestibility coefficient. (%)	85.2[a]	86.9[a]	81.3[b]	79.6[b]	81.9[b]

[1] Feed intake: Total daily feed intake (dry)/(total fish at start + total fish at the end) × 0.5.
[2] Feed efficiency: 100 × (Weight gain (g)/feed intake (g)).

rate was unaffected. The highest daily growth rate was observed in the fish fed the basal (zero shrimp head meal) diet, but this was not significantly better than the diet with 10% shrimp head meal. The apparent protein digestibilities of the basal and 10% shrimp head meal diets were similar (85% cf 87%, respectively) and significantly better than for diets with higher inclusions of shrimp head meal. This indicates that humpback grouper have some, though limited, capacity to digest the chitin-protein complex of shrimp head meal. Chitin is a long chain polysaccharide which is not well digested by marine carnivorous teleosts (Saleh et al., 1998; Angka and Suhartono, 2000). In parallel with changes in the protein digestibility of the diet, feed efficiency and protein efficiency ratio deteriorated as shrimp head meal was used at fishmeal substitution rates above 10%. Surprisingly, the reduced digestibility of high shrimp head meal diets did not stimulate a compensatory increase in feed intake. Instead, average feed intake decreased with increasing shrimp head meal inclusion, thus compounding a depression of fish growth rate.

Conclusion

- Shrimp head meal is not well digested by humpback grouper and its use as a replacement for fishmeal should be limited to no more than 10% of the diet.

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Development of Formulated Feeds for Grow-out Culture of Grouper (*Epinephelus coioides*) — Tank and Field Studies

O.M. Millamena and J.D. Toledo

Southeast Asian Fisheries Development Centre Aquaculture Department staff checking growth of groupers fed experimental diets in replicate cage/pond trials.

Introduction

The availability of a practical diet for grouper is a major constraint to grow-out production. Nutritional studies on grouper include dietary protein to energy ratio (Serrano and Apines 1996; Shiau and Lan 1996), optimum dietary lipid level (New 1987), essential fatty acid requirement (Millamena and Golez, unpublished data) and vitamin requirements (Boonyaratpalin 2002). This information was used as a basis in developing a formulated diet for juvenile grouper. The objective of this study was to compare the performance of a Southeast Asian Fisheries Development Centre formulated diet with a commercial feed for grow-out culture of grouper and to transfer technology on grouper diet developed at SEAFDEC to industry.

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Methods

Experimental diets

The percentage composition of a SEAFDEC formulated diet and proximate analyses of diets are shown in Tables 1a and 1b. In the tank trial, the SEAFDEC diet was prepared at the SEAFDEC Feed Laboratory, while a commercial feed miller prepared the commercial feed. In field trials, both the SEAFDEC diet and commercial feed were compounded into feeds by a commercial feed mill.

Tank Study

Grouper *E. coioides* juveniles were reared in 12 units of 150 L circular fibreglass tanks at 15 fish per tank with four replicates per treatment. Tanks were supplied with sand-filtered seawater in a flow-through system with adequate aeration and cut PVC pipes as shelter for the fish. Fish were fed the diets at a feeding rate of 5–6% of body weight (BW) and trash fish at 10–12% BW per day for 60 days. The tanks were cleaned of excess food and faeces before feeding each morning. Every 20 days, fish were bulk weighed to determine weight gain, which was used as the basis for adjustment of the feed ration. At the end of culture, the parameters used to determine diet efficiency were growth, expressed as percentage weight gain, specific growth rate (SGR), survival and food conversion ratio (FCR). The essential amino acid composition of the diets and commercial feed were compared with essential amino acid profiles of grouper juveniles.

Field Study

In the SEAFDEC Dumangas Brackishwater Station feeding trial, fish stocked were variable in size and grouped into two size groups. Treatments were arranged in a randomised complete block design with size groups as block. Each size group was stocked in two replicate, or a total of four replicate, cages per dietary treatment. Grouper juveniles, with initial BW of around 50 and 100 g, were reared 12-units of 2 m $\times$ 2 m $\times$ 1 m deep net cages installed in brackishwater ponds at six fishes per net cage. Thirty six fishes were stocked per size group or a total of 72 fishes for the 12 cages. Formulated feeds were given twice a day. Daily feeding rates were 5–6% of BW for the feeds and 10% of BW for trash fish. Fish were sampled every 20 days to determine weight gains for adjustment of the feed ration. The field trial was terminated after 120 days.

