Australian Government

Australian Centre for International Agricultural Research

Final report

project

Promising indigenous fish species and bioremediation for barramundi aquaculture in northern Australia

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1 Acknowledgments

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The enthusiasm and professionalism of Robert Alphonse Kaiyun, Kaupa Kia, Waum Elamnop and Johnson Karl of Western Province, and Havini Vira, Irene Kamang, and Noel Tonko of Ok Tedi Development Foundation ensured the project met it's milestones.

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Cec Langton of the Tablelands Restocking Group based in Atherton Queensland, assisted in the visits by the PNG staff to Australia by welcoming the visitors to his hatchery and sharing his knowledge.

Carole Wright of the Queensland Department of Employment Economic Development and Innovation (DEEDI) conducted the statistic analysis.

2 Executive summary

This project was a continuation of the ACIAR-funded scoping study *Development of capacity for aquaculture of indigenous fish species in Papua New Guinea*. The project aim was to assist inland aquaculture development in Papua New Guinea (PNG) and Australia in an environmentally and cultural sensitive manner. Aquaculture has been identified for its potential to provide dietary protein for PNG people. The PNG National Fisheries Authority (NFA) in conjunction with the PNG project partners, Ok Tedi Mining Limited (OTML) and the Western Province government, identified the potential of native fish species for aquaculture. Developing farming systems built on herbivorous fish to negate the need for expensive feeds, and avoiding exotic species that may cause deleterious ecosystem impacts, were the aims of this research. Native fish species are a favoured food of inland people in the Western Province / Ok Tedi region because of their cultural significance (Smith 2000). As there are 35 species of freshwater fish common to northern Queensland and the PNG Western Province, the knowledge and experience in the biology and culture of these species among staff of the Department of Primary Industries and Fisheries (DPI&F)[1] could be transferred to PNG.

Water availability and quality of the discharge water required to meet environmental standards are limiting factors to aquaculture development. Recycling of water through aquatic plants back to fish ponds can reduce the volume of water required to operate, and remove the nutrients prior to discharge to natural waterways. Water re-use will allow legislative compliance and can lead to greater profitability. Although barramundi were used as the culture species in the trials, the culture systems used would allow other species to be cultured. The aquatic plants used are common to PNG and Australia, allowing the technology to be transferred to other culture species in both countries.

Objectives of the project were to:

- Develop robust hatchery and grow-out technologies for native fish species (including barramundi) for sustainable aquaculture development in Queensland and PNG.
- Evaluate the potential of pond-based freshwater crustacean culture in PNG and Queensland, building on technologies already developed in Queensland for these species.
- To assess the acceptance by farmers of new species/techniques and to develop appropriate husbandry packages.

Following a mid-term review of the project, some of the objectives were either modified or ceased. As DEEDI closed its Walkamin freshwater aquaculture centre with loss of critical staff, and OTDF was undergoing a restructure, ACIAR decided to terminate the project in December 2009, 18 months before the scheduled completion date.

With assistance from ACIAR to supply specialist aquaculture equipment, OTML constructed a functional aquaculture facility complete with hatchery and six ponds. Western Province government constructed six ponds as well, and although constructed a hatchery building it was not operational. Water and power supplies were required to be connected to make the hatchery functional. Confidence and capacity building to operate the facilities was achieved by periodic visits by DEEDI staff to PNG and visits to DEEDI facilities in Australia by the PNG staff. These visits to Australia allowed the PNG staff to participate in and view the farming practices that were to be developed in PNG. Technologies to breed various species of fish were demonstrated and applied in PNG for the following species: Fly River herring (*Nematalosa papuensis)*, sleepy cod (*Oxyeleotris selheimi*), eel-tailed catfish (*Neosilurus ater*) and sooty grunter (*Hephaestus fuliginosus*) were all bred.

[1] Since this study the department has been restructured and renamed the Department of Employment Economic Development and Innovation (DEEDI).

Investigation into plant-based treatment systems to remediate barramundi pond water was carried out using two different plants, duckweed *(Spirodela punctata* and *Wolffia angusta)* and lotus (*Nelumbo nucifera*). Water savings of 22% and 62% were achieved with duckweed and lotus, respectively, in the trials. Nutrient removal by the aquatic plants of 45% (NH_3-N), 35% (total nitrogen), 19% (total phosphate) and 32% (total suspended solids) were achieved. Growth rates of the fish were not effected by water re-use.

Successful trials were carried out in Australia by James Cook University into culture of *Macrobrachium rosenbergii*. The research demonstrated successful techniques for breeding and larval rearing. The life cycle was completely closed using hatchery-bred broodstock. Due to difficulties experienced in PNG, crayfish could not be cultured in PNG during the period of this project. The mid-term review recommended that all *Macrobrachium* work be discontinued as the review team considered the objectives too ambitious. The review team recommended that resources be concentrated on overcoming the problems experienced in culturing crayfish.

Five farmers were provided with training in sleepy cod culture and their farms stocked with this species. Results were not encouraging because of poor site selection of the farmers' ponds and stocking with fodder fish(Fly River herring) had not taken place. The project was terminated before these factors could be addressed. Two farms were stocked with herring, but again, no data could be collected from these farms before the project was terminated.

This project was aiming to create an industry where none existed previously. Results showed real potential of native fish aquaculture with minimal inputs, but further work is required to realise its full potential. The restructured section of OTML, renamed Ok Tedi Fly River Development Program (OTFRDP) and Western Province government have demonstrated a willingness to see this work succeed and are continuing to work on the aquaculture of native fish after the termination of this ACIAR project.

Glossary of Acronyms

ACIAR	Australian Centre for International Agriculture Research
DEEDI	Queensland Department of Employment Economic Development and Innovation
DPI&F	Queensland Department of Primary Industries and Fisheries
JCU	James Cook University
LARDEC	Lowlands Aquaculture Research, Development & Extension Centre
MARFU	Marine and Aquaculture Research Facilities Research Unit
NFA	National Fisheries Authority
OTFRDP	Ok Tedi Fly River Development Program
OTDF	Ok Tedi Development Foundation
OTML	Ok Tedi Mining Limited
PNG	Papua New Guinea

3 Background

PNG component

Malnutrition of the Papua New Guinea (PNG) people in rural areas is widespread (Bourke et al. 2000). Consistent with this an earlier ACIAR project on inland aquaculture in PNG (Smith 2007) identified that malnutrition is a major problem in the Eastern Highlands Province.

Fish farming in PNG has a long history, with reports of more than 25 fish introductions into PNG starting in 1949 (Smith 2007). Most interest in aquaculture has been with exotic species, although Haines (1979) and Smith (2000) suggest indigenous species as having potential for aquaculture in PNG. Interest in fish farming is strong, with 11 000 fish farms reported in inland PNG (Smith 2007). The PNG people are embracing fish farming as a source of protein in their diets, as well as a source of income (Smith 2007).

The development of mining in the highlands, rapid population growth (or demographic shifts) and a history of protein deficiency, have led to food insecurity as an important issue.

Ok Tedi Mining Limited (OTML) is scheduled to cease its mining activities in the Western Province (WP) in 2013, although exploration is being carried out to extend the mine life if possible. As part of the mine closure program and its moral obligation to the affected people, OTML, by funding the Ok Tedi Development Foundation (OTDF), has created a Rural Development Section within OTDF, the aim of which is to help the local people sustain themselves once the employment and economic activity generated by the mine abates with the mine closure. Projects undertaken by OTDF Rural Development section include rice, vanilla bean, rubber, poultry, forestry and a pilot study on cage culture of barramundi.

Fish farming is relatively new to Western Province, where there has been little exposure to non-native aquaculture species, such as carp and tilapia, that are grown elsewhere in PNG. The deleterious effect of the spread of some exotic species, such as the climbing perch (*Anabas testudineus*) from West Papua has been observed in the Fly River by PNG authorities. Allen (2008) states “Contrary to popular opinion, the biggest threat facing the native freshwater fish fauna of Papua New Guinea is neither mining nor logging, but rather the uncontrolled introduction and spread of introduced fishes”. Government officials from both the Western Province government and NFA, along with OTDF researchers, identified local native fish species as having potential for aquaculture. Native species have the advantage of being well-adapted to the local conditions, and are familiar to the local people, who have a traditional connection with native species (Smith 2000).

Prior to this project, an ACIAR-funded scoping study *Development of capacity for aquaculture of indigenous fish species in Papua New Guinea* (C2003/149) (Herbert 2006) into the potential for aquaculture of native fish species was undertaken in collaboration with OTDF as the in-country partner. Dr. Brett Herbert from DPI&F and Mr. Boga Figa from OTDF undertook this scoping study. This project continued on from the work of the scoping study.

There are 35 native freshwater fish species common to both the Fly River and northern Queensland (Allen et al. 2008). The knowledge gained in Australia of breeding these species could be transferred to PNG by DEEDI researchers. The scoping study demonstrated the potential of native fish aquaculture, and the potential for a successful partnership between PNG and Australian agencies.

OTDF had already committed resources to aquaculture, with trials on barramundi cage culture in the Fly River as well as trout stocking in the highlands. OTDF was well positioned to partner with ACIAR to develop aquaculture in PNG as OTDF has the staff and logistics in place to benefit from expert assistance. The scoping study was a pioneering project, with ACIAR partnering with a private company rather than government agencies. This initial aquaculture project has been followed by other ACIAR/OTDF projects including forestry and poultry feeds. This project also linked directly into the aims and aspirations of PNG government agencies.

NFA and the Western Province government had already recognised the potential of native species for aquaculture and become project partners, and committed resources to the work.

The Western Province is very remote with no road connection to the rest of the country. Basic fish feed ingredients such as copra meal, fish meal and rice bran, which are available in other areas of the country, are not generally available. Often farmers do not have the resources to purchase feeds even when available. The approach of this project was to grow primary consumers (herbivores), such as herring (*Nematalosa papuensis*) and redclaw crayfish (*Cherax quadricarinatus*), for human consumption and/or as food for other carnivorous fish such as sleepy cod (*Oxyeleotris selheimi)*, eel-tailed catfish (*Neosilurus ater)* and sooty grunter (*Hephaestus fuliginosus).* This approach may negate the need for expensive or unattainable fish feeds. The approach of culturing fish without processed feeds in PNG is not new (Glucksman 1969, Reynolds n.d.).

Australian component

Reducing environmental impacts and increasing ecological sustainability are important elements of aquaculture in Queensland. Water re-use and water discharge quality to the natural environment are factors currently concerning the aquaculture industry in Queensland with barramundi farming being the largest inland aquaculture industry in Queensland. Environmental requirements in Queensland are becoming more stringent and farmers are required to move towards zero discharge to the environment under both State and Federal policy and legislation. The Great Barrier Reef Marine Park Authority (GBRMPA) is increasingly scrutinising farming practices adjacent the GBR Marine Park. In 2009 an updated reef water quality protection plan was endorsed by the Queensland and Australian governments. The goal of the plan is to halt and reverse the decline of water quality from land-based activities, and prevent this water from entering the reef lagoon. The Australian Barramundi Farmers Association (ABFA) has identified water re-use and water discharge issues as a major research area. With greater demand on fresh water resources, water re-use is becoming an important component to greater productivity and profitability.

Prior to this project, issues of water quality and ecological sustainability of aquaculture were being investigated with a grant from the Rural Industries Research and Development Corporation (RIRDC). This project investigated the potential of lotus (*Nelumbo nucifera*) to remove nutrients and solids from barramundi aquaculture waste water. The by-product of the process, the lotus plant, may then become a saleable commodity for the farmer.

DEEDI Bribie Island Aquaculture Research Centre was also undertaking bioremediation trials on municipal wastewater treatment for release to natural waterways using duckweed, and were developing bacterial floc and mechanical treatment systems (Willett and Morrison 2006). Under the current ACIAR project, evaluation of duckweed for barramundi pond effluent bioremediation was set up at Walkamin to extend the trials that had already been undertaken by DEEDI.

A better understanding of husbandry techniques and water quality parameters for barramundi culture will help improve the viability of the fledgling PNG aquaculture industry. Water re-use and the production of usable by-products from the waste water will also help the PNG industry.

4 Objectives

Objectives as set out in the project proposal

Objective 1

Develop robust hatchery and growout technologies for indigenous fish species (including barramundi) for sustainable aquaculture development in Queensland and PNG.

Objective 2

Evaluate potential of pond based freshwater crustacean culture in PNG and Queensland, building on techniques already developed in Queensland for those species.

Objective 3

To assess the acceptance by farmers of the new species/techniques and to develop appropriate husbandry packages.

Changes to the project

A mid-term review was carried out in Goroka PNG in November 2008 by Dr. Ken Menz and Professor Peter Edwards, supported by Dr. Geoff Allan and Mr. Jacob Wani. Table 1 summarises the recommendations made by the review team.

All aquaculture activities at Walkamin ceased in June 2009 as DEEDI ceased all operations at the aquaculture centre, with only one staff member (Peter Graham) remaining to finalise activities at Walkamin and to continue the overseas component of this ACIAR project.

A section of OTML (OTDF) underwent an organisational restructure of its community work at the end of 2009 with a new entity, Ok Tedi Fly River Development Program (OTFRDP) taking over these and other activities in 2010.

In light of these changes and uncertainties, as well as changes in key personnel, ACIAR decided to terminate the project in December 2009, 18 months before its scheduled completion date.

Although the ACIAR project has ceased, the Western Province government has committed to continue the work. OTFRDP has also committed to supporting aquaculture as it moves forward.

Table 1. Changes to activities as recommended by the mid-term review.

Activities as per Project document	Activity changes recommended by review	Comments
Activity 1.1: Assist OTML and Western Province to develop two functional aquaculture facilities including design, construction and staff training	No change	The review noted that OTML hatchery is completed and second hatchery is under construction.

in all operational aspects.		
Activity 1.2: Transfer and adaptation to PNG of techniques developed in Queensland for production of freshwater species with initial emphasis on sooty grunter, eel-tailed catfish, and sleepy cod as models.	The review team recommended the project should concentrate efforts on fewer species and clearly articulate how technology would be demonstrated to farmers	The review team felt there were too many species and while the overall approach of transferring technology, evaluating breeding on station and then on farm was appropriate, the chance of successfully completing research with all species was small.
Activity 1.3: Investigate plant-based bacterial floc based, and/or mechanical treatment systems for improving barramundi production and management in freshwater aquaculture systems in Queensland.	Omit the words relating to bacterial floc and mechanical treatment systems and focus on plant-based systems as well as those based on naturally occurring bacteria and phytoplankton.	The review team noted that the activity as written was too specific and some farmers in Queensland were unlikely to be in a position to adopt biofloc technology or mechanical filtration.
Activity 1.4: Assess effectiveness of current settlement pond efficiencies in nutrient assimilation.	Consider adding a separate activity relating to the evaluation of farmers' own remedial systems along with provision of advice for their improvement	As the activity is not clear on its intention, the review team misunderstood the activity was proposing to do what they have suggested. The project was terminated before this activity commenced.
Activity 2.1: Collect freshwater crayfish from Fly River and evaluate growth and production characteristics.	No change (note that difficulties have been experienced with collecting redclaw juveniles because of unusually high river levels).	The research with redclaw is supported by the review team as proposed.
Activity 2.2: Distribute crayfish juveniles to selected farmers for growout trails in production conditions, monitor production and issues and address these as they arise.	Remove this activity and constrain all research with redclaw to that described in the revised Activities 1.2 and 3.1.	
Activity 2.3: Determine growout characteristics of *Macrobrachium* collected from Fly River recruitment runs at OTML/Western Province managed facilities.	It is recommended that all future work under this objective be dropped.	The review team considered the *Macrobrachium* component in this objective to be of a magnitude equal to a stand-alone ACIAR project and thus too ambitious in the current context and should not be

		pursued.
Activity 2.4: Investigate hatchery characteristics, production performance and aquaculture potential of two strains of *Macrobrachium rosenbergii* in Australia.	The Australian component has been completed	
Activity 3.1: Establish model farms for trialling and demonstration of culture techniques for various species.	This activity is primarily a demonstration/evaluation of technological feasibility - trials will be essentially 'researcher managed' (i.e., farmers will act under the strong direction of the research team). If this is successfully achieved, a further adaptive research phase would be required to interactively tailor the technology to the socioeconomic conditions faced by the farmers.	The review team felt that this objective needed clarity.
Activity 3.2: Distribute crayfish juveniles and/or fish fingerlings to selected farmers for growout trials in production conditions.	Omit as no longer relevant (once activity 2.2 is omitted).	
Activity 3.3: Assess performance and develop husbandry packages based on most appropriate production methodologies.	No comment was made on this activity by the review team	Project terminated before this activity commenced
Activity 3.4: Improve skills of provincial extension officers and NGOs in aquaculture techniques, under guidance from NFA, to extend results of the project beyond the life and footprint of the project.	No comment was made on this activity by the review team	Project terminated before this activity commenced

5 Methodology

5.1 Study sites

The project was carried out in four sites: two in PNG and two in Australia. The two PNG sites were both in Kiunga (Fig. 1), one the OTML site and the other the Western Province Government site. In Australia, pond-based studies took place at the DEEDI Research Station in Walkamin (north Queensland), and laboratory-based studies at James Cook University (JCU) in Townsville.

5.1.1

5.1.2 OTML site

The initial scoping study (described in Section 3 above) was conducted at the OTML Agriculture Resource Centre in Tabubil (Fig. 1). This project was initially planned to continue at the same location, but just as the project was about to commence, OTML decided to relocate to a site in Kiunga.

The aquaculture facility was constructed in Kiunga at an OTML compound commonly referred to as the 'Trust Yard'. Although space was limited, this site had access to a good source of bore water, electricity supply and above all very good security. A basic hatchery was built quickly under an existing roof structure and ponds were constructed. The site also had all other services such as telephones, e-mail and transport facilities supplied by OTML. This facility was planned to be a temporary site, with the equipment to be transferred to the nearby Western Province government site at Kiunga (see below) when OTML withdrew from this work as the mine closed down.

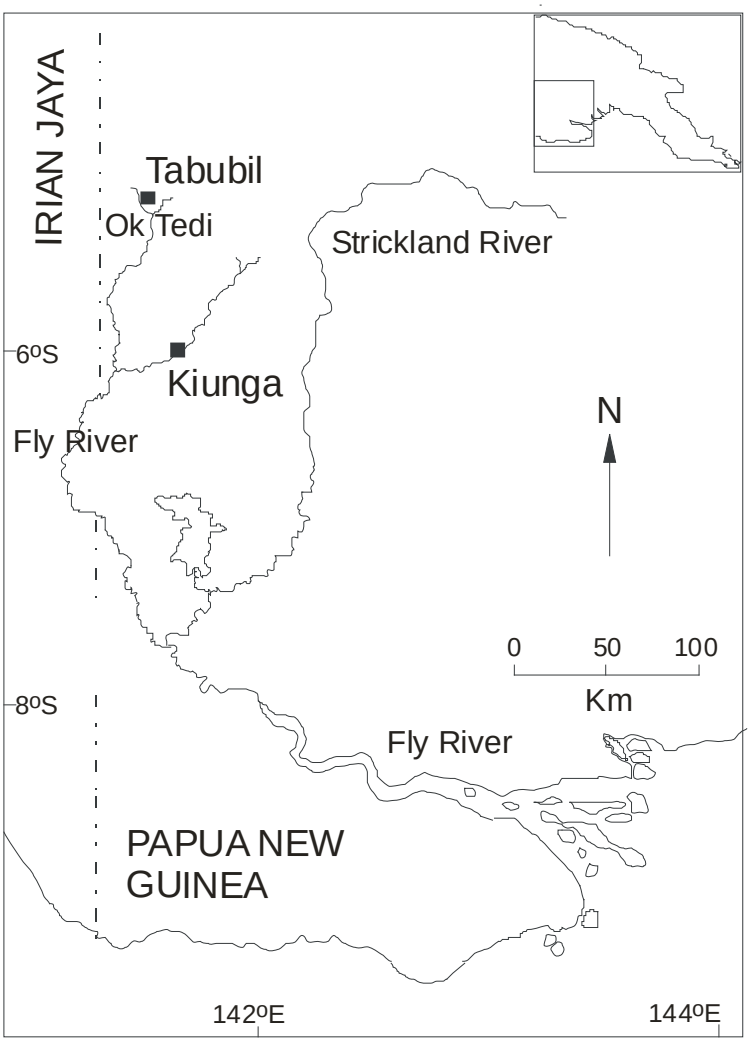

Figure 1. Tabubil, the planned original site for aquaculture development, is on the Ok Tedi, near the Ok Tedi mine site. Kiunga is on the Fly River.

5.1.3 Western Province government site (LARDEC)

The Western Province government aquaculture facility, Lowlands Aquaculture Research, Development & Extension Centre (LARDEC) was located at Samagos, on the outskirts of Kiunga. The construction of LARDEC had commenced prior to the development of the ACIAR project, as the Western Province government had already committed to developing aquaculture. LARDEC had some offices and accommodation for staff, but communications and transport were lacking.

5.1.4 DEEDI site

The major part of the Australian component of the project was carried out at the DEEDI research station at Walkamin, on the Atherton Tablelands west of Cairns. This site has an existing aquaculture facility, complete with laboratories and ponds designed for replicated trials. Growout trials on *Macrobrachium* conducted by JCU, as well as the water re-use trials were conducted at this facility.

5.1.5 James Cook University site

The larval rearing component of the work on *Macrobrachium* was carried out at JCU in Townsville. A project investigating the larval rearing of *Macrobrachium* by a John Allwright Fellowship-sponsored MSc student, which was already underway, was linked to this ACIAR project.

5.2 Personnel

Boga Figa of OTML and Brett Herbert of DEEDI prepared the project proposal, with input from Robert Alphonse Kaiyun from the Western Province government. Guidance on the formation of the project was given by Jacob Wani of the PNG National Fisheries Authority (NFA).

There were significant changes to personnel over the course of the project. Boga Figa left the project and was replaced by Havini Vira in 2006, as team coordinator at OTML. Brett Herbert resigned from DEEDI at the end of 2006 and was replaced by Evizel Seymour in April 2007.

From 2006 onwards OTML staff consisted of Havini Vira as the team leader with Irene Kamang as a fisheries officer. Noel Tonko was a temporary fisheries officer based at the Trust Yard and the main person doing the practical work.

The Western Province team was headed by Robert Alphonse Kaiyun with Kaupa Kia as the fisheries officer, assisted by Waum Elemnop and Johnson Karl.

The Australian team at Walkamin was led by Evizel Seymour with three technicians to conduct the Australian research component of the project and to assist the PNG partners in their activities. Peter Graham assisted with the PNG component as well as the Australian research. Clarita Agcopra was involved with the Australian research specialising in the water chemistry analysis, assisted by Karen Willows.

Dr. Chaoshu Zeng at JCU in Townsville supervised Malwine Lober in a John Allwright Fellowship MSc on *Macrobrachium* hatchery production.

All positions were funded by the respective organisations except for Karen Willows, who was funded through the ACIAR project.

5.3 Delivery of the project

5.3.1 Logistics

As OTML has the resources and infrastructure in place, they were the main agency to supply the logistics for the project. Travel into and out of PNG was facilitated on the OTML charter flights direct from Cairns to Tabubil, PNG. As part of their project budget commitment, OTML supplied all transport, accommodation and meals for visiting DEEDI staff. DEEDI staff welfare and security were also catered for by the OTML infrastructure. Safety briefings were conducted at the beginning of each visit, with DEEDI staff ensuring all safety requirements OTML were adhered to.

DEEDI staff conducted four to five visits a year, each of 10-14 days duration. The objectives of the visits were to review progress from the last visit, to advise on future activities, and to work alongside the PNG staff demonstrating and reinforcing skills and procedures.

5.3.2 Methodology

Objective 1 Develop hatchery and growout technologies for indigenous fish species for sustainable aquaculture development in Queensland and PNG

Activity 1.1 Assist OTML and Western Province government to develop two functional aquaculture facilities.

A hatchery was constructed at Tabubil (Fig. 1) as part of the scoping study but required further development and fittings. Pond facilities and a small laboratory were constructed to complete the hatchery facility at the Trust Yard. Ponds and a hatchery were also constructed at the Western Province site at LARDEC,Samagos. The LARDEC hatchery was not completed by the time the project was terminated.

As facilities were being constructed, the next phase was to ensure that staff were conversant with fish handling and maintenance, pond management and spawning techniques to ensure the hatcheries were functional. Training was delivered in the form of 'learning by doing' in the following activities.

Activity 1.2 Transfer and adoption in PNG of techniques developed in Queensland for production of freshwater species with initial emphasis on sooty grunter, eel-tailed catfish and sleepy cod.

An integral part of the breeding program was to transfer techniques to partner country staff and adapt techniques used in Queensland to the PNG situation. Four staff (two from OTML and two from Western Province) travelled to Queensland to participate in an exchange visit to develop their skills, abilities and confidence in handling and spawning native fish. The staff also viewed operational aspects of fish farming in Australia. Broodstock at both facilities were regularly assessed for breeding condition, to develop skills in handling, health monitoring and spawning. Larvae produced were reared in tanks and ponds. All activities were carried out repeatedly to develop confidence and abilities. These activities produced fingerlings for growout both on hatchery sites and at participating demonstration farms in PNG.

Activity 1.3 Investigate plant-based, bacterial floc based, and /or mechanical treatment systems for improving barramundi production and management in freshwater aquaculture systems in Queensland.

In response to the mid-team review, investigations of the bacterial floc and mechanical treatment systems were discontinued. Two trials were carried out on plant-based systems.

Plant-based treatment was used to investigate the capacity of these systems to assimilate nutrients from the water of barramundi fish farms in order to reduce the water requirements. Paired ponds were used at Walkamin to replicate partition systems, with one pond holding the fish and the other acting as the water treatment pond. The treatment ponds were planted with duckweed and lotus, both native to PNG and Australia. Water was pumped from the production pond to the treatment pond, and gravity fed back to the production pond after a residence time in the treatment pond. Each system was replicated. The experimental control on water quality was ponds of fish without water re-use, with water quality monitored and managed by water exchange as required to maintain fish health. Barramundi growth and food consumption were monitored along with water usage.

Objective 2 Evaluate the potential of pond-based freshwater crustacean culture in PNG and Queensland building on techniques already developed in Queensland for these species.

Activity 2.1 Collect freshwater crayfish from the Fly River catchment and evaluate growth and production characteristics.

Freshwater crayfish were collected from the Fly River near Kiunga and stocked into a pond at the OTML Trust Yard at Kiunga. The pond was fenced with a barrier to prevent the crayfish escaping, and the crayfish provided with habitat in the pond. A tank breeding trial was also carried out.

Activity 2.2 Distribute crayfish juveniles to selected farmers for growout trials in production conditions, monitor production and issues and address these as they arise.

This activity was scheduled in the project proposal to start in year 3, month 9-12, so the activity had not started before the mid-term review. The mid-term review recommended this activity be dropped.

Activity 2.3 Determine growout characteristics of seed stock Macrobrachium collected from the Fly river recruitment runs at OTML/Western Province managed facilities.

This activity was scheduled in the project proposal to start in year 3, month 3, so the activity had not started before the mid-term review. The mid-term review recommended this activity be dropped.

Activity 2.4 Investigate hatchery characteristics, production performance and aquaculture potential of two strains of *Macrobrachium rosenbergii* in Australia

Broodstock management, larval culture and nursery culture of post-larvae of the Australian strain of *M. rosenbergii* were conducted at the Marine and Aquaculture Research Facilities Unit (MARFU) at JCU. Nursery culture of two batches of post-larvae and grow-out trials were conducted at the ponds at Walkamin Research Station with JCU personnel monitoring the growth of the prawns.

Objective 3 To assess the acceptance by farmers of the new species/techniques and to develop appropriate husbandry packages.

Activity 3.1 Establish model farms for trialling and demonstration of culture techniques for various species.

Five farmers near Kiunga and two near Tabubil were selected by Western Province staff to develop as model farms. These farmers were given training by Western Province staff and supplied with fish fingerlings. Further assistance and advice was supplied by visits from staff.

6 Achievements against activities and outputs/milestones

Objective 1: To develop robust hatchery and growout technologies for indigenous fish species (including barramundi) for sustainable aquaculture development in Queensland and PNG

no.	activity	outputs/ milestones	completion date	comments

1.1	Assist OTML and Western Province to develop two functional aquaculture facilities.	Reliable, repeatable methods for spawning and fingerling production of native fish applied in PNG.	OTML hatchery completed and fully operational (February 2008) LARDEC hatchery building completed (December 2009)	A functional temporary hatchery was constructed at the OTML Trust Yard. It consisted of three 2-tonne fibreglass tanks, three 75-litre *Artemia*/egg hatching tanks with aeration, and a good bore water supply to the tanks. A small wet laboratory was also built, equipped with the necessary equipment. The LARDEC hatchery building was not completed until December 2009 due to the slow provision of funds from the WP Government administration. Water and power supply had not been connected by the end of the project. Six 2-tonne fibreglass tanks, five 75-litre *Artemia*/egg hatching tanks, an air blower and other ancillary equipment were supplied by ACIAR on site. Visits to Walkamin Research Station for work experience by OTML and WP staff. Quarterly visits by DPI&F staff to PNG, to work alongside PNG staff reinforcing and refining skills and knowledge. Successful hormone-induced spawning of sooty grunter and eel-tailed catfish, with 83 sooty grunter fingerlings distributed to farmers. Eel-tailed catfish spawned but only 3 fingerlings were raised. Sleepy cod and Fly River herring were successfully spawned in ponds. Required specialist aquaculture equipment was supplied via ACIAR to in-country partners.
		Confidence and capacity built for breeding native fish species in Papua New Guinea	Ongoing until early termination of project (December 2009)	

1.2	Transfer and adaptation to PNG of techniques developed in Queensland for production of freshwater species with initial emphasis on sooty grunter, eel-tailed catfish and sleepy cod.	Expansion of use of transferred technologies beyond OTML staff Confidence and capacity built in PNG to explore production of native fish species independently.	Ongoing until early termination of project in Dec 2009 Ongoing until early termination of project in Dec 2009.	A training workshop was held by WP staff, supported by OTML, for local farmers. Ongoing farm visits by WP staff, with farm visits arranged for visiting DPI&F staff. Herring and sleepy cod successfully bred in WP ponds. 2 000 herring and 2 000 sleepy cod produced with some stocks transferred to farmers' ponds. Successful spawnings of sooty grunter and eel-tailed catfish (*Neosilurus ater*) at OTML's Trust Yard, but limited success in fingerling production.
1.3	Investigations of plant-based, bacterial floc based, and/or mechanical treatment systems for improving barramundi production and management in freshwater aquaculture systems in Queensland.	Water extraction and discharge reduced substantially. Fish production economics improved through efficiencies in handling, feeding and protection. Model for requirements for partition systems developed.	May 2009	Two trials were carried out using plant-based treatment systems. Additional trials were not possible due to changes in delayed staff commencement dates and the early termination of the project There was no adverse effect on fish growth and survival by water re-use through plant-based water treatment. The plant-based treatment systems were effective in treating the water but further research is required to evaluate and compare the other treatment systems and to further on-farm management practices.
1.4	Assess effectiveness of current settlement pond efficiencies in nutrient assimilation.	Understanding of properties of settlement ponds conducive to nutrient and waste assimilation.		Project terminated prior to activity commencing this activity.

Objective 2 To evaluate potential of pond based freshwater crustacean culture in PNG and QLD, building on techniques already developed in QLD for these species.

no.	activity	outputs/ milestones	completion date	Comments
2.1	Collect freshwater crayfish from Fly River catchment and evaluate growth and production characteristics.	Crayfish growth and reproduction potential in PNG determined.	December 2009	Breeding was successfully achieved in tanks, but broodstock disappeared when stocked into ponds. A study to identify the cause(s) (death or emigration) was devised, but the project was terminated before this was completed. This work should continue under the new OTML structure.
2.2	Distribute crayfish juveniles to selected farmers for growout trials in production conditions, monitor production and issues and address these as they arise.	Juveniles ready for distribution and farmers selected and trained.		Mid-term review recommended this activity be removed.
2.3	Trial growout seed stock collected from identified Fly River recruitment runs of *M. rosenbergii.*	Assessment of growth rates and aquaculture potential of PNG stocks of *Macrobrachium*.	Work had not commenced on this activity as efforts were confined to the difficulties in breeding redclaw.	Mid-term review recommended that all future work under this objective be dropped (possibility to be considered as a separate project).

2.4	Investigate hatchery characteristics, production performance and aquaculture potential of two strains of *M. rosenbergii* in Australia	Assessment of hatchery and growth potential of genetically distinct stocks of *Macro-brachium* in Australia. ACIAR sponsored student develops skills base required for *Macro-brachium* production.	October 2008	Reproductive seasonality, conditioning, fecundity as well as induction of out-of-season spawning and the attempt to close the life cycle although induction of out-of-season spawning by temperature and photoperiod manipulation achieved relatively limited success. Although out-of-season spawning was unsuccessful, the life cycle of the Australian *M. rosenbergii* was fully closed in 2008. All hatchery production in this year came from broodstock harvested from DPI&F ponds. In 2008, four hatchery runs (27 Feb to 16 May) produced larvae hatched from females stocked in the DPI&F pond as postlarvae in 2007, thereby closing the life cycle. Survival was generally lower than 2007 when wild broodstock were used. Survival was recorded at between 40-68% and postlarvae harvested on 25 to 35 days after hatch. A total of 65 000 PL were produced; these are currently under nursery culture and will be used for stocking ponds in northern Queensland Barramundi farm. (Further information on 2008 research is attached as Appendix 1) Ms. Malwine Lober, an ACIAR-sponsored MSc student from Samoa has involved in all aspects of *M. rosenbergii* production, particularly broodstock management, larval and nursery culture and has learnt all skills needed for the production. She also has a first authored paper being accepted for publication in 'Aquaculture' and two papers under preparation. Additionally, there were other students and a visiting scholar from Indonesia, Mexico and Japan involved in the project and received training, broadening the training impacts beyond the original plan.

Objective 3: To assess the acceptance by farmers of the new species/techniques and to develop appropriate husbandry extension packages.

no.	activity	outputs/ milestones	completion date	comments
3.1	Establish model farms for trialling and demonstration of culture techniques for various species	At least five model farms in the project area and at least two in highlands Farmers fully conversant with subsistence aquaculture techniques for their area and willing to share knowledge	Ongoing until project termination (December 2010); post-project activities are continuing.	Two farms assisted in the higher altitude and Tabubil areas; both stocked with Fly River herring. Five farms were assisted in the Kiunga area. Only sleepy cod were stocked, crayfish could not be introduced due to initial difficulties faced in breeding. Farmers were trained but require further guidance. Provincial government fisheries has an active extension program that will continue after the project's completion.
3.2	Distribute crayfish juveniles and/or fish fingerlings to farmers for growout trials in production conditions	Farmers, including model farmers throughout the project area and further afield, are farming native species Aquaculture produce available in local markets Farmers trained and applying that knowledge in fish farming.		Omitted in accordance with mid-term review recommendation.

3.3	Assess performance and develop husbandry packages based on most appropriate production methodologies	Defined best-practice techniques adapted to specific regional peculiarities identified and adopted. Manual (for extension officers in NGOs, NFA, NDAL and provincial DAL) produced with guidelines on farm production methodologies		Project terminated before commencement of this activity.
3.4	Improve skills of provincial extension officers and NGOs in aquaculture techniques under guidance from NFA, to extend results of the project beyond the life and footprint of the project.	A base of trained extension people (NGO and provincial government) in provinces outside the immediate project area. Network of capable farmers developed to provide model farm examples for prospective fish farmers in conjunction with FIS/2001/083.		Project terminated before commencement of this activity

7 Key results and discussion

7.1 Activity 1.1: Assist OTML and Western Province government to develop two functional aquaculture facilities.

OTDF constructed six 5m x 10m ponds and a functional hatchery at their site at the Trust Yard. Equipment supplied as part of the scoping study was used to fit out the hatchery. This included three 2-tonne fibreglass tanks, three 75-litre *Artemia*/egg hatching tanks and an air blower, which supplied air to both the hatchery and the ponds. The hatchery and ponds were supplied with bore water through existing facilities at the site. A small air-conditioned laboratory room was also constructed.

Six 10m x 20m ponds water storage and water supply reticulation to the ponds were constructed at the Western Province facility (LARDEC) at Samagos. A weir was constructed across a water course allowing water to be gravity fed to the ponds, negating the need for pumps. The completion of the ponds and water supply in the early part of the project allowed the ponds to be utilized to hold and breed fish. A hatchery building was completed, although it was not in operation as the water and power supplies were not connected. This did not constrain the project as a functioning hatchery was available at the nearby Trust Yard. Six 2-tonne tanks, five 75-litre *Artemia*/egg hatching tanks, an air blower and other ancillary equipment were supplied to Samagos through the project to equip the hatchery.

7.2 Activity 1.2: Transfer and adaption to PNG of techniques developed in Queensland for production of freshwater species with initial emphasis on sooty grunter, eel-tailed catfish and sleepy cod.

Training and skills transfer

To assist in confidence and capacity building in PNG, two staff members each from OTDF and the Western Province government attended 10-day visits to Walkamin Research Station. Noel Tonko of OTDF and Kaupa Kia of Western Province government visited in November 2007 while Irene Kamang of OTDF and Waum Elemnop of Western Province government visited in November 2008. Havini Vira and Tike Kulingim of OTDF visited Walkamin under the scoping study in March 2005 with Havini Vira awarded a John Dillon Fellowship to visit Australia from September to November 2006. These visits to Walkamin allowed PNG staff to view and participate in farming activities that the project was aiming to achieve in PNG. The PNG personnel gained practical experience in farming and harvesting redclaw crayfish, successful hormone-induced spawning of eel-tail catfish fish and sooty grunter, and were part of the daily routine of feeding and water quality monitoring. With the assistance of the Atherton Tablelands Stocking Group, which spawned sooty grunter prior to the visit by PNG personnel, the visitors were able to view the larval rearing and feed production techniques of sooty grunter in the latter stages of their culture. Visits to commercial barramundi and redclaw farms were also included in their program.

