FRDC FINAL REPORT

DEVELOPMENT OF COMMERCIAL PRODUCTION SYSTEMS FOR MUD CRAB (*Scylla serrata*) AQUACULTURE IN AUSTRALIA: HATCHERY & NURSERY

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July 2008

FRDC Project No. 2000/210

Northern Territory Government

Australian Government

Fisheries Research and Development Corporation

Queensland Government

Department of Primary Industries and Fisheries

ISBN 978-0-7245-4730-2

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Title Page

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Table of Contents

List of Tables

List of Figures

FRDC Project No. 2000/210 **DEVELOPMENT OF COMMERCIAL PRODUCTION SYSTEMS FOR MUD CRABS *(Scylla serrata)* AQUACULTURE IN AUSTRALIA: HATCHERY & NURSERY**

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Objectives

1. Develop a commercial scale larviculture system for mud crab megalops.
2. Develop a commercial scale nursery system for crablet production.
3. Produce manuals for larviculture and nursery rearing of the mud crab, *Scylla serrata*.

Non Technical Summary

Outcomes Achieved

a. Developed a hatchery system to mass produce megalops of *Scylla serrata*.

b. Developed options for nursery systems to mass produce crablets of *Scylla serrata*.

c. Gained a greater understanding of how to control water quality and bacteriology in mud crab larval rearing systems.

d. Identified a number of bacteria that were demonstrated to be virulent against mud crab larvae.

e. Established technology which is now supporting indigenous and non-indigenous development of crab farming in the Northern Territory and Queensland.

f. Undertook a series of workshops to assist in transferring hatchery and nursery technology to the private sector, producing and providing a CD of presentations for participants and to assist industry development.

Executive summary

Developed a hatchery system to mass produce megalops of *Scylla serrata*.

Commercially viable techniques for rearing *Scylla serrata* larvae through to megalops stage have been developed at both the centres involved in the project, the Darwin Aquaculture Centre (DAC) and the Bribie Island Aquaculture Research Centre (BIARC). The methods developed in an earlier ACIAR project (FIS/1992/017) were not generally reliable enough on a larger scale to be considered able to support commercial production, although much valuable information was generated. The methods developed in this project are suitable for use in commercial scale larval rearing of mud crabs and will be able to support the initial development of mud crab grow-out. The techniques were developed throughout the project by carrying out a series of experiments at each centre. These experiments led to the development of an accepted Standard Procedure which has been shown to be a reliable method of producing commercial quantities of mud crab megalops.

The research groups at DAC and BIARC collectively identified three alternate systems of reliably combating catastrophic losses of mud crab larvae that were found to be associated with bacteria during the rotifer feeding phase of crab larval rearing.

The first method is based on the combination of larval rearing vessels that incorporate design features that keep larvae and food continuously well mixed and suspended, combined with strict hygiene requirements. This included daily manual cleaning of tank surfaces and significant water exchange.

Secondly it was demonstrated that the prophylactic use of oxy-tetracycline (OTC) could be used to control bacterial larval disease. Using OTC as a tool, various operational parameters were investigated to optimise production.

Thirdly, a method was developed where the rotifer feeding phase was replaced by the use of decapsulated *Artemia cysts* as larval feed for the first few days of culture.

Developed options for nursery systems to mass produce crablets of *Scylla serrata*.

The production of larger numbers (tens of thousands) of megalops made possible the development of techniques for settlement and metamorphosis of megalops to first stage crablets. The ability to research the crablet production stage of the process on a suitable scale had been previously hampered by the limited supply of megalops prior to this project. The techniques for the production of crablets from megalops that were developed do not require sophisticated facilities and are suitable for adoption by the private sector.

Key findings of nursery systems investigated were as follows:

- Megalops to crab (C1) yields of 20–40 per cent could be achieved at densities up to 2300 m^{-2} .
- During the early crab stags of nursery production provision of shelters⁄3-D structures are critical to reduce cannibalism.
- Hapa nets floating in ponds, equipped with shelter⁄3-D structures can provide a cost effective way of rearing megalops–crablets (C5–C8) suitable for release to grow-out systems.

Gained a greater understanding of how to control water quality and bacteriology in mud crab larval rearing systems.