Results and Discussion

Tank Study

After 60 days of feeding, grouper juveniles attained weight gains of 215% (SEAFDEC diet), 118% (commercial feed) and 222% (trash fish), respectively (Table 2). Survival was 73%, 68% and 63%, respectively. Correspondingly, the FCRs were 1.5, 1.83 and 1.62. The commercial feed gave significantly lower growth, survival and FCR compared with SEAFDEC diet and trash fish control. The commercial feed had low protein content (Table 1a) that is below the established protein requirement of juvenile grouper

Table 1a. Proximate analysis (%) of experimental diets on dry matter (Tank study).

Diets	Moisture (%)	Crude Protein	Crude Fat	Crude Fibre	NFE[1]	Ash
SEAFDEC	4.64	44.06	7.22	3.22	33.35	12.15
Commercial	3.98	38.98	11.51	4.50	33.37	11.70

Table 1b. Proximate analysis (%) of experimental diets on dry matter (Field study).

Diets	Moisture (%)	Crude Protein	Crude Fat	Crude Fibre	NFE[1]	Ash
SEAFDEC	4.64	44.06	7.22	3.22	33.35	12.15
Commercial	3.98	44.74	7.54	3.48	32.02	12.02

[1] NFE, nitrogen free extract.

Table 2. Weight gain, survival, specific growth rate and food conversion ratio (FCR) of juvenile grouper fed the experimental diets for 60 days (Tank study).

Diet	Weight gain (%)	SGR	Survival (%)	FCR
SEAFDEC	215 ± 31[a]	1.88 ± 0.2[a]	73 ± 7[ab]	1.50 ± 0.05[bc]
Commercial	118 ± 14[b]	1.29 ± 0.1[b]	68 ± 6[ab]	1.83 ± 0.04[a]
Trash fish	222 ± 70[a]	1.85 ± 0.3[a]	63 ± 5[a]	1.62 ± 0.10[ab]

Figures are presented as mean ± SE. For each column, values with different superscripts are significantly different ($P < 0.05$).

at 44% protein. The feed was also grossly deficient in four essential amino acids: methionine, isoleucine, lysine, and threonine (Table 3). Levels of these essential amino acids were relatively low compared with the amounts that were present in grouper juveniles. The commercial feed formulator was then informed of the results of chemical (proximate and amino acid) analyses and advised to improve the feed formulation to achieve the desired protein levels and amino acid composition.

Table 3. The essential amino acid content of the SEAFDEC diet, commercial feed and grouper juveniles (g/100g sample).

Amino Acid	Grouper juvenile	SEAFDEC	Commercial
Arginine	1.02	1.70	1.68
Histidine	0.43	0.66	0.05
Isoleucine	0.75	1.30	0.93
Leucine	1.75	2.32	1.74
Lysine	1.59	1.79	1.28
Methionine	0.62	0.57	0.32
Phenylalanine	0.87	1.46	1.05
Threonine	0.98	1.21	0.85
Tryptophan	0.03	—	—
Valine	0.65	1.21	1.11

Field Study

After 123 days of culture, the mean values of percent weight gains and SGR in two size groups are: SEAFDEC diet (504% and 2.58), commercial feed (445% and 2.49), and trash fish (522% and 2.78) (Table 4). Correspondingly, the feed conversion ratios were 3.52, 3.84, and 3.50. Survival rates were high in all treatments at 100%, 96% and 96%, respectively. Results of field trials at grow-out ponds did not show significant differences in growth performance, survival and FCR of grouper juveniles fed with the diets. The proximate composition of diets used in field studies was found to be similar in levels of crude protein, fat, fiber, ash and nitrogen free extract (NFE) (Table 1b). Both the SEAFDEC diet and commercial feed conformed to the established protein requirement of juvenile grouper. It should be noted that the present commercial feed had a higher protein content compared with the formulation that was pre-tested in tanks. This could explain the marked improvement in growth performance of grouper fed with the commercial feed.