Results in PNG showed that the project was starting to build the capacity to develop an aquaculture industry in the Western Province. Facilities were constructed by OTDF and Western Province government staff, broodstock successfully collected from the river and stocked into ponds. Visits to Walkamin Research Station by PNG staff were invaluable in exposing the staff to successfully operating facilities that were conducting the same activities that the project was aiming towards in PNG, thereby giving credibility to the Australian team members in the eyes of PNG staff. These visits not only demonstrated the husbandry skills but showed practical skills in constructing and adapting materials into required equipment that they could implement themselves. This experience led to the construction of practical facilities in PNG such as the hatcheries and ponds.

Periodic visits from DEEDI staff allowed the skills and knowledge to be built on over time. By being present and working along side, but allowing the PNG staff to take the lead role in activities, the DEEDI staff guided and assisted to build confidence in the PNG staff in their own abilities. Through the extended visits by DEEDI staff the importance of the daily routine, observations and record keeping by the PNG staff was instilled. Also by being on site Australian staff could view activities and correct misguided practices.

Fly River Herring (Nematalosa papuensis)

Introduction

Although Fly River herring was not among the species proposed for this project, it was included because several attributes make it an easy species with which to learn fish farming skills. Fly River herring breed naturally in ponds, are prolific breeders and feed mainly on algae and detritus, negating the need to be fed expensive processed feeds. Herring can be used directly for human consumption (wild-caught herring are currently sold in Kiunga markets) or used as a feed source for carnivorous fish such as sooty grunter or sleepy cod. Research supported by ACIAR has been carried out to investigate the harvesting of herring from the lagoons of the Fly River to produce fish meal. Possibilities exist for the culture of herring in high organic-content water such as at intensive pig farms to produce herring for fish meal or to feed fresh to pigs and chickens as suggested by Reynolds (n.d.).

Methods

Broodstock fish were collected from the oxbow lakes off the Fly River near Kiunga and transported back to the ponds at LARDEC. OTDF provided the logistics to undertake the field trips to collect the broodstock, while OTDF and Western Province staff ensured all landholder issues were resolved. Herring do not transport well and need careful handling, well-aerated water and minimum time in transit to survive transportation. To maximise chances of successful collection, in-country staff undertook this activity rather than local fishermen.

As herring breed naturally in ponds and do not need hatchery facilities for breeding, they were allocated to the LARDEC ponds where the hatchery was built but not functional. Western Province staff provided constant water supply to the ponds to ensure high dissolved oxygen levels for the fish. As the ponds were constantly flushed, fertilisation of the ponds was not considered necessary for increasing productivity of the pond. The water from the supply weir provided food and nutrients for natural pond production to sustain the herring.

Results

From June 2008 to June 2009, approximately 2000 herring fingerlings were produced ranging in size from 25 to 75 mm. Most of this production was achieved in the first half of 2009. Due to a severe water shortage in the later part of 2009 most of these fingerlings were lost.

Discussion

Due to transport constraints Western Province staff were unable to distribute herring stocks to farmers' ponds for evaluation. An impediment to aquaculture of herring is that they need careful handling to transport live. When catching, contact with the fish needs to be kept to a minimum as scales are easily dislodged, and transport water needs to be well aerated.

A prolonged dry spell and leaks in the supply weir resulted in a water shortage in late 2009. Western Province staff were repairing the leaks with the aid of OTML as the project ended. Power was also about to be supplied to the ponds, allowing their aeration and thereby reducing the water required to maintain high dissolved oxygen levels in the ponds. Less flushing of the ponds would allow them to be fertilised, leading to greater herring productivity.

Reference

Reynolds L. F. (no date, but late 1960s) Fish culture in fertilized ponds. Department of Agriculture, Stock and Fisheries, Fisheries Research, Kanudi, PNG. *Sighted in* Aquaculture in Papua New Guinea. Reports from 1950 to 2005, accompanying disc for ACIAR monograph no. 125.

Sleepy Cod (Oxyeleotris selheimi)

Introduction

Sleepy cod were selected as it breeds naturally in ponds and is highly regarded as a food fish. As sleepy cod are carnivorous, the aquaculture potential was considered to be in co-culturing herring with sleepy cod. Herring would supply a food source for the cod, with excess production of herring in the pond providing additional production. Due to their placid behaviour, sleepy cod are easily transported and handled. This species can be stocked at very high densities and is very suited to grow-out in recirculation aquaculture systems.

Methods

Western Province staff facilitated the collection of broodstock by purchasing fish from local fishermen or collecting fish themselves. As hatchery facilities were not required to breed sleepy cod, the broodstock were kept in ponds at LARDEC. The total number of broodstock stocked into the pond was three males and two females. The sex ratio of the broodstock was dictated by the fish collected.

Breeding was achieved by using practices adapted from Herbert and Graham (2004), who provided spawning structures and removed the eggs from the broodstock pond. In this project the fish spawned adhesive eggs onto surfaces in the pond such as the vertical surfaces of the concrete outlet structure. The eggs were not removed from the broodstock pond but left to hatch; the larvae were then grown in the broodstock pond. As the broodstock were left to breed naturally, spawning was continuous when environmental conditions were suitable. Feeding of the broodstock and larvae was through natural production of plankton and aquatic insects, and by herring stocked into the pond. Water was constantly supplied to maintain high dissolved oxygen levels and to replace water lost to leakage. This also supplied nutrients and plankton from the supply weir to the pond.

Results

From June 2008 to June 2009, approximately 2000 sleepy cod fingerlings were produced ranging in size from 25 to 100 mm. Most of this production was harvested from the pond in June

2009 and stocked into a cage in the pond. Due to a severe water shortage in the later part of 2009 most of these fingerlings were lost.

Although the ACIAR project was terminated, the broodstock fish remained in the ponds and continued breeding, but no further information was available on activities.

Discussion

Sleepy cod bred well in the ponds with minimum care, demonstrating their suitably for village-based farming. The need to buy and transport fingerlings from hatcheries is negated. Greater productivity could be achieved by stocking the eggs into fertilised ponds to ensure greater larval survival.

As reported in the herring discussion above, the water shortage was caused by a severe dry period and leaks developing in the supply weir. Western Province staff with support from OTML were endeavouring to repair the leaks.

Reference

Herbert B., Graham P. (2004). Breeding and fecundity of the endemic Australian gudgeon, sleepy cod *Oxyeleotris lineolatus* (Steindachner 1867) (Eleotridae). *Aquaculture* 236, 241-252.

Eel-tailed catfish (Neosilurus ater)

Introduction

Haines (1979) and Smith (2000) identified the aquaculture potential of eel-tailed catfish. It is highly regarded in PNG as a food fish and has high cultural significance. They feed on insects, crustaceans and molluscs. Although not yet tested, co-culture with crayfish would seem a suitable approach to allow the catfish to feed on recruitment from crayfish breeding, producing a mixed crop of crayfish and catfish.

Methods

Broodstock fish were purchased from local fishermen, facilitated and funded by OTDF. As eel-tailed catfish require hormone induction to spawn, the broodstock were kept in ponds at the Trust Yard where a functional hatchery was available. Nine females and three males were held in an aerated pond. The sex ratio was dictated by what was available for purchase. Chicken pellets and floating barramundi pellets were fed each afternoon to supplement the natural production in the ponds. Hormone dose rates and spawning techniques were adapted from Cheah and Lee (2000), while larval rearing experience with other species (sooty grunter) was utilised for larval rearing of catfish. Further information on larval rearing was also gained from experience at Walkamin when PNG staff visited.

Five hormone-induced spawnings were attempted but only one attempt produced larvae. This spawning was the result of hand stripping of milt and eggs as conducted by Cheah and Lee (2000). Previous attempts to achieve natural spawning in tanks were unsuccessful.

Although unsuccessful, these attempts to produce larvae provided an important training exercise. The repetition of the procedure reinforced the skills and planning required, as well as giving valuable knowledge on the spawning condition of the fish, which can only be gained from practical experience. No information exists, either published or anecdotal, on predicting spawning condition from the oocytes of catfish. The only known reasonably reliable method is the degree of distension of the female's abdomen. Before each spawning attempt a sample of oocytes was cannulated from the female and observed under a dissecting microscope with an average oocyte diameter calculated.

In September 2009 successful spawning and larval production were achieved. After anesthetising with AQUI-S and cannulating, two females with the largest oocyte diameter were injected with 1ml/kg Ovaprim while two males were injected at 0.5ml/kg at 09:00h. One female and one male were each stocked into a static, aerated 2-tonne tank with the water temperature maintained at 28°C. After 24 hours the fish were anaesthetised, hand stripped and fertilised by Noel Tonko using his experience of stripping rainbow trout. After measuring the volume and diameter of the water-hardened eggs, the fertilised eggs were stocked into an aerated 2-tonne tank with continuous water flow at approximately 2 litres/minute. The aeration was not strong enough to lift the eggs in suspension as the eggs are negatively buoyant.

The number of eggs was calculated from the egg diameter and volume following Hogan (1990). Prior to hatching, viable egg percentage was calculated, allowing the predicted number of larvae to be estimated. After hatching, the tank was drained and the larvae transferred to 50-l conical tanks and supplied with *Artemia* nauplii each day, then inspected for signs of feeding. On day 5 after hatching the larvae commenced feeding on the *Artemia*. Five days later the larvae were stocked into a fertilised pond.

Results

The results of the spawning are summarised in Table 2.

Table 2. Eel-tailed catfish (*Neosilurus ater*) spawning

Date	Oocyte diameter	Egg volume	Egg diameter	Calculated no. of eggs	% fertilisation	No. of larvae
8 Sept 2009	1.8 mm	200 ml	2.0 mm	28 000	2%	500

Mortalities were experienced in the hatching tank after the eggs had hatched, but no data are available on how many surviving larvae were stocked into the pond. When the pond was drained on 2 December 2009 only three fingerlings were recovered, with an average total length of 75 mm after 10 weeks.

Discussion

Poor broodstock conditioning due to poor nutrition, or above-optimal water temperatures may have led to reduced quality and quantity of oocytes, leading in turn to the very low fertilisation rate. This may indicate the surviving larvae were of poor quality, resulting in the low survival to fingerling stage. Maximum water temperature reached during 2009 was 36.2°C with a minimum of 25.7°C and an average of 31.0°C. No data are available on optimum or limiting water temperatures for eel-tailed catfish. Oocyte diameters of only 1.8 mm were recorded in this study, compared to oocyte diameters of 1.91 to 2.0 mm reported by Cheah and Lee (2000). Spawning of Australian catfish at Walkamin during visits of PNG staff resulted in fish spawning naturally in the tank, oocyte diameters of 2.2 mm and a latency period of less than 24 hours.

Good-quality broodstock is the key to successful breeding. Larger pond size may assist with better conditioning of the broodstock, allowing for lower stocking densities. This would allow the fish to feed on natural production of aquatic insects etc giving a more nutritional diet. Larger ponds would also have a moderating effect on the maximum water temperatures.

It is not known if this growth rate of eel-tailed catfish would still be achieved at higher fingerling densities (there is no published information on this). The growth rate of the small sample harvested is encouraging, as compared with barramundi extensively reared in ponds reported to

reach 20-30 mm total length after about 3 weeks (Rimmer and Russell 1998). Hogan (1990) reported sooty grunter grows to 40-50 mm TL in 40-50 days.

Knowledge gained from cannulating the female broodstock prior to hormone injection has led Noel Tonko to predict that an oocyte diameter of greater than 1.8 mm may result in successful hormone induction spawning of eel-tailed catfish.

References

Cheah M. S. H., Lee C. L. (2000) Induced ovulation of the Australian eel-tailed catfish *Neosilurus ate*r (Perugia) with Ovaprim. *Asian Fisheries Science* 13, 87-96.

Haines A. K. (1979) Highland Aquaculture. *Sighted in* Aquaculture in Papua New Guinea. Reports from 1950 to 2005, accompanying disc for ACIAR monograph no. 125

Hogan A. E. (1990). Notes on the production of sooty grunter (*Hephaestus fuliginosus*). Fisheries Branch Department of Primaries Industries Unpublished report.

Rimmer M. A., Russell D. J. (1998) Aspects of the biology and culture of *Lates calcarifer*. In Tropical Mariculture, De Silva S. S. (ed.) Academic Press. pp 449- 476.

Smith P. (2000) Report on: Inland Fisheries Research Project in Papua New Guinea. Stage 1. University of Western Sydney. *Sighted in* Aquaculture in Papua New Guinea. Reports from 1950 to 2005, accompanying disc for ACIAR monograph no. 125. Canberra 2007.

Sooty Grunter (Hephaestus fuliginosus)

Introduction

Haines (1979) identified sooty grunter as having aquaculture potential in PNG due to its omnivorous diet. It is generally hardy and easy to maintain. Breeding trials carried out in the scoping study were therefore continued with in this project. Sooty grunter has been bred in Queensland since the 1980s for stocking into impoundments for recreational fishing, initially by DEEDI but more recently by community-based stocking groups. It has not been farmed commercially in Queensland.

Methods

Broodstock were purchased from local fishermen on the Elevala River, a tributary of the Fly River. OTDF facilitated the purchase and travel to collect the fish from the fishermen. Twelve broodstock fish consisting of 9 females and 3 males were housed in two ponds at the Trust Yard. They were fed floating barramundi pellets each afternoon.

Spawning-induction techniques were adapted from Hogan (1990). Ovaprim was used in this project at 1 ml/kg for female and 0.5 ml/kg for males.

Four hormone-induction spawning attempts were undertaken on sooty grunter at the Trust Yard hatchery, with two attempts producing larvae. The unsuccessful attempts were an important training exercise. The continued repetition of the procedure reinforced the skills and planning required as well as giving valuable knowledge on the spawning condition of the fish, which can only be gained from practical experience. No information exists, either published or anecdotal on predicting spawning condition of sooty grunter. The only known reasonably reliable method is the degree of distension of the female's abdomen.

On 25 March 2008 two females and two males were selected after anaesthetising with AQUI-S. The males were selected on the ability to extract milt with gentle squeezing of the abdomen, while the females with the larger abdomens were selected. The fish were injected with hormone

at 10:00h and stocked into two static 2-tonne tanks. Water temperature was maintained at 28°C and a water exchange was carried out 24 hours later. The fish were checked for spawning 31 hour after injecting, at 17:00h, and rechecked at 07:00h the following day.

Results

One female spawned with the eggs being removed from the tank with a plankton net. Egg volume and diameter were recorded. The eggs were placed into a 50-litre hatching vessel with aeration sufficient to keep the eggs in suspension. Prior to hatching, viable egg percentage was calculated allowing the number of larvae expected to be estimated. The results of the spawning are summarised in Table 3.

Table 3. Sooty grunter *(Hephaestus fuliginosus)* spawning

Date	Egg volume	Egg diameter	Calculated No. of eggs	% fertilisation	No. of larvae
25.Mar.2008	300 ml	1.9 mm	50 000	5%	2 500
25.Nov.2009	200 ml	2.0 mm	28 000	10%	2 800

Forty-eight hours after hatching, *Artemia* nauplii were stocked into the larval tank and the larvae monitored for feeding. Three days after hatching all surviving larvae were feeding. The larvae were fed *Artemia* for five days before stocking approximately 1000 surviving larvae into a bloomed pond. No fish were recovered when the pond was drained 8 weeks later.

On 25 November 2009 another spawning was undertaken. The methods used were the same as the previous spawning with two females and two males being injected. The results of the spawning are summarised in Table 3.

After feeding for seven days on *Artemia* in the hatching tank, approximately 1000 larvae were stocked into a fertilised pond. When the pond was harvested on 8 February 2010, 83 fingerlings, average total length 40 mm were harvested from the pond. These fish were distributed to farmers in the Kiunga area. No further information was available on these fish due to the organisational changes and the early termination of the project.

Discussion

The very low fertilisation rates (5 and 10 percent) may have been the result of inadequate female spawning condition, which may have been affected by poor nutrition or high water temperatures. Maximum water temperature reached during 2009 was 36.2°C with a minimum of 25.7°C and an average of 31.0°C. No data are available on optimum or lethal water temperatures for sooty grunter. Experience from Australia is that sooty grunter broodstock need to be kept at low densities in ponds to develop into breeding condition. The Trust Yard ponds were small (sufficient only to hold four broodstock fish), and larger pond size may assist in moderating the high water temperatures, resulting in better broodstock conditioning. They may also reduce fish densities, and therefore interactions, as sooty grunter can be aggressive towards each other (Hogan 1990).

A pilot study conducted at Walkamin Research Station showed sooty grunter grew from an average of 0.4 g to 66 g in one year (unpublished results). One thousand six hundred fish were stocked into a 0.03 Ha pond and fed a commercial native fish pellet. Maximum weight gained was 160 g with a minimum of 14 g. These preliminary results suggest sooty grunter is not an attractive aquaculture species.

References

Haines A. K. (1979) Highland Aquaculture. Sighted in Aquaculture in Papua New Guinea. Reports from 1950 to 2005, accompanying disc for ACIAR monograph no. 125.

Hogan A. E. (1990). Notes on the production of sooty grunter (*Hephaestus fuliginosus*). Fisheries Branch, Department of Primaries Industries. Unpublished report.

7.3 Activity 1.3: Investigate plant-based, bacterial floc based, and /or mechanical treatment systems for improving barramundi production and management in freshwater aquaculture systems in Queensland.

Introduction

Water conservation, reuse and release to the environment are increasing issues to be addressed in aquaculture. The Australian Barramundi Farmers Association (ABFA) has identified water re-use and water discharge issues as a major research area.

Queensland government is becoming more stringent, and aquaculture farmers are required to move towards zero discharge to the environment under both State and Federal policy and legislation. The Great Barrier Reef Marine Park Authority (GBRMPA) is increasingly scrutinising farming practices adjacent the Great Barrier Reef Marine Park. In 2009 an updated reef water quality protection plan was endorsed by the Queensland and Australian governments. The goal of the plan is to halt and reverse the decline of water quality from land-based activities, and prevent this water from entering the reef lagoon.

The purpose of this study was therefore to investigate the effectiveness of plant-based treatment systems to remove nutrients and suspended solids from barramundi aquaculture pond water before returning the water to the culture system. The objectives of the trial were to reduce the water requirements of the culture system by recirculating the water, while not adversely affecting the growth and behaviour of the fish, and to measure the effectiveness of the plants in removing nutrients and suspended solids.

Prior to the early termination of this project, two trials were completed, one using duckweed *(Spirodela punctata* and *Wolffia angusta)* and the other using lotus (*Nelumbo nucifera*). The trials were carried out at Walkamin Research Station, on the Atherton Tablelands, north Queensland. The trial using lotus commenced in January 2009 and terminated prematurely in May 2009 when the decision was made to suspend aquaculture research at Walkamin Research Station.

Methods

The commencement of the bioremediation research was delayed until the appointment of the new project leader in April 2007 to replace Brett Herbert. A very severe winter was experienced in 2007 during the first trial with duckweed, causing fish mortalities. The ponds were restocked with fish and the trial recommenced in January 2008. This trial was terminated in December 2008 due to the onset of summer storms and the possibility of frequent power supply interruptions. It was determined there was an animal welfare issue since the emergency aeration system in place was potentially insufficient for the biomass of fish in the ponds.

The same experimental design and pond configuration were used for both the duckweed and lotus trials with adaptation to accommodate the two plant types (as discussed later in the aquatic plant management section), but with the results analysed separately for the two trials.

Trials with both duckweed and lotus consisted of two treatments with three replicates per treatment. *Treatment 1*: Water from the fish culture pond recycled through a pond containing aquatic plants for treatment. This treatment is referred to as “water re-use treatment” and consists of two ponds per replicate: one to culture the fish (referred to as the fish culture pond), and one to treat the water before returning to the fish pond (referred to as the treatment pond). *Treatment 2*: Fish ponds with static water except for water exchanges to maintain water quality for fish health (NH_3-N less than 1 mg/l following Rimmer 1995).This treatment is referred to as ‘static’ ponds and consists of one fish culture pond but no water re-use pond.

Pond configuration

Walkamin Research Station has 24 ponds designed for replicated research, all lined with high-density polyethylene (HDPE) and predator proofed with bird netting over the ponds. Each pond is 0.034 ha in size, 250 000 litres volume and a maximum depth of 1.7 metres. The ponds are in four rows of six ponds with each row slightly lower than the one above. Two adjoining rows of ponds were required for each trial.

The fish culture ponds for the trial were grouped in one row of six ponds and randomly assigned a treatment method. If a pond was assigned the water re-use treatment, it was paired up with the pond above as the water-treatment pond. The water from each fish culture pond in the water re-use treatment was treated by the paired pond immediately above (Figs. 2, 3).

Water was pumped from the lower fish pond, to the pond above for treatment (Fig. 2). The static water treatment does not have a treatment pond connected to the fish pond and the ponds located above the static water treatment fish ponds were not used in the trial (Fig. 3).

The treatment ponds were partitioned with three internal baffle walls (Fig. 2) constructed of weed matting. As water flows around alternate ends of the baffles, it increases the contact time of the water in the pond.

The baffles also minimise the disturbance of the duckweed mat by wind by reducing the area the duckweed is contained in (Figure 4). A large area of duckweed allows the wind action to push the duckweed to one end of the pond, rather than spreading evenly over the surface (Zirschky and Reed 1988, Skillicorn et al. 1993, Willett 2005).

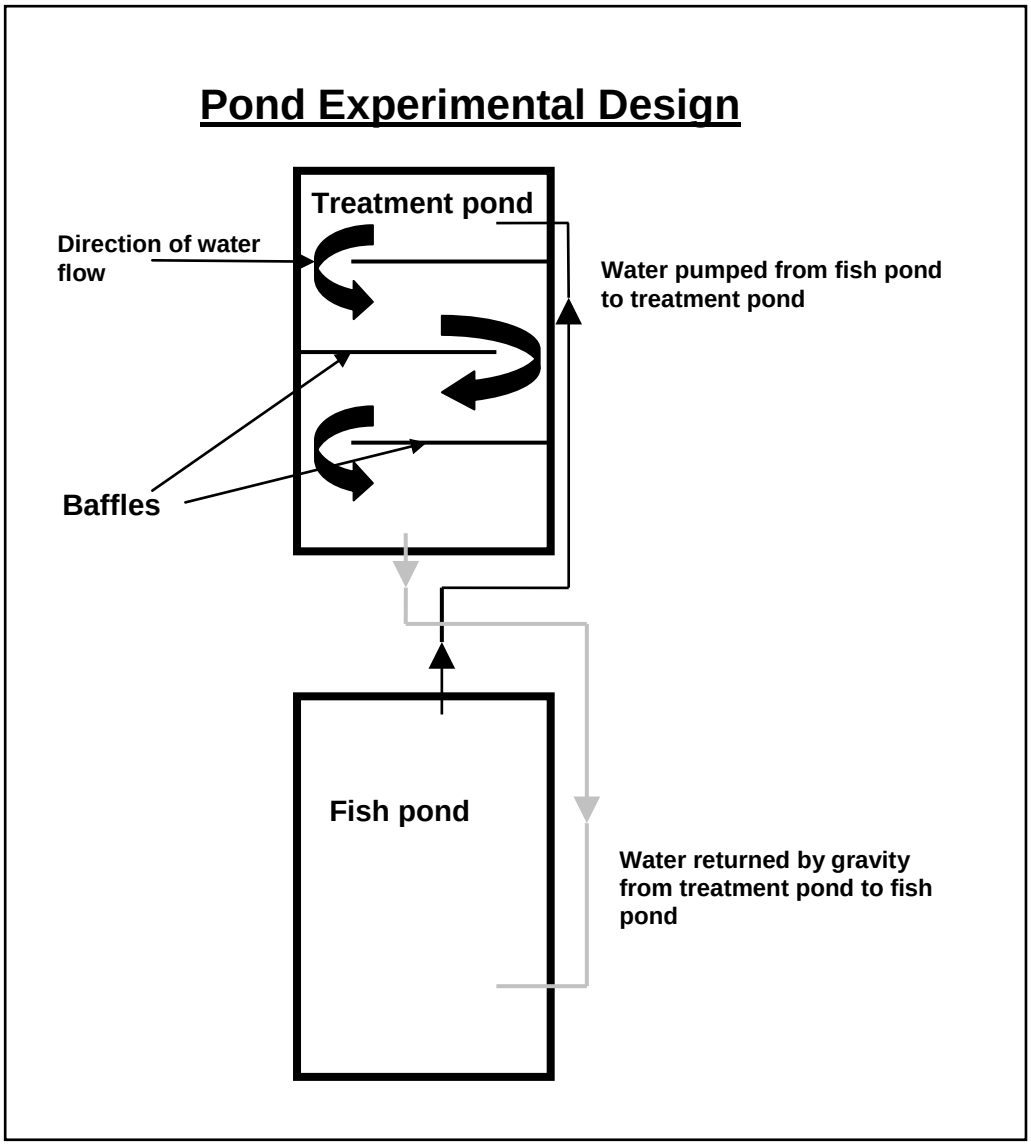

Figure 2. Diagram showing the pond configuration for the water re-use treatment.

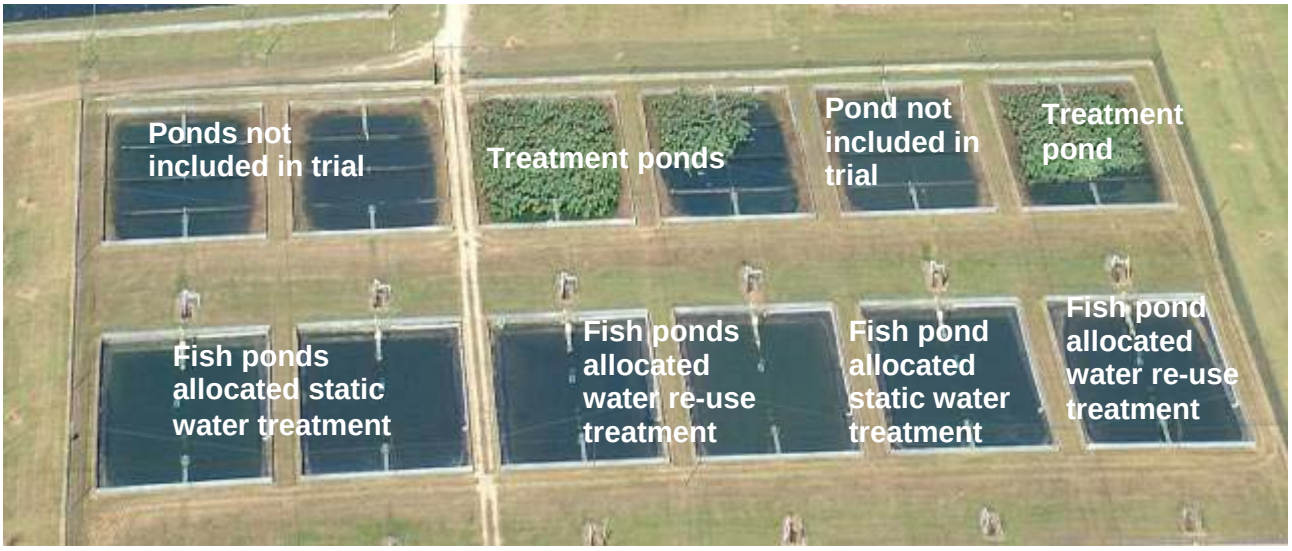

Figure 3. Pond configuration for the lotus trial.

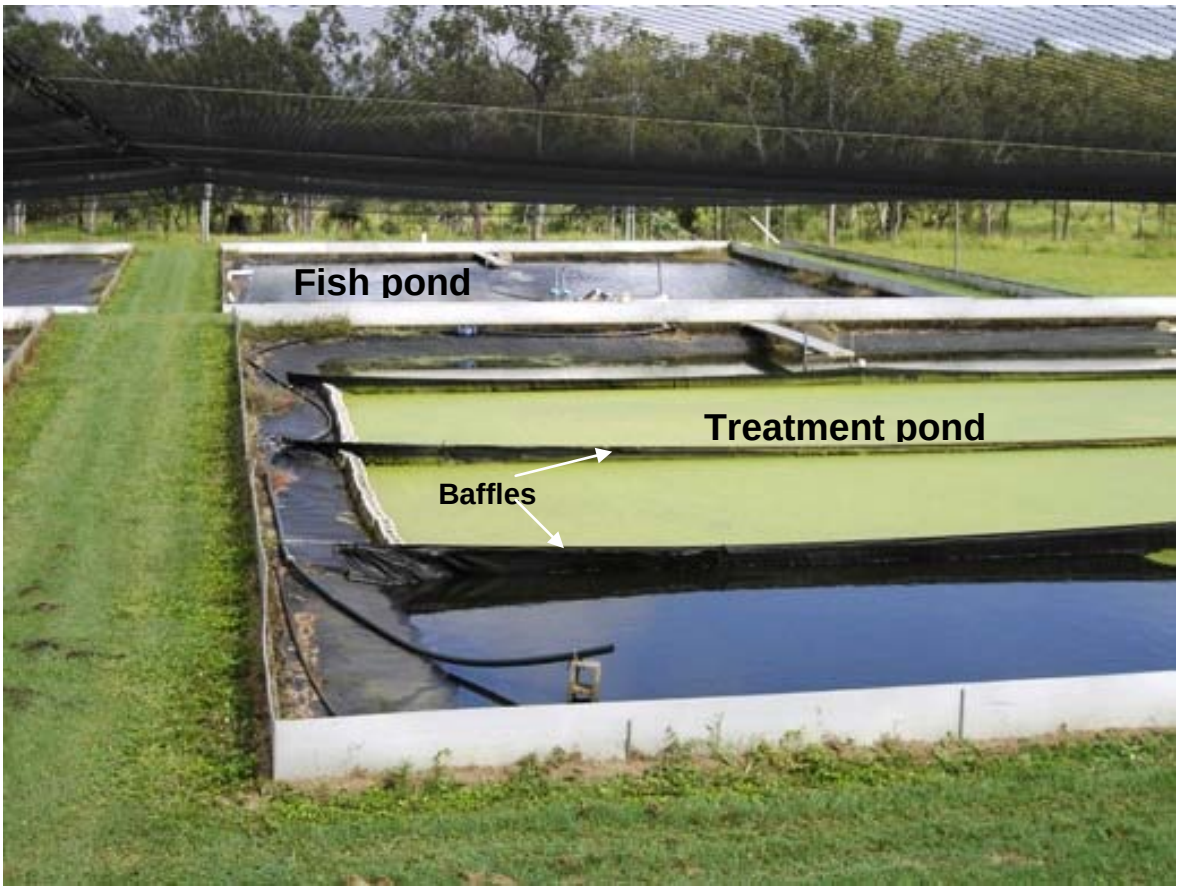

Figure 4. The experimental unit with the treatment pond using duckweed in the foreground and the fish pond below (rear).

Twenty-eight percent (approximately 70 000 litres) of the treatment pond volume was exchanged each day for both duckweed and lotus trials, giving a retention time of the water of 3.5 days. This exchange rate has been reported to reduce total nitrogen by 38% (Willett 2005).

Pumping was from 17:00h to 23:00h daily to transfer the required volume of water from the fish ponds to the treatment ponds. Flow meters were monitored daily to ensure the volume pumped between the replicates was equal. The pump intake was located approx 75 cm below the water surface. The water returning from the treatment ponds to the fish ponds was sourced from approximately 10 cm below the surface of the treatment pond by an internal stand pipe. Fig. 5 illustrates this stand pipe with the water level lowered to allow the stand pipe to be viewed.

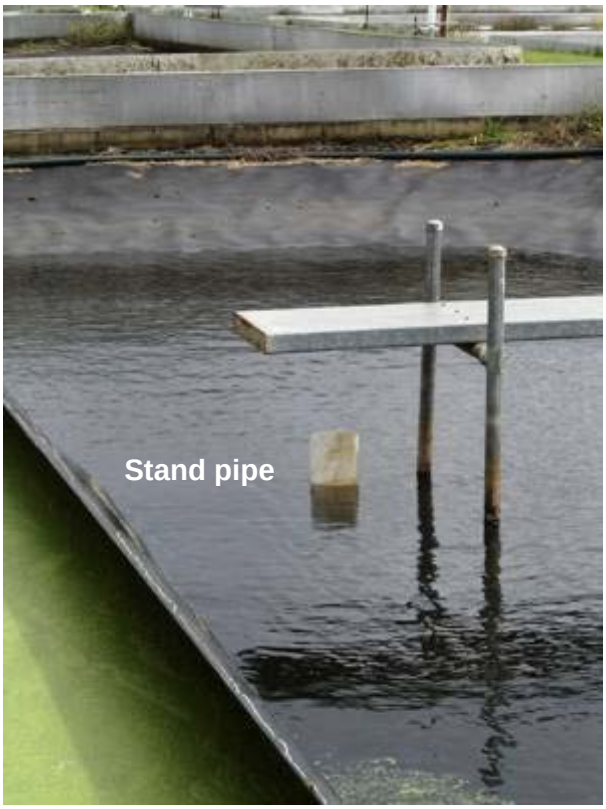

Figure 5. The internal standpipe, the top of which is 10 cm below the water surface, taking the water to be returned to the fish pond from the surface of the treatment pond. The water level has been lowered to allow the stand pipe to be exposed for the photo.

Figure 6. The external stand pipe structure allowing water to overflow from the treatment pond back to the fish pond.

The water was gravity-fed from the treatment pond back to the fish pond via an external stand pipe (Fig. 6) This stand pipe controlled the water level in the treatment pond and allowed the water to flow back to the fish pond as the water level rose in the treatment pond with pumping,

Experimental Procedure

Although they were different experiments, the same experimental procedure was used in both the duckweed and lotus trials. Each pond in the duckweed trial was stocked with 594 barramundi (average weight 0.412 kg), while the lotus trial ponds were each stocked with 300 barramundi (average weight 2.2 kg). The barramundi were fed to satiation once daily in the afternoon using a commercial floating barramundi diet. The weight of food fed to each pond each day was recorded. Constant aeration via an air blower was provided to all fish ponds to maintain oxygen levels and to prevent temperature stratification in the ponds. Additional aeration when required was provided, usually overnight, by 1.1 kW aspirators. The water treatment ponds were not aerated.

The barramundi were stocked into the ponds and left to settle for three weeks prior to initial weights and measurements being recorded; the trial then commenced. The fish were measured for growth bi-monthly when the water temperature was above 25^{o}^{C}. (Growth slows at water temperatures below 25^{o}^{C} and feeding ceases at 20^{o}$^{C: Rimmer 1995}$.) To reduce stress and avoid disease problems, the fish were not handled when the water was cool. The fish were not fed the day before handling to reduce the chance of injury or stress. For the duckweed trial, total length, standard length and weight were recorded for 50 individual fish randomly captured from the population of 594 fish in each pond. For the lotus trial, 20 fish were randomly sampled from the population of 300 fish per pond, with the same measurements recorded. The fish were anaesthetised with Aqui-S prior to being measured and given a salt bath at 10 ppt before returning to the pond. All fish mortalities during the trial were recorded.

Oxygen, pH, temperature and turbidity were measured in the morning (between 08:00h and 08:30h) and afternoon (between 15:00h and 16:00h) every day using a TPS 90FLT meter. Readings in the water treatment ponds were made at the surface, 20 cm below the surface, and at the bottom (1.4 m below the surface). The bottom reading was taken 30 cm above the actual pond bottom to prevent the probes coming in contact with the bottom sediments to avoid false readings. These data collection points are referred to as “surface” and “bottom” in the results. Only one reading was taken in the fish ponds as there was no stratification due to the constant aeration. Temperature loggers were installed in all ponds, one logger in each of the fish ponds while the water treatment ponds had one logger on the bottom (1.7 metres) and another 0.2 metre below the water surface. As with the water quality data, these data collection points are referred to as “surface” and “bottom” in the results. The loggers recorded temperature each hour, and daily maximum, minimum and average temperatures were extracted.

Water lost from the ponds through evaporation or leakage was replaced and recorded by refilling using flow meters. Water exchanges (releases and refills) undertaken in the static ponds to maintain water quality were also measured and recorded. No water was released from the water re-use ponds into the surrounding environment.

Water chemistry analysis was carried out fortnightly on water samples collected at 08:00h. One sample was taken from each of the fish ponds beside the pump intake, and one sample from each of the treatment ponds taken beside the top of the internal stand pipe returning the water to the fish pond. Parameters analysed were suspended solids, total nitrogen, total phosphorus, ammonia (NH_3-N), nitrate and nitrite. Analysis of alkalinity, total calcium and total hardness was carried out each month. These parameters were analysed at Walkamin using a spectrophotometer and Hach brand reagents. All water samples were filtered to 0.45µm prior to analysis except for total nitrogen and total phosphorus. Supplementary NH_3-N samples were taken if it was considered necessary to monitor fish health. Methodologies such as sample collection and storage of samples prior to analysis, were checked for errors and spectrophotometer accuracy confirmed by creation of standard curves.

Water for the ponds was sourced from Lake Tinaroo via the irrigation channel. As the alkalinity of the channel water was only 30 mg/l, 42 kg of agricultural lime was spread in each fish culture pond prior to filling.

Water exchanges in the static fish ponds to reduce NH_3-N to less than 1 mg/l to maintain fish health (Rimmer 1995) were carried out after the fish had been fed. The pond water level was reduced by 20 cm and required approximately 37 000 litres to refill (approximately 15% water exchange). The ponds were refilled overnight using a flow meter to measure the volume required to refill the pond. It was judged that lowering the pond water level further than 20 cm to facilitate higher volumes of water exchange may stress the fish and lead to reduced food intake.

Aquatic Plant Management

Duckweed

The duckweed to inoculate the water re-use treatment ponds was sourced from a local barramundi farmer's ponds. The initial inoculation rate was approximately 0.2 kg of duckweed per square metre of culture bay surface using two species of duckweed *Spirodela punctata* and *Wolffia angusta* (identified by the Queensland Herbarium). Seed stock sourced locally will be well adapted to the local climate and two or more species of duckweed will increase the range of environmental conditions within which the crop will grow (Skillicorn et al. 1993).) It is not necessary or desirable to plan for a monoculture of a particular species (Zirschky and Reed 1998).