Consistent achievement of high yields of vigorous healthy megalops, and hence crablets was found to be critically dependent on a combination of optimised equipment design, operation and husbandry protocols as summarised below:

1. Sophisticated larval rearing vessels:
    - Maintained larvae at optimum DO, pH, salinity (30ppt at Z1 declining to 25ppt at megalops), very stable temperature (± 0.5°C within optimum range 27–32°C)
    - Gentle non-turbulent continuous dispersion of larvae and live food
    - Fast, efficient transfer of larvae between containers
    - Cleaning of settled solids
    - High daily rates of water exchange and removal of suspended and surface organic solids comprising residual feed, micro-algae, dead larvae and faeces
2. Pre-treatment of replacement water for larval culture including sand filtration, followed by various mixtures of settlement, secondary filtration, foam fractionation and UV filtration.
3. Restriction of rotifer feeding to only feed Z1 and Z2 larval stages, plus hygienic provision of *Artemia* .
4. Restricted use of OTC administered under veterinary direction.
5. Provision of mixed microalgal cultures (*Nannochloropsis*, PSII and *Tetraselmis*.)

Identified a number of bacteria that were demonstrated to be virulent against mud crab larvae.

Whilst this outcome was achieved, the failure of the PhD student (employed on the project) to complete their thesis, resulted in limited analysis of this component of the project.

Established technology which is now supporting indigenous and non-indigenous development of crab farming in the Northern Territory and Queensland.

Technology developed for hatchery and nursery systems has been utilised by both pioneering farms in both the Northern Territory and Queensland. Results from this study have also helped with the development of farming the blue swimmer crab in Queensland.

In the Northern Territory indigenous communities have and continue to explore opportunities to grow-out mud crabs in both ponds and mangrove enclosures.

Extension of R&D to the aquaculture industry

The results of the research carried out have been disseminated by well attended workshops that were conducted to explain the techniques developed and to pass on general information on all aspects of mud crab aquaculture. The information from the workshops is contained on a CD that is available to industry and the public, and forms a component of this final report.

Keywords

Scylla serrata, mud crab, aquaculture, larviculture, zoea, megalops, crablets.

Acknowledgements

We would particularly like to recognise the support of our commercial partners in this project, McRobert Aquaculture Systems and Meridian Aquatic Technology, manufacturer of Aquamats®. Both companies provided equipment for use and testing which assisted with our development of systems for mud crab aquaculture.

This project was only made possible through the dedicated work of the technical staff supporting it. Our sincere thanks go the technical staff at the Darwin Aquaculture Centre (DAC) : Keith Newman, Rachael Naylor, Cameron Moir, Evan Needham, Brian Dalliston, Vincent Saffi,; and at the Bribie Island Aquaculture Research Centre (BIARC): Beverley Kelly, Tom Asakawa, Trent Lindsay, and Shannon McBride.

The manager of the DAC, Murray Barton, is acknowledged for the support he provided the project in the areas of construction, logistics, finance, human resource management, transportation, maintenance and administration. All other professional and technical staff at the DAC are thanked for their support and constructive input into the project, especially for covering crab staff occasionally during weekends and holidays.

The current head of the DAC, Glenn Schipp is also thanked for his assistance in editing various drafts of this document.

The students who contributed to the material presented in this report; in particular Luke Neil (Curtin University of Technology), and various work experience Aquaculture TAFE course students.

Special recognition of the Berrimah Veterinary Laboratory is made in recognition of the excellent support and outstanding service it provided to this project, in particular Dr John Humphrey.

In particular the partnerships formed and strengthened between and within institutions during this project includes those with:

Charles Darwin University
Curtin University of Technology
Queensland University of Technology
Berrimah Veterinary Laboratory

This project provided significant, indirect support for a PhD student, Tuan Anh Vu from Vietnam, who was funded by AusAid and the Australian Centre for International Agricultural Research (ACIAR). The research team at the DAC provided support for Tuan and gained a better understanding of crab nutrition from the work he undertook in Darwin. His involvement in the project also helped in developing new international linkages with Vietnam. The staff at BIARC have also benefited from collaborative interactions with Vietnam and Indonesia, through the ACIAR funded project and acknowledge the cross-fertilisation of ideas and concepts that occurred between it and this project.

Background

The mud crab *Scylla serrata* is widely distributed across the northern half of Australia and is the basis of well established fisheries in both the Northern Territory and Queensland, which have recently been described by Hay *et al*. (2005). Mud crabs are a premium product when handled and packaged properly, with a high level of market recognition and acceptance.