Conclusions

- In tank trials, the poor performance of commercial feed was attributed to the low protein content and deficiencies in essential amino acids as confirmed by analysis of the amino acid composition.
- Improvement in growth performance of the commercial feed was achieved in field trials by increasing the dietary protein level and improving the amino acid composition to match that of grouper juveniles.

Table 4. Weight gain, survival, specific growth rate and food conversion ratios (FCR) of juvenile grouper fed the experimental diets for 123 days (Field study).

Diet	Weight gain (%)	SGR	Survival (%)	FCR
SEAFDEC	504 ± 146[a]	2.58 ± 0.3[a]	100 ± 0[a]	3.52 ± 0.71[b]
Commercial	445 ± 81[a]	2.44 ± 0.1[a]	96 ± 4[a]	3.84 ± 0.59[a]
Trash fish	522 ± 127[a]	2.78 ± 0.3[a]	96 ± 4[a]	3.50 ± 0.42[a]

Figures are mean ± SE. For each column, values with different superscript are significantly different ($P < 0.05$).

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SECTION 4
THE ASIA-PACIFIC GROUPER NETWORK

M.A. Rimmer, M.J. Phillips and S.Y. Sim

The Asia-Pacific Marine Finfish Aquaculture Network, which was established (as the Asia-Pacific Grouper Network) in 1998, has grown rapidly. Network activities have contributed to improving the overall progress of developing sustainable grouper aquaculture in the Asia-Pacific region by supporting improved communication and providing opportunities for enhanced cooperation between participating agencies. Technology transfer has been a major focus for the network, with innovative use of modern electronic communication strategies and direct technology transfer through technical training. The outcome of these activities has been improved information access for researchers and industry and the development of mechanisms to spread project impacts widely throughout the Asia-Pacific region, beyond the agencies directly involved in projects.

Introduction

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

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

Recognising the importance of marine fish farming in the Asia-Pacific region, senior government representatives at the NACA 13th Governing Council Meeting in 2002 absorbed the grouper network into NACA's core program, to ensure its long-term sustainability. The coverage of the network was also expanded to include other species such as sea bass, snapper,

Students in the Gondol grouper hatchery training course being shown broodstock management techniques.

cobia, tuna and marine ornamentals and the name was changed to the Asia-Pacific Marine Finfish Aquaculture Network (APMFAN).

Demonstration of tank management and feeding techniques.

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

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

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

Communication

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

Electronic communication

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

The network produces two e-newsletters:

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

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

Course participants observe the preparation of live feeds culture.

Students obtain 'hands-on' experience in the grading and sorting of juveniles.

Workshops

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

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

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

On completion of the course participants were presented with an official certificate of accomplishment.

Publications

Publications developed by the network are listed in Appendix 2. An excellent example of the strength of the networking approach to developing extension information is the Husbandry and Health Manual for Grouper. Access to network participants provided the coordinating agency, SEAFDEC AQD, with information and

experience from grouper aquaculture researchers and practitioners throughout the Asia-Pacific region. Following publication of the original English version, network participants provided translation into local languages: Filipino, Indonesian, Mandarin, Thai and Vietnamese. The result was a high-quality publication of direct application to farmers in the major grouper farming countries of Southeast Asia.

Staff exchanges

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

Research, development and extension coordination

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

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

1 Production technology
   1.1 Broodstock
   1.2 Larviculture
   1.3 Nursery
   1.4 Grow-out
   1.5 Post-harvest
2 Environment
3 Marketing and Trade
4 Food safety and certification
5 Socio-economics and coastal livelihoods
6 Fish health
7 Training and extension

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

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

Technology uptake

APMFAN has a strong focus on 'hands-on' training to facilitate technology uptake by farmers. An example of this is the Regional Grouper Hatchery Production Course, run at the Gondol Research Institute for Mariculture, Bali, Indonesia, for the last two years. The Gondol course provides hands-on training for a limited number (~15) of participants at a centre renowned for its excellence in developing production technology for marine finfish, particularly groupers.