Duckweed was introduced into the two middle bays of the partitioned water treatment ponds (Fig. 4). As the water was pumped into the top or shallow end of the treatment pond, duckweed was not inoculated into this bay as the incoming water would disturb the duckweed mat. The bay at the deep (discharge) end of the pond was not inoculated to avoid the possibility of the duckweed blocking the pipes returning the water to the fish pond.

An optimal base density of duckweed is required for nutrient recovery. Willett (2005) recommended an optimal base density of 1 kg/m^2 equating visually to a complete coverage of the surface by a single layer of duckweed fronds, and a bay or cell size of 16 to 50 m^2 for the growth of duckweed. As the bay size in this trial was 93 m^2, larger than recommended by Willett (2005), a larger base density of 1.5 kg/m^2 duckweed was selected to provide a complete cover with greater wind action over the larger bay size. The total area of the two bays populated with duckweed was 186 m^2 or 55% of the pond area.

The drained wet weight of duckweed in the pond was estimated bi-monthly by collecting and weighing six 0.25 m^2 subsamples per bay. Once the base density of duckweed reached 1.5 kg/m^2 excess duckweed above this biomass was removed and weighed. Removal of excess duckweed above the base density allowed the duckweed to continue growing and remove nutrients. At the end of the trial the total amount of duckweed in each pond was estimated by subsampling.

Lotus

Native lotus seed was sourced from Ross River in Townsville, Queensland. The seeds were germinated and planted into approximately 0.2 m depth of soil over the HDPE lining of each lotus treatment ponds. Lotus plants were allowed to cover the entire area of the re-use pond.

As the trial commenced the re-use treatment ponds had an almost complete covering of lotus plants. The only removal of plant material from the lotus ponds during the trial was the minimum required to ensure the water outlet from the treatment ponds remained unblocked.

A 48-hour monitoring of the nutrient levels was undertaken in February 2009 during the lotus trial to ascertain if there was diurnal variation in the dissolved nutrients in the fish ponds. Water

samples were taken every 2 hours and the following parameters measured: dissolved oxygen, pH, temperature, NH_3-N and NO_2-N. All six fish ponds were monitored.

Results

Duckweed trial

For reasons unknown, the first two attempts to inoculate the treatment ponds were unsuccessful. Duckweed was successfully introduced into the treatment ponds in May 2008 and the first harvest of duckweed took place in September 2008, 20 weeks later. A total wet weight of 4245 kg of duckweed including the base density was produced over the life of the trial. The growth rate was an average of 60.5 gm/m^2/day from the base density of 1.5 kg/m^2, measured from when the duckweed reached the desired base density.

Analysis by *t*-test showed no significant differences in individual fish weight (and therefore growth) (p=0.823), biomass (p=0.420) or survival (p=0.314) between the water re-use and static water pond treatments. Average individual fish weight across the replicates of 1886 g was achieved in the water re-use ponds, and 1880 g in the static water ponds. In the water re-use ponds, final survival was 95.8% and average pond biomass 33.4 tonnes per hectare equivalent. In the static water ponds final average survival was 92.9% and average biomass 32.4 tonnes per hectare equivalent (Table 4). Analysis showed survival over the winter period was significantly lower than the other three weigh periods (ANOVA, p=0.012).

Increase in total fish biomass over the course of the trial is shown in Table 4. The average food conversion ratios (FCR) achieved over the course of the trial—1.38:1 for the water re-use treatment and 1.35:1 for the static water ponds—was not statistically significant (p=0.848) (Table 5). Table 5 presents the FCRs for the individual fish weigh periods.

Table 4. Average weight per fish and equivalent biomass in static and re-use ponds.

Date	**Biomass (t/ha)**		**Av. wt. (g)**	
	Re-use	Static	Re-use	Static
08-Jan-08	7.64	7.62	414	410
06-Mar-08	15.99	15.80	868	853
25-Sep-08	26.05	23.76	1460	1360
20-Nov-08	31.53	30.07	1800	1728
09-Dec-08	33.37	32.40	1886	1880

Table 5. Cumulative survival and food conversion ratio (FCR) for barramundi in the static-water and reused-water treatments.

Time interval	**Cumulative survival (%)**		**FCR**	
	Re-use	Static	Re-use	Static
8.Jan – 6.Mar.08	99.8	99.8	1.01	1.01
6.Mar – 25.Sep.08	96.6	94.1	1.59	1.90

25.Sep – 20.Nov.08	96.3	93.7	1.52	1.30
20.Nov – 09.Dec.08	95.8	92.9	1.37	1.19

The average total water use for the static-water ponds over the course of the trial was 1 727 547 litres and for the re-use ponds 1 351 786 litres, 21.7% less water than the static water ponds.

Analysis of the nutrient data between each water re-use pond and its paired treatment pond showed the duckweed treatment ponds significantly reduced the levels of total ammonia, nitrite, nitrate and total suspended solids compared to the fish culture ponds (p<0.001). The average reductions after treatment for these nutrients were 52.3%, 55.7%, 61.8% and 38.1%. The average reduction in the level of phosphate was 13.7% but this was not a statistically significant difference (p=0.308). The average level of total nitrogen actually increased by 19.1% but again this was not significantly different (p=0.308). The variation between replicates of phosphate and nitrogen produced the non-significant result.

Table 6 presents the maximum, minimum and average values of the nutrients measured in the duck weed treatment ponds and the paired fish culture ponds. The percentage nutrient removed by the duckweed treatment pond is also presented. The data were averaged across the replicates for the duration of the trial.

Table 6. Water quality values obtained in the duckweed treatment ponds and its paired fish ponds.

	NH_3-N (mg/l)		**NO_2-N** (mg/l)		**NO_3-N** (mg/l)	
	Fish	Treatment	Fish	Treatment	Fish	Treatment
Max	2.40	0.91	0.19	0.14	1.75	1.01
Min	0.07	0.00	0.00	0.00	0.04	0.03
Mean	0.54	0.16	0.05	0.02	0.47	0.20
Mean % removed	52.23 (p<0.001)		55.69 (p<0.001)		61.82 (p<0.001)	

Table 6. continued.

	Total N (mg/l)		**PO_4** (mg/l)		**TSS** (mg/l)	
	Fish	Treatment	Fish	Treatment	Fish	Treatment
Max	6.55	12.12	0.41	0.31	85.33	35.33
Min	0.18	0.00	0.05	0.02	8.62	4.19
Mean	2.99	3.38	0.20	0.13	25.68	11.97
Mean % removed	-19.11 (p=0.308)		13.67 (p=0.185)		38.10 (p<0.001)	

The duckweed treatment ponds were stratified for the duration of the trial. The average difference in daily water temperature for the three ponds from the surface (20 cm below the surface) to the bottom (1.7 metres below surface) was 1.6°C with a maximum of 3.9°C (Fig. 7).

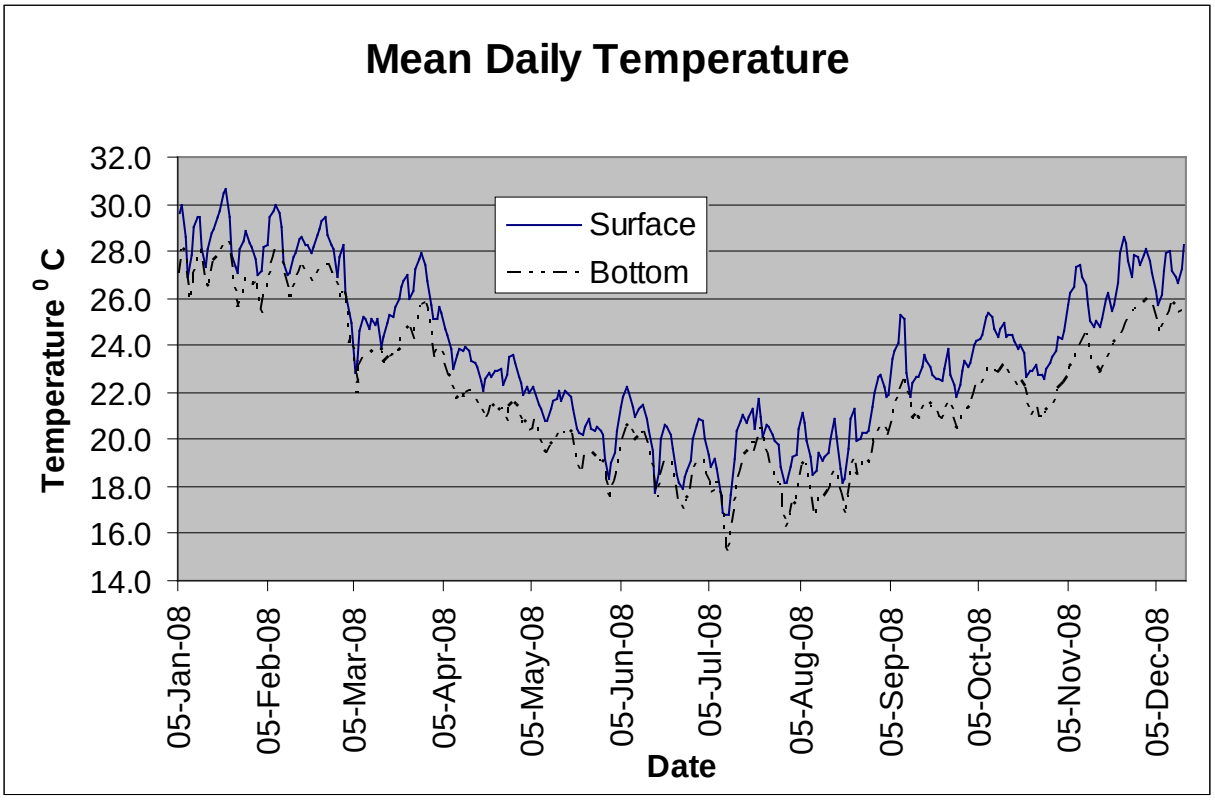

Figure 7. Mean daily water surface and bottom temperature in the duckweed treatment ponds.

Average daily water temperature in the water re-use and static water fish ponds were extremely similar (Fig. 8), indicating that the water treatment did not have any effect on the pond temperatures.

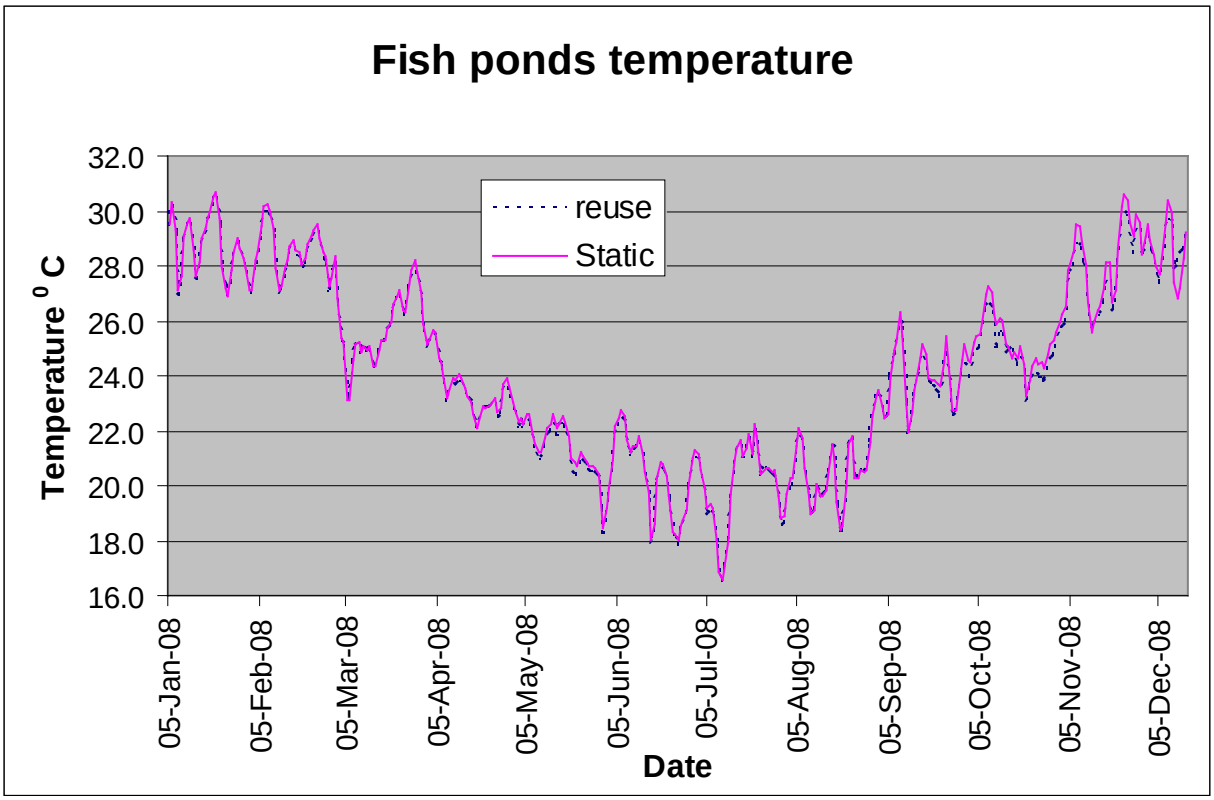

Figure 8. Mean daily water temperature for the duckweed water re-use and the static water fish ponds.

Pond stratification increased over the course of the study, as demonstrated by dissolved oxygen (DO) levels (Fig. 9). Stratification became more pronounced as the duckweed cover grew to its maximum 55% pond coverage. There was a marked decrease in the bottom oxygen level from approximately 8 mg/l at the beginning of August to approximately 2 mg/l at the beginning of September when the duckweed mat was complete and the first duckweed removal took place. As the duckweed mat consolidated, from 1 November to the end of the trial, the bottom DO average was 0.4 mg/l with a maximum of 1.0 mg/l and a minimum of 0.1 mg/l. The average daily afternoon surface dissolved oxygen was 15.1 mg/l, with a maximum of 27.6 mg/l (Figure 9). A saturated DO level of 8.8 mg/l at 18°C and the altitude at Walkamin of 594 metres indicates the duckweed treatment pond surface water was over-saturated in the afternoons.

The pooled replicate surface pH values of the duckweed treatment ponds demonstrated a large diurnal fluctuation especially towards the end of the trial with a maximum pH of 10.5 (Fig. 10). The maximum daily pH change from morning to afternoon was 3.4 with and an average daily pH change of 1.3.

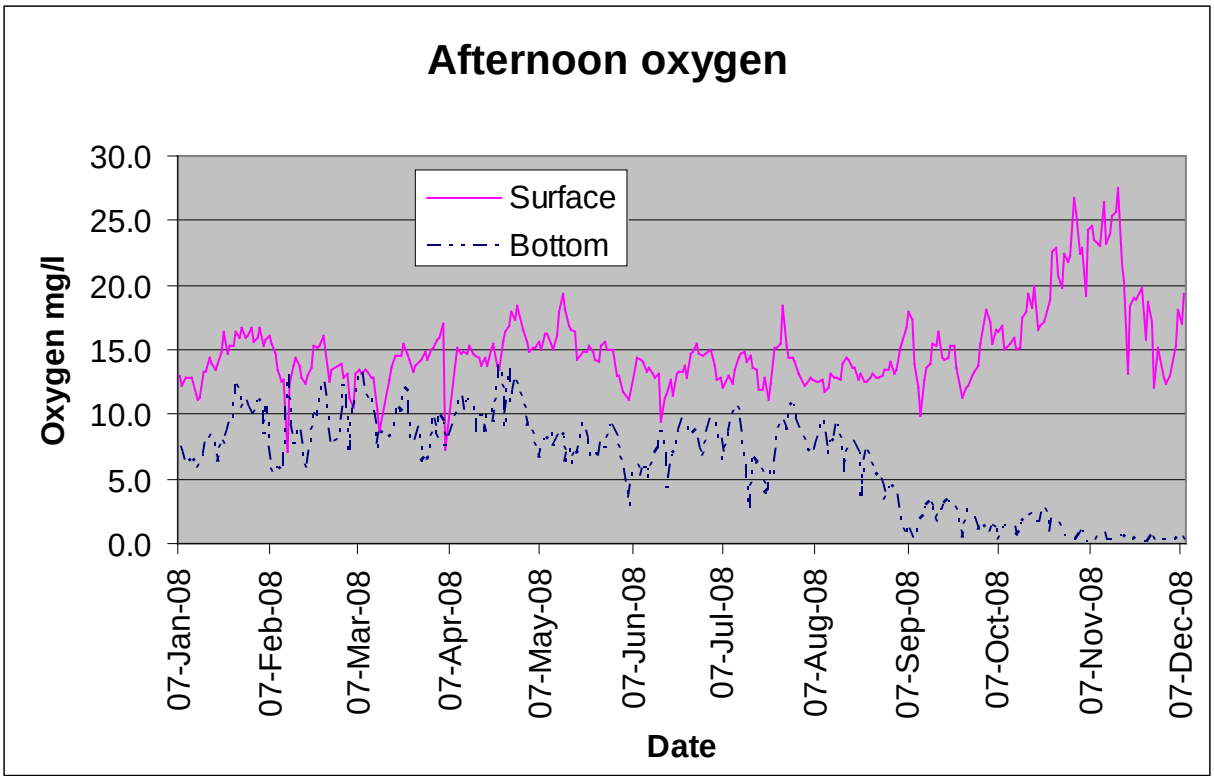

Figure 9. Daily afternoon surface and bottom dissolved oxygen in the duckweed treatment ponds.

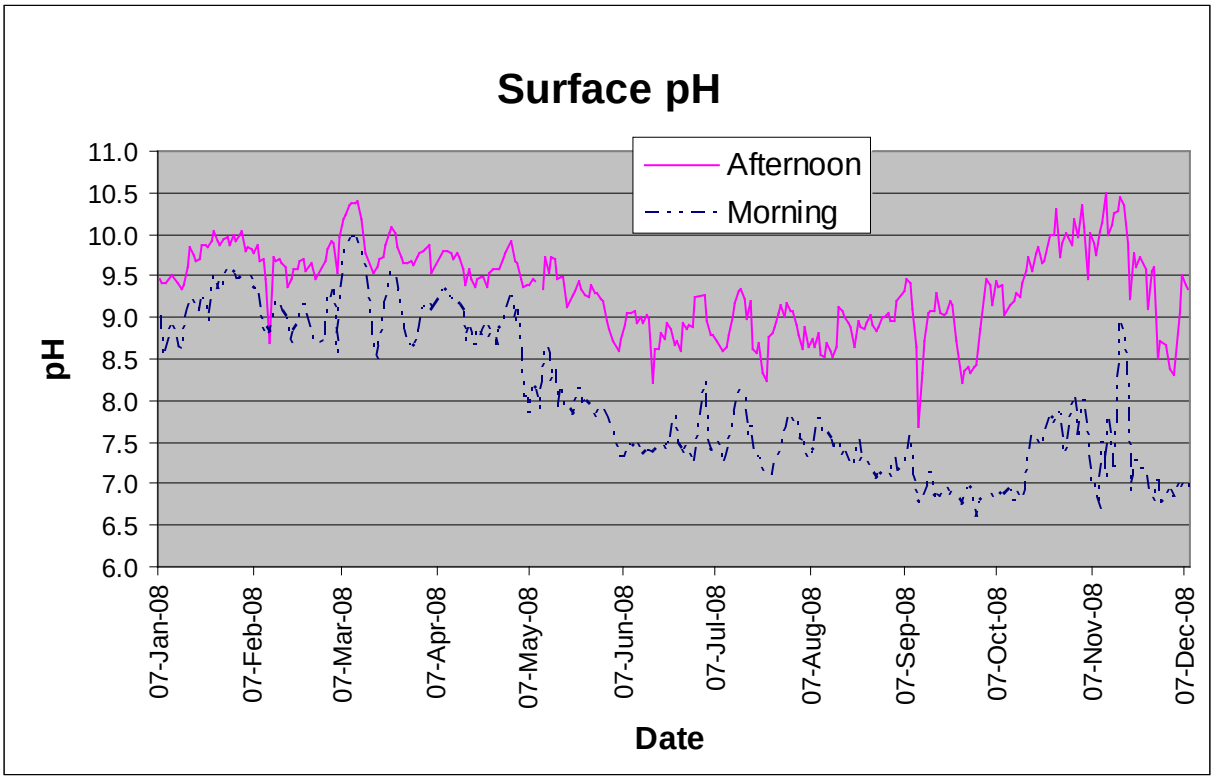

Figure 10. Daily surface pH values taken morning and afternoon in the duckweed treatment ponds.

Water quality values recorded in the fish culture ponds for the two treatments over the period of the trial (Table 7) showed no statistical differences between the treatments (p>0.05). All parameters listed in the table displayed a wide range of values, reflecting the changing circumstances the ponds experienced over the course of the trial. The temperature range of 14.4 to 32.8°C reflects the changing seasons from summer to winter. NH_3-N, NH_3 and NO_2-N ranged from zero to 6.92, 2.41 and 0.31 mg/l respectively, as fish biomass increased. The maximum oxygen level of 25.85 mg/l was well above dissolved oxygen saturation levels demonstrating algal photosynthesis in the ponds. This is supported by the maximum pH of 10.35.

Table 7. Maximum, minimum and mean water quality values experienced in the grow-out of the barramundi during the duckweed water re-use trial.

	NH3-N (mg/l)		**NH3** (mg/l**)**		**NO2-N** (mg/l)		**TSS** (mg/l)	
	Re-use	Static	Re-use	Static	Re-use	Static	Re-use	Static
Max	3.13	6.92	0.51	2.41	0.21	0.31	108.00	148.00
Min	0.01	0.00	0.00	0.00	0.00	0.00	8.20	4.00
Mean	0.54	1.15	0.11	0.20	0.04	0.09	25.68	44.17

Table 7 - continued.

	Alkalinity (mg/l)		**Oxygen** (mg/l)		**pH**		**Temp** (°C)	
	Re-use	Static	Re-use	Static	Re-use	Static	Re-use	Static
Max	105.00	95.00	25.85	21.42	10.18	10.35	32.5	32.8
Min	35.00	10.00	1.87	1.90	6.69	5.65	14.4	14.5
Mean	60.82	52.25	9.57	9.36	8.11	7.92	24.1	24.3

Lotus trial

There were no significant differences in individual fish weights, (p = 0.884), biomass in ponds, (p = 0.910) or survival (p = 0.391) between the treatments of water re-use and static water. Average individual fish weight across the replicates of 3155 g was achieved in the water re-use ponds, while the static water fish ponds achieved 3181 g. In the water re-use ponds a final survival of 99.7% and average pond biomass of 29.5 tonnes per hectare equivalent were achieved. In the static ponds a final average survival of 99.4% resulted in an average biomass of 29.7 tonnes per hectare equivalent. Table 8 presents the cumulative survival data for the fish weigh periods and the FCR values. Table 9 presents the fish growth measured over the period of the trial with a calculated biomass.

The food conversion ratios (FCR) achieved over the total period of the trial was 1.60:1 for the water re-use treatment and 1.75:1 for the static water ponds. This difference was not significant (p = 0.666).

Table 8. Survival and food conversion ratio (FCR) for the individual weigh periods for the two treatments of the lotus trial.

Interval	**Cumulative survival**		**FCR**	
	Re-use	Static	Re-use	Static

15.Jan – 12.Mar.2009	99.8%	99.8%	1.60	2.14
12.Mar – 7.May.2009	99.7%	99.4%	1.61	1.48

Table 9. Average individual fish weight and equivalent biomass for each of the two treatments of the lotus trial.

Date	**Biomass** (t/ha)		**Average Wt.** (g)	
	Re-use	Static	Re-use	Static
15.Jan2009	20.5	21.3	2183	2273
12.Mar.2009	25.0	24.8	2668	2648
7.May.2009	29.5	29.7	3155	3181

The average total water usage of the replicates for topping up water in the re-use ponds was 167 449 litres, and the static water ponds water changes used 446 683 litres. Thus, the water re-use ponds used 62.5% less water than the static water ponds.

Analysis of the nutrient data showed that the lotus treatment ponds significantly reduced the levels of all nutrients from the paired fish ponds. The average reductions of NO_2-N and NO_3-N were 87.5% and 91.9% respectively, while NH_3-N, and total nitrogen reduced by 45.4% and 35.7%. The reduction in PO_4 and total suspended solids was less: 19.6% and 33.0% respectively. Table 10 presents the maximum, minimum and average values, as well as the mean percentage of nutrient removed from the lotus treatment ponds and the paired fish culture ponds.

Table 10. Water quality values obtained in the lotus treatment ponds and the paired fish ponds.

	NH_3-N (mg/l)		**NO_2-N (mg/l)**		**NO_3-N (mg/l)**	
	Fish	Treatment	Fish	Treatment	Fish	Treatment
Max	2.63	2.33	0.08	0.02	1.60	0.22
Min	0.214	0.02	0.00	0.00	0.18	0.03
Mean	0.77	0.46	0.03	0.00	1.60	0.05
Mean % removed	45.39 (p<0.001)		87.48 (p<0.001)		91.90 (p<0.001)	

Table 10 - continued

	Total N (mg/l)		**PO_4 (mg/l)**		**TSS (mg/l)**	
	Fish	Treatment	Fish	Treatment	Fish	Treatment
Max	11.69	7.14	1.96	2.16	37.50	28.40
Min	0.00	0.00	0.71	0.52	5.60	0.80
Mean	3.47	1.69	1.22	0.96	14.14	7.33
Mean % removed	35.68 (p=0.030)		19.59 (p=0.005)		32.95 (p=0.058)	

The temperature in the lotus treatment ponds demonstrated stratification, but this decreased towards the last month of the trial (Fig. 11). The difference in average daily water temperature

for the 3 ponds from the top (0.2 metres below the surface) to the bottom (1.7 m below surface) was 0.8°C with a maximum of 2.1°C.

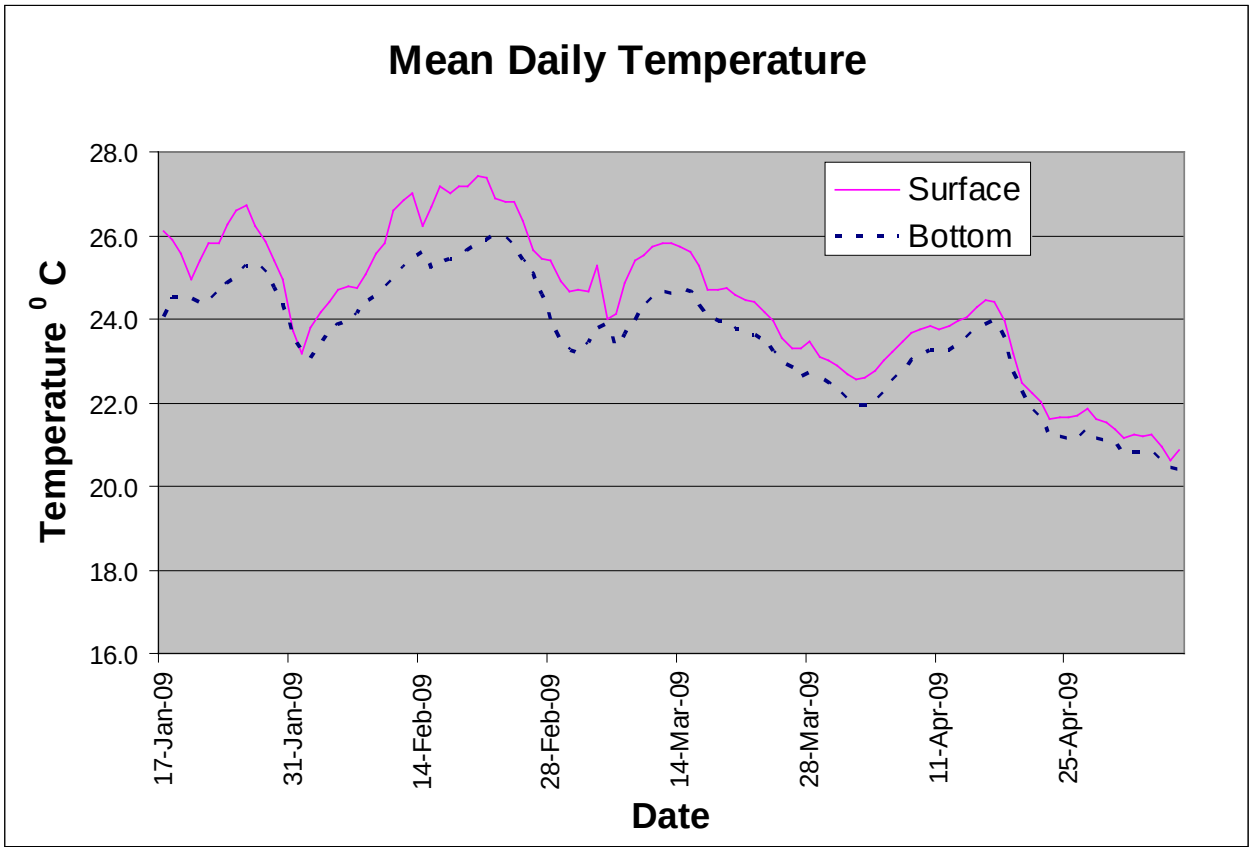

Figure 11. Mean daily surface and bottom temperature in lotus treatment ponds.

The pooled replicate afternoon dissolved oxygen data (Fig. 12) demonstrates stratification in the lotus treatment ponds. The average daily difference between top and bottom was 2.4 mg/l with a maximum difference of 6.1 mg/l. The maximum bottom dissolved oxygen during the trial was 0.6 mg/l with a minimum of 0.1 mg/l. The average was 0.3 mg/l. The maximum afternoon surface dissolved oxygen was 6.3 mg/l, demonstrating that oxygen over- saturation did not occur.

The pooled replicate surface pH values in the lotus treatment ponds (Fig. 13) demonstrated only a small diurnal fluctuation especially towards the end of the trial. The maximum pH daily change was 0.8, with an average change of 0.2. The maximum pH in the lotus treatment ponds for the duration of the trial was 7.5.

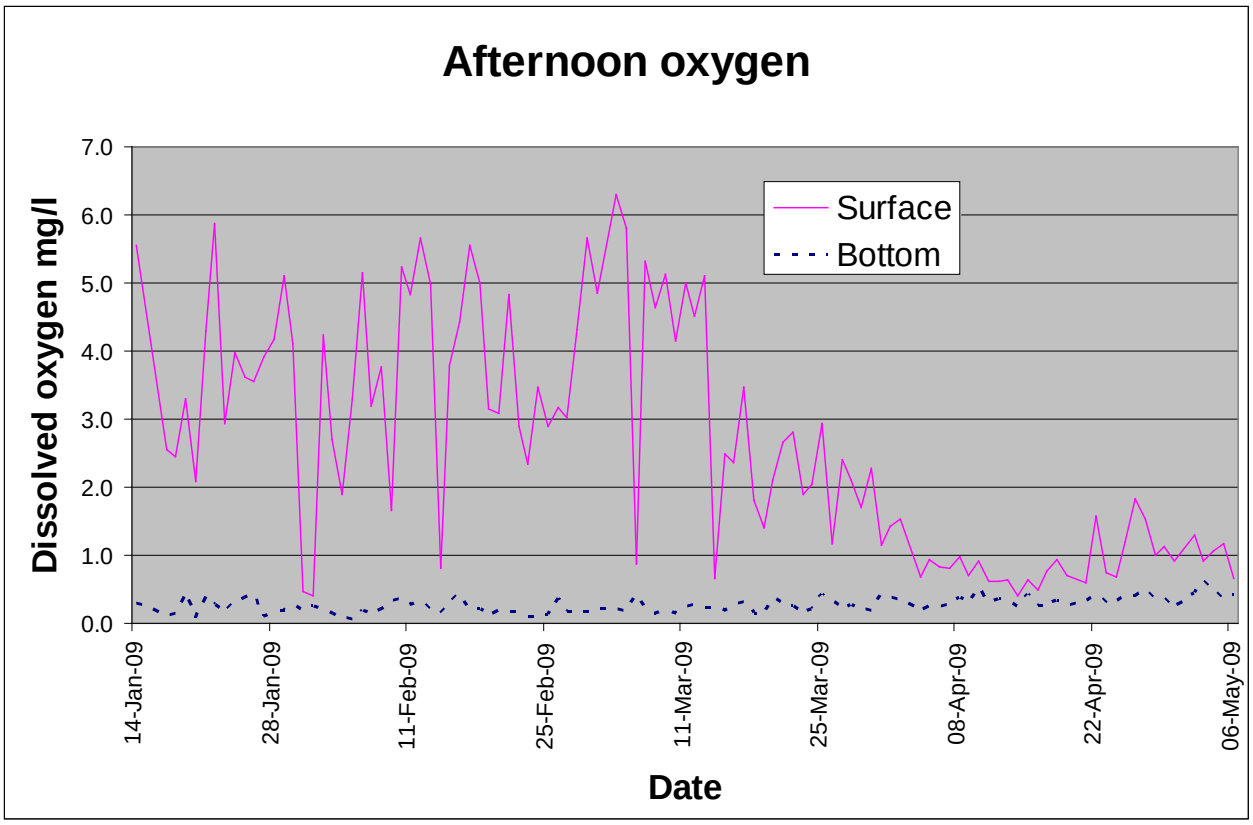

Figure 12. Daily afternoon surface and bottom dissolved oxygen in the lotus water treatment pond.

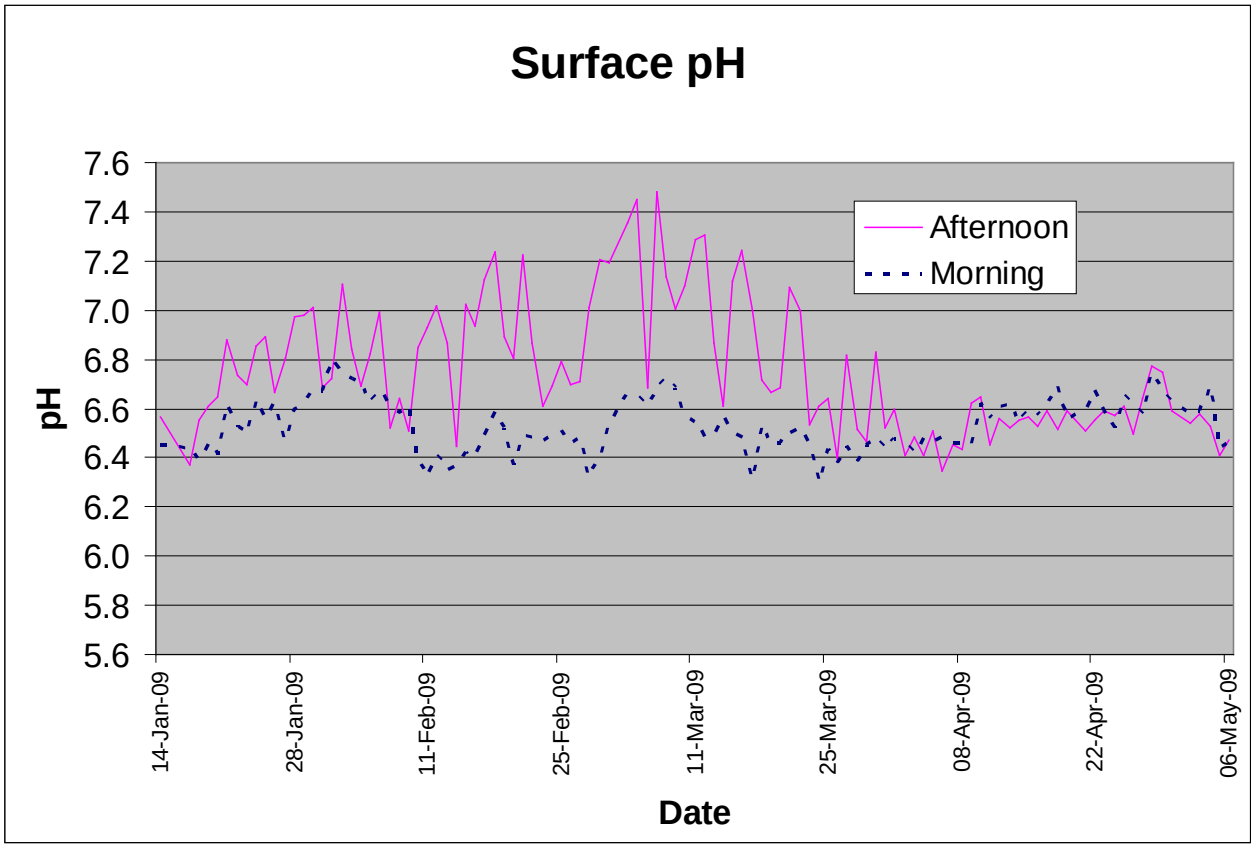

Figure 13. Surface daily pH measured morning and afternoon in the lotus treatment ponds.

Daily average temperature in the water re-use and the static fish ponds are shown in Fig. 14. Minimum, maximum and mean daily temperature differences between the two pond types were 0.2°C, 1.1°C and 0.7°C, respectively.