Mud crab farming, based on juveniles caught from the wild, has been carried out in Asia for many years. Farmer's reliance on wild caught juveniles has limited the scale and continuity of production. In some areas removal of juveniles has contributed to the crash of some crab fisheries. In Australia, legislation prevents the use of wild caught juveniles for stocking farms. So if mud crab farming is to develop in Australia, juveniles must be sourced from hatchery operations, so that the only dependence on farming crabs from the wild fishery is through the provision of broodstock.

In Australia, mud crab aquaculture has been researched by Northern Territory and Queensland government research groups since 1992. An Australian Centre for International Agricultural Research (ACIAR) funded project (FIS/1992/017) brought together the Department of Primary Industries (Queensland), the Department of Primary Industry and Fisheries (Northern Territory) and Philippine institutions to form a research team of sufficient critical mass to develop basic farming technologies (Anon 1988, Keenan & Blackshaw 1999). At a research scale survival rates of 70–90 per cent of zoea to megalops were obtained, but was not repeated at a larger scale. Two major issues were identified, the microbiology of larviculture systems and the related low, variable, survival of zoea to megalops to crablet in larviculture systems.

Prior to this project being developed, industry was keen to see the positive results of mud crab culture at an experimental scale, transferred through to full commercialisation. Economic modelling demonstrated that mud crab culture showed a significant potential to develop into a profitable industry sector for Australian aquaculture (Cann & Shelley 1999).

Preliminary pond grow-out trials of mud crab grow-out in Australia indicated that *S. serrata* could grow to 800g within six months, with an average size of 600g. These results reinforce those of Trino *et al*. (1999) working in the Philippines.

In addition to development of industrial aquaculture technology, there is also significant interest in work from Sarawak which has identified simple enclosures in mangroves as a low technology grow-out system. Such systems could form the basis for development of mud crab farming in Aboriginal communities in Australia, who have tenure over significant tracts of coastal land in northern Australia.

The aim of this project was to commercialise hatchery and nursery production of megalops and crablets respectively, underpinning the establishment of a new aquaculture industry for Australia.

Need

This project provided the opportunity to develop mud crab farming as a new aquaculture sector for tropical and sub-tropical Australia.

Crab farming provides a significant new opportunity for farmers with access to marine water, in particular for those who have already invested in infrastructure for other forms of aquaculture.

McRobert Aquaculture Systems was interested in being involved with the supply of crablets using its innovative tank systems. To that end the company was keen to see its new tank system fully tested for mud crab larviculture, so that it could be marketed on a sound, scientific basis.

Aboriginal groups across northern Australia have expressed great interest in becoming involved with mud crab aquaculture development. This project was to provide the technology to support their aspirations.

Both the Northern Territory and Queensland government agencies have dealt with a steady stream of inquiries regarding mud crab farming development and the availability of crablets for farming. This project was designed to assist these governments meet their demands for industry development of this sector.

The fisheries for mud crab in northern Australia are seasonal, resulting in variable quantities of crabs at different times of the year. Developing mud crab farming systems will provide for more consistent and reliable, year round supply of high quality crabs to the market. Farming crabs also provides the opportunity for the development of a wider range of marketable products such as soft shell crabs, crabs of various sizes and a range of crab meat products. Farmers, unlike fishermen, would not be legally limited to marketing crabs over a specified size (to address fisheries management issues), as their product is not part of the wild population.

Any animal which is farmed intensively will encounter a range of health challenges. Identifying the disease agents affecting mud crab larviculture and developing effective management strategies to counter them is the key to their commercialisation. Control of bacteria in larviculture systems was identified in the ACIAR project between Australia and the Philippines, as a key barrier to overcome in commercialisation of mud crab culture.

When this project was designed it had a major prawn company as a commercial partner that wanted to secure a reliable supply of crablets for their operation. Although the company's priorities changed and the collaborative arrangements were terminated, other prawn farming companies have expressed interest in diversifying to include mud crabs.

The project was a vital first step in the development of a mud crab farming sector in Australia. Further to this study, work on mud crab nutrition and grow-out systems design will be required to fully commercialise mud crab farming. Whilst ACIAR is already funding preliminary nutrition work, the private sector has itself invested in grow-out system design. In the Northern Territory, government and indigenous groups are also investing in pilot grow-out of mud crabs in both ponds and mangrove enclosures.