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

Other network partners have also incorporated recent research results into their training courses. For example, SEAFDEC AQD has incorporated recent technological improvements

in grouper hatchery production into their regular Marine Finfish Hatchery course, and the Department of Primary Industries and Fisheries, Queensland, has run a series of workshops for farmers interested in grouper aquaculture in Australia. The Gondol Research Institute for Mariculture has run several courses in Indonesia for local farmers and fisheries officers.

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

Conclusion

The coordinated and structured approach adopted by the network has proved to be effective in supporting research in marine finfish aquaculture, and in translating some of the research outcomes to development activities. APMFAN will continue to share knowledge and experience across the region. It is presently building its scope of activities to cover a wide range of marine fish and other species. Further work is also being undertaken on formalising the participation of institutes within the network. The model is also being considered for other mariculture species and commodities, thus providing a wide range of mariculture options for coastal development in the region.

The building of further partnerships with government, the private sector and NGOs will be essential to continue the success of the network, as part of a concerted Asia-Pacific regional collaborative effort to address unsustainable fishing practices and poverty in coral reef and other coastal areas through responsible marine fish aquaculture development.

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APPENDIX 1

Development of Sustainable Marine Finfish Aquaculture in the Asia-Pacific Region

Needs Evaluation

To support the widespread dissemination of project results, the ACIAR project organised a *Regional Workshop on Sustainable Marine Finfish Aquaculture for the Asia-Pacific*, held at HaLong City, Vietnam, from 30 September to 4 October 2002. The workshop was funded by ACIAR, the Australian Academies of Technological Sciences and Engineering (through the Department of Education Science and Training, Frontiers of Science and Technology Missions and Workshops element of the Innovation Access Program) and by the Government of Vietnam.

The workshop attracted over 80 participants from Australia, Brunei Darussalam, China, Hong Kong SAR, India, Indonesia, Malaysia, Myanmar, Philippines, Solomon Islands, Thailand, Vietnam, and Europe, including representatives of the Asia-Pacific Economic Cooperation Fisheries Working Group, Food and Agriculture Organisation of the United Nations, International Marinelife Alliance, Marine Aquarium Council, Network of Aquaculture Centres in Asia-Pacific, The Nature Conservancy, and The WorldFish Centre.

The overall objectives of the workshop were to:

1. Provide detailed technical results of ACIAR project *FIS/97/73 Improved hatchery and grow-out technology for grouper aquaculture in the Asia-Pacific region*.
2. Provide a forum for young researchers involved in the development of sustainable marine finfish aquaculture in the Asia-Pacific region to present their results and interact with other researchers.
3. Review the R&D needs for sustainable marine finfish aquaculture development in the Asia-Pacific region.
4. Identify potential collaborative projects to assist the development of sustainable marine finfish aquaculture development in the Asia-Pacific region.

To achieve the latter two objectives, the workshop participants formed discussion groups to:

- Identify constraints to the development of sustainable marine finfish aquaculture in the Asia-Pacific region;
- Identify activities required to address these constraints/issues (these may include: research and development, policy development, training, extension, etc.);
- Prioritise these activities (assigned high (H), medium (M) or low (L) priority).

Discussion Group	Chair	Rapporteur
Hatchery		
Broodstock	Joebert Toledo	Elizabeth Cox
Larval rearing	Ketut Sugama	Mike Rimmer
Larval feeds	Kevin Williams	Richard Knuckey
Grow-out		
Nursery	Clarissa Marte	Elizabeth Cox
Grow-out	Le Thanh Luu	Mike Rimmer
Environment	Yvonne Sadovy	Mike Phillips
Other issues	Pedro Bueno	Mike Rimmer

During the subsequent plenary sessions, the results of each discussion group were presented to the workshop for further discussion and clarification. As well, workshop participants identified the institutions which are currently undertaking or are prepared to undertake research in each topic area.

The outcomes of this review are listed here to provide an overview of regional research needs to support the continued development of sustainable marine finfish aquaculture, and to indicate where research, development and extension efforts are already taking place in regard to these topics.