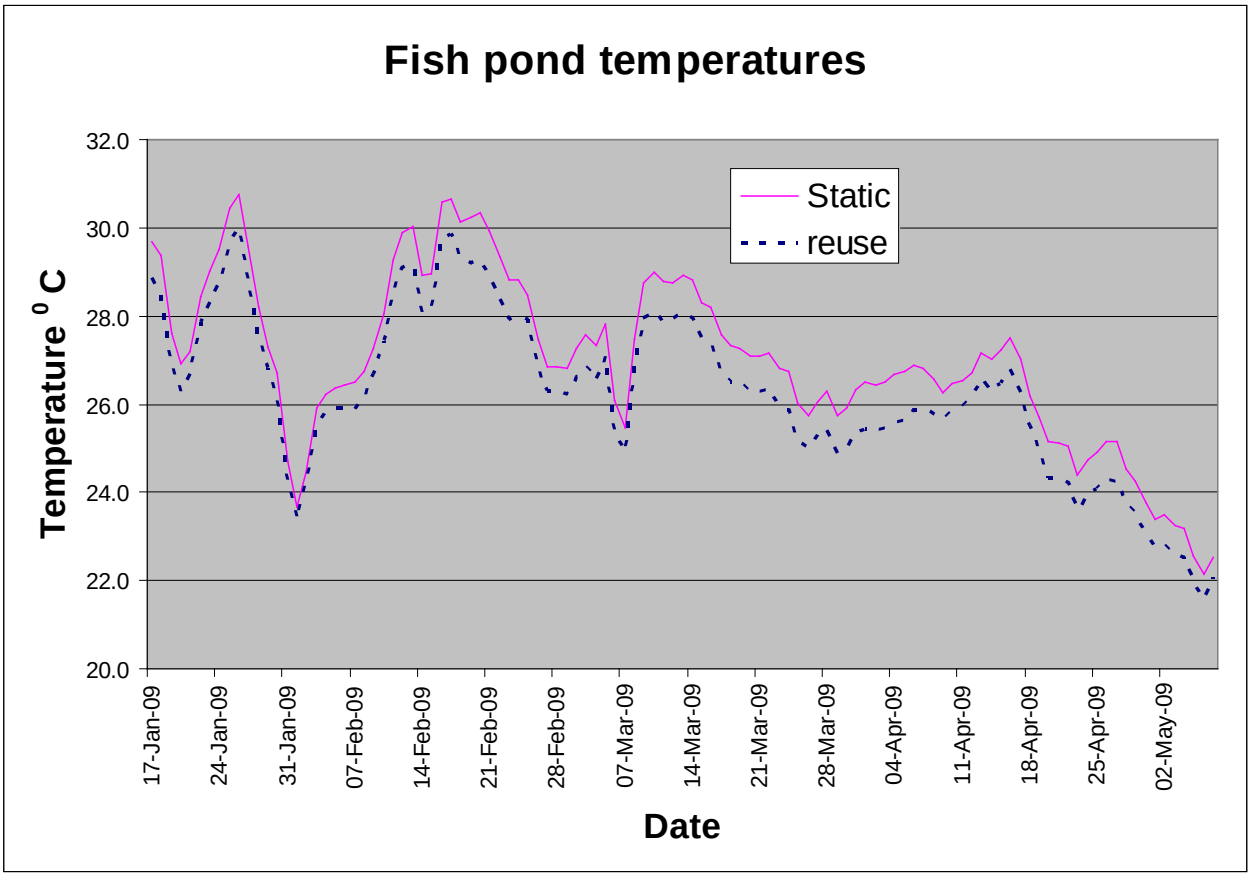

Figure 14. Mean daily water temperature for the lotus water re-use fish ponds and the Static fish ponds.

The average daily temperature of the pooled lotus treatment ponds was 1.9°C lower than the paired water re-use fish ponds. The maximum was 3.3°C cooler in the lotus ponds to the fish ponds demonstrating the cooling effect of water treatment by shading by the lotus on water temperature of the ponds.

Water quality values experienced in the fish culture ponds for the treatments over the period of the trial are listed in Table 11. Statistical analysis demonstrated there were significant differences for NH_3, NO_2-N, TSS and alkalinity, while NH_3-N, dissolved oxygen, pH and temperature were not significantly different. The maximum values attained of 2.6 mg/l for the re-use treatment and 6.3 mg/l for the static treatment demonstrate less variation in the re-use system than in the static system. Maximum oxygen values in both the water re-use fish ponds and the static water fish ponds of 20.8 mg/l and 20.0 mg/l respectively demonstrate algal activity in the water as these values are above dissolved oxygen saturation levels. The value of 20.8 mg/l oxygen in the water re-use fish ponds contrasts with the maximum dissolved level of 6.3 mg/l in the lotus treatment ponds (Fig. 11).

Table 11. Maximum, minimum and average water quality values experienced in the growout of the barramundi in the lotus trial.

	NH_3-N (mg/l)		**NH_3** (mg/l)		**NO_2-N** (mg/l)		**TSS** (mg/l)	
	Re-use	Static	Re-use	Static	Re-se	Static	Re-use	Static
Max	2.67	6.34	0.27	0.49	0.08	0.86	37.50	60.00
Min	0.05	0.01	0.00	0.00	0.00	0.01	5.60	9.50
Mean	0.70 (p=0.110)	1.05	0.05 (p=0.013)	0.10	0.03 (p<0.001)	0.12	14.14 (p<0.001)	31.16

Table 11 – continued.

	Alkalinity (mg/l)		**Oxygen** (mg/l)		**pH**		**Temp** (°C)	
	Re-use	Static	Re-use	Static	Re-use	Static	Re-use	Static
Max	70.00	45.00	20.77	20.07	10.15	10.24	31.2	32.9
Min	30.00	20.00	4.10	2.72	6.54	6.36	20.4	21.1
Mean	45.42 (p=0.042)	35.42	7.85 (p=0.085)	8.76	7.52 (p=0.546)	7.61	26.3 (p>0.05)	27.00

48-hour nutrient monitoring trial

Results of the 48-hr monitoring showed there was a clear 24-hr cycle for NH_3-N and NH_3, but no clear cyclic trend was detected for NO_2-N. NH_3-N peaked 10-14 hours after the fish were fed while NH_3 peaked shortly after the feed at 16:30. Full results are presented in Appendix 2.

Discussion

Duckweed

The results presented show there was no difference in fish growth, survival or FCRs between water re-use through duckweed ponds and static fish ponds, demonstrating that water re-use did not have any effect on the fish. Water use savings of 21.7% was achieved by water re-use, demonstrating that water re-use can be used to make water savings without comprising fish production.

The growth rates of the barramundi was comparable to industry standards (Michael Heidenreich, pers. comm.), and the biomass of 33.3 t/ha achieved in the water re-use treatment was above industry average yields of 20.7 t/ha in 2007-2008 (Lobegeiger and Wingfield 2009)

FCRs were comparable to research standards and less than commercial farm conditions of 1.6-1.8 (Barlow et al. 1996). The FCR values of 1.9:1 recorded during the winter period of this trial is comparable with Barlow et al. (1996) who reported that FCRs often exceed 2.0:1 during winter.

The high level of survival demonstrated that the barramundi coped well with pond conditions. The proportionally greater drop in survival occurred during the winter period indicating that temperature may have the greater influence on mortalities. Cheong (1989 citing Kungvanki et al. 1984) found mortalities to rise when temperatures drop below 20°C over prolonged periods, and for barramundi to die within minutes when water temperature drops to 15°C (Cheong 1989 citing Wu pers. comm. 1989). The minimum temperature in this trial was 14.4°C (Table 3), the daily average temperature was below 20°C for 3 months (Fig. 9). Despite this, the lowest

survival in the treatments at the end of the trial was still 92.9%, indicating the fish survived these low temperatures well.

The wide pH range of 5.65 to 10.35 experienced in this study contrasts with Rimmer and Russell (1998) optimum ph range for barramundi of 7.5 – 8.5 with a minimum limit of >4, with an upper limit not quoted. The average pH recorded in this study was 8.01, which is within the optimum range quoted.

The average NH_3-N value for the duration of the trial in the static ponds was 1.15 mg/l which corresponds to water changes being undertaken when NH_3-N values were >1 mg/l.

The maximum toxic NH_3 (un-ionised ammonia) recorded in this study was 2.41 mg/l with an average 0.20 mg/l in the static ponds, which is well above the recommended upper limit of <0.46 and an optimum of 0 mg/l (Rimmer and Russell 1998). Since the level of NH_3 in the water increases with increasing pH and temperature (Boyd 1979), the amount of NH_3 will vary in a diurnal cycle. Thus the fish would not have been continually exposed to the maximum level of NH_3 in the water.

Rimmer and Russell (1998) quote a turbidity limit value of <10 ppm. This study had total suspended solids (TSS) maximum of 148 ppm with an average of 44.17 ppm. The TSS in the fish ponds was mainly due to algae which also lead to the wide range of pH values and high maximum dissolved oxygen levels in the fish culture ponds.

The health and condition of the fish relate directly to their ability to cope with suboptimal conditions. The fish in this study were in healthy condition throughout the trial, including the winter period when the fish are more susceptible to health issues. The development of better nutrition may allow the fish to cope with colder water temperatures (Williams et al 2006). Better nutrition may also assist the fish's ability to tolerate a wider range in other water quality parameters.

Water savings of 21% were achieved in this trial, even though water losses through leakage/evaporation were greater in the water re-use ponds due to double the pond area compared with static ponds (Fig. 4). Greater water savings would also be made the longer the fish were cultured. Experimental procedure required water re-use to be implemented from the beginning of the trial, even though water quality was still in acceptable limits. On a commercial farm water/energy savings can be made by modifying water management practices following those used here. At the beginning of the crop when fish biomass is low, natural plankton in the pond have sufficient capacity to assimilate toxic ammonia. Implementing water re-use only when water quality deteriorates will save on pumping costs and limit evaporation/leakage losses from the water treatment area.

Duckweed production of 60 g/m^2/day in this study is well below the 175 gm/m^2/day from a base density of 1 kg/m^2 reported by Willett (2005). Skillicorn et al. (1993) reports growth of between 50 and 150 g/m^2/day from a base density of 600 g/m^2, comparable with the results of this study. Duckweed growth is limited by availability of nutrients, as well as environmental and water quality conditions. The published studies used municipal waste waters to grow the duckweed, which could have different water quality parameters to the aquaculture waste water used in this study, giving different growth results.

A diurnal pH peak of 10 is possible in ponds only partially covered with duckweed because of algal activity (Zirschky and Reed 1988). A complete duckweed crop cover suppresses algal growth, which minimizes CO_2 production from algal respiration and its elevating effect on pH (Skillicorn et al. 1993). The results of this trial (Fig. 8) showed a maximum pH of 10.5 and a daily average pH variation of 1.3 indicating algal growth. This is supported by the afternoon dissolved oxygen levels above saturation (Fig. 7). Algal production in the duckweed ponds would have contributed to the nutrient removal and results may be different with a complete duckweed cover.

Figures 9 and 10 demonstrate dissolved oxygen was over-saturated in the afternoon and pH fluctuated daily up to 3.4 units, indicating algal activity in the duckweed treatment ponds. Since water samples were not filtered prior to total N and P analysis (see Methods), the analysis of the

samples would include algae contained in the sample. This may explain the increase of N by 19% (Table 6) from the fish pond to the treatment pond.

Removal of N in duckweed-covered systems can be attributed to N uptake by duckweed, microalgae in the water column and the attached biofilm on the duckweed and walls of the system, as well as coupled nitrification-denitrification by these biofilms (Korner et al. 2003). Zirschky and Reed (1988) found that most of the biological activity in a duckweed pond is caused by the microbial and other flora suspended in the water column. This supports the recommendation of the mid-term review team to investigate naturally occurring pond blooms for bioremediation.

Most of the references published on duckweed water treatment systems relate to treating effluent from municipal systems, intensive livestock systems and processing industries for returning waters back to natural systems. Little work has been carried out on a botanical approach to the treatment of aquaculture wastewaters (Redding et al. 1997). Because of this emphasis on municipal wastewater discharge to the environment, research has focused on the removal of total nitrogen and phosphorus. Although this would also be the case if aquaculture effluent water were to be released back to the natural waterways, water returned to fish culture systems require other water quality parameters to be monitored, such as NH_3-N, which can be toxic to fish at higher levels. While Willett (2005) concentrated on retention time for removal of total nitrogen, Porath and Pollock (1982) reported a retention time of less than 48 hours to remove 80% of ammonia using duckweed in tank recirculating aquaculture systems. As retention time is controlled by the volume of the treatment pond, a shorter retention time required will allow for a smaller volume of treatment in relation to the fish culture pond. This would be an area for further research.

Further research into duckweed as a water treatment for aquaculture is required to answer points raised in this discussion. A full cover of duckweed may lead to better nutrient absorption, less water evaporation and a stable pH. However, it may also lead to high hydrogen sulphide, low dissolved oxygen and lower water temperatures. The addition of a treatment area to aerate the water before returning to the fish culture pond may overcome these water quality issues. Research into retention times along with the ratio of treatment area to biomass of animals could lead to greater profitability of the system.

Uses for duckweed as an animal feed is well documented. Bio-Tech Waste Management Pty Ltd (1998) trialled duckweed as a feed for chickens, ducks, sheep, fish and abalone. While numerous papers promote duckweed as a low-cost feed for tilapia (Hassan and Edwards 1992, Skillicorn et al. 1993), none have actually compared costs of duckweed production to other feed sources. In developing countries where feed ingredients may be unobtainable and labour is the only commodity, duckweed may be a viable feed source to provide protein. This may be relevant to highland PNG, where protein supplies are short.

If duckweed is to be considered as a treatment option for aquaculture water, a viable means of harvest and use of the freshly harvested product would need to be developed.

Lotus trial

The lotus trials showed that water re-use using lotus does not affect the growth, survival and food consumption of the fish but significantly reduces water usage.

As with the duckweed trial, the lotus trial produced a fish biomass greater than industry standards of 20 tonne/ha (Lobegeiger and Wingfield 2009). FCRs of 1.6 were above research standards of 1.0 - 1.2:1 but comparable with commercial farm conditions of 1.6 - 1.8 (Barlow et al. 1996). The initial starting weight of the fish was 2.2 kg with a final weight of 3.1 kg. The literature does not report data on FCRs of barramundi grown this large.

Although survival was very high (>99%) over the course of the trial, the trial did not continue over a winter period and the minimum water temperature was above 21°C; the fish were therefore not subjected to the stresses of cooler water experienced in the duckweed trials.

Water savings of 62.5% were made in this trial by water re-use through the lotus treatment ponds. Some 14% of these savings were due to reduced evaporation because of lotus plant coverage of the water surface (Seymour et al. 2009).

The lotus treatment ponds were about 2°C cooler than the paired water re-use fish ponds, possibility due to shading of the water by the lotus vegetation. Daily pH variation was low, especially towards the end of the trial. The lotus cover completely shaded the pond, reducing algal growth, as shown by the constant level below saturation of dissolved oxygen, indicating the lack of algae, and consequently photosynthesis in the water body. As this trial did not continue through a winter period, further research would be needed to ascertain the water temperature differences during winter and the effect on barramundi growth and survival. Pumping times may need to be altered to prevent the water temperature in the fish pond being lowered by cooler water from the treatment pond; for example, pumping early morning when the temperature difference of all the ponds is least.

Kanabkaew and Puetpaiboon (2004) report after a retention time of 5.4 days lotus removed 52% total nitrogen, 80% NH_3-N, 55% total phosphorus and 67% of suspended solids from domestic wastewater. Removal from aquaculture water for these same nutrients in this study were 57%, 40%, 24% and 31% respectively, while Seymour (2009) reported 32%, 28%, 35% and 44% respectively. The greater nutrient removal reported by Kanabkaew and Puetpaiboon (2004) may be the result of greater retention time—3.5 days in this study and for Seymour (2009).

The significant differences in the mean of NH_3, NO_2-N and TSS between the water re-use and static fish ponds demonstrate the treatment ponds are efficient in removing these nutrients. The difference in alkalinity demonstrates the flushing effects of the water changes in the static ponds. Although the means of NH_3-N are very similar, the maxima and minima show greater variation in the static ponds than the re-use ponds. This is the result of the static fish ponds receiving batch water changes periodically when required to replace evaporative water loss, while the re-use fish ponds received a water treatment every day. The mean of NH_3-N in the static ponds of 1.05 mg/l compares with water changes being carried out when NH_3-N was above 1.0 mg/l.

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7.4 Activity 2.1: Collect freshwater crayfish from the Fly River catchment and evaluate growth and production characteristics.

Introduction

Redclaw crayfish are very suited to aquaculture, especially for subsistence-based cultures, as they are herbivores and can be cultured on inexpensive and locally available feeds. They are prolific breeders and breed naturally in ponds without the need for expensive facilities. They are less aggressive than other species of crayfish, allowing them to be cultured in high densities. If kept cool and moist they will live out of water for 24 to 48 hours allowing for easy transport to market. A small industry exists in Queensland producing 100 tonne per annum (Lobegeiger and Wingfield 2009).

Uses of crayfish at village level are for direct consumption by the farmer, sale at markets, or as a feed source for other fish species. As refrigeration is generally unavailable in PNG, having an aquatic animal that survives out of water has great benefits as it will not perish when taken to market for sale.

Methods

Broodstock were purchased from local fishermen at Kiunga by OTDF staff and stocked into a pond at the Trust Yard. Procedures for the culture of the redclaw crayfish were adapted from Jones (1990). The perimeter of the pond was fenced with a sheet metal barrier to prevent the crayfish walking from the pond. The pond was provided with aeration overnight and for 2 hours starting at midday to prevent temperature stratification. Fifteen habitat structures were also provided. The structures consisted of six 40-mm diameter PVC pipes, 300 mm in length bundled together lengthwise, with one pipe filled with concrete to weigh the structure to the

bottom. As the pond was being filled approximately 10kg of dry grass was put into the pond. Supplementary feeding to the natural pond production was supplied with locally sourced chicken pellets. Feed trays were used to judge feeding requirements. Dissolved oxygen and pH were measured daily with alkalinity measured when required, such as after heavy rain. Temperature loggers were installed to record the water temperature. After 3 to 4 months the pond was drained to ascertain the level of recruitment.

Particular attention was paid to the alkalinity of the pond water to ensure it was above 40 mg/l, and pH remained in the 6 to 9.5 range. Close observation was made each morning to detect crayfish mortalities.

Results

Three attempts were made to establish a breeding population of redclaw in a pond at the Trust Yard. Only limited numbers of broodstock were available from the local people due to adverse river conditions at the time broodstock were required. The first pond stocking was with a breeding nucleus of approximately 20 crayfish. The male to female ratio was not recorded. Four months after stocking the pond was drained revealing no stock left in the pond and no exoskeletons visible from deceased crayfish. After two failed attempts possible reasons for the failures were investigated. Possible causes were: theft, removal by predators, escapes due to inadequate pond fencing and outlet screening, poor water quality and high water temperatures.

To determine whether water quality was the problem a population of crayfish was held in a 2-tonne hatchery tank supplied with bore water used to fill the ponds. Water temperature was recorded and heaters installed to keep the water temperature above 24°C. The tank was stocked with 10 females and 6 males in January 2009. Periodic inspection of the crayfish revealed females becoming berried and juvenile crayfish being produced. The tank trial was terminated at the beginning of September 2009. The maximum water temperature reached in the tank was 30.4°C with a minimum of 24.2°C and an average of 27.6°C.

Prior to stocking the pond with broodstock crayfish for a third time, the other possible reasons for a population failing to establish were assessed. As the Trust Yard has a strong security presence and fish in the neighbouring ponds did not disappear, theft was unlikely. Predatory birds were not observed at the ponds by OTDF staff or security officers. Water rats (*Hydromys chrysogaster*) were considered a possibility, but there was no evidence of these—for example, crayfish shells at feeding stations. A mammal trap was set for two weeks at different locations around the pond and compound, but nothing was caught.

All possible escape areas around the walkway and inlet pipes in the 300 mm high metal fence surrounding the pond were checked and reinforced as required. A robust outlet screen was constructed and fitted to the internal stand pipe of the pond.

The pond was restocked on 4 September 2009 with 13 females and 10 males along with 30 juveniles weighing 5 to 10 g bred in the tank trial.

When the pond was drained on 27 November 2009 no crayfish were recovered from the pond. Only one mortality was recorded five days after stocking. No other evidence of mortality or predation was observed. The mammal trap was set in the bottom of the empty pond directly after draining and the pond bottom was inspected in the morning after draining for evidence of mammal footprints without success. With no evidence of predation and no crayfish exoskeletal remains, and strong security, the cause of disappearance remains unresolved.

Discussion

Maximum water temperature reached during this period was 36.2°C with a minimum of 25.7°C and an average of 31.0°C. Jones (1990) suggests 34°C as the upper limit for good growth in *C. quadricarinatus* in Australia. As the scoping study was coming to an end OTML staff stocked

crayfish into ponds leased by OTML at Tabubil. These crayfish survived and reproduced in the ponds, which attained maximum water temperatures of 32°C. Due to landholder issues, however, these ponds became unavailable to the project staff. The survival of crayfish in Tabubil under different environmental conditions than at Kiunga would suggest the nil survival in ponds experienced in Kiunga may be due to geographic variation in environmental conditions, especially water temperature.

To solve the mystery of the disappearing crayfish, the plan was to contain the crayfish in submerged wire mesh cages, and to prepare a pond at the Western Province location at Samagos to culture crayfish as well. However, as the project was terminated prematurely these activities did not eventuate. Clearly, the fate of the crayfish must be resolved for crayfish farming to have any prospects of success.

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7.5 Activity 2.4: Investigate hatchery characteristics, production performance and aquaculture potential of two strains of *Macrobrachium rosenbergii* in Australia

Introduction

This activity linked in with a John Allwright Fellowship granted to Ms. Malwine Lober who researched improving hatchery and nursery techniques for the Australian strain of *Macrobrachium rosenbergii*.

Methods

Broodstock were collected by DEEDI Walkamin staff from Mitchell River, north Queensland, and sent to the Marine and Aquaculture Research Facility Unit (MARFU) at the James Cook University (JCU) Townsville campus.

Broodstock management, larval and nursery culture for several hatchery runs of the Australian strain of *M*. *rosenbergii* were conducted at MARFU. Nursery culture of two batches of postlarvae and growout trials were conducted in ponds at Walkamin Station; JCU personnel travelled regularly to the site to monitor crayfish growth.

Results and Discussion

Broodstock maintenance and conditioning.

Research was conducted on reproductive seasonality, conditioning, fecundity and nodavirus disease of *M*. *rosenbergii*. Out-of-season spawning and an attempt to close life cycle were also researched. The life cycle of *M*. *rosenbergii* was fully closed in 2008, since hatchery production in 2009 came from broodstock that was of last year's hatchery-produced postlarvae reared in the DEEDI ponds at Walkamin.

Attempts to induce of out-of-season spawning of *M. rosenbergii* by temperature and photoperiod manipulation achieved relatively limited success.

Larval culture

Hatchery-based research focused on comparison of 'green water' vs 'clear water', and the effects of different algal concentrations in 'green water' culture. Three other factors were also investigated: *Artemia* enrichment, larval stocking density and water exchange procedures. Optimal procedures for acclimation of postlarvae to freshwater was also researched. A trial on culture of larvae at extra low salinity (~3-5 ppt) has shown that it has limited impact on larval survival.

Hatchery-based research focused on comparison of 'green water' vs 'clear water', and the effects of different algae concentration in 'green water' culture. Results showed that larval survival to post larvae (PL) at the two higher algae concentration of 12.5 and $25x10^5$ cells/ml was 70.8% and 63.3%. This was significantly higher ($P<0.05$) than those of lower algae concentrations of 2.5 and $6.25x10^5$ cells/ml and the 'clear water' treatments (survival of 26.7, 35.0 and 30.0% respectively). *Artemia* enrichment vs. no enrichment was tested with the results showing enriched artemia did not affect survival to PL.. Optimal procedures for acclimation of postlarvae to freshwater was also researched. A trial on culture of larvae at extra low salinity of 3-5 ppt has shown that it has limited impact on larval survival.

This research has resulted in two published papers and two papers in preparation.

Published papers:

Lober M. and Zeng C. 2009 Effect of microalgae concentration on larval survival, development and growth of an Australian strain of giant freshwater prawn *Macrobrachium rosenbergii. Aquaculture* 289, 95-100. (Appendix 3)

Owens, L., La Fauce, K., Juntunen, K., Hayakijkosol, O., Zeng, C. (2009) *Macrobrachium rosenbergii* nodavirus disease (white tailed disease) in Australia. *Diseases of Aquatic Organisms* 85, 175-180. (Appendix 4)

Papers in preparation:

Effect of stocking density on growth and survival of Australian Macrobrachium rosenbergii (de Man) newly settled post larvae. (Appendix 5)

The effect of enriched *Artemia* nauplii on survival and development of an Australian strain *Macrobrachium rosenbergii.* (Appendix 6)

Hatchery production

Three hatchery runs in 2007 (15 Feb to 20 April, 2007) produced 150,000 postlarvae using broodstock from the wild, and also achieved the following:

- High survival rates of > 85%.
- Substantially shorter hatchery cycle compared to Western strains (which originated from S.E. Asia and have been cultured worldwide), 22-26 days vs. 32-35 days.
- Synchronized larval development, on the day of harvesting only 1-2% remained at larval stages.
- High productivity, outputs of postlarvae (PL) as high as 170 PL/litre culture water.

In 2008, four hatchery runs (27 Feb to 16 May) were conducted producing larvae hatched from broodstock produced from the Walkamin ponds the previous year. Thereby closing the life cycle using hatchery bred broodstock. However, survival (40-68%) was generally lower than 2007 when wild broodstock were used. Postlarvae were harvested 25 to 35 days after hatching, whereas in 2007 it was 22 to 26 days. Inbreeding could have significantly impacted on the larval quality. A total of 65,000 postlarvae were produced. These will be used for stocking ponds at a barramundi farm to test growth and survival in farm conditions.

Growout

Growout conducted at DEEDI Walkamin ponds in 2007 showed that the Australian *M. rosenbergii* strain can survive temperatures as low as 15°C and is capable of reaching a maximum weight of about 200 g in a 10-month growout period. This included 4 winter months of average temperatures around 20°C or lower, when little growth is expected. Another interesting finding is that the majority of prawns appear to form two very distinct size groups of large size of average 70 g and small size of average of 4 g; this is different from what has been reported for the Malaysian strain, and suggests that better management is likely to further improve the productivity.

7.6 Activity 3.1: Establish model farms for trialling and demonstration of culture techniques for various species

Introduction

Western Province staff took the lead role, with support from OTDF staff, in setting up the model farms, as the OTDF staff did not want the project to be perceived as a solely Ok Tedi project. It was also planned that the Western Province government would take over this area of work as the mining company reduced its input leading up to the planned 2013 mine closure. The in-country staff would transfer the skills and knowledge acquired by culturing fish in the Samagos and Trust Yard ponds to the model farmers.

Methods

Five model farms were selected by Western Province staff and the farmers attended workshops hosted by the in-country partners, in preparation for becoming the model farms. A preliminary workshop was held in December 2007 by Western Province and OTDF staff at Kiunga to highlight the process, roles and responsibilities of all involved. The workshop focused on explaining the objectives of the ACIAR native fish project, the expected outcomes and the role of the model farms.

This preliminary workshop was followed up with a four-day workshop conducted by the in-country staff at Samagos in March 2008 to train the five selected farmers in native fish aquaculture. All aspects of fish farming were covered with an evaluation questionnaire completed by the participants on completion of the workshop. Reports on the two workshops prepared by in-country staff are attached as Appendix 7.

Fish stocks bred at Samagos were used to supply farmers with stock for their ponds. Sleepy cod were distributed by Western Province staff to four farmers in the Kiunga area in February 2009. Due to transport difficulties and other logistical issues, herring were not stocked in the ponds as a food source with the sleepy cod.

Fly River herring were distributed to two farmers in the Tabubil area by OTDF staff in June 2009. These farmers were not original model farmers, but it was considered beneficial to also select farmers away from rivers where the need for farming is greater than near rivers where

wild fish are still available. These herring stockings were to be future broodstock for further breeding in the ponds. Locations and fish stocking is presented in Table 12.

Table 12. Fish stocking details on six farms in PNG.

Village	Date	# farms	Pond size	Species	# stocked per pond
Gii	Feb 2009	3	5 x 10 m	Sleepy cod	50
Grengas	Feb 2009	1	5 x 10 m	Sleepy cod	50
Wangbin	Jun 2009	1	8 x 10 m	Herring	20
Kwiloknai	Jun 2009	1	10 x 15 m	Herring	20

Results and Discussion

A site visit was made in December 2009, just prior to the early completion of the project, by Kaupa Kia, Johnson Karl, Noel Tonko and Peter Graham to Dasi Tukowre and Moki Sipe farms at Gii. The sleepy cod were harvested with survival rates of 37% and 77% recorded respectively. From an average total length (T.L.) of 30 mm at stocking, average T.L. of 125 mm and 113mm were recorded respectively. Growth was very poor with the fish growing a maximum of 95 mm in approximately 10 months. Water quality was not ideal, with pH values of 5.3 and 4.75 respectively on the two farms at the time of the visit. The farm with the lower pH experienced higher survival but slower growth.

Dasi's pond also contained 20 purple spotted gudgeons (*Mogurnda* sp.), 2 redclaw crayfish and 3 swamp eels (*Ophisternon* sp.), while Moksi's pond contained a 50mm T.L. snakehead (*Channa striata*). These fish would have entered the ponds during heavy rain from nearby natural water courses.

Information on the herring stockings was difficult to collect as the decision to end the project was made and transport to visit these farmers was unavailable. In December 2009, the Wangbin farmer reported to OTML staff that he had recently observed the herring in his pond, but it was unknown if the herring had bred there. No information was available from the Kwiloknai farmer on the status of the herring stocked to his pond.

Poor site selection of the model farmers' ponds was the main impediment to more successful results. The ponds were constructed in low-lying swampy ground surrounded by sago palms. The water supply to one pond was from ground water seeping into the pond. As previously reported the pH of the pond water was low. Failure to stock the herring with the sleepy cod as food also inhibited sleepy cod growth. With hindsight greater guidance from the Australian project staff was required to ensure greater success of the farm stockings. Better guidance with site selection would have increased the success of fish culture as well as ensuring that the ponds were located in areas where the people would benefit the most. Greater guidance with the fish stockings and husbandry would have also led to better production from the ponds, although at the time of the early termination of the project transport difficulties was an unresolved problem. These issues were identified, and if the project had continued, changes would have been made to increase the achievements by the original project finish date.

7.7 General Discussion

Evaluating the suitability of several fish species for aquaculture in the PNG village setting, as used in this study, is useful for giving first evidence of the suitability of fish species for aquaculture in Papua New Guinea. Future technical assistance in the Western Province should use the results of this study to focus on the species that are best suited. In the rearing trials of

carnivorous species, it was intended to feed these species with Fly River herring co-cultured in the same ponds. This was not successful because of high pond mortality of the herring during dry periods, or in transport by farmers to the model farms.

It therefore has not been established whether co-culture of carnivorous species with their food species is feasible; for example, it is not known whether herring production in ponds can keep up with predation rates to maintain production of the carnivorous species (e.g., sleepy cod). Unless co-culture can be achieved, future pond culture efforts in PNG village communities should focus on omnivorous or herbivorous species, since pond culture of carnivorous fish species in the PNG village context may be difficult because of the scarcity of available animal proteins for either direct feeding or for pelletised feeds.

It is evident also that the technical, logistic and/or social problems associated with developing fish farming had not been sufficiently overcome by the project prior to engaging with villagers to set up the model farms. Poor site selection of the model farmers' ponds was the main impediment to more successful results. The ponds were constructed in low-lying swampy ground surrounded by sago palms. The water supply to one pond was from low-pH ground water seeping into the pond. Failure to stock the herring with the sleepy cod as food also inhibited sleepy cod growth. Greater guidance from the Australian project staff was required to ensure greater success of the farm stockings. Greater guidance with the fish stockings and husbandry would also have led to better production from the ponds, although at the time of the early termination of the project, transport difficulties was an unresolved problem.

At least some of the poor successes with fish spawning and larval production were the result of inadequate husbandry. Poor broodstock condition was a major factor inhibiting successful hormone-induced spawnings. Inadequate nutrition and high stocking densities were probable causes in many cases, and these issues need to be overcome to improve success in the future. Excessively high water temperatures (recorded as high as 37.7°C in redclaw ponds, found to be lethal to redclaw in Australian conditions) were also a likely contributing factor in some cases.

Several other constraints and challenges were encountered that need to be addressed for aquaculture to be successful. Progress was constrained by the inability of Western Province government staff to meet their project commitments because funds that were allocated to the project were slow to be released through the government processes. Thus, for example, although a hatchery building was constructed at LARDEC, funds were not available to connect a water or power supply to it. Similarly, the ability of Western Province government staff to travel was greatly curtailed because of lack of transport. OTDF was relied on greatly for the supply of materials and transportation to progress the project. For example, OTDF supplied materials to facilitate the construction of the weir to provide a water supply to the ponds. Communication between Western Province staff and the Australian partners was very difficult and sometimes almost non-existent due to the lack of reliable communication infrastructure. Information had to be passed through OTDF contacts.

Security was a problem. Although staff were housed near the ponds at Samagos to assist with security, there were still problems with theft: temperature loggers installed in the ponds disappeared, disrupting the collection of data. A power supply 200 metres from the ponds could have been used to power an air blower installed to supply air to the ponds, but because of the perceived risk of theft the network of air pipes was not built.

Incidents of this sort can spell failure for aquaculture development initiatives because they may lead to significant mortalities and lost production, and more generally to a loss of motivation among villagers to continue. Any future aquaculture development interventions need to address these problems.

8 Impacts

8.1 Scientific impacts – now and in 5 years

PNG component

The aim of the PNG component of the study was to build local expertise and knowledge in the various aspects of fish farming with the assistance of the Queensland members of the project team. As such there was no scientific impact of this component.

Australian component

Despite the increasing use in Australia of freshwater macrophytes in constructed wetlands for effluent treatment (Greenway 2005), there has been very little study here of the use of native aquatic macrophytes in managed recirculation aquaculture systems. This project has documented for the first time the effect of aquatic macrophytes on water quality in tropical freshwater pond aquaculture. This research recorded the levels of nutrients assimilated by the aquatic plants and the water savings made by these systems. This research also documented barramundi growth rates and the water quality parameters the fish experienced over an extended period of time, which has not been previously published.

Research findings suggest there is no technical impediment to the aquaculture of the Australian strain of *M*. *rosenbergii*. It has also been shown that the Australian strain of *M. rosenbergii* possesses biological traits different than those of the Malaysian strain.

The project has so far produced two papers that have been published in aquaculture journals and at least two more papers are forthcoming (see section 8.5 and Appendices 4 to 7).

8.2 Capacity impacts – now and in 5 years

The most valuable aspect of this project is the improved capacity and desire of PNG partners to continue the work commenced by this project. The Ok Tedi Fly River Development Program (OTFRDP) has replaced OTDF and has employed Noel Tonko to continue aquaculture activities. OTFRDP has the financial resources to continue the work started by this project and not to allow the momentum to fade. The Western Province government aquaculture program will continue to function and promote the objectives of this project, in cooperation with OTFRDP.

This project has allowed PNG staff the opportunity to gain valuable skills and experience, which would otherwise have been extremely difficult to acquire. The visits to Australia allowed PNG staff to view what is possible in aquaculture, using species common to both countries. The hands-on experience has given them the confidence to tackle the challenging task of developing aquaculture in PNG. The skills acquired during this project will be transferrable between native and exotic fish species.

Equipment supplied through ACIAR in this project will provide support in continuation of aquaculture activities in PNG. It will be available for demonstration and training facilities for potential aquaculture farmers in the future.

Through their participation in the project, the freshwater prawn project at JCU has helped train various personnel from different backgrounds and countries; this is expected to yield impacts beyond the original scope of the project.

Personnel training:

Ms. Malwine Lober, Senior Fisheries Officer, Ministry of Agriculture, Samoa: Malwine undertook a Master of Science research degree at JCU and was associated with the project. She worked on improving hatchery and nursery techniques for the Australian strain freshwater prawn, *Macrobrachium rosenbergii* and was supported by a John Allwright Fellowship.

Mr. Haruo Tsuji, a JCU Master of Applied Science student from Japan, conducted a minor project comparing 'Green water' and 'Clear water' larval culture methods for *M. rosenbergii* in 2007.

Mr. Ifran Abas, Fishery Department, Hasanuddin University, Makassar, Indonesia: With funding from his university, Mr. Abas undertook a short training course on hatchery techniques for *M. rosenbergii* at JCU between March and April 2008.

Mr. Cristian Bernstorff, a JCU aquaculture student from Mexico, undertook training on broodstock management and nursery culture of *M. rosenbergii* in 2008.

Although DEEDI has suspended its freshwater aquaculture research at the Walkamin Research Station, the knowledge and experience gained in the methodology and application of experimental procedures in conducting water re-use trials is available for assisting future research activities, should suitable projects be developed.

8.3 Community impacts – now and in 5 years

8.3.1 Economic impacts

The project did not achieve positive economic impacts during its term. This was a ground-breaking project for PNG that sought to develop pond-based aquaculture in a region and with communities where it did not previously exist. It is therefore understandable that progress was slow and successes modest; the hurdles to achieving prosperous, efficient pond-based aquaculture take time to overcome.

Aquaculture in the Western Province is unlikely to develop much beyond its current nascent state without further assistance and support. Viable, productive fish farming in the Western Province will require:

(i) continuing development of and support for the Western Province government facility LARDEC, at Samagos, to continue research and dissemination of skills to villagers

(ii) better site selection for ponds, and training for farmers to develop their skills

(iii) continuing research and review of species suitability for different environmental conditions.

Culturing fish that do not have high input costs, such as for processed feeds, will reduce the level of economic impact should the crop fail. The low level of start-up costs would also enable more people to be involved in native fish farming. In subsistence communities with little or no access to cash or credit, fish production that relies on purchased feeds faces strong difficulties. The concept of farming herbivorous fish that either do not need to be fed (relying on natural in-pond primary production) or can be fed terrestrial plant residue has therefore raised awareness of possibilities of aquaculture at the village level.

Reduced water usage and compliance costs will greatly enhance the profitability of aquaculture farms in Australia. Further research is needed, but bioremediation using aquatic macrophytes research shows that significant nutrient removal can be achieved and significant water savings made through the technologies tested.

8.3.2 Social impacts

As the project was terminated 18 months early and the concept of farming native fish was only just being introduced to farmers, this project was yet to have a positive social impact. Nevertheless, there was a high level of interest in the project in the community as the work progressed with the PNG in-country partners.

8.3.3 Environmental impacts

This project was building towards having positive environmental impacts in both the Australian and PNG components. Although some authors (Haines 1979, Smith 2000) have advocated using native species in PNG this is the first project to investigate the potential of native fish for aquaculture in PNG. Future decisions on the direction of aquaculture and research priorities will be influenced by the knowledge gained from this work. Through the use of local native species of fish for aquaculture, the environmental threat posed by exotic species is in some respects reduced, since a major threat facing native freshwater fish is the uncontrolled introduction and spread of introduced fishes (Allen et al. 2008). Although native-fish farming may be preferred for biodiversity conservation reasons, 'the Fly now faces an onslaught of exotics from across the Indonesian border' (Allen et al. 2008) where introduced fishes are now prevalent. The conservation imperative to not farm carp or tilapia in the Fly River may therefore be lessened.