Objectives

1. Develop a commercial scale larviculture system for mud crab megalops.
2. Develop a commercial scale nursery system for crablet production.
3. Produce manuals for larviculture and nursery rearing of the mud crab, *Scylla serrata*.

All objectives of this project were achieved.

General Introduction

The mud crab *Scylla serrata* is the largest of the four species of mud crab (*Scylla* spp.) found throughout the Indo-West Pacific (Keenan 1999) and is an important fisheries product for tropical and sub-tropical Australia. Its catch is strictly controlled by fisheries management plans in the Northern Territory, Queensland and Western Australia. Internationally there is a strong demand for mud crabs. To expand production of mud crab in Australia to meet market demand, and to provide a new development opportunity for coastal tropical and sub-tropical Australia, the Northern Territory and Queensland governments first invested in the research of mud crab aquaculture technology in the early 1990's. This work followed encouraging pioneering work carried out by the private sector in the late 1980's.

In south-east Asia, until recently, mud crab aquaculture development has been based entirely on collection of seed-stock from the wild and its subsequent rearing in ponds or enclosures. In Australia, collection of seed-stock was not an option to support farming as it was considered there could be a detrimental effect on the wild fishery and ecosystems. Therefore, for mud crab aquaculture to take place in Australia, culture systems would have to be developed to provide hatchery produced seed-stock, commonly referred to as crablets, which would not impact on fishery resources.

Following initial work at the Darwin Aquaculture Centre (DAC) and Bribie Island Aquaculture Research Centre (BIARC), both organisations collaborated with the Philippine institutions the South East Asian Fisheries Development Centre (SEAFDEC) and the University of the Philippines in the Visayas (UPV) in developing core technologies to support mud crab aquaculture development. The project was supported with funds from the Australian Centre for International Agricultural Research (ACIAR). A series of workshops and publications have disseminated the major outcomes of that project (Anon. 1998, Keenan & Blackshaw 1999, Anon 2001). Whilst the ACIAR project demonstrated what could be done in terms of, crab husbandry, basic larval rearing, nursery production and grow-out, the technology was not fully commercialised to a point where it was ready for industry to pick up and run with. However, the technology developed during the ACIAR project was still seen as a significant step forward and ACIAR backed up their initial investment with a further project designed to transfer the technology to Vietnam and Indonesia. A review of ACIAR's investment in mud crab aquaculture in Vietnam and the Philippines was carried out by Shelley (2005). Technology transfer and industry development was found to have been particularly successful in Vietnam. A further economic assessment of ACIAR's investment has recently been carried out by Lindner (2005).

At the end of the ACIAR project the major issue was unreliable and inconsistent production of crablets. Successful production of mud crab zoea and their subsequent settlement and metamorphosis through the megalops stage to crablet was demonstrated to be highly dependent on good water quality, and in particular, control of bacterial contamination of cultures.

This FRDC project (Project No. 2000/210) was designed to address the unreliable and inconsistent production of crablets, using a mixture of experimentation and industrial design to develop commercially robust hatchery and nursery systems for the production of *Scylla serrata* in Australia.

Larviculture Introduction

Historical and geographical effort

Interest in the development of new larviculture techniques to support development of mud crab aquaculture is not new in Australia. Work was undertaken by Heasman and Fielder (1983) who successfully reared *S. serrata* larvae to crablets on a small-scale. Whilst research has been undertaken in many countries where *Scylla* spp. occurs, research which has already led to, or is leading toward closed-cycle aquaculture, has occurred in China (Ding, Zhou *et al*. 2001), Vietnam (Nghia 2001), the Philippines, (Fortes, 1999) Indonesia (Cholik,1999) and India. This project is the first aimed at commercialising mud crab larviculture technology in Australia, and follows on from the successful development of basic mud crab culture technology through collaborative research between Australia and the Philippines (ACIAR project #9217).