Hatchery

Topic	Constraint/issues	Activities required	Priority (H/M/L)	Institutional commitment
Broodstock				
Broodstock supply	History of wild caught broodstock unknown (age, reproduction longevity, does spawning and/or egg quality decrease in older fish?)	• Document stock mortalities, age and record reproductive history of individuals across centres • Database, sharing of information between centres; long-term goals due to nature of collecting data • Centralisation of otolith reading to standardise results	H	
	Identification of species selection criteria; use existing selection criteria matrices	• Individual countries to develop criteria most suitable to local markets/conditions; exchange of these criteria between countries? (The mechanism for exchange needs to be determined). Assistance from for example NACA • Importance of economic surveys in species selection	H	
	Impact of fishing pressure on spawning aggregations in the wild How will this affect the supply of broodstock for captive breeding?	• Policy — protection for some spawning aggregations (Note: this is a broader fisheries sustainability issue — the discussion here focused on aquaculture aspects) • Possible source to access spermiating males (collect milt and return to wild)	H	
	Difficulty of accessing males of some species	• Techniques already developed for some species • Further work may be required with specific species	L	
Broodstock management	Development and assessment of captive breeding populations Important for future sustainability to reduce our reliance on wild caught fish as broodstock	• Husbandry techniques — feed and feeding, holding systems, disease prevention and control • Water quality management	Very high	

Topic	Constraint/issues	Activities required	Priority (H/M/L)	Institutional commitment
	Implications of maintaining genetic diversity in captive breeding programs Option to exchange captive bred brooodstock between centres to maintain genetic diversity (Note issues regarding disease transfer and limited knowledge regarding genetic populations across regions) Benefit to reduce the need for individual institutions to hold large numbers of broodstock	• Population genetics across geographic areas to identify different strains within species	M	
Spawning	Optimising hormone induction techniques — dosages, frequency, sexes induced	• Collation/dissemination of reproduction techniques used	H	
	Control of seasonal reproduction techniques	• Environmental control of reproduction	H	
	Cryo-preservation of sperm			
Egg supply/ quality	Effect of broodstock nutrition on egg quality	• Expansion into other areas (to be identified) of nutrition required	H	
	Development of egg quality criteria	• Certification of standards (for egg sales)	M	• Note: Existing work (fatty acids) on snapper, grouper at SEAFDEC.
Disease	Develop techniques/protocols to acquire and maintain pathogen-free broodstock	• Research required to increase our understanding of susceptibility to and transmission of pathogens • Training and dissemination regarding collection and handling • Study on vertical transmission of passive immunity from broodstock to larvae	H	
	Need for coordination of viral disease testing protocols	• Coordinated facility(ies) for nodavirus testing; one central facility to provide expertise, primers, etc.		Current APEC involvement in disease issues: Refer to APEC Regional Research Program on Grouper Virus Transmission and Vaccine Development — needs to be taken forward.
Larval rearing				
Pre-feeding stages	Egg quality Yolk absorption rate — should have yolk left when mouth opens	• Broodstock nutrition improved	H	
	Handling of eggs and larvae	• Information on egg handling needs to be transferred to private sector — training, extension	H	

Topic	Constraint/issues	Activities required	Priority (H/M/L)	Institutional commitment
Early feeding stage (rotifer)	Suitable natural feed for initial feed Some countries — difficulty in producing SS-strain rotifers	• Technology transfer for SS-strain rotifer culture — training, extension	L	
	Difficulty in producing suitable numbers of copepods New species (Napoleon wrasse, coral trout, blue tang) — lack of suitable first feed organism	• Research on copepod culture technology	H	
Late feeding stage (*Artemia*)	*Artemia* expensive Nutrition — requires enrichment Nutritional requirements may change through development stages	• Research: optimal enrichment method	H	
	Rearing tank management	• Training in rearing procedures		
Metamorphosis	Cannibalism	• Research to reduce cannibalism, for example grading, feeding frequency, exercising, fish density — behavioural studies • Training in grading, management techniques	H	
Disease	VNN	• Optimal management will reduce incidence of VNN • Research on nodavirus • Improve immune response — research • Long-term research: vaccine	H L	
	Bacterial diseases	• Probiotics • Research on egg washing (ozone, iodine, UV) — effects on eggs and embryos	H	
	Parasites in extensive pond larval rearing	• Research on prevention/control of parasites in ponds	H	
Larval biology, nutrition		• Research in larval biology • Research in larval nutrition	L H	
Chemical use	Deformities in larvae/juveniles Chemicals (and antibiotics) used prophylactically	• Training and extension on use of chemicals	H	
		• Policy development/education on responsible use of chemicals and antibiotics — good practice guidelines/standards	H	
		• Research on chemical application	M	
Larval Feeds				
Rotifers	Need for improved management	• Contact Europeans (and others) who have developed intensive rotifer culture	H-immediate need	
	Identify local small strains (SSS-rotifer)	• Develop culture method that continually harvests the smallest rotifers		
	Overcome problems in countries where rotifers are sourced from open ponds	• Transfer of existing technology, extension • Disease transfer, disinfection methods for all live-prey species		