The preliminary research into nutrient removal from aquaculture water has given the freshwater aquaculture industry in Queensland information to allow it to apply methods of ameliorating discharge water. Water re-use can lead to greater water efficiencies, reducing the water extraction from the environment. Results of this research into practical, environmentally sustainable methods to ameliorate aquaculture pond water and water re-use may lead to long-term sustainability of aquaculture.

8.4 Communication and dissemination activities

Agcopra C. Water Quality Fact Sheet presented at the Australian Barramundi Farmers Association Conference, Townsville, August 2009.

Graham P., Willows K. Poster presentation addressing the PNG component of the project at the Australasian Aquaculture 2008 International Conference, Brisbane, August 2008.

Juntunen, K., Owens, L., Lafauce, K., Hayakijkosol, O., Endo, K., Zeng, C. *Macrobrachium rosenbergii* nodavirus (white tailed disease) in Australia. Oral presentation at aquaculture American 2009, Seattle, Washington, USA, February 2009.

Seymour E. Aquaculture Potential of Macrobrachium. Oral presentation to Aquaculture Association of Queensland (AAQ) Childers July 2008.

Seymour E. Aquaculture water re-use research. Oral presentation to the Barramundi Farmers meeting, Darwin, November 2008.

Zeng, C., Lober, M. 2007. Development of hatchery techniques for an Australian strain giant freshwater prawn *Macrobrachium rosenbergii*. - Oral presentation at 'Asia-Pacific Aquaculture 2007', Hanoi, Vietnam, 5-8 August 2007.

8.5 List of publications produced by the project

Lober, M., Zeng, C. (2009) Effect of microalgae concentration on larval survival, development and growth of an Australian strain of giant freshwater prawn *Macrobrachium rosenbergii*. Aquaculture, 289, 95-100.

Owens, L., LaFauce, K., Juntunen, K., Hayakijkosol, O., Zeng, C. (2009) *Macrobrachium rosenbergii* nodavirus disease (white tailed disease) in Australia. Diseases of Aquatic Organisms, 85, 175-180.

Lober, M., Zeng, C. (In preparation) Effect of stocking density and shelter on survival and growth of newly settled postlarve of an Australian strain of *Macrobrachium rosenbergii* (de Man).

Lober, M., Zeng, C. (In preparation) The interactions of *Artemia* enrichment and microalgal addition on larval survival and development of an Australian strain of *Macrobrachium rosenbergii.*

9 Conclusions and recommendations

Papua New Guinea

Although aquaculture in Papua New Guinea is relatively new, there is widespread, strong interest by villagers in adopting it. The various skills needed for successful fish farming—husbandry, spawning induction, hatchery production, nutrition—take time to learn, and over the course of this project the training that was imparted by the project team, both in-country and at the Walkamin Research Station, led to some level of proficiency in fish farming by Western Province villagers and project partners. It is evident, however, that the skills and experience needed to deal with the day-to-day running and trouble-shooting of fish aquaculture have, in general, yet to be achieved.

This was partly because of the substantial shortening of the project, which was terminated 18 months before its scheduled end date because of the unforeseen suspension of aquaculture activities at the Walkamin Research Station, the loss of key project staff from Walkamin, and a restructuring of the Ok Tedi Development Foundation.

The objectives of the PNG component of the original project were overly ambitious, as identified in the mid-term review. The broad range of activities prevented a focus of resources on fewer objectives to deliver better outcomes. Any future projects need to set realistic and achievable goals that do not underestimate the time taken for skills transfer and incorporation of pond-culture activities into the daily routines of villagers.

As described in section 7.7, fertilisation success, larval survival and growth rates were often low, and these can in general be attributed to poor husbandry; more particularly to poor nutrition and suboptimal conditions in the ponds. This illustrates the importance for any future projects of providing thorough training in the various husbandry skills needed. Just as important is the need for careful oversight, guidance and assistance by skilled aquaculture practitioners who are also experienced in engaging with peoples in the rural village milieu.

The several species that were evaluated for their aquaculture potential in PNG provided a range of successes and problems that may provide guidance to future aquaculture assistance interventions. Perhaps the main challenge is to select the most appropriate species for culture. Attempts to rear carnivorous species (sleepy cod, eel-tailed catfish, sooty grunter) were not very successful, partly because of the inability to provide Fly River herring as co-cultured prey within the pond environment. The project was unfortunate to have experienced drought conditions, leading to fish deaths, so that important questions about the extent to which co-cultured prey species may reduce the need to provide feed remain unanswered. This is important because access to capital to purchase high-protein feeds, or to feed directly with animal protein, is not available in many rural areas.

Although ‘food web polyculture’ has been applied in Yonki Reservoir (Eastern Highlands Province), where several non-native fish species were introduced to establish the various trophic levels required to support a population of the target species, it remains to be demonstrated that this approach could work well in small ponds.

Herbivorous or omnivorous species therefore seem the most suitable candidates for small-pond aquaculture, at least until the co-culture question has been resolved.

Australia

As environmental regulations become more stringent, aquaculture water treatment and re-use will become a necessity. The freshwater aquaculture industry in Queensland has been informed of the results of the Walkamin component of this study, but there have been no subsequent surveys to determine the extent to which farming practices have been modified.

Further research is required to maximise the efficiency of the water treatment systems for aquaculture applications and to modify on-farm management practices. The range of species already trialled or suggested for bioremediation is large, with Redding et al. (1997) comparing the bioremediation efficiency of free-floating, submerged and emergent aquatic plants. The mid-term review team recommended the efficiency of naturally established systems be investigated. Proposals to use herbivorous fish such as *Nematalosa erebi* and *Clupeoides papuensis* to improve water quality by consuming the natural production in treatment ponds (B. Herbert, pers. comm.) have not been evaluated. There may be benefits to farmers as the fish could be fed back to the farmed species (such as barramundi), reducing feed costs. This sort of system has yet to be evaluated.

10 References

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11 Appendixes

11.1 Appendix 1: *Macrobrachium* research 2008 annual ACIAR report

11.1.1 Milestone

Development of the hatchery

1. Broodstock maintenance and conditioning, reproductive biology:

Research has been conducted on Australian strain *M. rosenbergii* reproductive seasonality, conditioning, fecundity as well as induce of out-of-season spawning and the attempt to close life cycle etc. while induce of out-of-season spawning by temperature and photoperiod manipulation has achieved relatively limited success, the life cycle of Australian strain *M. rosenbergii* has been fully closed in 2008 as all hatchery production this year came from broodstock of last year stocked hatchery produced postlarvae in DPI pond.

2. Larval culture

• Hatchery technique development focused on comparison of 'green water' vs 'clear water'; effects of different algae concentration in 'green water' culture; Artemia enrichment vs. no enrichment; different larval stocking density and water exchange schemes as well as optimal procedure for acclimation of postlarvae to freshwater. A trail on culture of larvae at extra low salinity of about 3-5 ppt has shown that it has limited impact on larval survival. 2007 hatchery runs (15 Feb to 20 April, 2007; 3 runs, produced 150,000 PL) using broodstock from wild has consistently achieved:

- High survival rates of > 85%.
- Substantially shorter hatchery cycle compared to 'western from' strains (22-26 vs. 32-35 days).
- Synchronized larval development, on the day of harvesting, only 1-2% remained at larval stages.
- High productivity, outputs of postlarvae (PL) as high as170 PL/liter culture water.

2008 hatchery runs (27 Feb to 16 May, 4 runs, produced 65,000 PL) using pond grow broodstock has shown a trend of lower survival (40-65%), higher cannibalism rates and longer larval duration (25-35 days) as compared to last year, whether this is a result of inbreeding would need further investigation. Nevertheless, the result is still compatible to that reported for Malaysian strain.

macrobrachium hatchery & grow out (how many times, how many animals, number juveniles stocked)

In 2008, four hatchery runs (27 Feb to 16 May) were conducted with larvae hatched from females stocked in the DPI pond as postlarvae in 2007, therefore, the fully close of life cycle has been achieved. However, the survival was generally lower than 2007 when wild broodstock were used. Survival were recorded at between 40-68% and postlarvae harvested on 25 to 35 days after hatch. A total of 65,000 PL were produced which is currently under nursery culture and will be used

for stocking ponds in DPI and a Barra farm.

Growout

Growout conducted at DPI pond in 2007 has showed that the *M. rosenbergii* strain can survived temperature as low as 15 C and capable of reach a max weight of about 200g in 10 month growout period (including 4 winter months of average temperatures around 20 C or lower, little growth is expected during the period). Another interesting finding is that the majority of prawns appear to form two very distinct size groups of large size of average 70g and small size of average of 4 g, this has been different from what has been reported for Malaysian strain and suggested that better management scheme are likely to further improve the productivity.

11.1.2 Impacts

Scientific impacts – the research findings so far has clearly suggested that the Australian strain of *M. rosenbergii* possess different biological straits from that of Malaysian counterpart. Research results also suggested that some current hatchery practices for Malaysian *M. rosenbergii* may be further improved. It is expect that at least two papers will be generated out of research conducted so far.

Capacity Impacts – Through their participation in the project, the freshwater prawn project conducted at JCU have helped training many personnel from different backgrounds and countries (see Training Activities section), which is expected to bring on impacts beyond the original scope of the project.

11.1.3 Training Activities

Listed following are main participators, there were more students involved in shorter term participation of the project.

- Ms. Malwine Lober, Senior Fisheries Officer, Ministry of Agriculture, Samoa: Ms. Lober is undertaking MSc (by research) degree at James Cook University since project started. She has been working on improving hatchery and nursery techniques for the Australian strain freshwater prawn, *Macrobrachium rosenbergii* and is founded by John Allwright Fellowship study award.

- Mr. Ifran Abas, Fishery Department, Hasanuddin University, Makassar, Indonesia. Mr. Abas has undertaken a short training course on hatchery techniques for freshwater prawn, *Macrobrachium rosenbergii* at James Cook University between 10 March and 26 April 2008. He is funded by Hasanuddin University, Indonesia.

- Mr. Haruo Tsuji, a JCU Master Applied Science student from Japan, who has conducted a minor project on hatchery culture of *M. rosenbergii* in 2007.

- Mr. Cristian Armbruster Bernstorff, a current JCU aquaculture student from Mexico, who has volunteered for training on broodstock maintenance and nursery culture of M. rosenbergii since early 2008. Mr. Bernstorff has keen interest in developing freshwater prawn aquaculture in Mexico upon his return.

11.1.4 Variations of future activities

Problems & opportunities

Problems: Ms. Malwine Lober is expected to complete her MSc degree by the end of 2008. This will have substantial impacts on the project, which has already under consideration constraints for lacking of the manpower.

Conference presentations

Zeng, C., Lober, M. 2007. Development of hatchery techniques for an Australian strain giant freshwater prawn Macrobrachium rosenbergii. ¬Oral presentation at 'Asian¬Pacific Aquaculture 2007', Hanoi, Vietnam, 5¬8 August, 2007.

Water quality in barramundi culture

Fact sheet

48 hour barramundi pond water quality monitoring

Queensland Primary Industries and Fisheries has been working on a research project with the Australian Centre for International Agricultural Research to gain information on bioremediation for barramundi aquaculture.

This part of the project is to learn the trends and cycles of the water quality parameters deemed most important for barramundi freshwater culture in a 24 hour period.

The information gathered will assist farmers to schedule farming activities such as fish handling, water exchange, aeration and timing of testing for toxic waste products for maximum benefits. It will also give the farmer the knowledge of when emergency situations caused by poor water quality are most likely to occur.

Maintaining optimum pond water quality is key to a successful aquaculture venture. Knowledge gained from this 48 hour monitoring will assist the farmer in establishing an effective water quality maintenance regimen.

Methods

Monitoring was performed between 27 and 29 April, 2009 at the Freshwater Fisheries and Aquaculture Centre, Walkamin Research Station, Walkamin. The six fish ponds in a large trial to assess Lotus, *Nelumbo nucifera*, in wastewater bioremediation were used.

Three of the six ponds had recirculating water and the other three were static - the currently more common industry practice. The inclusion of the recirculating ponds is to determine if there is a difference in the 24-hour trends.

Over 48 hours, pond water samples were taken every two hours to monitor total ammonia nitrogen (NH_3-N) and nitrite nitrogen (NO_2-N) levels. Dissolved oxygen (DO), total dissolved solids (TDS), pH, temperature and turbidity were also measured. The levels of the toxic form of ammonia (unionised NH_3) were calculated using standard procedures.

The ponds
Surface area: 0.032 hectares
Volume: 0.250 mega litres

Fish were fed to satiety, once daily, between 3pm and 4pm. Water pumps for the recirculating ponds were turned on daily for six hours, from

11.2 Appendix 2: Fact sheet of results of 48 hour nutrient monitoring

5pm to 11pm. A blower supplied 24-hour aeration and an additional aspirator for every pond was turned on after the feed from 5pm to 10am the following day. Average pond biomass was equivalent to 29.6 tonnes/ha and average fish size was 3.2 kg.

Results and discussions

Results are expressed as average values for the three ponds in each recirculating and static ponds.

These results are specific for the time of the year that the work was done. Seasonal changes can change the time of peaks and lows for some parameters.

Key points:

- There was a clear 24-hour cycle for both forms of ammonia (NH_3 and NH_3-N), temperature, pH and dissolved oxygen. No clear 24-hour cyclic trend was seen on NO_2-N, TDS and turbidity.

- Unionised NH_3 (Fig. 1), which is the more toxic form to fish, peaked at 4.30pm (shortly after the feed) – the highest level observed was 0.06 mg/L. Minimum levels were between midnight and 8.30am; whereas total ammonia nitrogen (NH_3-N) (Fig. 2) peaked between 2.30am to 6.30am (10-14 hours after the feed) – the highest value being 0.4 mg/L and minimum values were observed around 3pm. Knowledge of toxic ammonia levels straight after the feed could lead to better timing for water exchange or, for recirculating systems, better pumping schedule.

- Highest nitrite-nitrogen (NO_2-N) (Fig. 3) observed was 0.02 mg/L. The results in this two-day monitoring suggest that Lotus, as a bioremediation plant, works well in reducing nitrite levels, however, conclusions are reserved pending analysis of the main trial.

- Temperature (Fig. 4) peaked at 3.30pm, and lowest at 7.30am. This has implications in timing water/heat exchange in winter, and in areas where summer temperatures can exceed optimum levels for barramundi culture. This knowledge can also be used to schedule time of feeding to maximise food intake in winter.

- Dissolved oxygen and pH (Figures 5 and 6) peaked at 2.30pm (18 ppM for DO and 9.6 for pH); Minimum dissolved oxygen was between 8.30pm to 6.30am (5 to 6 ppM); minimum pH was between midnight and 4.30am (7.02). This knowledge on DO allows the farmer to determine the best time to aerate ponds. DO and pH outside the minimum range can be lethal to fish. pH can also be an indicator of algal blooms, which also affects dissolved oxygen values and trends.

- On the first day of monitoring, recirculating ponds were fed an average 1.7 kg/pond and static ponds 1.9 kg/pond; and on the second day, 2.5 kg/pond for recirculating ponds and 1.3 kg/pond for static ponds. These figures were very much lower than the average 4.2 kg/pond/day for the 109 days duration of the main trial, perhaps affected by the monitoring activity in and around the ponds and also by the time of the year (mid-autumn).

- There was no significant difference between the trends in the recirculating and static ponds. It has to be taken into account that water exchange between the fish and the treatment ponds was done only for six hours.

- The consistently higher total ammonia nitrogen readings for the recirculating ponds can be attributed to the large amount of decaying plant material in the bioremediation ponds. This indicates that it is important for farmers with recirculating outdoor ponds to clean out bioremediation ponds of decaying organic material.

- The consistently lower temperature readings in the recirculating ponds could be attributed to the pumping time (5pm to 11pm). The treated water flows back into the fish pond but gets exposed to the cooler air in two sections before it goes back into the fish pond.

- A marked difference between the recirculating and static ponds was that the recirculating ponds had visually clearer water; this observation is also supported by the difference in turbidity (Fig. 7) and TDS (Fig. 8) values. Preliminary analysis of the main trial results suggests that pond clarity can be manipulated by the volume of water pumped for bioremediation by the Lotus plant. This will help in attaining lower fluctuations in pH and DO in a 24-hr cycle.

The following graphs of average values of the parameters discussed for the recirculating and static ponds tell the story.

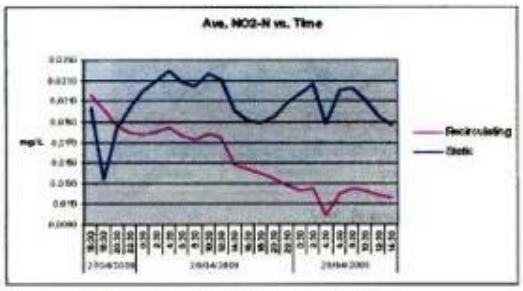

Figure 3. Nitrite Nitrogen (NO_2-N)

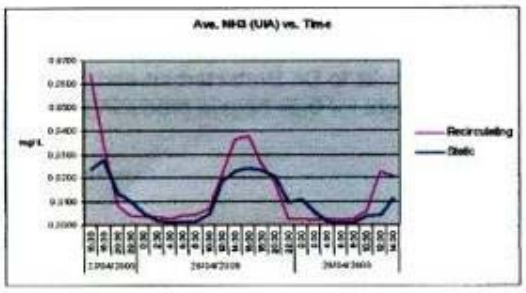

Figure 1. Unionised Ammonia (NH_3)

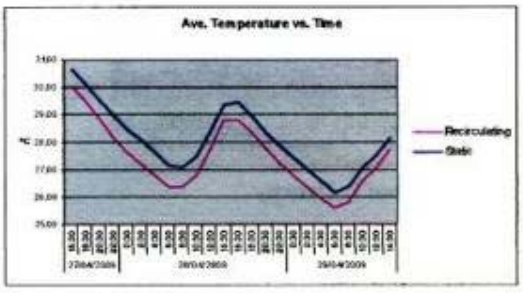

Figure 4. Temperature

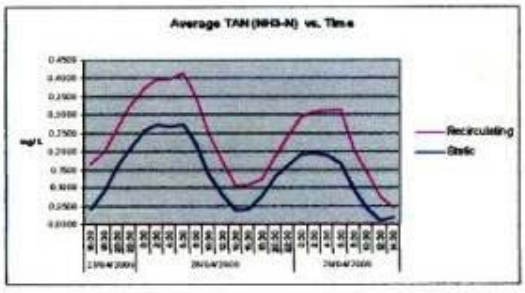

Figure 2. Total Ammonia Nitrogen (NH_3-N)

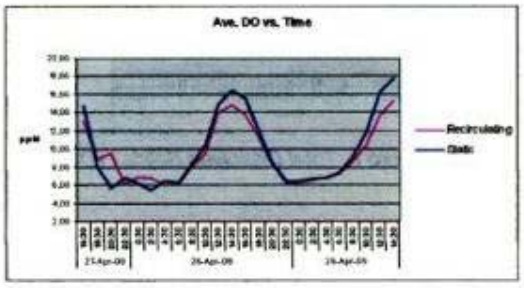

Figure 6. Dissolved Oxygen (DO)

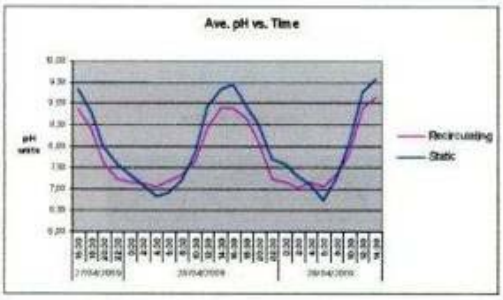

Figure 6. pH

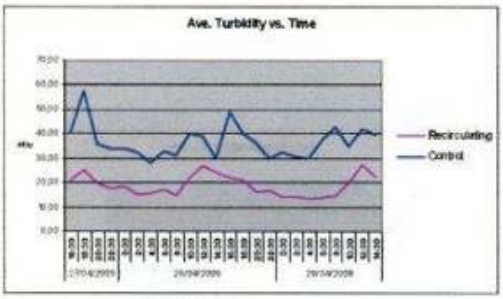

Figure 7. Turbidity

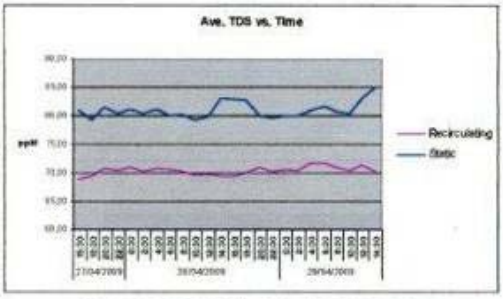

Figure 8. Total Dissolved Solids (TDS)

The staff
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Special thanks to Dr. Brett Herbert and Dr. Carole Wright for their help in producing this fact sheet.

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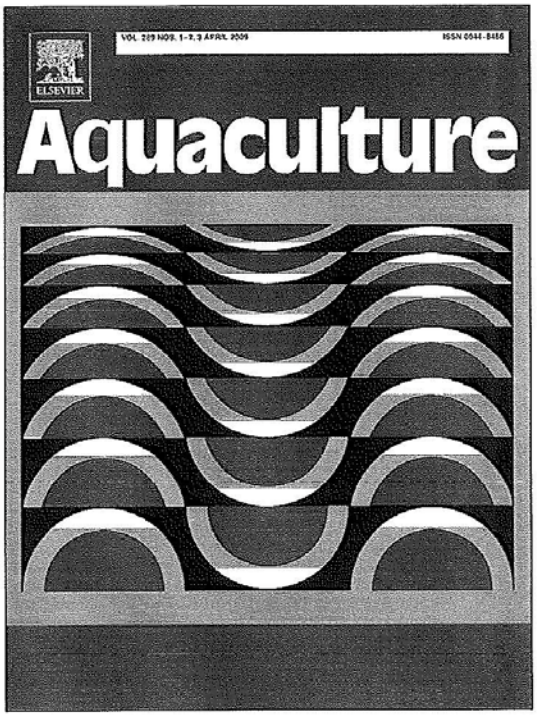
11.3 Appendix 3: Effect of microalgae concentration on larval survival, development and growth of Australian strain of giant freshwater prawn macrobrachium rosengergii. (Published paper)



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Effect of microalgae concentration on larval survival, development and growth of an Australian strain of giant freshwater prawn *Macrobrachium rosenbergii*

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ABSTRACT

The present study investigated the effects of microalgae *Nannochloropsis* sp. addition and concentration on larval survival, development and growth of an Australian strain of *M. rosenbergii* (lineage II). Newly hatched larvae were reared to postlarval (PL) stage under the condition of no algae addition ('clear water') and four *Nannochloropsis* concentrations of 2.5, 6.25, 12.5 and 25×10^5 cells/ml. All treatments were in quadruplicate and each replicate had 30 larvae stocked in a 5L vessel. Larvae were fed 3 *Artemia*/ml throughout with 100% water exchange daily. The results showed that larval survival to PL at the two higher algae concentrations of 12.5 and 25×10^5 cells/ml (70.8 and 63.3%, respectively) were significantly higher ($P<0.05$) than those of lower algae concentrations of 2.5 and 6.25×10^5 cells/ml and the 'clear water' treatments (26.7, 35.0 and 30.0%, respectively). Meanwhile, the fastest mean development to PL (30.6 days) registered at the highest algal density was 14 days shorter than that of the 'clear water' treatment (44.3 days). Larval development at the two higher algal densities were significantly shorter than that of the 'clear water' treatment and larval development of the highest algal density was further significantly faster than those of the two lower algal densities (40.1 and 40.0 days) ($P<0.05$). The mean dry weights of newly settled PL of the two high algal density treatments were also significantly heavier ($P<0.05$) than those of the lowest algal density and the 'clear water' treatments. The results have shown that the addition of *Nannochloropsis* sp. at appropriate levels substantially improved performance of larval culture of the Australian strain of *M. rosenbergii*, suggesting that the Australian native strain has a promising potential for aquacultural development.

1. Introduction

The giant freshwater prawn, *Macrobrachium rosenbergii*, has long been part of the diet of the peoples of Asia and the Pacific who greatly value its flavour. The extensive farming of *M. rosenbergii* employing various traditional methods has a long history in Southeast Asia where it occurs naturally (Ling, 1969). The development of modern culture techniques for the species in the 1960's and 70's has led to the introduction of the species to many countries and farming of the species is now wide spread throughout the world wherever appropriate climate and freshwater impoundments exist (New, 2002, 2005).

The natural distribution of *M. rosenbergii* extends from Pakistan across to Southeast Asia, south to Papua New Guinea, northern Australia (De Bruyn et al., 2004a) and in some Indian and Pacific Ocean islands (Mather and De Bruyn, 2003). In Australia, the giant freshwater prawn is widely distributed throughout the tropical northern regions and endemic to the west of the Great Dividing Range (Short, 2004). Several attempts in the past to grow local *M. rosenbergii* commercially in the country failed, reporting various problems, including low hatchery survival, excessive cannibalism, lack of technical expertise and infrastructure to consistently produce postlarvae (Cantrelle, 1988) as well as diseases that have affected commercial operations (Bergin, 1986; Owens and Evans, 1989). As a consequence of such failed attempts and strict government regulations that prohibit introduction of foreign species/strains to Australia for aquaculture, commercial freshwater prawn farming is currently non-existent in the country.

The worldwide upsurge in *Macrobrachium* culture in the past decade has prompted research interest again in Australia, particularly on the problematical hatchery phase. These new efforts were further encouraged by recent reports, which through the analyses of 16sRNA, divided natural populations of *M. rosenbergii* into 'eastern' and 'western' forms, demarcated by Huxley's Line, a biogeographic barrier running between Borneo and Sumatra extending north into the Philippines (De Bruyn et al., 2004a). Based on this finding, *M. rosenbergii* in Australia belong to the 'eastern' form, differing from the widely cultured 'western' form of mainly Malaysian origin (De Bruyn et al., 2004a). Further analysis of mitochondrial DNA revealed that within Australia, wild stocks of *M. rosenbergii* can be categorized into four genealogically distinguished lineages, i.e. Western Australia

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(lineage I), Gulf of Carpentaria/Northern Territory (lineage II), Irian Jaya (lineage III) and Papua New Guinea/North east Cape York (lineage IV) (De Bruyn et al., 2004b).

In line with these new findings, in the attempts to assess the aquaculture potential of various Australian native strains of *M. rosenbergii*, wild broodstock of Lineage II were sourced from the Flinders River system, North Queensland and larval culture trials were carried out based on techniques derived from the 'western form' (New, 2002). As both the 'clear water' and 'green water' methods have been used for larval culture of the 'western form' of *M. rosenbergii* and no clear verdict has been made as to which was superior (New, 2002), both methods were trialled during several larval culture runs for the Australian strain of *M. rosenbergii* in identical tanks to compare their relative merits. Interestingly, contrary to the current trend of commercial hatcheries overseas opting for the 'clear water' method (New, 2002), results from our trials suggested that the 'green water' method consistently produced significantly better results for the Australian strain of *M. rosenbergii*. Anecdotal observations further suggested that larval performance was linked to the density of microalgae added. Hence, the present study was designed to quantitatively assess the effects of algae addition on larval survival, development and growth of the Australian strain of *M. rosenbergii*.

2. Materials and methods

2.1. Source of broodstock and larvae

Broodstock prawns were collected from the Flinders River system and its tributaries, near the Gulf of Carpentaria (latitude 17°52.522; longitude 140°46.837), North Queensland, Australia. The prawns were transported overnight in 200 L black plastic drums with aeration to the Marine and Aquaculture Research Facility Unit (MARFU), James Cook University (JCU), Townsville, Queensland. Upon arrival at MARFU, broodstock prawns were held in recirculating 2500 L tanks at a female to male ration of 4–5:1. The prawns were fed daily in excess on shrimp, mussel, squid and formulated feed (36% crude protein; 6% crude fat and 3% fibre) designed for black tiger prawn *Penaeus monodon* (Ridley Aqua-feed, Australia). Samples of these wild prawns were sent to Queensland University of Technology (QUT), Brisbane for identification and were confirmed as Lineage II from rivers flowing into the Gulf of Carpentaria (De Bruyn et al., 2004b).

The development of ovaries and spawning of the female prawns were monitored closely. Berried females were transferred to 5–8‰ brackish water for incubation and embryonic development monitored to predict the date of hatching. On the day of hatching, broodstock females were removed from the hatching tank after larvae had hatched and salinity in the tank was increased to 12‰. Newly hatched larvae were held for 1 h at 12‰ before being collected and transferred to experimental containers for the experiment.

2.2. Experimental design and setup

Five larval culture treatments were setup with concentrations of green microalgae *Nannochloropsis* sp. set at five levels of 0, 2.5, 6.25, 12.5 and 25×10^5 cells/ml. All treatments were in quadruplicate where each replicate consisted of a 5 L round clear plastic container (diameter=215 mm; depth=177 mm) stocked with 30 randomly selected newly hatched larvae in 12±1‰. Replicates of various treatments were organised in a random block design inside water baths set at 30.0±1.5 °C. Each container was covered with a clear plastic lid to prevent larvae jumping out during the late larval stages and gentle aeration was provided to each container via a fine-tipped glass pipette inserted through the lid. Photoperiod was set at 14.5 L: 9.5D throughout the experiment and larvae were cultured from the day of hatching (day 0) until they either reached postlarval stage (PL) or death in all replicates.

Throughout the experiment, larvae were fed a ration of 3 *Artemia*/ml with 100% water exchange carried out daily. Water was exchanged in the morning where any mortality was recorded and the surviving larvae of each replicate were transferred to an identical container with freshly prepared food (3 *Artemia*/ml and designated concentration of *Nannochloropsis*) and 12‰ water. The water salinity was pre-adjusted by diluting natural seawater of 33–36‰ with dechlorinated tap water using a refractometer (Shibuya Salinometer S-10). Water quality parameters, including ammonia, nitrite, nitrate, pH and DO, were measured weekly. Over the period of the experiment, the ammonia, nitrite and nitrate ranged from 0–0.3, 0–0.1 and 0–10 mg/L, respectively, while pH fluctuated between 7.4 to 7.9 and DO between 5.7 to 6.3 mg/L.

Microalgae *Nannochloropsis* sp. was mass cultured in 3000 L tanks at JCU's algal culture facility using a commercially available fertiliser (AQUASOL, Yates Ltd, New South Wales, Australia). The *Nannochloropsis* cultures were generally re-inoculated every 7–10 days. During the experiment, a stock solution was prepared daily by selecting a *Nannochloropsis* sp. culture at its exponential phase of growth and diluted to 12‰ by mixing with dechlorinated freshwater in a 20 L container with strong aeration. Samples were then taken from the stock solution and the algal density counted using a hemocytometer under a high power microscope. The *Nannochloropsis* concentration of the stock solution was estimated by averaging the concentrations of five 1 ml samples. The volume of stock solution required to prepare a desired concentration of microalgae for each treatment was calculated using the equation:

$$C_1V_1 = C_2V_2$$

where C_1 was the designated algal concentration for a particular treatment and V_1 was the total volume required for daily water exchange of the treatment; C_2 was the algal density of the stock solution and V_2 was the volume of the stock solution required to prepare the designated algal concentration for the particular treatment. The required volume of the stock solution was subsequently measured and diluted with 12‰ brackish water to prepare the total volumes required for daily water exchange. Meanwhile, *Artemia* cysts (INVE Inc, Thailand) were hatched daily in 18‰ and newly hatched nauplii were harvested early morning on the following day and their density counted before being fed to the larvae directly without enrichment.

Every 3 days, 16 larvae (4 from each replicate) were randomly sampled from each treatment and their developmental stage identified under a microscope according to Uno and Kwon (1969). The larvae were placed in a small pool of water during the stage identification and returned to the original culture promptly after staging. Such a process has been shown previously not to lead to larval mortality when operated properly. Once postlarvae were found in a replicate during the daily check, they were removed from the cultures and euthanized by quick freezing. The larvae were then measured for their carapace length (mm) using a microscope equipped with a camera (Leica). They were subsequently dried individually in a 60 °C oven for 24 h before being weighed for dry weight using a Cahn C-33 microbalance (0.001 mg).

2.3. Data analysis

Based on results of larval staging, larval stage index (LSI) was calculated according to Manzi et al. (1977) and Mallasen and Valenti (2006):

$$LSI = (\sum S_i \times n_i)/N$$

Where S_i=larval stage (i=1–11; representing each larval stage); n_i=number of larvae in stage S_i; N=total number of larvae examined. The survival, mean development time from hatching to PL, mean carapace

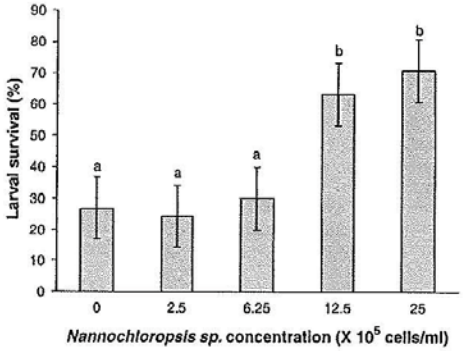

Fig. 1. Cumulative larval survival from hatching to postlarvae of an Australian strain giant freshwater prawn *Macrobrachium rosenbergii* (Lineage II), cultured under different concentrations of *Nannochloropsis* sp. Data are presented as mean±SD (n=4). Different superscripted letters indicate significant differences (P<0.05).

length of PL were analysed using one-way ANOVA while mean dry weights of newly settled PL were log transformed prior to analysis with one-way ANOVA. Tukey's test was employed to detect significant differences among treatments at the 0.05 significance level (Sokal and Rohlf, 1995). All data analysis was carried out using statistic's package, SPSS Version 16.0.

3. Results

3.1. Larval survival

Fig. 1 shows percentage survival of newly hatched larvae to PL stage of the different treatments. The effects of *Nannochloropsis* addition on larval survival were evident: the highest survival (70.8%) was obtained for the highest microalgae concentration of 25×10^5 cells/ml, followed by the second highest microalgae concentration treatment of 12.5×10^5 cells/ml (63.3%). Substantially lower survival was recorded from the 'clear water' treatment (30.0%) and two lower microalgae density treatments of 2.5 and 6.25×10^5 cells/ml (26.7% and 35.0%, respectively). Statistical analyses showed that larval survival of the two high algal treatments were significantly better than that of the other 3 treatments of lower or no algal addition (P<0.05), however, no significant differences was detected between the two highest algal treatments and among other three treatments (Fig. 1).

Interestingly, the plot of mean daily larval survival of various treatments showed that for the first 5–6 days, larval survival were all very high and not much different from each other. The major departing in larval survival occurred mainly over the period of day 7 to 20, during which larval survival of the two high algal treatments remained >80% while those of the low algal and 'clear water' treatments dropped substantially (Fig. 2).

Table 1
Mean development time to postlarval stage (PL), carapace length and dry weight of newly settled postlarvae cultured at different microalgae (*Nannochloropsis* sp.) concentrations

Nannochloropsis sp. concentration (cells/ml)	Mean development time from hatching to PL (days)*	Carapace length of newly settled PL (mm)*	Dry weight of newly settled PL (mg)*
0	44.3±4.0^c	3.03±0.44^a	0.676±0.177^b
2.5×10^5	40.0±5.2^{bc}	3.03±0.39^a	0.676±0.124^b
6.25×10^5	40.1±3.1^{bc}	2.92±0.31^a	0.704±0.156^{ab}
12.5×10^5	33.2±3.5^{ab}	3.06±0.20^a	0.852±0.241^a
25×10^5	30.6±2.1^a	3.05±0.25^a	0.852±0.204^a

*Different superscripted letters of a same column indicate significant differences (P<0.05).
Data are presented as mean±S.D. (n=4).

3.2. Larval development

Larval development, as measured by mean time required to develop to PL, showed a clear trend of improvement with increased algae addition (Table 1). The fastest development registered at the highest algal density (25×10^5 cells/ml) was about 14 days shorter than that of 'clear water' treatment. Statistics showed that larval development at the highest algal concentration was significantly faster than those of two lower algal concentrations (2.5 and 6.25×10^5 cells/ml) and the 'clear water' treatment while the larval development of the second highest algal treatment (12.5×10^5 cells/ml) was also significantly shorter than that of the 'clear water' treatment (P<0.05) (Table 1). Furthermore, larval development appeared to be more synchronised at the highest algae concentration of 25×10^5 cells/ml, as indicated by a substantially reduced standard deviation (Table 1).

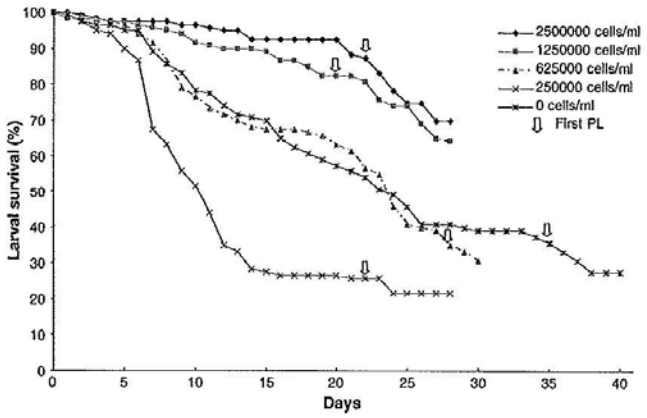

Fig. 2. Daily percentage survival of larvae of an Australian strain giant freshwater prawn *Macrobrachium rosenbergii* (Lineage II), cultured under different concentrations of *Nannochloropsis* sp. Note that postlarvae survival is not included and the survival line of a treatment terminated on the day that all larvae in the treatment became PL or were dead.