Topic	Constraint/issues	Activities required	Priority (H/M/L)	Institutional commitment
Copepods	Difficulty in maintaining culture	• Identify why copepod is better feed for larvae	M-H (long-term)	
	Difficulty in getting numbers of n1–n2 nauplii	• Try to compensate deficiency in rotifer		
	Species selection	• Develop mass culture technology		
Artemia	Not issue at the moment but still reliant on Great Salt Lake supply	• Training for local farmers on best practice use, decapsulation, nutritional enhancement etc. • Put in place an effective extension operation which can work with local bodies to design extension specific to the country, companies can be involved (INVE)	L	
Nutritional enhancement	Most enhancement geared around enrichment for temperate species; need more results for tropical species	• More nutritional information on requirements of target species	H	
	Best-practice for use of commercial products	• Extension		
	Use of bacteria, probiotics	• Protocol where you design specific enrichment composition		
Artificial feeds	Under-utilised	• Need information on how to wean onto artificial diets	M-H	
	Have to change total management of farm, water management, tank design	• Weaning methods and water quality management		
	Reluctance to use, high cost			
Microalgae	Quality control of microalgae, maintenance of cultures	• Use of algal concentrates — survey and assess available concentrates • Develop a protocol for their use	L	

Grow-out

Topic	Constraint/issues	Activities required	Priority (H/M/L)	Institutional commitment
Nursery				
Holding systems Stage: post-metamorphosis to 2–3 cm plus 'tinies'. Duration = approx. 2 months	Holding systems — high mortality of wild caught fry, no standardised management protocols	• Collation of existing practices and development of standardised procedures for handling and transport	M (easily done)	
	Mortalities during and after transportation (of hatchery bred fry and wild caught juveniles).	• Information is available for some species		
	No current management protocols, particularly for wild caught juveniles; no standardised nursery systems	• Desired outcome — preparation of a manual that addresses optimal and standardised procedures for transportation and holding system maintenance		
	Information about management of nursery systems is available	• Submit to APEC for possible funding		

Topic	Constraint/issues	Activities required	Priority (H/M/L)	Institutional commitment
Feeds	Difficulties weaning wild caught juveniles onto artificial diets	• Need to develop better weaning protocols	H	
	Farmers do not readily adopt artificial diets; there is a preference to continue feeding trash fish.	• Training/demonstrations to farmers on best practice weaning techniques	H	
	Feed availability/cost is an issue	• Need to develop an on-farm feed preparation method using local ingredients	M	
	Need to develop *Artemia* replacement diet for nursery phase	• Necessary to provide an information guide and training on feed composition, compile from existing information	H	
	Lack of information on some of the important micronutrients	• Further research is required to determine micronutrient requirements		
	Is wide size variation during nursery phase due to poor feeding management?	• Need further work on feed distribution and stocking density, shelters; work on feeding frequency has been done	H	
Cannibalism	Need to reduce the frequency of grading, stressful to fish	• Development of grading systems suited to species behaviour is needed; suggest assessment of available graders and associated mortalities	H	
	No knowledge about why cannibalism occurs	• Study behaviour of juveniles to assist in the development of effective solutions	H	
Disease	Transfer of diseases between centres/regions	• Develop quarantine procedures to address quality of seed for sale (local/import/export) • A future need to address certification of some hatchery operators in regard to developed quarantine protocols	H	
	Significant mortalities from disease outbreaks including viral and bacterial still occur			This has been addressed by the health and husbandry manual developed for grouper disease management.
	Grouper deformities — cause unknown	• Research focus may need to start in the hatchery phase, for example, with nutrition, physical handling	M	
	No prevention for viral diseases	• Development of vaccines and vaccination procedures and immuno-stimulants	H	
Grow-out				
Grow-out systems	Lack of information on pond grow-out (Indonesia, Vietnam and China have major grow-out systems) Remediation of effluent from pond culture	• Research: stocking density, pond management, water quality management; pond rotation, polyculture options	M	