Table 2
Larval stage index (LSI) for larvae cultured at different microglae (*Nannochloropsis* sp.) concentrations

Nannochloropsis concentration (cells/ml)	Day 2	Day 5	Day 8	Day 11	Day 14	Day 17	Day 20	Day 23**	Day 26	Day 29	Day32	Day 35	Day 38
0	2.8 ± 0.1^{a}	4.1 ± 0.5^{b}	4.9 ± 0.4^{b}	5.9 ± 0.5^{c}	6.4 ± 0.9^{b}	7.1 ± 0.9^{b}	7.7 ± 0.8^{c}	7.9 ± 1.0^{a}	7.9 ± 0.7^{b}	8.7 ± 0.7	8.9 ± 0.4	8.8 ± 0.6	9.5 ± 0.7
2.5×10^{5}	2.5 ± 0.7^{a}	4.3 ± 0.6^{b}	5.1 ± 0.5^{ab}	6.2 ± 0.3^{c}	7.5 ± 0.3^{ab}	7.8 ± 1.0^{ab}	8.3 ± 0.5^{bc}	8.9 ± 0.4	9.3 ± 0.2	9.8 ± 0.7	9.9 ± 0.5	10.1 ± 0.3	–
6.25×10^{5}	2.5 ± 0.7^{a}	4.8 ± 0.1^{ab}	5.3 ± 0.4^{ab}	6.8 ± 0.4^{bc}	7.4 ± 0.3^{ab}	7.4 ± 0.3^{ab}	7.7 ± 0.5^{c}	8.2 ± 0.7^{a}	8.9 ± 0.4^{a}	8.7 ± 0.4	9.1 ± 0.3	9.5 ± 0.5	–
12.5×10^{5}	2.8 ± 0.2^{a}	4.8 ± 0.4^{ab}	5.7 ± 0.1^{ab}	7.3 ± 0.4^{ab}	7.9 ± 0.5^{a}	7.9 ± 0.5^{ab}	9.0 ± 0.5^{ab}	9.2 ± 0.6	9.6 ± 0.3	10.4 ± 0.6	9.9 ± 0.8	–	–
25×10^{5}	3.0 ± 0.0^{a}	5.1 ± 0.1^{a}	6.1 ± 0.7^{a}	7.9 ± 0.5^{a}	8.3 ± 0.4^{a}	8.3 ± 0.4^{a}	10.1 ± 0.1^{a}	10.2 ± 0.4	10.4 ± 0.4	10.6 ± 0.3	10.5 ± 0.3	–	–

*Different superscripted letters of a same column indicate significant differences ($P<0.05$).
**After first appearance of PL, treatments were excluded from the statistical analysis and the data presented after the first PL are for reference only as all PL were removed from culture and not included for the calculation of LSI.

The larval stage index (LSI) calculated based on larval samples taken throughout the culture showed a general trend of higher LSI value with increased algal concentration. Significant differences of LSI occurred as early as day 5, when the LSI of the highest algal concentration was significantly higher than those of the lowest algal concentration and the 'clear water' treatments (Table 2). The first PL appeared on day 20 and 22 for higher algal treatments of 12.5 and 25×10^{5} cells/ml, respectively, it was on day 28 and 35 that the first PL was found in the lower algae concentration of 6.25×10^{5} cells/ml and the 'clear water' treatment, respectively (Fig. 2). At 2.5×10^{5} cells/ml, the first and second PL's were observed relatively early on day 22 and 23, respectively, however, unlike in other treatments where subsequent PL's were found on the following days, the 3rd PL of the treatment appeared only 7 days later, on day 30. The LSI data showed that the first metamorphosis to PL generally occurred at LSI ≤9 for all treatments except that it was 10 for the highest algal density treatment, further suggesting a more synchronised development under the culture condition.

3.3. Postlarval dry weight and carapace length

The dry weight of newly settled PL generally increased with elevated microalgae concentration (Table 1). Dry weights of PL from the two high algal concentrations were the same at 0.852 mg, about 20.6% heavier than the lowest dry weight (0.676 mg) recorded for the lowest algal density and the 'clear water' treatments. The dry weight of PL from the 6.25×10^{5} cells/ml treatment was intermediate at 0.704 mg. Statistics showed that dry weights of the two high algal concentration treatments were significantly higher ($P<0.05$) than those of the lowest algal density and the 'clear water' treatments. In contrast, no significant differences in mean carapace lengths of PL was found among all treatments (Table 1).

4. Discussion

The current study clearly demonstrated that the addition of *Nannochloropsis* sp. at appropriate levels led to significantly improved larval survival, development and growth of the Australian strain of *M. rosenbergii*. Larval survival to PL was significantly higher at the two higher algal levels of 12.5 and 25×10^{5} cells/ml, suggesting that between the algal levels of 12.5×10^{5} cells/ml and 6.25×10^{5} cells/ml, lies a critical threshold. At the highest algal concentration of 25×10^{5} cells/ml, development to PL was the shortest and significantly faster than all other treatments except that of the second highest algal density of 12.5×10^{5} cells/ml. This was also reflected in the LSI values, with generally higher LSI recorded for the higher algal concentrations. Significant differences in LSI were registered as early as on day 5, suggesting that the beneficial effects of algae started rather early.

Furthermore, the improved growth at the two higher microalgae levels was manifested by significantly heavier PL dry weights. It is worth noting that the carapace length of PL was not significantly different among treatments, suggesting that carapace length is not a good indicator for larval growth. Based on the present results, it is recommended that for larval culture of the Lineage II of *M. rosenbergii*, microalgae *Nannochloropsis* sp. should be added at a level $\geq12.5\times10^{5}$ cells/ml.

There are several possible explanations for the observed beneficial effects of adding high levels of *Nannochloropsis* to larval culture of the Australia strain of *M. rosenbergii*. Firstly, the addition of *Nannochloropsis* may provide better nutrition to the larvae. Manzi et al. (1977) reported that algal cells were found in the gut of larvae of the 'western form' of *M. rosenbergii*, however, they were unsure whether those cells were actively consumed or accidentally ingested. These authors further pointed out that it appeared there was no evidence of any overt assimilation of the algal cells and direct nutrition to the larvae (Cohen et al., 1976; Joseph, 1977). Judging by the carnivorous nature of *M. rosenbergii* larvae, the nutritional benefits of direct ingestion of algal cells by larvae is probably limited.

Alternatively, larvae may benefit nutritionally through ingesting *Artemia* grazing on abundance of algae. *Artemia* are the major live feed for *M. rosenbergii* larvae, however, *Artemia* is known to lack some essential nutrients, particularly the highly unsaturated fatty acids (HUFA), such as decosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), that are important for the survival and development of fish and crustacean larvae (Léger et al., 1986). The HUFA content of *Artemia* therefore often determines the food value of *Artemia*. Despite lack of DHA, *Nannochloropsis* sp. is known to be rich in EPA (Volkman et al., 1989), which may contribute to better nutritional content and quality of *Artemia* as food for the freshwater prawn larvae. However, to what extent such contribution was is unknown as *Nannochloropsis* sp. is not considered a particularly suitable diet for *Artemia* nauplii due to toughness and the indigestibility of its cell wall (Dhont and Lavens, 1996).

Secondly, the green microalgae *Nannochloropsis* may provide a culture condition that is less stressful to *M. rosenbergii* larvae and at the same time, may aid their feeding. There is ample evidence that underwater lighting conditions and tank colour could play an important role in foraging success of fish larvae (Ostrowski, 1989; Tamazouzt et al., 2002), far less attention has been paid to crustacean larvae, probably because of the fact they are capable of feeding in the darkness, therefore, implying a feeding mechanism by either random encounter or chemosensory detection (Rabbani and Zeng, 2005). However, such ability does not preclude the possibility that in the presence of light, crustacean larvae may utilise visual cues to enhance their foraging and feeding efficiency (Rabbani and Zeng, 2005). Such a hypothesis is supported by two recent reports that showed tank colour significantly impacted larval survival of the 'western form' of *M. rosenbergii* (Yasharian et al., 2005) and the mud crab *Scylla serrata* (Rabbani and Zeng, 2005). Interestingly, Yasharian et al. (2005) reported that for the 'western form' of *M. rosenbergii*, when the 'clear water' method was used, larvae cultured in red and green tanks had the best and significantly higher survival than those reared in white and blue tanks. This agrees with the finding of AQUACOP (1977) who reported best larval survival from dark green tanks. It was suggested that as visual ability and active predatory behaviour increases with development, the ability to effectively pinpoint the food items become

increasingly important; the green background probably provided better contrast or silhouette for the food items, hence improved larval feeding success, survival and growth (Yasharian et al., 2005). The addition of green algae *Nannochloropsis* may have a similar effect on larval feeding as the green coloured tanks. This is also in agreement with the present results that significant improvement in larval performance mainly occurred when high concentrations of *Nannochloropsis* were added. In a separate study, Lin and Omori (1993) found that feeding rates of larval *M. rosenbergii* decreased with the increase in lightness of the rearing container. They found that the speed and distance of horizontal movement of the larvae increased by 3 times in the white containers compared to those in black containers. Yasharian et al. (2005) suggested that such excess 'excitation' behaviour may contribute to lower feeding rates and that white background may represent a chronic stressor to the larval prawn. Similarly, the addition of *Nannochloropsis* may serve to reduce the stress and allow larvae to feed more readily and effectively.

Finally, adding microalgae to larval culture may help improve water quality via a reduction in ammonia and other nitrogen wastes in the culture medium (Cohen et al., 1976; New, 2002). However, considering that the water used in the current experiment was totally exchanged daily, a built up of toxic nitrogen compounds seems less likely to be a major limiting factor. However, there is also a possibility that microalgae may secrete unknown bioactive chemicals that inhibit various pathogens or directly benefit the larvae. Obviously, the beneficial effects of adding *Nannochloropsis* to *M. rosenbergii* larval culture could be multiple with added-on or synergistic effects, which is an interesting area that warrants further research. Whatever the underling mechanisms may be, the results of this study clearly show that larvae reared in high microalgae densities performed significantly better for the lineage II Australian strain of *M. rosenbergii*. It strongly suggests that microalgae addition is important to larval culture of this strain of *M. rosenbergii* and there is a threshold level to be reached before such beneficial effects become apparent.

Microalgae have also previously been reported to have a significant role in the success of the larval culture of the 'Western form' of *M. rosenbergii*. The pioneer work of Fujimura (1966) and Fujimura and Okamoto (1972) highlighted the importance of using microalgae and *Artemia* nauplii in larviculture as it increased larval survival and reduced the time to achieve PL. Other researchers also confirmed that unialgal supplements, such as *Isochrysis galbana* and *Tetraselmis suecica*, in combination with *Artemia* nauplii improved larval growth and survival (Wickins, 1972). Manzi et al. (1977) further reported that algal supplements, particularly those of the Chrysophta family (i.e. *Isochrysis galbana*, *Pseudoisochrysis paradoxam* and *Phaedactylum tricornutum*) significantly increased survival and PL production in both static and recirculating culture systems. Unfortunately, the optimal level of microalgae was not established experimentally by these experiments. For example, algal concentrations were reported to range from 20,000 to 1,200,000 cells/ml with an average of 340,000 cells/ml by Manzi et al. (1977). More recently, New (2002) recommended that *Chlorella* sp. to be added at a range of 750,000 to 1,500,000 cells/ml for green water hatchery culture of *M. rosenbergii* larvae. Overall, these ranges of algal levels used for *M. rosenbergii* larvae culture are comparatively higher than those used for marine shrimp, such as *Penaeus stylirostris* and *P. vannamei*, for which diatom *Chaetoceros gracilis* concentrations between 30,000 and 100,000 cells/ml have been reported to assure good growth and survival of the zoeal larvae (Simon, 1978).

Despite earlier research showing beneficial effects of the 'green water' method for the 'Western form' of *M. rosenbergii* larvae, commercial hatchery operations have reportedly moved away from the 'green water' method in favour of the 'clear water' method (New, 2002). A possible explanation for this trend is probably the perceived extra requirements for facilities, labour and expertise for the 'green water' method as well as its management complexity. Another reason could be that, as demonstrated by this study, if the algal concentration did not reach a required threshold, its beneficial effects is relatively limited, which could well sway a hatchery manager to abandon the practice. There were however indications that the adaptation of 'green water' culture may be more crucial for the 'eastern form' of *M. rosenbergii* as 'clear water' generally produced <30% larval survival for the lineage II larvae in our cultures while substantially higher survival has been reported for the 'western from' of *M. rosenbergii* with 'clear water' culture (AQUACOP, 1983). Whether strain differences exist, a systematic reassessment of the pros and cons of the 'clear' vs 'green water' methods for the hatchery culture of the 'western form' of *M. rosenbergii*, including identifying optimal concentrations, may be worthwhile.

Our results clearly showed that hatchery culture of the Australian strain of *M. rosenbergii* is technically feasible when the 'green water' method is adopted. The establishment of the 'green water' method based on microalgae *Nannochloropsis* sp., a euryhaline, hardy species suitable for large scale outdoor culture, could also prove beneficial. Overall, the larval survival and development of this strain are comparable to those of the 'western form'. For example, using the 'green water' method, Manzi et al. (1977) reported survival ranging from 75 to 82% for the 'western form' of *M. rosenbergii*. First PLs were observed in the current cultures from day 20 to 35 in various treatments, similarly, 18 to 35 days were reported for the 'western form' (Manzi and Maddox, 1977; Manzi et al., 1977; Malecha et al., 1980). The slightly lower survival recorded in the current experiment is most likely due to differences in culture conditions, such as substantially smaller culture vessels used (1 L vs. 60 L) and high stress levels caused by regular handling during daily water exchange and regular larval staging in the current experiment. In fact, in our larval culture trials using large 400–500 L tanks, survival has consistently been higher than 85% with PL production as high as 170 PLs/L culture water. PLs also appeared as early as day 18 with >95% larvae reaching PL by day 24–26. The promising larvae culture results of this Australian strain of *M. rosenbergii* not only show potential for the development of a freshwater prawn aquaculture industry in Australia, but also possible cross breeding programmes with other strains/form for the improvement of productivity of *M. rosenbergii* worldwide (New, 2005).

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11.4 Appendix 4: *Macrobarchium rosenbergii* nodavirus disease (white tailed disease) in Australia. (Published paper)

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DISEASES OF AQUATIC ORGANISMS
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Macrobrachium rosenbergii nodavirus disease (white tail disease) in Australia

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ABSTRACT: The index case of white tail disease (WTD) is presented in adult broodstock prawns *Macrobrachium rosenbergii* from the Flinders River in western Queensland, Australia, in mid-2007. Histological examination revealed extensive myonecrosis with massive infiltration of myonuclei and some haemocytes. Juveniles from the same broodstock but not from 3 other families displayed white muscle lesions. Low-grade chronic mortalities approaching 100% over 1 yr occurred. Reverse transcriptase polymerase chain reactions (RT-PCR) were attempted for both *M. rosenbergii* nodavirus (*Mr*NV) with 2 sets of primers and for the satellite virus, extrasmall virus (XSV). All 3 PCRs generated amplicons of the expected sizes. Basic local alignment search tool (BLAST) analyses of the 3 consensus sequences identified a 91% match with *Mr*NV viral capsid protein gene, 96% match with *Mr*NV RNA-directed RNA polymerase gene, and a 99% match with *M. rosenbergii* XSV capsid protein gene. The clinical signs, histopathological lesions and RT-PCR amplicons could be reproduced in *M. rosenbergii* inoculated with cell-free extracts fulfilling River's postulates. We conclude that this is an endemic strain of *Mr*NV as the sequences are dissimilar to strains of *Mr*NV circulating around Asia and the Americas. This case only poorly meets the Office International des Epizooties (OIE) case definition for WTD due to the age of the prawns involved and the nature of the inclusion bodies. Perhaps the OIE case definition needs broadening.

KEY WORDS: White tail disease · WTD · *Macrobrachium rosenbergii* · Broodstock · *Macrobrachium rosenbergii* nodavirus · *Mr*NV · Extrasmall virus · XSV

INTRODUCTION

The giant freshwater prawn *Macrobrachium rosenbergii*, sometimes called cherabin in Australia, or scampi internationally, is farmed throughout Asia and the Pacific mainly for domestic consumption. It has been distributed widely around the tropical regions of the globe (Qian et al. 2003, New 2005). During aquaculture of the giant freshwater prawn, diseases have arisen, including the widespread white tail disease (WTD; Arcier et al. 1999, Tung et al. 1999, Qian et al. 2003). The causative agent of WTD has been identified as *Macrobrachium rosenbergii* nodavirus (*Mr*NV), which is a small, icosahedral, non-enveloped virion, 26 to 27 nm in diameter, containing 2 segments of RNA.

In Australia where *Macrobrachium rosenbergii* is endemic west of the Great Dividing Range, a number of attempts have been made to grow this species commercially. All attempts have ultimately failed due to disease problems. The first recorded failure of a farm established in northern Queensland was due to microsporidian infections that made the product unpalatable (Bergin 1986). Later, mortalities from mid-cycle disease caused by Gram-negative bacteria, mostly *Enterobacter aerogenes* and *Vibrio alginolyticus*, affected commercial farms in Western Australia and northern Queensland, which forced their closure (Owens & Evans 1989).

Recently, the worldwide upsurge in interest in *Macrobrachium* culture has prompted research in Australia, particularly on the problematical hatchery phase. Re-

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cent analysis of 18S rRNA gene sequences suggested that *M. rosenbergii* can be divided into 'eastern' and 'western' lineages separated by the Huxley's Line biogeographic barrier (De Bruyn et al. 2004). Based on these findings, Australian *M. rosenbergii* belong to the 'eastern form' and differ from the widely cultured 'western form'. The research further found that within Australia, *M. rosenbergii* distributed throughout the northern tropical and sub-tropical regions could be further categorised into 4 genetically distinguished lineages (Lineages I to IV; De Bruyn et al. 2004).

Wild broodstock belonging to Lineage II were sourced from the Flinders River area of western Queensland and were used to establish 4 families in hatchery facilities at James Cook University. Adults of one family started to display white muscle and mortalities during the cold winter months. Incidental observations suggested that nearly all broodstock infected with WTD died. However, for early juveniles that showed minor WTD symptoms, which subsequently subsided, their continued growth was reduced substantially compared to normal juveniles. Here we describe the histopathology, experimental infections, incorporating reverse transcriptase polymerase chain reaction detection of *Mr*NV and genome sequencing.

MATERIALS AND METHODS

Source of *Macrobrachium rosenbergii* broodstock. Wild broodstock were collected from the Flinders River system and its tributaries, near the Gulf of Carpentaria region (17° 52.522' S; 140° 46.837' E), Queensland, Australia. Samples of the prawns were sent to Queensland University of Technology, Brisbane, Australia, for lineage identification and were confirmed to belong to Lineage II from the rivers flowing into the Gulf of Carpentaria (De Bruyn et al. 2004). Broodstock were held in several 2500 l tanks subject to ambient changes in temperature.

Histology. The cephalothoraxes of all prawns were prepared for histology by splitting laterally. The cephalothoraxes were placed in Davidson's fixative for 48 h, and the tail of each prawn was stored in 95% ethanol for RT-PCR. After 48 h, fixed tissues were transferred to 70% ethanol and processed for histology using standard methods before being embedded in paraffin wax. Sections were cut at 5 µm and stained with Mayer's haematoxylin and eosin. Special stains including methyl-green pyronin, Feulgen's and phloxine tartrazine were used to check for inclusion bodies. Light microscopy (Olympus E C microscope) was used to view the sections. Photographs were taken using an Olympus Camedia 5.0 Megapixel Digital Camera with a C-5050 zoom.

Preparation of inoculum. Tail muscle tissues from the index case of *Macrobrachium rosenbergii* WTD were homogenised in phosphate-buffered saline (PBS) in a stomacher bag with a hammer. The homogenate was partial clarified by centrifugation at 7000 × *g* in an Eppendorf 5424 centrifuge. A 0.45 µm syringe filter was then used to make a cell-free extract.

RNA extraction. Total RNA was extracted from tail muscle of 10 prawns from the diseased family and from experimental prawns using the SV Total RNA Isolation System (Promega) according to the manufacturer's instructions, and RNA was used immediately for reverse transcription-polymerase chain reaction (RT-PCR).

RT-PCR. As the histopathology was consistent with WTD (see 'Results' below) and not consistent with other viruses found naturally or experimentally in *Macrobrachium* spp., PCRs targeted only *Mr*NV and extra small virus (XSV). cDNA was synthesised using the SuperScript III First-Strand Synthesis System for RT-PCR (Invitrogen). cDNA was amplified using *Mr*NV primers described by Sri Widada et al. (2003) and Yoganandhan et al. (2006) and for XSV by Sri Widada et al. (2004). The sequence of Sri Widada et al.'s (2003) oligonucleotide primers were forward (1A775) 5'-CCA CGT TCT TAG TGG ATC CT-3' and reverse (1B690) 5'-CGT CCG CCT GGT AGT TCC-3'. The oligonucleotide primers from Yoganandhan et al. (2006) were *Mr*NV (no name) forward 5'-GAT ACA GAT CCA CTA GAT GAC C-3' and *Mr*NV (no name) reverse 5'-GAC GAT AGC TCT GAT AAT CC-3', whilst the XSV primers from Sri Widada et al. (2004) were (XS-1) forward 5'-GGA GAA CCA TGA GAT CAC G-3' and (XS-5) reverse 5'-CTG CTC ATT ACT GTT CGG AGT C-3'.

Each PCR mixture contained 1× *Taq* buffer (750 mM Tris-HCl pH 8.8, 200 mM $[NH_4]_2SO_4$, 0.1% Tween 20), 2.5 mM $MgCl_2$, 0.75 U *Taq* polymerase (MBI Fermentas), 200 µM each dNTP, 50 pmol of each primer and 20 to 50 ng of DNA template. The PCR volume was adjusted with sterile distilled water to a final volume of 25 µl. Amplification was performed in an Eppendorf Mastercycler Gradient Thermocycler with a PCR profile consisting of an initial 94°C for 7 min, 35 cycles at 94°C for 45 s, 55°C for 45 s and 72°C for 1 min, and 5 min at 72°C on the last cycle. The PCR products were analysed by electrophoresis in 1.0% agarose gels containing 0.5 µg ml^{-1} ethidium bromide.

Cloning and sequencing. RT-PCR amplicons were purified from agarose gels using the Wizard® SV Gel and PCR Clean-Up System (Promega), ligated directly into the pGEM-T® Easy Vector (Promega) and transfected into *Escherichia coli* JM 109 cells. Blue/white screening was used to identify *E. coli* cells containing recombinant plasmids that were purified from 4 white

colonies using the Wizard® Plus SV Minipreps DNA Purification System (Promega), according to the manufacturer's instructions. Plasmid DNA was digested with *Spe*I, followed by electrophoresis in 1.0% agarose gels to screen for DNA inserts. Plasmids containing DNA inserts were sent to Macrogen Inc. for sequencing using M13 universal primers. Three forward and 3 reverse sequencing reactions were performed on each clone. Sequencher™ software (Gene Codes Corporation) was used to analyse and align overlapping sequences for each clone. Sequence results were compared to the GenBank database using the basic local alignment search tool (BLAST) at the National Center for Biotechnology Information (NCBI) (www.ncbi.nlm.nih.gov/).

The 861 nucleotides (nt) consensus sequence for *Mr*NV using Sri Widada et al.'s (2003) primers was generated from 12 overlapping reads. With Yoganandhan et al.'s (2006) primers, the 682 nt consensus sequence was generated from 5 reads. The 508 nt consensus sequence for XSV using Sri Widada et al.'s (2004) primers was from 8 reads. *Mr*NV sequences from GenBank were trimmed to be the same number of nt as the Australian isolate, and ClustalW2 with a neighbour-joining algorithm was then used to produce the phylograms.

Experimental infections. Juvenile *Macrobrachium rosenbergii* approximately 3 to 4 cm in length and 2 g in weight were obtained from one uninfected family at the Marine & Aquaculture Research Facilities Unit (MARFU), James Cook University. Prawns were divided at random into 3 treatment groups: (1) control, (2) per os exposure, and (3) inoculated, each with 3 replicates of 10 prawns. Water exchanges were undertaken daily to maintain water quality.

Control prawns were fed only commercial pelleted food. After being starved for 24 h, prawns in the feed exposure were fed muscle tissue of an individual displaying WTD clinical signs at 5% of body weight on Day 0 and fed thereafter on pelleted food. For the inoculated treatment, the cell-free extract was diluted with PBS, and 25 µl were inoculated intramuscularly into the first abdominal segment. The experiment was terminated on Day 30 post injection.

Statistical analysis was undertaken using the Statistical Package for the Social Sciences version 14 with a 1-way analysis of variance (ANOVA) conducted after testing for normality. The significance level was set at $p < 0.05$.

RESULTS

Inoculated *Macrobrachium rosenbergii* and, to a lesser extent, fed prawns developed the same gross clinical signs consistent with WTD (Fig. 1). Prawns from the uninfected family possessed normal muscle tissues (Fig. 2). The index case adults had extensive myonecrosis with massive infiltration of myonuclei and some haemocytes (myositis; Figs. 3 to 5). Similarly, prawns fed infected tissue and prawns injected with muscle extract showed the same myonecrosis of the tail and myositis. Approximately half (10/25 fed and 15/23 inoculated) of the exposed prawns showed mostly limited

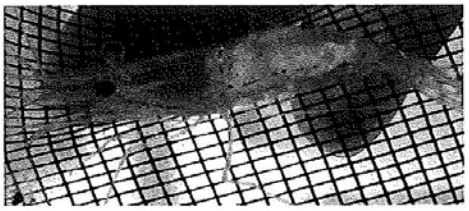

Fig. 1. *Macrobrachium rosenbergii*. Gross signs of white tail disease in a juvenile on Day 25 after injection with a cell-free extract

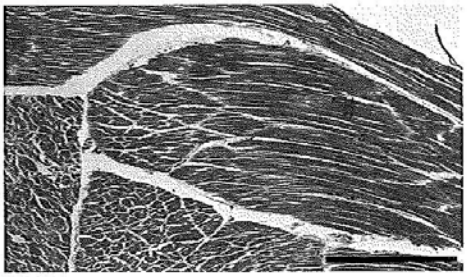

Fig. 2. *Macrobrachium rosenbergii*. Normal abdominal muscle of a control (scale bar = 125 µm)

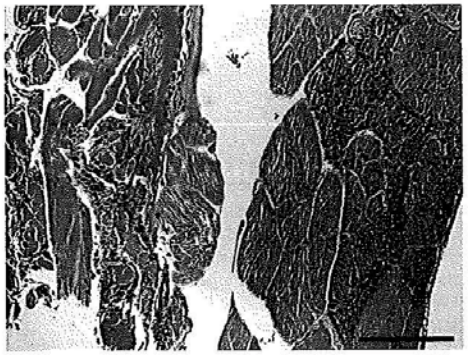

Fig. 3. *Macrobrachium rosenbergii*. Cellulitis in abdominal muscles of index case broodstock (scale bar = 250 µm)

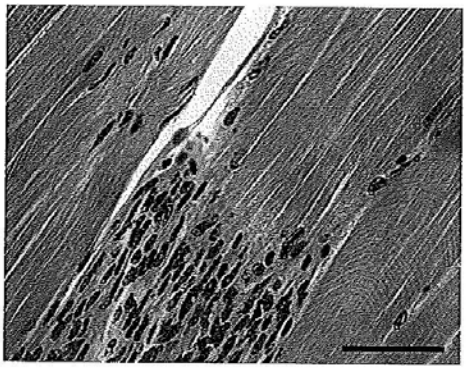

Fig. 4. *Macrobrachium rosenbergii*. Myonecrosis and myolitis of abdominal muscles of index case broodstock (scale bar = 50 µm)

Fig. 5. *Macrobrachium rosenbergii*. Myonecrosis and myolitis of abdominal muscles of the index case broodstock (scale bar = 50 µm). Inclusion bodies of unknown significance were present in many of the haemocytes (arrows)

muscle necrosis and myositis but some displayed extensive areas of lesions. With methyl green pyronin, nuclear debris stained green whilst some cytoplasmic inclusion bodies stained pink (pyroninophilic; data not shown).

When the challenge experiment was finalised at 30 d, the control prawns had the highest survival (90%, 27/30), while the per os exposed prawns had slightly lower survival at (83%, 25/30) and the injected prawns had the lowest survival (77%, 23/30). However, these differences between exposure treatments were not significant ($p > 0.05$). As prawns that died were cannibalised with only the exoskeleton being found the next morning, histology and RT-PCR were not possible on these remains. Of interest, approximately 400 juvenile *Macrobrachium rosenbergii* displaying WTD in a holding tank slowly perished over 12 mo.

All RT-PCRs produced amplicons of approximately the size expected (see below). Seven of 10 progeny were positive with the Sri Widada et al. (2003) primers for *Mr*NV; 6/10 of the same prawns were positive with the Yoganandhan et al. (2006) primers and 5/10 of the same prawns were positive for the XSV (Fig. 6).

The 861 nt viral capsid protein gene (RNA-2) consensus sequence obtained from the PCR amplicons produced with the Sri Widada et al. (2003) primers (expected size 859 nt) had 91.0% identity to *Mr*NV from the French West Indies and 90.9% identity to *Mr*NV from China (the highest 2 matches in BLAST searches; Table 1). This sequence for the Australian isolate of *Mr*NV has been logged in GenBank (accession FJ379531). The 682 nt RNA polymerase gene (RNA-1) consensus sequence (GenBank FJ379530) obtained from the PCR amplicons using Yoganandhan et al.'s (2006) primers (expected size 681 nt) for a different part of the genome had 95.9% identity to *Mr*NV from India and 95.6% identity to *Mr*NV from the French West Indies. The 508 nt consensus sequence (GenBank FJ379532) obtained from the PCR amplicons for XSV with Sri Widada et al.'s (2004) primers (expected size 507 nt) had 99.4% identity to XSV from 2 separate XSV sequences from Thailand.

The phylograms gave similar results in that the Australian isolate of *Mr*NV is the most distant from all other isolates with both the sequences of the RNA-dependent RNA polymerase gene (Fig. 7a) and the capsid gene (Fig. 7b). Due to how concurrent nt changes are weighted in ClustalW, the phylogenetically closest isolate to the Australian isolate was from China (AY231436, AY231437).

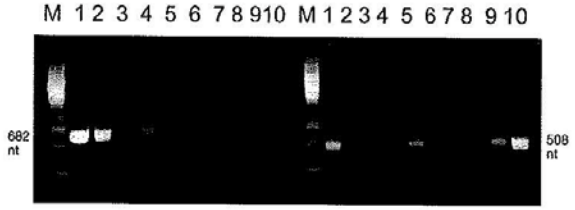

Fig. 6. *Macrobrachium rosenbergii*. RT-PCR amplicons of 10 randomly selected juvenile progeny from infected broodstock for *M. rosenbergii* nodavirus (*Mr*NV) using the primers of Yoganandhan et al. (2006). Lanes labelled M contain the 250 nucleotide (nt) ladder markers (Fermentas). Sizes of the amplicons are marked. Left half of the gel depicts amplicons using primers for *Mr*NV; right half of the gel depicts amplicons using primers for extra small virus produced by the primers of Sri Widada et al. (2004)

Table 1. Top 2 alignments giving the highest scores for sequence identity between Australian *Macrobrachium rosenbergii* nodavirus (*Mr*NV) and other geographical isolates

Primer set	Location	Accession no.	Description	Identity (%)
Sri Widada et al. (2003)	French West Indies	AY222840.1	*Macrobrachium rosenbergii* nodavirus segment RNA-2, complete sequence	91.04
	China	AY231437.2	*Macrobrachium rosenbergii* nodavirus capsid protein gene, complete coding sequence	90.89
Yoganandhan et al. (2006)	India	DQ146969.1	*Macrobrachium rosenbergii* nodavirus RNA-directed RNA polymerase gene, partial coding sequence	95.92
	French West Indies	AY222839.1	*Macrobrachium rosenbergii* nodavirus segment RNA-1, complete sequence	95.58
Sri Widada et al. (2004)	Thailand	EU150133.1	*Macrobrachium rosenbergii* XSV isolate M23 capsid protein gene, complete coding sequence	99.38
	Thailand	EU150132.1	*Macrobrachium rosenbergii* XSV isolate M308 capsid protein gene, complete coding sequence	99.38

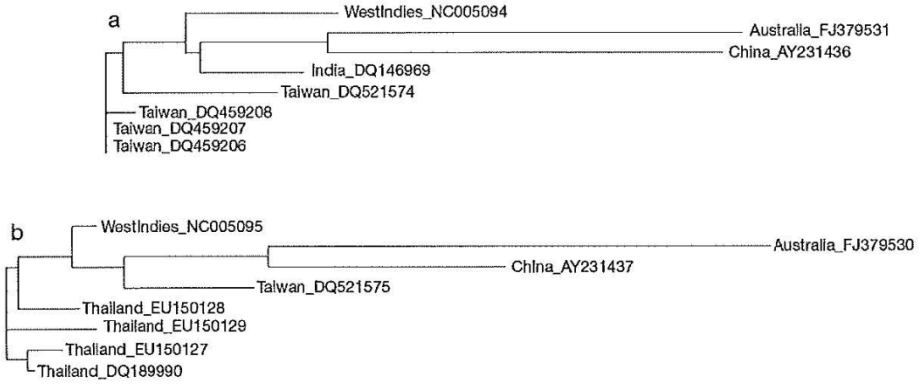

Fig. 7. Phylograms of the sequence the Australian isolate of *Macrobrachium rosenbergii* nodavirus (*Mr*NV) compared to other isolates of *Mr*NV using both sequences from RNA-1 (a) 861 nt and RNA-2 (b) 692 nt. Phylograms were produced by ClustalW2

The RT-PCR results for prawns from the experimental infections were poor, and only 2 inoculated and 1 fed prawn of the 48 exposed prawns that remained generated PCR amplicons using the primers from Yoganandhan et al. (2006). All of the 27 control prawns remaining alive were negative by RT-PCR and histology for evidence of *Mr*NV.

DISCUSSION

Due to the small size of the juvenile prawns, the inoculum was both diluted and reduced in volume so that the mortality due to the injection procedure would be minimised. This may have reduced the mortality rate and the number of positive PCRs from the experimental groups. Other explanations for the limited effect of the inoculum are possible, such as the life stage of the prawns, virulence of the virus and the sensitivity of the PCR. Further research should disclose the probable causes.

The data reported here are consistent with WTD in Australian *Macrobrachium rosenbergii* caused by *Mr*NV. The gross clinical signs, the histopathology, both of which are not pathognomonic for WTD, the genomic RNA sequence relationships and the ability to fulfil River's postulates support the conclusion that *Mr*NV is present in Australia, which was previously declared free of this disease (Ernst 2007). WTD is a notifiable disease to the Office International des Epizooties (OIE) and as such, this case was reported to government authorities and confirmed by analyses undertaken at the Australian Animal Health Laboratories. OIE was notified formally in February 2008.

This case does not quite meet the criteria defined currently in the OIE WTD disease card, mainly due to

the age of the prawns involved. Most previous reports have involved postlarvae or very young juveniles, whereas this case involved adults and older juveniles. The gross signs, the histopathological lesions in muscle and the confirmatory genome sequence are all consistent with the OIE definitions. The mortality in experimental prawns was chronic and limited over the time frame of the study, and inclusion bodies were not seen in connective tissue but rather in the muscle, staining more like RNA (pyroninophilic). Based on the findings in this case, perhaps the OIE definition needs to be broadened somewhat to include adult life stages, and the staining characteristics should be reevaluated.

WTD has been observed in many countries including Taiwan (Tung et al. 1999), French West Indies (Arcier et al. 1999), China (Qian et al. 2003), India (Sahul Hameed et al. 2004) and Thailand (Yoganandhan et al. 2006). This study adds Australia to the list of countries that have reported WTD.

The preliminary data herein suggest that the Australian isolate of *Mr*NV is most closely related to the Chinese isolate. At this time, this suggests that the Australian isolate was introduced at some time by human activities rather than being a reflection of the ancient zoogeographical spread of the virus with its natural host, *Macrobrachium rosenbergii*.

The level of nucleotide sequence identity (91 to 96%) between the genome sequences of the Australian *Mr*NV strain compared to strains from other geographic localities suggests that *Mr*NV has been in Australia for some time and that it is therefore now endemic rather than very recently introduced. However, only sequencing of the full genome of Australian *Mr*NV will determine the complete similarity. This will allow a full analysis of the amino acid changes and a better understanding of the true phylogenetic relationships.

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11.5 Appendix 5: Effect of stocking density on growth and survival of Australian *Macrobrachium rosenbergii* newly settled post larvae. (Paper in preparation)

Effect of stocking density on growth and survival of Australian *Macrobrachium rosenbergii* (De Man) newly settled post larvae

Abstract

Introduction

Initial grow-out studies of freshwater prawn, *Macrobrachium rosenbergii*, were carried out in ponds in Malaysia (Ling & Costello, 1979) after the development stages of the larvae were detailed by Ling & Merican (1961). Commercial development of Malaysian freshwater prawns in Hawaii saw the development of mass rearing techniques for commercial scale production of post larvae for stocking into ponds (Fujimura & Okamoto, 1972). Since then several studies on grow-out of the species has taken place and the Malaysian prawn has been translocated for experimental research and commercial purposes (New, 2000). Technologies to improve management of grow-out has advanced since then with inputs to enhance production such as appropriate stocking densities, substrates, size grading and selective harvesting, either in tropical to temperate regions.

Stocking densities used in grow-out of *M. rosenbergii* are generally extensive, 1 -4/ m², with production at 500kg/ha/yr and semi-intensive, 4–20/ m² and production of 500 – 5000kg/ha/yr for the Western form (Valenti & New, 2000). Intensive production is relegated to experimental use only (Sagi, Ra'anan, Cohen & Wax, 1986; Sebastian, Mathew & George, 1992) and has yet to be economically feasible.

Extensive grow-out is generally practiced with little management intervention and low productivity; semi-intensive practices are employed in commercial operations with management interventions such as feeding, fertilisation, water quality management in ponds and is commonly utilised worldwide (Lee & Wickins, 1992). Intensive culture monoculture in cages showed higher production in all male populations (473 g / m^3) than mixed (260 g / m^3) and all female populations (248 g / m^3) however called for more efforts for research (Sagi *et al*., 1986). Similar results in monosex culture in tanks showed increasing yield and marketable prawns with increasing density and best performance recorded from the all-male population (Siddiqui, Al-Hafedh, Al-Harbi, & Ali, 1997).