Topic	Constraint/issues	Activities required	Priority (H/M/L)	Institutional commitment
	Reluctance of small operators to invest in intensification (cages) — high capital cost Lack of data on such systems Lack of appropriate technology? Land-based aquaculture — tanks	• Extension and technology transfer from other countries or regions • Research: information for cost-benefit analysis • Technology transfer, for example from Europe	L	
	Availability of suitable cage construction material for poor farmers	• Assess suitability of various locally-available materials	H	
	Australia: limited availability of se cage sites Vietnam: sites for coastal cages limited Resource use conflicts, for example aquaculture and tourism, capture fisheries, traditional fisheries	• Assessment of site suitability — GIS, planning • Integrated coastal/marine planning • Transfer of methodology from, for example, Sabah study — after evaluation of success	M	
	Need to identify beneficiaries of aquaculture development	• Socio-economic evaluation of aquaculture	M–H	
Feeds	Cost of artificial diet high	• Research: more cost-effective feeds • Utilise locally-available ingredients • Further optimise feeds to maximise cost-effectiveness • Determine requirements for all fish up to harvest size • Feed management, FCR tables, especially for dry diets	M	
	'Trash' fish issues: for example pollution, waste	• Need to shift farmers from using 'trash' fish to artificial feeds • Better utilisation of 'trash' fish farmer-made feeds • Extension of research results to feed companies and farmers	H	
	Diversity of marine finfish culture: may need different feeds for different species	• Research: identify nutritional requirements of various groups, for example groupers, snapper	M	
	Need for culture of lower trophic level fish species, eg milkfish, tilapia	• Technology transfer	L	
	Dependence on fish oil	• Research: alternatives for substitution	M	
	Quality issues – for example high fat levels in gut cavity	• Tailor-made (consumer-focussed) feeds.		
Disease	Ectoparasites	• Training for farmers	H	
	Viral diseases: nodavirus	• Research: disease prevention, control, vaccination • Coordination of existing research efforts. APEC viral disease program • Immune stimulants (vit. C)	H	
	Chemical use issues	• Education of farmers/extension	H	

Topic	Constraint/issues	Activities required	Priority (H/M/L)	Institutional commitment
Post-harvest				
Transport systems — live product	Sea transport for live fish — high mortality, high cost	• Research: improved transport	M	
	Chemical (anaesthetic) use in transport	• Extension, education	L	
	Product quality at point of sale	• Research need	L	
Chilled and frozen product	Product quality	• HACCP implementation • Value-adding opportunities	L (no immediate need)	
Product	Impact of feed substitution on product quality	• Research: product development and evaluation	L	
	Fish colour – market demand	• Research: optimise environmental conditions, feeds	L	
Environment				
Planning	Need for equity among resource users, access to common property Need to identify proper places, potential areas, zones, and resource allocation	• Govt policy to deal with equitable planning: including mapping of suitable areas (including bio-physical, social, economic, environmental, and legal/ institutional aspects)	H	
	Need for awareness of environmentally sound planning and operational practices at both govt policy and producer level	• Planning stages to incorporate carrying capacity, and environmental assessment, take account of other sectors (land and water based activities that may affect aquaculture) and promote integrated planning	H	
	Lack of cooperation/coherence between policy makers, private sector and researchers	• Consider community based management options	H	
	Lack of capacity to develop and implement planning and management for coastal mariculture	• Information on good practices for planning, extension of such practices	H	
	'Clustering' behaviour and impacts	• Prepare a set of recommendations on good planning practice guidelines for adoption by APEC economies	H	
Carrying capacity *(capacity of area to sustain cages, and limits of coastal environment to sustain mariculture)*	Lack of assessment methodologies for practical application in tropical fish culture	• Develop principles and practical guidelines (rules of thumb) for applying carrying capacity in tropical environments	H	
	Use of trash fish: (1) price, industry sustainability; (2) impacts on wild fisheries; (3) water pollution	• Extend feed research results to feed companies	L/M	
	Lack of understanding/ consideration of biological limits to marine fish culture (feed, wild juvenile supply)	• Promote awareness of unsustainability of wild juvenile supply	L	
	Deterioration of coastal environments	• Monitoring of aquaculture development	M	
		• Licensing based on suitability and carrying capacity	H	

Topic	Constraint/issues	Activities required	Priority (H/M/L)	Institutional commitment
Impact assessment	EA is tedious and costly to apply, particularly for small-scale developments	• Promote continuous monitoring	M/H	
	Lack of information on nutrient loadings — characterisation of waste	• Assessment of nutrient loadings and budgets from marine fish culture	H	
		• Clarify responsibilities and scope/ requirement for EA (ideally within the planning process)	H	
Disease	Disease outbreaks, particularly viral diseases (VNN, iridovirus)	• Development and use of hatchery reared SPF stock • Research on vaccines	H (long-term)	
	Trans-boundary spread of pathogens	• Implement Asia regional guidelines on health management and responsible movement	H	
	Lack of knowledge	• Further extension efforts on health management based on 'good husbandry practices' (health management manual)	H	

Socio-economics, marketing

Topic	Constraint/issues	Activities required	Priority (H/M/L)	Institutional commitment
Socio-economics, livelihoods	Need to identify beneficiaries of aquaculture development Willingness of people in coastal communities to accept alternative livelihoods	• Socio-economic evaluation of aquaculture • Need to ensure that there is a technology transfer phase • Encourage network participants to share experiences in this area as part of the STREAM APEC study on mariculture to provide alternative livelihoods		
Aquaculture/ capture fisheries interactions	Seed supply — competition between fisheries and aquaculture sectors	• Better documentation on seed transportation		
	High levels of mortality in seed capture and transportation Destructive/wasteful fishing practices	• Extension material to improve seed handling techniques • Policy development: local seed used locally (increases appreciation of value of resource)		
	Use of 'trash' fish	• Policy development: promotion of compounded feed use, for example licence condition.		
Market	Certification, eco-labelling	• Voluntary codes of practice	M	
	Better meeting market requirements	• Market demand information • Forecasting	H	

Topic	Constraint/issues	Activities required	Priority (H/M/L)	Institutional commitment
	Need to improve market chain	• Increase communication and interaction between producers and market end • Develop farmer cooperatives to improve bargaining power • Promote aquacultured fish as higher quality, ciguatera free product	H	
	Lack of understanding/uncertainly on long-term market demand for marine finfish	• Include market assessment for non-live fish markets		
	Consumer perception regarding quality of aquaculture/wild product, especially fat quality	• Feeds development research to incorporate assessment of end-product quality (see grow-out)		
	Focus has been on high-value species	• Need to focus on other species that are maricultured • Market study for full range of marine fish in A-P region		
GMOs	Attitude to GMO technology Market access versus improved productivity		L	
General recommen-dations on networking	Information	• Prioritise activities of network — seek funding from donor agencies for specific activities/projects (APEC, ACIAR) • Hold additional marine finfish aquaculture workshop in Vietnam (invitation of Vietnamese Government) • Ensure that information is further disseminated to national/regional extension services		
	Training, technical exchanges	• (many covered in recommendations)		
	Institutional commitment Private sector involvement Many fish farmers are small-scale operations, cannot attend large regional workshops	• Need to ensure that research results are extended to private sector • Need to ensure that small-scale fish farmers are kept informed of technological improvements		

APPENDIX 2
Project and Asia-Pacific Marine Finfish Aquaculture Network Publications

Asia-Pacific Grouper Network Publications

APEC/NACA/BOBP/GOI (2002). Report of the Regional Workshop on Sustainable Seafarming and Grouper Aquaculture, Medan, Indonesia, 17–20 April 2000. Collaborative APEC Grouper Research and Development Network (FWG 01/99). Network of Aquaculture Centres in Asia-Pacific, Bangkok, Thailand. 224 pp.

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