Semi-intensive grow-out at higher stocking rates tends to increase production (4,700 vs 3,828 kg/ ha, lower stocking) but survival (73 % vs 66.5 %) and average harvest weight were higher (16.2 g vs 8.5 g) in lower stocking rates and projected higher crop value because of larger animals (Sandifer, Smith, Stokes & Jenkins, 1982).

Productivity has improved with the combination of appropriate stocking densities with other grow-out technologies and appropriate management.

The cannibalistic nature of *M. rosenbergii* (Cohen, Ra'anan & Brody, 1981) is reduced by addition of substrate (shelters) for moulted individuals (Sandifer & Smith, 1977; Cohen *et al*., 1983) increases the surface area in three dimensions in the water column (Tidwell & D'Abramo, 2000; Tidwell, D'Abramo, Coyle & Yasharian, 2005) and improve production yield (Cohen, Ra'anan, Rappaport & Arieli, 1983; Tidwell *et al*., 1998). Substrate orientation, either vertically or horizontally, did not affect survival and average weight of ungraded juveniles after 60 days in nursery culture (Tidwell, Coyle, Van Arnum & Weibel, 2002). The physical characteristics of the type of substrate had little impact on performance of the Malaysian *M. rosenbergii* and cheaper materials can be used to save on costs (Tidwell & Coyle, 2008). An added benefit of substrate use was improved feed efficiency (17%) possibly through increased surfaces for periphyton production (Tidwel *et al*., 1999; Tidwell *et al*., 2005)

The duration of the prawn nursery phase increases size variation requiring size grading to disrupt the socially induced differential growth rates by removing faster growing individuals and allowing other prawns to compensate for their slow growth (Tidwell *et al*., 2005). This social hierarchy is established early in the life stages of prawns, where faster growing individuals retard the growth of other prawns in the population (Karplus, Hulata, Wohlfarth & Halevy, 1986).

Grading of juvenile prawns has shown improved production in grow-out. Graded (0.30 g) and ungraded (0.33 g) prawns stocked into earthen ponds for five months of grow-out, showed increased mean harvest size and mean yield of graded populations by 37.3% and 45.6% over ungraded prawns (D'Abramo, Malecha, Fuller, Daniels & Heinen (1991). Stocking different size fractions of graded populations, especially upper grade, increases total production, average weights and marketable harvest sizes, 20 and 30 g (Tidwell, Coyle & Dasgupta, 2004).

Since grow-out of the Australian freshwater prawn has not been detailed, this study looked at adopting some of the strategies utilised for culture of the Malaysian prawn. Four strains of *M. rosenbergii* have been identified in Australia distributed throughout the northern regions. Strains are classified according to lineages I, II, III and IV conforming to the western form (De Bruyn *et al*., 2004). Lineage II or Gulf strain has been continuously produced at the JCU hatchery. In this study, we investigated the effect of stocking density and subtrate on survival, mean final weights, biomass, dry weights and relationship of length and weight of this strain.

Method

(i) Source of Post larvae

Broodstock of Lineage II, were collected from ponds at DPI, Walkamin were used in hatchery culture to produce post larvae using the greenwater method. Newly settled *M. rosenbergii* post larvae were held in nursery tanks for one week then transferred to experimental conditions for acclimatisation for one week. Post larvae were weighed (0.012 ± 0.004 g) prior to stocking into experimental tanks.

(ii) Experimental design

Experimental aquaria (100 L) were connected to a freshwater recirculation system with two biofilters in laboratory. Aeration was connected to each aquaria. Heaters (75 W) were placed in the water sump to maintain water temperature at 28 ± 2 °C. Photoperiod was set at 14.5L: 9.5D.

Post larvae were stocked into randomly into aquaria at 500, 1000 and 2000 PL/ m^3 (New, 2002) in a nested design without substrate and with substrate. A black plastic mesh was used as substrate (20 mm diagonal length), weighted down with terracotta squares. The surface area of provided by substrate increased the bottom surface area by 66 %. Treatments were in quadruplicate.

Juveniles were fed with a 43% crude protein commercial prawn grower pellet (Ridley's Aquafeed) at 10% body weight. Uneaten feed and waste was siphoned out daily. Water quality (DO, pH, NH_3/NH_4^+, NO_2^-, NO_3^- mg/ L) was checked weekly with 100 % exchange fortnightly. Duration of the study was 60 days.

(iii) Data Analysis

Growth and survival were estimated by a sub-sampling (20 post larvae per replicate) to collect wet weight (g) using an Ohaus digital scale, total length (mm) using vernier callipers and survival (%) at the end of the study. Average weight gain, mean final weights and increase in biomass were also recorded at the end of the study. Sampled juveniles were placed in dryer for 48 h after which were then recorded for dry weights (µg). Data was analysed with one-way ANOVA and Bonferroni's test for significance. Final wet weight and total length were collected at the end of the study and expressed as linear regression.

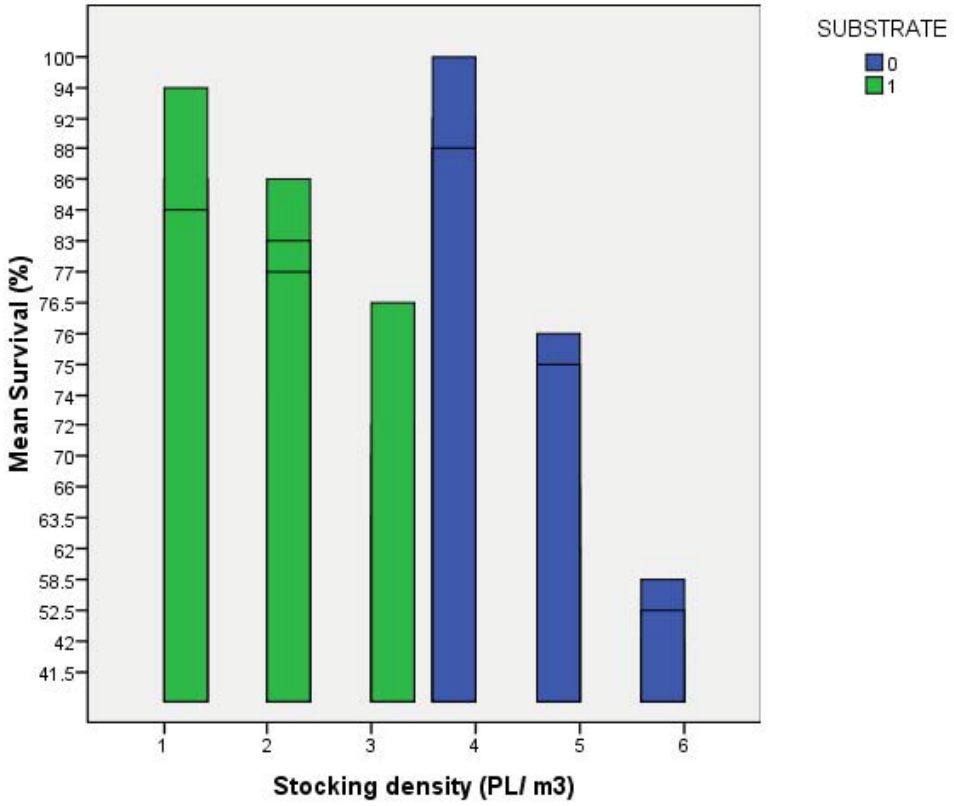
Results

Water quality

Table 1 Water quality parameters for juvenile prawn culture in aquaria.

Water temperature (°C)	27.17 – 30
Dissolved oxygen (mg/ L)	5.52 – 7
pH	6.4 – 8.2
Ammonia (NH_3/ NH_4^+) (mg/ L)	0 – 0.25
Nitrite (NO_2^-) (mg/ L)	0
Nitrate (NO_3^-) (mg/ L)	0.1 – 5

Water quality monitored over the course of the culture period of 60 days as shown in a range of the data recorded (Table 1). Levels of ammonia (NH_3/ NH_4^+ mg/ L), (nitrite NO_2^- mg/ L) and nitrate (NO_3^- mg/ L), pH, dissolved oxygen (mg/ L) were all within favourable conditions.



Survival

Figure 1: Mean survival of *Macrobrachium rosenbergii* juveniles after 60 days of culture, with and without substrate.

NB: 1 &4 = 500, 2& 5 =1000, 3& 6= 2000 PL/ m^3

Table 2: Mean survival of *Macrobrachium rosenbergii* juveniles after 60 days of culture.

Stocking density (PL/ m_3)	Mean Survival (%)
500 (Substrate)	$87.50 \pm 4.43_a$
1000 (Substrate)	$80.00 \pm 5.48_b$
2000 (Substrate)	$70.5 \pm 5.40_{ac}$
500	$92.0 \pm 5.66_{cd}$
1000	$69.75 \pm 6.85_{ad}$
Stocking density (PL/ m_3)	Mean Survival (%)
500 (Substrate)	$87.50 \pm 4.43_a$
1000 (Substrate)	$80.00 \pm 5.48_b$
2000 (Substrate)	$70.5 \pm 5.40_{ac}$
500	$92.0 \pm 5.66_{cd}$

2000	48.63 ± 4.16^{abc}

Values with the same superscripts are significantly different ($p<0.05$).

The highest survival recorded from the study was in the non-substrate, lowest density treatment, 500 PL/ m^3 (92.0 ± 5.66 %) which was significantly ($P<0.05$) higher than all other treatments except the substrate 500 and 1000 PL/m^3 (87.50 ± 4.43 and 80.00 ± 5.48 %) treatments respectively.

Survival in the highest density, non-substrate treatment, 2000 PL/ m^3, was significantly ($P<0.05$) lower than all the other treatments (48.63 ± 4.16 %). Similarly, its equivalent density in the substrate treatment was significantly lower (70.5 ± 5.40 %) from other treatments except the substrate 1000 PL/m^3 (80.00 ± 5.48 %).

There was a significant interaction effect ($P<0.05$) between stocking density and substrate. However survival in both treatments showed consistently higher survival in the different densities with substrate while variable results were recorded in the different densities of the non-substrate treatments.

The substrate treatments generally had high survival overall as compared to the non-substrate treatments which was highly variable. This variability was reflected in the lowest and highest survival recorded in the 500 and 2000 PL/ m^3 (48.63 ± 4.16 and 92.0 ± 5.66 %) densities without substrate, respectively. With increasing stocking density, survival showed a decline with a more marked reduction in survival in the treatments without substrate especially at high densities where cannibalism and competition for space and food would be most intense. Addition of substrate into the culture environment provided shelter from competition and predators more available surface area in 3-dimensions for juveniles to maximise the utilisation of the water column (Tidwell *et al*., 2005).

Table 3: Performance parameters of Australian freshwater prawn, *Macrobrachium rosenbergii* , at different culture densities with and without substrate.

Treatments	Average weight gain (g)	Mean final weight (g)	Increase in Biomass (g)	Dry weights (µg)
500/ m²	0.076 ± 0.079^{a}	0.114 ± 0.031	2.783 ± 0.478^{a}	0.033 ± 0.010
1000/ m²	0.081 ± 0.003	0.118 ± 0.029	4.508 ± 0.992^{b}	0.034 ± 0.010
2000/ m²	0.103 ± 0.011^{ab}	0.139 ± 0.060^{a}	6.149 ± 2.023^{abc}	0.036 ± 0.008
500/ m² (Substrate)	0.079 ± 0.008^{b}	0.122 ± 0.305^{a}	3.118 ± 0.375^{c}	0.033 ± 0.010
1000/ m² (Substrate)	0.088 ± 0.002	0.131 ± 0.036^{a}	6.069 ± 0.683^{c}	0.036 ± 0.010
2000/ m² (Substrate)	0.109 ± 0.024	0.149 ± 0.135	11.592 ± 2.626^{c}	0.033 ± 0.012

Values with similar superscripts are significantly different (P<0.05).

Average weight gain

Significant average weight gained for juvenile prawns were recorded in treatments without substrates, 500 and 2000/ m^2, (0.076 ± 0.079 and 0.103 ± 0.011 g) and the lowest stocking density 500/ m^2 (0.079 ± 0.008) with substrate (Table 2). There were no significant differences in weight gain in other substrate treatments. The average weight gained increased with increasing density, with the highest gains recorded in the 2000/ m^2 densities with substrate (0.109 ± 0.024 g) and non-substrate (0.103 ± 0.011 g) treatments. It could be said that the average weight gained was highest in the substrate treatments versus their non-substrate density equivalent.

Mean final weights

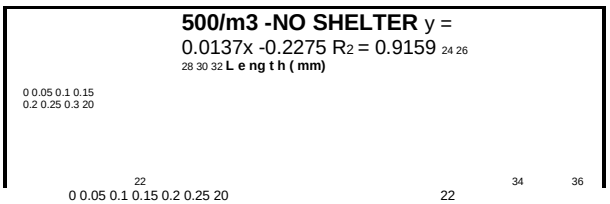
The mean final weights of juveniles were significantly ($p<0.05$) heavier in substrate treatments, 500 and 1000/ m^3, (0.122 ± 0.305 and 0.149 ± 0.135 g) respectively and the highest stocking density, 2000/ m^3, without substrate (0.139 ± 0.060 g) (Table 2). The mean final weights increased with stocking density

however the substrate treatments (500, 1000, 2000/ m^3) showed heavier post larvae (0.122 ± 0.305, 0.131 ± 0.036, 0.149 ± 0.135 g) respectively. The heaviest post larvae were recorded from the highest stocking densities (2000/ m^3) in treatments with substrate (0.149 ± 0.135 g) and without substrate (0.139 ± 0.060 g). The mean final weights were generally heavier in the substrate treatments versus their non-substrate equivalent possibly owing to the effect of added substrate.

Increase in Biomass

Significant ($p<0.05$) increases in biomass were recorded in all the stocking densities, 500, 1000 and 2000 PL/ m^3 with substrate and without substrate (Table 2). The highest increase in biomass (11.592 ± 2.626 g) was recorded in the highest stocking density, 2000/ m^3, in the substrate treatment as compared to the equivalent density in the non-substrate treatment (6.149 ± 2.023 g). Within the non-substrate treatments, 2000/ m^3 showed significant biomass increase (6.149 ± 2.023 g) while significant increases were recorded amongst all substrate densities 500, 1000 and 2000 PL/ m^3
(3.118 ± 0.375, 6.069 ± 0.683 and 11.592 ± 2.626 g respectively). The added artificial substrate possibly has had an effect on biomass with all the substrate treatments having significant augmentation in their biomass.

Dry weights



There were no significant differences ($p>0.05$) in dry weights of juveniles at the different stocking densities with or without substrate (Table 2).

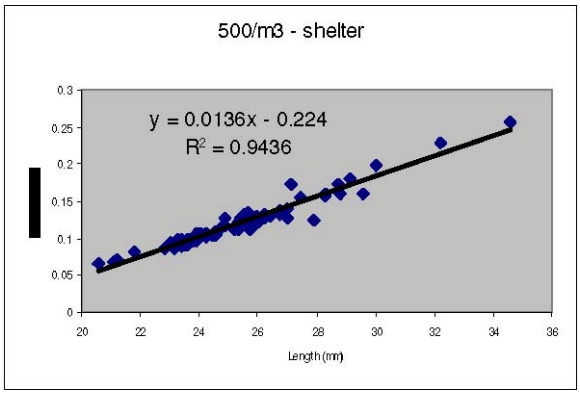
		1000/m3 - (x 10^6 cel
10 + Enrichment	20.50 ± 0.15 a	0.863 ±
10	21.46 ± 1.60 ac	0.848 ±
2.5 + Enrichment	25.76 ± 2.40 b	0.928 ±
2.5	26.96 ± 4.05 bc	0.659 ±
0 + Enrichment	27.28 ± 1.34 b	0.934 ± ab
0	-	-

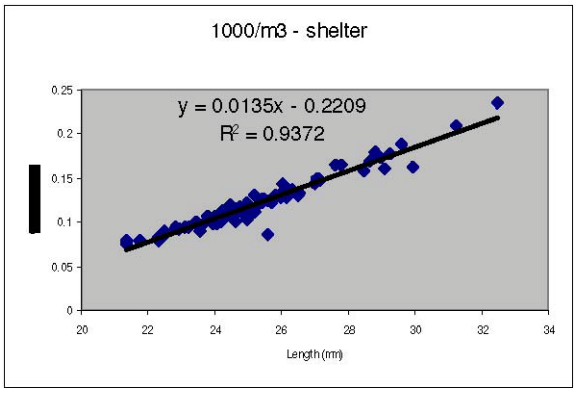
Length-weight

The length-weight relationship of juvenile prawns at the end of the study period showed a strong correlation and regression (Fig.2) between the measured parameters in all the different densities, 500, 1000 and 2000 PL/ m^3 without shelter. Similarly in the treatments with substrate, length-weight relationship showed very strong correlation and regression (Fig. 3) between the two parameters.

Also obvious from the graphs, Figs. 2 & 3, there were outliers with higher values for length-weight. This is possibly owed to the heterogenous individual growth (HIG) being established early in the social hierarchy of the juveniles in culture whereby the biggest individual, possibly male has assuming itself as the dominant individual within the population.

Figure 2: Linear regression of juvenile *Macrobrachium rosenbergii* at 60 days of culture.





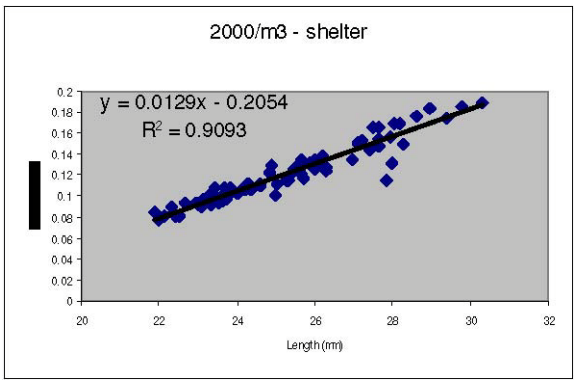


Figure 3: Linear regression of juvenile *Macrobrachium rosenbergii* after 60 days of culture.

Discussion

The performance parameters of the Australian *M. rosenbergii* has shown good results under culture conditions. Average weight gained, mean final weights and increase in biomass, high survival and strong correlation and regression show compelling performance traits. These traits were most enhanced in the higher stocking densities of 2000 PL/ m^3 with the exception of survival which diminished with density. Relatively high stocking densities were used in this study, which was also recommended by New (2002) at 1000 to 2000 PL/ m^3 for primary nurseries with substrates at higher rates when holding for a short period. These were used to determine the performance of the Australian strain in nursery culture. In a period of just over 60 days, including acclimatization, the mean final weights ranged from 0.114 ± 0.031 g in lowest density, 500 PL/ m^3, without substrate to 0.149 ± 0.135 g highest density 2000 PL/ m^3 with substrate. Similarly biomass increase ranged from 2.783 ± 0.478 in 500 PL/ m^3 to 11.592 ± 2.626 g in 2000 PL/ m^3. Given these increases in weights and biomass in a relatively short time, post larvae can be stocked at high densities to before stocking juvenile sizes into grow-out ponds.

Clearly, the added substrate enhanced these performance parameters for the Australian strain which without, produced relatively weaker results of their equivalent density. These high stocking densities, probably demonstrated the efficacy of the substrate in improving production, as suggested by Raanan, Cohen, Rappaport & Zohar (1984). Substrates provide a place to hide after moulting, possibly to avoid competition for space, evade cannibalism from other prawns as well as providing more available surface area to maximise the use of the water column as has been suggested by previous authors. Since only 66% of the bottom tank surface area was increased in this study with addition of substrate, perhaps adding more than 66 % surface area as in this study, would increase survival particularly at the high stocking densities to maximise on production from the culture tanks and reduced operational costs.

The heavier juveniles recorded from the high stocking densities 2000 PL/ m^3 whether with or without substrate probably had much to do with the increased surface area. The added substrate possibly improved feed efficiency producing heavier, larger juveniles as suggested earlier by Tidwell *et al*., (1999). With the added substrate, prawns in these treatments had the advantage of increased surface area and more hiding and perching surface space which possibly produced their larger sizes than juveniles in

stocking densities without substrate. This is probably further intensified by competition for food and space in a densely populated confined area given the cannibalism nature of these prawns, contributes as another source of nutrition besides the artificial feed which all together contributed to heavier prawns.

In studies of the ubiquitous Malaysian prawn, which has been translocated extensively and studied, show similar results to this study. Increasing stocking density saw a parallel increase in total production but average prawn size was reduced and similarly production of market size prawns (Tidwell *et al*., 1999). However, increased stocking densities significantly reduced survival (Siddiqui *et al*., 1997; Coyle *et al*., 2003; Tidwell et al., 2003; Marques *et al*., 2003) as has also been shown in this study. With added substrate, it improved total production (Tidwell *et al*., 1999), increased average weight (Cohen, et al., 1983) and is suggested to be more efficient with intensive stocking densities (Ra'anan *et al*., 1984). Tidwell *et al*. (1999) however did not find a significant interaction between stocking density and substrate but nevertheless significantly improved total production. Various studies including this one show similar results with high stocking densities and reduced survival, with enhanced production, higher averager weights and final weights and improved survival with the added substrate although other findings deviate from these.

High stocking densities for nursery culture of the Australian freshwater prawn has shown relatively good results for a 60 day holding period. In such a short time, heavier juveniles were achieved at high densities in both substrate and without substrate, despite its lower survival in the former, results were relatively good. Also post larvae stocked at high densities with substrate will probably be more efficient in improving production to reduce time and cost of maintenance in the nursery and to reach juvenile sizes for grow-out culture.

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11.6 Appendix 6: The effect of enriched *Artemia* nauplii on survival and development of Australian strain Macrobrachium rosenbergii larvae. (paper in preparation)

The effect of enriched *Artemia* nauplii on survival and development of a Australian strain *Macrobrachium rosenbergii* (De Man) larvae.

Abstract

Introduction

The closure of the freshwater prawn life cycle in Malaysia followed by the development of techniques for commercial farming in Hawaii has led to the wide spread distribution and cultivation of *Macrobrachium rosenbergii* (New, 2000). The species is found from Pakistan to Vietnam, SE Asia, south to Papua New Guinea, northern Australia (De Bruyn et al. 2004a) and some Indian and Pacific Ocean Islands (Mather & De Bruyn, 2003). The species has been divided into two separate forms on either side of Huxley's Line where freshwater pawns west of Huxley's barrier were assigned as 'western' and 'eastern' form for those occurring in the opposite side. (De Bruyn et al., 2004a). Given this description, Australian *M. rosenbergii* are of the eastern form and are distributed throughout the tropical northern regions, endemic to the west of the Great Dividing Range (Short, 2004). Within the Australian *M. rosenbergii* fauna several lineages exist namely, Western Australia (lineage I), Gulf of Carpentaria/Northern Territory (lineage II), Irian Jaya (lineage III) and Papua New Guinea/North east Cape York (lineage IV) (De Bruyn et al. 2004), prospective resources for further improving the performance of the species (Mather & De Bruyn, 2003).

Larvae of Lineage II from the Gulf of Carpentaria were experimentally cultured at JCU utilising both clear and green water methods where the latter utilised four different microalgae concentrations to rear newly hatched larvae to settlement. Growth, survival and development of larvae were superior in the green water method consistently and more so when a threshold of 12.5 x 10^5 cells/ml of microalgae is surpassed before performance can be substantial and the benefits to the larvae are more pronounced. Though at the highest density 25 x 10^5 cells/ml performance was even more enhanced which suggests investigation of higher concentrations and the effect on growth, development and survival.

Microalgae clearly had beneficial contribution to the performance of *M. rosenbergii* larvae culture and we speculate this to be nutritional through *Artemia* nauplii grazing on algae. Earlier attempts to understand the role of microalgae in freshwater prawn larval culture deduced algal cells were found in the gut of larvae (Manzi et al, 1977) but may not have direct nutritional benefit to the larvae (Cohen et al. 1976; Joseph, 1977). Even more, typical fatty acids in microalgae were not manifested in prawn larvae but may possibly have a “species-oriented” lipid metabolism for conversion of microalgae fatty acids (Joseph, 1977). The nutritional role of algae has shown to improve growth in larval crustaceans, fish and molluscs particularly the fatty acid content. A high ratio of *n-3/ n-6* polyunsatrurated fatty acids is generally utilised as an index for high nutritional value provided fatty acids in the microalgae are known. Generally, high concentrations of eicosapentaenoic acid (EPA) 20:5n-3 and docosahexaenoic acid (DHA) 22:6n-3 determines high nutritional value of the microalgae species. Larval stages of crustaceans and fish, all bivalve stages are fed microalgae and is important as food for zooplankton which are fed to late larval and juveniles of fish and crustaceans (Volkman et al, 1989).

The growth and survival of the Australian *M. rosenbergii* larvae showed very good results when cultured at high concentrations of at least 1.25 x 10^6 cells/ml utilising *Nannochloropsis* sp. in a previous study by this research group which suggested high concentrations of microalgae were beneficial for the larvae however the dynamics behind it were obscure. Several investigators speculated on the benefits of increased microalgae concentrations though few have actually studied various concentrations and its effect on larval performance. The microalga, *Nannochloropsis* sp. is known to be rich in 20:5 n-3 eicosapentaenoic acid (EPA) however it is deficient in 22:6 n-3 decosahexaenoic acid (DHA) (Volkman et al, 1989) both vital to growth of crustacean larvae. The determining component of the nutritional value of *Artemia* is the quantity of essential fatty acid 20:5 n-3 eicosapentaenoic acid (EPA) (Léger *et al*., 1986) and docohexanoic acid (DHA) 22:6 n-3, another important essential fatty acid for larvae of brackishwater organisms was almost lacking in *Artemia* (Watanabe *et al*., 1993). Given the limitations of the microalgae and zooplankton, this study investigated the effect of culturing larvae at higher microalgae concentrations and the enrichment of *Artemia* nauplii with DHA on the growth, development and survival of freshwater prawn larvae.

Method

(i) *Source of broodstock* Broodstock for spawning was collected from the Flinder's river system and its tributaries, near the Gulf of Carpentaria region including Armstrong creek (latitude 17°52.522; longitude 140°46.837). Samples were identified as Lineage II from rivers flowing into the Gulf of Carpentaria. Wild broodstock were utilised in hatchery spawning in the previous year and stocked into outdoor ponds at Department of Primary Industries and Fisheries (DPI&F), Walkamin for research purposes. Berried females were collected from outdoor ponds at DPI&F, Walkamin and transported to the James Cook University Marie and Aquaculture Facility Unit (MARFU), Townsville. Broodstock were kept in tanks at a ratio of 4 -5 females: male, in 3000L recirculating freshwater tanks and monitored for spawning. Once berried females were found in tanks they were transferred to 5 – 8 psu holding tanks with aeration until hatching and larvae held for one hour prior to collection for experimentation.

(ii) *Experimental design and set up* Three microalgae concentrations of *Nannochloropsis* sp. 0, 2.5 and 10 were set up into two-factor experiment where *M. rosenbergii* zoea larvae were either fed enriched or non-enriched *Artemia* nauplii. Larvae were randomly distributed into 5L containers at 30 larvae per replicate, containing the designated microalgae concentration and feeding regime where treatments were in quadruplicate. Culture water for the 'no algae' clear water treatment, 0 x 10^6 cells/ml, was prepared daily by adjusting filtered seawater to 12psu with dechlorinated freshwater. Culture containers were randomly distributed inside two water baths (28 ± 1 °C) with photoperiod set at 14.5L: 9.5D. Culture containers were closed with punctured lids to prevent escapees and to relieve air pressure inside the container and placed inside two water baths with fine aeration via fine glass pipettes inserted through lids. Water exchange (100%) was carried out daily for all treatment replicates and similarly feeding regime.

Hatching of *Artemia* cysts (INVE Inc, Thailand) was carried out daily in 18 psu water and newly hatched nauplii were harvested early morning on the following day and divided into two batches, for daily feeding and enrichment. Density was counted before being fed to the larvae directly without enrichment. The second batch was enriched with enrichment medium (DC-DHA) prepared according to manufacturer's instructions and *Artemia* was introduced and cultured for a further 24 hrs and harvested. For the first experimental day, enriched *Artemia* was prepared a day ahead for feeding of larvae in the enriched treatments. The gut of *Artemia* nauplii was checked under microscope for presence of enrichment media

prior to feeding to zoea larvae at 3 *Artemia* nauplii/ml.

Microalgae *Nannochloropsis* sp. was mass cultured in 3000 L tanks at JCU's algal culture facility using a commercially available fertiliser (AQUASOL, Yates Ltd, New South Wales, Australia). The *Nannochloropsis* cultures were generally reinoculated every 7-10 days. During the experiment, a stock solution was made up daily by selecting a *Nannochloropsis* sp. culture at its exponential phase of growth and diluted to 12 psu by mixing it with dechlorinated freshwater in a 20 L container with strong aeration. Samples were then taken from the stock solution and the algal density counted using a hemocytometer under a high power microscope. The *Nannochloropsis* concentration of the stock solution was estimated by averaging the concentrations of five 1 ml samples. The volume of stock solution required to make up a desired concentration of microalgae for each treatment was calculated using the equation:

$C_1V_1 = C_2V_2$ where C_1 was the designated algal concentration for a particular treatment and V_1 was the total volume required for the treatment for daily water exchange; C_2 was the algal density of the stock solution and V_2 was the volume of the stock solution required to make up the designated algal concentration for the particular treatment. The required volume of the stock solution was subsequently measured and diluted with pre-adjusted 12 psu brackish water to make up the total volumes required for daily water exchange.

Every 3 days, sixteen larvae (4 from each replicate) were randomly sampled from each treatment and their developmental stages identified under a microscope according to Uno and Kwon (1969). The larvae were placed in a small pool of water during the stage identification and returned to the original culture promptly after staging. Such a process has been shown previously to not lead to larval mortality when operated properly. Once postlarvae were found in any replicate during the daily check, they were removed from the cultures and euthanazed by quick freezing. The larvae were then measured for their carapace length (mm) using a microscope equipped with a camera (Leica). They were subsequently dried individually in a 60°C oven for 24 h before being weighed for dry weight using a Cahn C-33 microbalance (0.001 mg).

Based on results of larval staging, larval stage index (LSI) was calculated according to Manzi et al. (1977) and Mallasen et al. (2006):

$$LSI = (\sum S_i \times n_i)/N$$

Where, S_i = larval stage (i=1-11; representing each larval stage); n_i = number of larvae in stage S_i; N = total number of larvae examined. The cumulated percentage survival from hatching to postlarval stage (PL) was determined by dividing the total number of successfully metamorphosed PL by the initial number of larvae stocked in each replicate.

Mass cultures of the six different treatments were carried out separately in 10L containers under similar microalgae concentrations, feeding regime and daily water exchange with culture conditions kept uniform as that of the experiment. Upon settlement, post larvae were harvested and snap frozen in liquid nitrogen and stored in -80°C for fatty acid analyses.

Lipids were extracted from samples with chloroform/methanol by the method of Folch *et al* (1957). Total lipid was determined gravimetrically on an aliquot of the extract by drying for 4 hours at 80°C in a pre-weighed glass vial. Aliquots of sample lipid extracts were taken for fatty acid analysis. The lipid fatty acids in the extracts were derivitised to their fatty acid methyl esters (FAME) using 14% boron trifluoride-methanol (Van Wijngaarden, 1967). FAME were analyzed on an Agilent Technologies 6890 gas chromatograph using split injection with helium carrier gas and a flame ionization detector. The column

used was a DB23 fused silica capillary column, 30m x 0.25mm, with a 0.25µm coating (Agilent Technologies, USA). Column oven temperature was held at 140°C for 5 minutes and then elevated at 3°C/minute

to 210°C where it was held until all FAME of interest had been eluted. FAME were identified by comparing their retention times with those of authentic standards (Sigma-Aldrich Co, USA), and were quantified by comparison with the response of an internal standard, heneicosanoic acid.

Data Analyses

Based on results of larval staging, larval stage index (LSI) was calculated according to Manzi et al. (1977) and Mallasen et al. (2006):

$$LSI = (\sum S_i x n_i)/N$$

Where, S_i = larval stage (i=1-11; representing each larval stage); n_i = number of larvae in stage S_i; N = total number of larvae examined. The cumulated percentage survival from hatching to postlarval stage was determined by dividing the total number of successfully metamorphosed PL by the initial number of larvae stocked in each replicate.

Larval stage index (LSI) data were analysed using Two-way ANOVA and Tukey's test was used to detect significant differences between treatments at the 0.05 level of significance. Survival, mean development time, dry weights and total lengths were analysed using Kruskal-Wallis test and significant effects between factors were detected with the Mann-Whitney test. All data was analysed using statistics package, SPSS Version 16.0.

Results

3.1 Larval survival

Larval survival (Fig. 1) in the no algae clear water treatment without enrichment started tapering off in early larval culture finally reaching total mortality on day 16. On the other hand, the 'no-algae' clear water enriched treatment showed significantly ($p<0.05$) lower survival (18%) than all the other treatments. The clear water enriched treatment had lower survival than the microalgae treatments and was not significantly different from the non-enriched 2.5 x 10^6 cells/ml but had significantly lower survival than the enriched 2.5 and both 10 x 10^6 cells/ml enriched and non-enriched treatments.

Survival to post larvae in the enriched 2.5 x 10^6 cells/ml was higher (80%) than the non-enriched 2.5 x 10^6 cells/ml (55%) but were not significantly different from each other. In the higher microalgae treatments, enriched 10 x 10^6 cells/ml (74%) and non-enriched 10 x 10^6 cells/ml (75%) showed similar survival but were not significantly ($P<0.05$) different from each other. Non-parametric results showed that microalgae concentration appeared to have significantly ($P<0.05$) affected survival of post larvae while enrichment did not.

Mean daily survival of larvae (Fig. 2) in the enriched and non-enriched no algae clear water treatments started to drop considerably after Day 9 but more drastically for the non-enriched clear water treatment. Total mortality was reached in the non-enriched clear water treatment on day 16. The mean daily survival of larvae in the first five days of culture was consistently high in the enriched and non-enriched microalgae treatments of 2.5 and 10 x 10^6 cells/ml. The non-enriched 2.5 x 10^6 cells/ml showed a gradual decline in the mean daily survival however it showed an extensive duration of larval culture, owing to a single larva that eventually reached mortality at Day 54. Highest survival was recorded in the enriched 2.5 x 10^6 cells/ml treatment (80%) also showed much higher mean daily survival than the higher microalgae cultures of 10 x 10^6 cells/ml however the latter attained post larvae earlier in day 18 in the enriched 10 x 10^6 cells/ml. Post larvae were attained shortly after in day 19 for both non-enriched 2.5 and 10 x 10^6 cells/ml; on day 20 for the non-enriched 2.5 x 10^6 cells/ml and the enriched clear water on day 23.

3.2 Larval development

There was a general trend of decreasing development time to metamorphose to post larvae with increasing microalgae concentration (Table 1). The mean development time to post larvae was significantly shortest in the highest microalgae concentration of 10 x 10^6 cells/ml for both enriched (20.5 days) and non-enriched (21.5 days) which were not significantly different ($P<0.05$) from each other. These two higher algal treatments were significantly different from other treatments except for the non-

enriched 2.5 x 10^6 cells/ml (26.96 days) which was not significantly different from the non-enriched 10 x 10^6 cells/ml. Mean development time for the enriched (25.76 days) and non enriched (26.96 days) microalgae treatments 2.5 x 10^6 cells/ml were not significantly different ($P>0.05$) from each other as well as the no algae treatment (27.28 days). Statistically, microalgae seemed to have some effect on development time to post larvae while enrichment did not.

Differences in the larval development were evident in the first three days of culture (Table 2). Larvae reared in the enriched and non-enriched 10 x 10^6 cells/ml microalgae treatments were significantly ($P<0.05$) advanced in their stages of development than the clear water treatments. LSI in the enriched 2.5 x 10^6 cells/ml was also different from the clear water treatments however the non-enriched 2.5 x 10^6 cells/ml was not. The microalgae and the enriched no clear water algae treatments showed consistently significant advanced development than the non-enriched clear water treatment throughout culture. There was no interaction effect detected between enrichment and concentration of microalgae however the concentration of microalgae appeared to have a consistent significant ($P<0.05$) effect on development throughout larval culture. Microalgae supplemented treatments of 10 x 10^6 cells/ml generally had a tendency to show higher LSI values throughout culture with the enriched 10 x 10^6 cells/ml showing higher faster development overall than other treatments.

3.3 Post larval dry weight and total length

Post larval dry weights were higher in the enriched treatments of 0 (0.934mg); 2.5 x 10^6 cells/ml (0.928 mg) and 10 x 10^6 cells/ml (0.863 mg) (Table 1). The non-enriched treatments showed lower dry weights in the 2.5 x 10^6 cells/ml (0.659mg) and 10 x 10^6 cells/ml (0.840 mg). Post larvae in the enriched (0.863 mg) and non-enriched (0.848 mg) 10 x 10^6 cells/ml were significantly different from both enriched and non-enriched the 2.5 x 10^6 cells/ml, but were not significantly different from each other as well as the clear water enriched treatment. Enriched 2.5 x 10^6 cells/ml (0.928 mg) was significantly different from all treatments with the exception of the enriched clear water which also produced the heaviest post larvae. The only treatment which showed lower post larval dry weights was the non-enriched 2.5 x 10^6 cells/ml (0.659 mg) and was significantly different from all other treatments. Non-parametric tests showed that microalgae density did not have an effect on post larval dry weights while enrichment significantly ($P<0.05$) affected post larval dry weight.

3.4 Fatty acid profile of post larvae

Postlarvae fed enriched *Artemia* showed much higher values for docohexaenoic acid (22:6n-3, DHA) with higher values when reared in combination with microalgae (Table 3). Non-enriched treatments showed a

decline in composition of DHA with increasing microalgae density. Eicosapentaenoic acid (20:5n-3, EPA) similarly showed higher values in post larvae fed enriched *Artemia* and lower quantities of EPA were recorded with increasing microalgae concentration. This was also a similar pattern with Arachidonic acid (20:4n-6, ARA). Linoleic acid (18:2n-6) showed higher values across all enriched treatments regardless of microalgae however this was reversed in Linolenic acid (18:3n-3) with highly inflated values in the non-enriched treatments. The total saturated fatty acids (ΣSFA), total polyunsaturated fatty acids (ΣPUFA) showed similar values regardless of enrichment and microalgae density. Total monounsaturated fatty acids (ΣMUFA) and total highly unsaturated fatty acids (ΣHUFA) on the other hand reflected higher values for post larvae fed enriched *Artemia* than the non-enriched post larvae where the latter seemed to show a decline in amount of total HUFA with increasing microalgae concentration.

Table 1: Mean development time, dry weights and total lengths of Lineage II *Macrobrachium rosenbergii* post larvae cultured at different concentrations of *Nannochloropsis* sp., fed enriched and non-enriched *Artemia* nauplii.

Treatment (x 10_6 cells/ ml)	Mean development time (days)	Dry weight (mg)	Total length (mm)
10 + Enrichment	20.50 ± 0.15 ^{a}	0.863 ± 0.033 ^{a}	10.63 ± 0.25 ^{ab}
10	21.46 ± 1.60 ^{ac}	0.848 ± 0.029 ^{a}	10.46 ± 0.06 ^{a}
2.5 + Enrichment	25.76 ± 2.40 ^{b}	0.928 ± 0.019 ^{b}	10.80 ± 0.22 ^{b}
2.5	26.96 ± 4.05 ^{bc}	0.659 ± 0.044 ^{c}	9.92 ± 0.42 ^{c}
0 + Enrichment	27.28 ± 1.34 ^{b}	0.934 ± 0.228 ^{ab}	10.56 ± 0.98^{abc}
0	-	-	-

Same superscripts in a column are not significantly different and values displayed are the mean ± SD.

Table 2: Larval stage index (LSI) of Lineage II *Macrobrachium rosenbergii*

Treatment	Day 3	Day 6	Day 9	Day 12	Day 15*	Da
0	2.5 ± 0.2 a	4.7 ± 0.4 a	4.9 ± 0.1 a	4.8 ± 0.4 a	5.4 ± 0.4	
0 + enrichment	2.6 ± 0.1 a	4.8 ± 0.3 a	5.8 ± 0.6 b	6.4 ± 1.0 b	6.8 ± 2.4	6.0
2.5 x 10_6 cells/ml	2.9 ± 0.3 ab	5.0 ± 0.2 a	6.0 ± 0.4 b	6.6 ± 0.9 b	8.3 ± 1.0 b	8.1 ±
2.5 x 10_6 cells/ml + enrichment	2.9 ± 0.1b	5.1 ± 0.3 a	5.9 ± 0.8 b	7.0 ± 1.3 b	8.9 ± 1.3 b	8.9 ±
10 x 10_6 cells/ml	3.2 ± 0.2 b	5.1 ± 0.1 a	5.9 ± 0.1 b	7.4 ± 0.5 b	8.4 ± 1.1 b	9.2 ±
10 x 10_6 cells/ml + enrichment	3.2 ± 0.4 b	5.1 ± 0.3 a	6.2 ± .6 b	7.0 ± 0.2 b	9.4 ± 0.9 b	10.7

0 treatment not included in two way anova.

Table 3: Total fatty acid profile of (%)

Fatty Acid (% in	0 x 10 cells/ ml Artemia	0 x 10 cells/ ml enriched	2.5 x 10 cells/ ml Artemia	2.5 x 10 cells/ ml enriched	10 x 10 cells/ ml Artemia	10 x 10 cells/ ml enriched
20:2n-6	0.5	1.06	0.5	1.06	0.6	0.71
20:3n-6	-	-	-	-	-	-
20:4n-6	2.3	2.23	1.9	2.30	1.6	2.38
20:3n-3	2.1	2.07	2.3	2.19	3.3	1.03
20:4n-3	-	-	-	-	-	-
20:5n-3	8.3	8.90	7.0	9.07	5.1	8.80
22:4n-6	-	-	-	-	-	-
22:3n-3	-	-	-	-	-	-
22:5n-6	0.2	0.31	0.2	0.33	-	0.40
22:5n-3	0.2	0.31	0.2	0.31	0.1	0.45
22:6n-3	2.3	6.86	0.8	7.20	0.6	7.44
ΣPUFA	**42.31**	**41.11**	**44.39**	**41.72**	**42.81**	**38.82**
n-3 PUFA	33.66	29.51	35.73	30.25	35.00	27.78
n-6 PUFA	8.66	11.61	8.66	11.48	7.81	11.04
n-3/n-6	3.89	2.54	4.13	2.64	4.48	2.52
ΣHUFA	**15.84**	**21.75**	**12.82**	**22.47**	**11.22**	**21.20**
14	1.6	1.28	1.6	1.28	1.81	1.69
15	0.8	0.56	0.3	0.65	0.48	0.49
16	15.8	19.39	14.9	19.22	15.7	17.02
17	1.0	1.02	0.9	0.98	0.9	1.02
18	11.4	9.90	10.2	9.78	11.8	8.01
19	-	-	-	-	-	-
20	0.3	0.23	0.3	0.23	0.3	0.30
22	0.3	0.23	0.4	0.23	0.3	0.47
24	0.3	0.16	0.3	0.17	0.3	0.36
ΣSFA	**31.62**	**28.51**	**29.53**	**28.25**	**30.28**	**29.37**
14:1n-5	-	-	-	-	-	-
16:1n-7	0.7	1.35	1.1	1.43	1.0	1.94
17:1n-8	-	-	-	-	-	-
18:1n-9	18.1	21.06	18.5	20.74	18.4	22.17
18:1n-7	6.6	6.71	5.9	6.61	6.8	6.09
20:1n-11	-	0.12	-	0.12	-	0.22
20:1n-9	0.6	1.13	0.6	1.13	0.7	1.24
20:1n-7	-	-	-	-	-	-
22:1n-11	-	-	-	-	-	-
22:1n-9	-	-	-	-	-	-
22:1n-7	-	-	-	-	-	-
24:1n-9	-	-	-	-	-	0.15
ΣMUFA	**26.07**	**30.38**	**26.08**	**30.03**	**26.91**	**31.81**
18:2n-6	5.6	8.00	6.1	7.78	5.7	7.55
18:3n-3	19.3	10.68	23.2	10.75	23.4	9.37
18:4n-3	1.5	0.70	2.3	0.73	2.5	0.69

Lineage II *Macrobrachium rosenbergii* post larvae cultured at different concentrations of *Nannochloropsis* sp., fed enriched and non-enriched *Artemia* nauplii.

Table 4: Fatty acid profile (mg/g of dry sample) of Lineage II *Macrobrachium rosenbergii* post larvae cultured at different concentrations of *Nannochloropsis* sp., fed enriched and non-enriched *Artemia* nauplii.

Fatty acid (mg/g of dry sample)	0 x 10 cells/ ml enriched	0 x 10 cells/ ml enriched	2.5 x 10 cells/ ml enriched	2.5 x 10 cells/ ml enriched	10 x 10 cells/ ml enriched	10 x 10 cells/ ml enriched
14	0.63	0.97	0.66	0.90	0.60	1.18
15	0.16	0.28	0.15	0.25	0.12	0.34
16	7.84	11.85	8.27	10.73	8.50	11.90
17	0.49	0.78	0.45	0.69	0.52	0.71
18	5.40	7.64	5.27	6.90	6.52	5.60
19	-	-	-	-	-	-
20	0.14	0.18	0.16	0.16	0.16	0.21
22	0.14	0.17	0.19	0.16	0.16	0.33
24	0.15	0.12	0.18	0.12	0.16	0.25
ΣSFA	**14.95**	**22.01**	**15.33**	**19.92**	**16.75**	**20.54**
14:1n-5	-	-	-	-	-	-
16:1n-7	0.35	1.04	0.57	1.01	0.54	1.35
17:1n-8	-	-	-	-	-	-
18:1n-9	8.57	16.26	9.58	14.63	10.17	15.50
18:1n-7	3.14	5.18	3.07	4.66	3.78	4.26
20:1n-11	-	0.09	-	0.08	-	0.16
20:1n-9	0.26	0.87	0.31	0.80	0.40	0.87
20:1n-7	-	-	-	-	-	-
22:1n-11	-	-	-	-	-	-
22:1n-9	-	-	-	-	-	-
22:1n-7	-	-	-	-	-	-
24:1n-9	-	-	-	-	-	0.10
ΣMUFA	**12.33**	**23.44**	**13.54**	**21.17**	**14.89**	**22.24**
18:2n-6	2.65	6.17	3.16	5.49	3.14	5.28
18:3n-3	9.11	8.24	12.04	7.58	12.96	6.55
18:4n-3	0.71	0.54	1.19	0.51	1.37	0.48

20:2n-6 0.25 0.82 0.24 0.75 0.32 0.50 20:3n-6 -----20:4n-6 1.10 1.72 1.01 1.62 0.86 1.66 20:3n-3 0.99 1.60 1.17 1.55 1.81 0.72 20:4n-3 -

----20:5n-3 3.92 6.87 3.61 6.40 2.82 6.15 22:4n-6 -----22:3n-3 -----22:5n-6 0.09 0.24 0.08 0.23 -0.28 22:5n-3 0.10 0.24 0.10 0.22 0.08

0.32 22:6n-3 1.09 5.29 0.43 5.08 0.32 5.20

ΣPUFA 20.00 31.73 23.04 29.42 23.68 27.14

n-3 PUFA 15.91 22.77 18.55 21.33 19.36 19.42 n-6 PUFA 4.09 8.96 4.50 8.09 4.32 7.72 n-3/n-6 1.26 1.39 1.24 1.38 1.22 1.40

ΣHUFA 7.54 16.78 6.65 15.84 6.21 14.82

TOTAL 47.28 77.18 51.92 70.51 55.31 69.91 "-" indicates <0.05mg/g

Figure Legends

Figure 1: Final survival of Lineage II *Macrobrachium rosenbergii* post larvae cultured at different *Nannochloropsis* sp. concentrations and fed either enriched and non-enriched *Artemia* nauplii.

Figure 2: Mean daily survival of Lineage II *Macrobrachium rosenbergii* during larval culture at different concentrations of *Nannochloropsis* sp. and fed either enriched or non-enriched *Artemia* nauplii

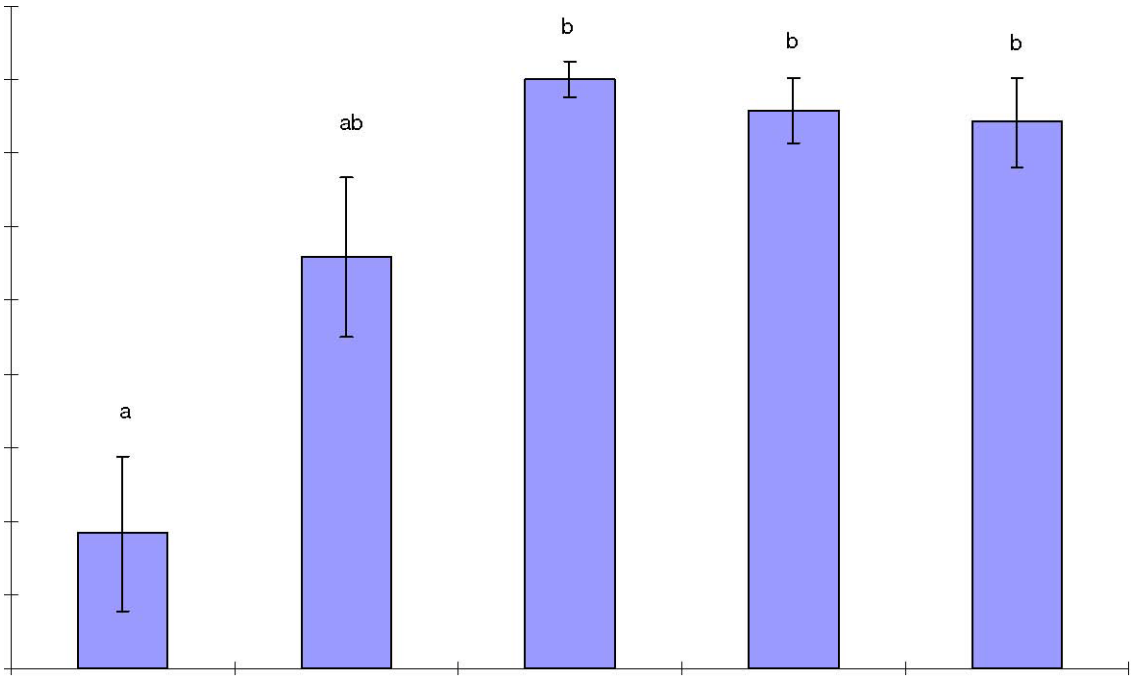

a
ab
b
b
b

Figure 1

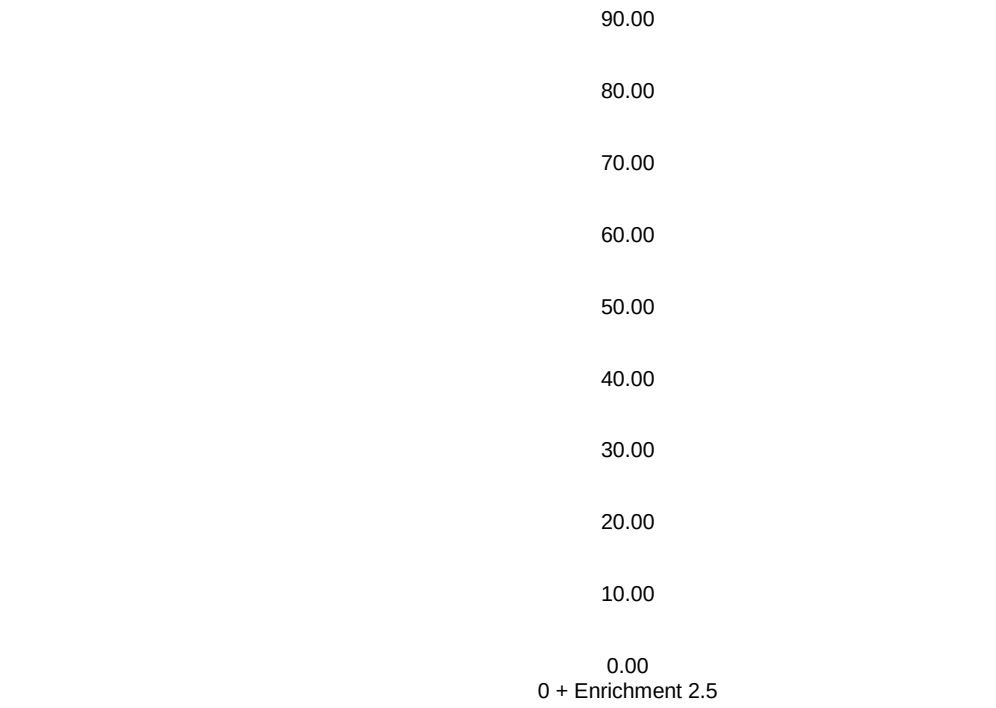
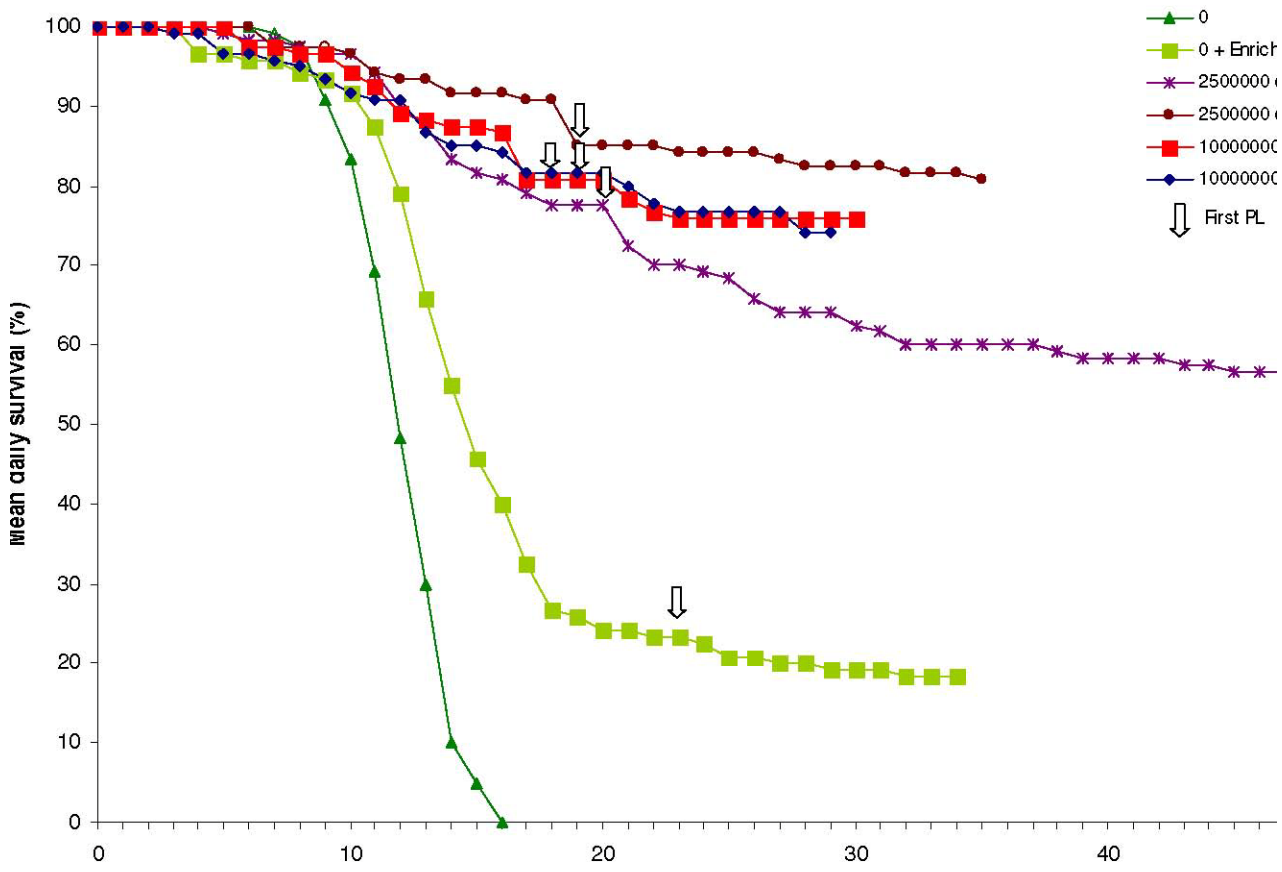

90.00
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
0 + Enrichment 2.5
Larval survival (%)
Nannochloropsis sp. (x 10cells/ml) ± Enrichment

Figure 2


Mean daily survival (%)
100
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
0
0 + Enrich
2500000
2500000
10000000
10000000
First PL

Time (Days

11.7 Appendix 7: Reports on farmer training in PNG

Aciar Native Fish Aquaculture Project

A Report on Model Farmers Workshop

23rd of December, 2007

Prepared By; OTDF Fisheries Program.

1.0 Introduction
The Aciar Native Fish Aquaculture project is implemented in collaboration with Queensland DPI&F and WPDAL. As one of the milestone activities in the project implementation schedule, interest farmers along the Kiunga/Bige area were selected as Model farmers to trial out aquaculture techniques developed from the project.

A total of five farmers were selected to model the project; four villagers from the Tutuwe CMCA trust region (Kiunga/Bige) and Montfort Technical School as the fifth model farm. A workshop on the objectives of the project was organized by OTDF fisheries program on the 13th of December.

2.0 Purpose of Workshop
The main purpose of the workshop was to explain the objectives of the Native fish aquaculture research project to the model farmers, and to explain to them the purpose of using them as model farmers in the project.

3.0 Workshop General
Prior to the main component of the session, everyone attending the workshop was given a chance to introduce themselves. A safety brief was made by the facilitator (Irene) before the program outline was read out and opening remarks made by Belden Dasa (OTDF Kiunga/Bige regional team).

4.0 Workshop focus
The workshop was focused on these key areas; An overview on the Aciar Native fish aquaculture project, the expected outcome, the current status of the project and where the model farmers come in, and the type of ponds required for demonstration in the model farms.

Havini took the participants through a presentation on the Native Fish Project. The presentation basically covered the objectives of the project and the expected outcome, and some pictures of native fish species that are researched.

Following Havini's presentation, a brief run down on the current status of project, and activities to be covered in the near future was covered by Irene. The five types of fish; sooty grunther, catfish, herrings, sleepy goby and crayfish were mentioned. Furthermore, the purpose of taking the farmers on as models to trial out the R&D project was deliberated upon. This covered what the project is expecting from the model farmers, and what it will give to them. Kaupa Kia also gave a brief on the status of the Lardec project and constraints involved in getting the facility ready on time. It was made clear that the project is in its third year and there's only three years left before it ends.

Noel Tonko took the participants through the technical part of pond construction; dimensions like the sizes, length and width, the depth of the pond, and materials required for building one was explained. It was stressed that materials supplied to the model farmers will only be the ones required for building the demonstration pond as specified

by the project, any additional requirements should be channeled through their respective trust.

5.0 Expectations

Kaupa Kia explained that the government and OTML are working together on this project; there is no demarcation between the two parties. Personal requests for materials can not be entertain as the government does not have the capacity to provided to all farmers, therefore extra request should go through your respective village planning committee for the trust to fund. Information materials, materials like pipes etc, and skills gained through training will remain with the farmers once the project terminates. Labor, time and commitment will be required from the farmers over the duration of the project in the next three years.

Furthermore, it was deliberated by Irene that CMCA projects are different from this aquaculture project; however this project will help develop a package that can be used as the basis for trust submissions for aquaculture development in their respective area.

6.0 Wrap-up Session

The farmers were given time to decide if they really want to participate as model farmers in the Native Fish Aquaculture project before their consents were solicited. Everyone guaranteed commitment for the next three years as model farmers, and also the WPDAL and OTDF officers made commitment that they will work together. Havini then summarized all discussion points, and it was agreed that the actual training will be conducted on the 11th to the 15th of February 2008.

The session ended with closing remarks from Kaupa Kia.

7.0 Appendix

ACIAR Model Farmers workshop

Participants

#	NAME	VILLAGE/INSTITUTION	STATUS
1	Freddy Panote*	Kiunga Monfort Tech High	Teacher
2	Waum Elamunop	NFDAL	Fisheries Officer
3	Jerry Kurapio*	Kwiloknai Vill.	Farmer
4	Jack Tuu*	Grengas Kona	Farmer
5	Siri Jack Tuu (Mrs)	Grengas Kona	Farmer
6	Simeon Supe*	Gii Village	Farmer
7	Dasi Tukwore*	Gii Village	Farmer
8	Moxy Sipe	Gii Village	Farmer
9	Irene Kamang	OTDF	Officer
10	Noel Tonko	OTDF	Officer
11	Kaupa Kia	NFDAL	Fisheries Officer
12	Havini Vira	OTDF	Officer
13	Nathaniel Dangona	OTDF	Officer

* = Model farmers to be under the project

1. Introduction - Irene

- Safety
- Program
- Names
- Opening remarks, welcome – Belden. Message: nobody brought you here; you brought yourself here through you effort in farming fish out of your initiative.

2. ACIAR Native Fish Presentation – Havini

A basic overview of the project, its objective and its expected outcomes.

- Tilapia and carp to continue; we will construct separate ponds for this purpose
- Redclaw are able to escape we will train on how to farm and keep from escaping
- We will not farm all at once; when we have developed techniques we will train and teach you and then build on from that knowledge.

3. Current Status - Irene

Where we are at this point in time and where we will go.

- We are now at year 3 we have 3 more.
- We are a little behind at LARDEC so if you farmers can commit yourselves and push us; you can gain the maximum benefit in the remaining years. The project will not wait for us, we have to move on.

4. Pond descriptions and materials - Noel

- Under the program, we will construct uniform ponds
- Description
- Cement harvest pit to avoid blockage of drain pipe

- We will do site selection for the new ponds; this will addressed in training.
- Materials required: 100mm x 3piece; 100mm x 2 elbow; screen; cement x 1 bag? gravel? Feed
- Stealing is a problem but at this point in time, it should not discourage from the project.

5. Expectations of the government. - Kaupa

- The govemment and OTML are together; there is no demarcation in fisheries work. If you go to OTML or government, it is the same.
- All your extra/expansion requests will have to go through your trust; our role is in training and advice and in supplying materials in this project only.
- Time is running, we cannot wait, we have to catch up.
- We will work together; whatever fish we want to trial will be done with you.
- Materials, feed, information, training will remain with you after the project.
- We will require your time and commitment
- LARDEC project should be launched in June/July
- Happy with the commitment from selected farmers to date; has been good.
- Plenty of farmers come asking for materials; we only assist a few farmers but cannot really commit.

6. Expectations of ACIAR - Irene

- We will only support the materials required for a pond
- Farmers will be required to supply free labor over the next 3 years.
- Package will be developed using model farmers to make submissions to trust.
- This training will focus on model farmer; extension is not your duty.
- Trust submissions are different from this project.
- We will develop techniques and ways to address

7. Summary - Havini

- This project is just one component of the fisheries program; work will continue as normal (i.e. Extension, advise, etc).
- At the end of the project life your ponds can be used for native fish or other species.
- Simeon – one pond to be converted if no space. Committed
- Jack & wife – committed
- Jerry (Simon) – is committed
- Freddy – can vocational school have a separate training for students and in their practical sessions build ponds? Ok. Is committed.
- Dasi - committed
- All to have wife at training session.
- Date for training: 2nd week of February (11/02/08 – 15/02/08); Samagos site

8. Closing Remarks - Kaupa

- You have to learn to be independent
- As the work grows, we will start to form fish farmer association
- Your commitment is acknowledged; apologies that funding is not coming through to assist farmers – budget problems.

- Thankyou OTDF for the support to date on the LARDEC project.
- Golgobip hatchery completed, 1st December opened.
- Feed is still a major issue; under this project, feed will be coming from Australia.
- To close this meeting thank you for your time and commitment.

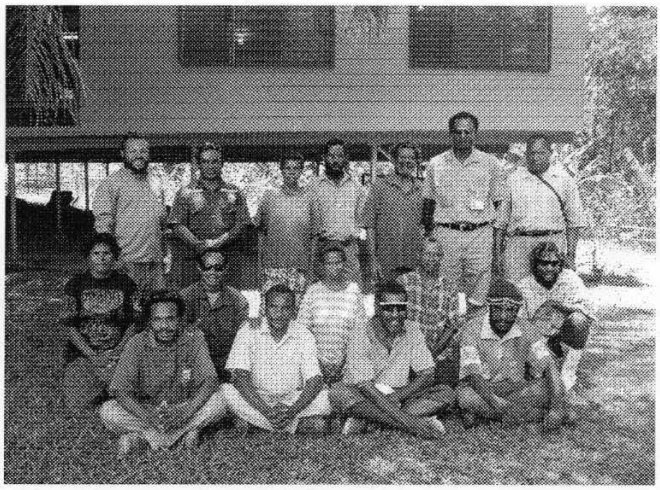
A REPORT

On

NATIVE FISH - MODEL FARMERS' TRAINING

3RD – 6TH MARCH 2008

DAL CONFERENCE ROOM – SAMAGOS, KIUNGA



FISHERIES DIVISION, WESTERN ROVINCIAL ADMINISTRATION AND

FISHERIES SECTION, OK TEDI DEVELOPMENT FOUNDATION &

ACIAR INDIGENOUS FISH PROJECT.

INTRODUCTION

The first 'Native Fish' model farmers' training was conducted at Kiunga. Five common carp (*Cyprinus carpio*) farmers were identified and selected to undergo training to do trial native fish farming at their respective places. Kaupa Kia of LARDEC Project, Western Provincial Administration and Noel Tonko, Ok Tedi Development Foundation (OTDF) of OTML facilitated the training while Irene Kamang, Fisheries Coordinator, OTDF catered for the logistical component. Three workers from LARDEC Project and two fisheries officers Mr. Waum Elemunop, Fisheries Division, North Fly District and Mr. Nathaniel Dangona from OTDF participated in the training. The training took four days.

PURPOSE OF THE TRAINING

This native fish farming training is the first of its kind for farmers not only in this province but also PNG. The training structure and topics were designed to direct the farmers understand the main concepts of farming the native species. Unlike the common carp, rainbow trout and tilapia farming which are more specific in terms of their water quality, pond structures, biology and other concepts of aquaculture, this training aimed to get the farmers to familiarize the general concepts of aquaculture and emphasized more deeply on the importance of environmental, biological and physical parameters required by different fish species. For example, in the case of red claw (*Cherax quadricarinatus*), which is a crustacean with a different feeding habit than the Sooty Grunter *(Hephaestus fuliginosus)* and other fin fishes?

The five common carp farmers have been selected to undergo this training as they have some experience in fish farming. These model farmers will be the ones who will be given the fish seeds (fingerlings) from the OTML Trust Yard Hatchery and LARDEC Project Samagos hatchery (yet to be built). They were taught to construct a uniform size and pond structure for data collection and assessment for the ACIAR Project. The ACIAR Project on culture in promising indigenous fish species falls in line and in time with the whole aim and purpose of LARDEC Project.

COURSE CONTENT

The course covers almost all the basics of fish farming (aquaculture) from fish biology, planning and site selection to farm management and its economics. As stated above, this training was aimed to target many different native species with specific water quality parameter requirements and the training introduces to the farmers to keep within the optimal range of each species as per and when recommended. Refer to **Appendix C** for the training course content. The content and variations in the topics are sufficient to equip the model farmers the basic techniques to improve and advance from their carp farming practice to the native fish farming stage.

COURSE MATERIAL

There was a preliminary workshop for these model farmers in December 2007 at the Brown Haus 2 Conference Room in preparation for this training. Apart from the stationery (exercise books, pencil and folders etc,) provided to participants, course notes were not ready at the time of the training. The notes are now being prepared as 'farmers manual' and should be distributed with their participatory certificates.

TIME FRAME

This training takes at least four days to cover. However, the 40-minutes interval is not sufficient to cover some areas that we would like to touch as well. Two afternoon practicals have been omitted due to unfavourable weather conditions. Minimum time for such training should be one week. As indicated in the evaluation questionnaire, some farmers felt that the four days training and the 40-minute interval was insufficient to have questions and discussions in between and during the lecture periods.

PARTICIPANT KNOWLEDGE

Four of the five participants have had some experience with common carp farming while the fifth one has his pond yet to stock. They either started from contact with other farmers or from government fisheries office. These initial knowledge and partial experience position them in a better stage to pursue with the native fish species breeding and farming trials

The Monfort Technical High School has no representation at the training although it is highly anticipated that such an educational institution involve. It can be included once the native fishes breeding program is successful.

PARTICIPANTS' RESPONSE

As this was the first farmers training on native fish, farmers expected what could be considered the contrary in the beginning. That is, they wanted specific details on various species which was not in line with the facilitators view. It would be tedious and most of the native species we intend to experiment for farm trials are new and yet to explore. However, they seem to understand most or if not all of the concepts the training proceeded. That was indicated during questions and discussions as well as during the evaluation. The training course was taught in 'tok pisin.'

FUTRURE TRAINING PLANS

As some farmers indicated in the evaluation, more training would be conducted after, as the native fish breeding progresses. Thus, there are many more carp farmers requesting training and which would include the native fish as well. We look forward to conducting more training as soon as the training manual the facilitators are compiling is complete.

EVALUATION RESULTS

Cumulative results as analyzed are included in the attached questionnaire (Appendix B). Variations in participants' answers to the questions are two-fold. That is, two fisheries officers' answers seem similar in trend compared to the farmers' answers that seem similar and different from the former.

OPINIONS AND DISCUSSION ON EVALUATION

The first evaluation question seems complicated in terms of the grading system which took more time to explain. This shall be modified to straight-forward answering mode for ease for farmers understanding and analysis.

The other questions are generally direct questions seeking farmers and participants' opinions on the length of time for training and how the training could be improved later.

Interestingly two questions, particularly Q8and Q9 were included to test the participants' seriousness or interest and commitment to actually invest their time, effort and resources. Apart from the officers' (participants) views indicating trainings and advices, farmers listed all the materials thy needed and also transportation. The last and final question seeks to identify underlying problems farmers and interested potential farmers face.

Some of the common obstacles as mentioned then are water supply, topography and materials. Water supply sources are mainly underground where farmers encounter difficulties with drainage and shortage during prolonged dry season. Topography is such that potential sites are at the higher level from the water supply source. Sourcing water supply using gravity is not applicable in this case.

FOLLOW UP ACTIVITIES

Fisheries Officers from the Fisheries Division, North Fly and OTDF will then visit and do feasibility study, especially site selection for these model farmers to construct a 10m by 5m pond. That is, all the five model farmers will have to construct a standard size pond which will be used to trial native seeds to be produced and distributed by the project. Certain construction materials such as some cement bags, PVC pipes and accessories would be provided as mentioned during the workshop prior to this training. There will be constant visit to these farmers to monitor their activities.

CONCLUSION

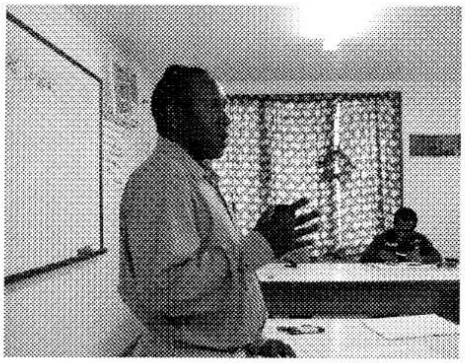
This was the first training of its kind on native fish farming in Western Province. The selected model farmers have expressed appreciation and satisfaction. Most of the topics covered are not species specific as the facilitators aimed to simplify as well as generalized to accommodate the many native species which will be researched for farming. A number of modifications would be considered basing on the evaluation questionnaire results.

RECCOMENDATION

Following are some recommendations that need considerations;

1. Experiment pond construction materials be purchased and supplied to the model farmers.
2. Facilitators produce and provide simplified native fish farming manuals for farmers.
3. Conduct a separate training and produce manual for extension and field officers

APPENDIX A. TRAINING PHOTOGRAPHPS



District Fisheries Officer opening the training

Noel Tonko during training presentation

Participants listening attentively

during practical session

APPENDIX B. EVALUATION QUESTIONNAIRE

Native Fish Model Farmers Training Course Evaluation

Q1. Evaluate each subject with the following 3-point grade system (3 or 2 or

A. Subject was interesting
B. Subject was useful
C. Course was difficult
D. Presentation was good

(Grade in order of your judgment e.g., if more interesting, indicate 3 or if boring 1)

No.	Subject	Interest	Useful	Difficult	Presentation
1	Fish biology				
2	Planning and site selection				
3	Pond construction				
4	Inlet & outlet construction				
5	Pond size & stocking density				
6	Water quality management				
7	Pond fertilization- Organic				
8	Feeding				
9	Pond management				
10	Brood stock management				
11	Fish health management				
12	Grading, sexing & maturity check				
13	Fish handling and transportation				
14	Economics of fish farming				
15	Practical				

Q2. Did this course help you?

Yes **ALL (100%)** No **Nil (0%)**

Q3. Would you recommend this course to someone else if we run it again in the future?

Yes **ALL (100%)** No **Nil (0%)**

Q4. Was this course too technical or too practical?

Too technical **(1/10)** Too practical Balanced **(9/10)**

Q5. Was time allocation appropriate in regards to area of topics covered?

Too short **(3/10)** Appropriate **(7/10)** Too long, should be shorter

Q6. Is there anything that you wanted to learn that we did not cover?

- Only practical ____

Q7. Is there any suggestion on how to improve this course?

- Training notes be prepared in Tok-Pisin,
- Notes are given to participants to follow
- Practical on all or most topics covered
- Punctuality needs to improve

Q8. What do you think the government should do to assist?

- Provide pond construction materials transportation, and training.
- More training and extension services,
- Subsidize farmers cost of fish farming,
- Provide technical advice

Q9. What do you think OTML should do to assist?

- Same as above

Q10. What is your biggest problem?

- Water supply, topography not suitable, construction materials, dig and level areas to increase area size, inlet construction etc.

(Please give us your honest answer)

APPENDIX C. TENTATIVE TRAINING SCHEDULE

Native Fish Farming – Model Farmers Training
Venue: DAL, Samagos Conference Room
Date: 3rd – 7th March 2008

	Time	Topic	Facilitators
	8:00-8:30	***Introduction*** to Native fish farming;	Robert Alphonse/ HaviniVira
	8:30-9:10	***Fish biology*** - Breeding - Feeding	Kaupa/Noel
	9:10-9.50	***Site Selection*** - Topography - Soil type - Water source	Kaupa/Noel
	9:50-10:10	**TEA BREAK**	
	10:10-10:50	***Continue site selection***	Kaupa/Noel
	10:50-11.30	***Pond Construction*** - Clearing - Marking out	Kaupa/Noel
	11:30-12:30	**L U N C H**	
	1:00-2:30	***Practical*** - Pond construction	Kaupa/Noel
	8:00-8:40	Inlet & outlet construction	Kaupa/Noel
	8:40-9:20	Pond size & stocking density	Kaupa/Noel
	9:20-10:00	Water quality control	Kaupa/Noel
	10.00–10.20	**TEA BREAK**	
	10:20-11:00	**Pond fertilization** - Organic	Kaupa/Noel
	11:00-11:40	**Feeding** - By schedule/Observation(Same spot)	Kaupa/Noel
	11:30-12:30	**LUNCH**	
	1:00-2:30	**Practical** - Pond construction	Kaupa/Noel
th	8:00-8:40	**Water quality - Filtration**	Noel/Kaupa
	8:40-9:20	**Pond management**	Noel/Kaupa
	9.20-10.00	**Brood stock management**	Noel /Kaupa
	10.00-10.20	**TEA BREAK**	
	10.20-11.00	**Fish health**	Noel/Kaupa
	11.00-11.40	**Fish grading, sexing and maturity check**	Noel/Kaupa
	11.40-12.40	**LUNCH**	
	1.00 – 2.30	**Practical** – Fish feeding	Noel/ Kaupa
	8.00 8.40	**Fish handling and transportation**	Irene
	8.40 – 9.20	**Economics of fish farming**	Kaupa
	9.20-10.00	**Evaluation**	Noel /Kaupa
	10.00-10.30	**CLOSING**	Havini/Robert

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3) OTML through OTDF, Fisheries Section.
4) Western Provincial DAL and the Farmers

We hope the next training will be much better than this one.