Feeds and feeding regimes

Many authors have studied larval feeding regimes for various mud crab species in an attempt to optimise survival (DuPlessis, 1971; (Brick 1974; Heasman and Fielder 1983); Jamari, 1992; (Marichamy and Rajapackiam 1992; Zainoddin Bin 1992); (Anil and Suseelan 1999) (Mann, Asakawa *et al*. 1999) (Baylon 2001; Baylon and Maningo 2001) (Mann *et al*. 2001; Quinitio, Parado-Estepa *et al*. 2001; Baylon *et al*. 2004)). Most of these used rotifers in combination with Artemia, often with success, but interestingly Brick (1974) found that *Artemia* fed alone (at up to 10 mL^{-1}), rather than in combination with either rotifers or zooplankton produced the best larval survival. Brick (1974) also suggested that zooplankton other than rotifers might be an alternate first feed for mud crab larvae. Different strains of *Artemia* have been demonstrated to have quite different performance as feed for mud crab larvae, with only two commercially available strains being able to provide adequate nutrition for the zoea to successfully moult to megalops (Mann *et al*. 2001). The phospholipid fraction was the component of the *Artemia* that was best correlated with mud crab larval survival.

Of the two species of mud crab found in Australia only one species of mud crab, *Scylla serrata*, is being developed for aquaculture. For this species, stocking densities of approximately 10–100 larvae L^{-1} have been used and live food, including microalgae, has been introduced at the time of stocking (Williams and Field 1999). Rotifers have been fed throughout the zoeal larval stages at approximately 10 mL^{-1} in conjunction with freshly hatched *Artemia nauplii* at 0.5 mL^{-1} from the second day of Z2 (Z2/2). Researchers in other Asian countries have utilised much higher larval stocking densities and incorporated similarly higher live food densities. Baylon (2001) stocked larvae at up to 100 L^{-1} in a green-water system where rotifers were included at 30 mL^{-1}, and *Artemia* added at 0.5 to 5 mL^{-1} as the larvae grew from Z1–Z5. (Djunaidah *et al*. 2001) also used a green-water rearing system with high larval densities up to 100 L^{-1} and up to 60 rotifers mL^{-1}. Baylon and Maningo (2001) also suggested a combined feeding regime of rotifers and *Artemia* to Z4 and a switch to *Artemia* alone at Z5. Nghia *et al*. (2001) reviewed larval rearing practises and made recommendations on how best to rear mud crab larvae in Vietnam. He recommended algal densities of 0.1–3 x 106 cells mL^{-1}, an (ICES) enriched rotifer density of 45 mL^{-1} for the first six days and an *Artemia* density of 20 mL^{-1} from day 4 onwards. Alternative micro-bound diets have been investigated (Genodepa *et al*. 2004; Genodepa *et al*. 2004) and demonstrated to have similar nutritional benefits to Artemia, decreasing inter-moult time compared to *Artemia* in one trial. The optimum sized micro-bound diet for different zoeal and the megalops stage of *S. serrata* were also quantified. It is also well established that commercially available prawn larval

and post larval diets used supplementary to *Artemia* can mitigate nutritional deficiencies associated with an *Artemia* only diet.

The nutritional quality of *Artemia* as a live feed for *S. tranquebarica* has been investigated (Kobayashi, Takeuchi *et al*. 2000). The results suggested that the fatty acids eicosapentaenoic acid (EPA) and docosahexanenoic acid (DHA) should be present in appropriate ratios in *Artemia* to promote survival and growth. They reported that optimal EPA and DHA levels of *Artemia* should be 1.3–2.5 per cent, and 0.46 per cent respectively, noting that DHA levels above this produced lower larval survival rates.

Similarly for *S. serrata*, it was found that elevated levels of EPA and DHA in larval diets could improve their growth and survival (Weng, Li *et al*. 2001). Through examination of starved larvae it was also determined that the diet provided to early stage crab larvae should have a high level of lipid and DHA (Weng, Li *et al*. 2002). In related work on *Portunus trituberculatus* it was found that the EPA levels in rotifers provided in larviculture should be in the range 0.9–1.7g per 100g on a dry weight of food basis for optimal survival (Takeuchi, Nakamoto *et al*. 1999), after earlier work had identified the need for rotifer enrichment as important for routine seed production in that species (Hamasaki, Sekiya *et al*. 1998).

Bacterial control

It has been understood for some time that successful rearing of mud crab larvae requires control of the bacteriology of larviculture systems. Brick (Brick 1974) found that antibiotics (penicillin-G & polymyxin-B) were necessary to rear larvae of *S. serrata* to the megalops stage, however survival from megalops to crablet was not affected by the their use. Kasry (1986) used the same mixture of antibiotics with some success on the same species. A variety of antibiotics or anti-bacterial chemicals have been used as experimental tools in mud crab larviculture. Using prefuran Anil & Suseelan (Anil and Suseelan 1999) obtained a larval survival rate of 23 per cent culturing S. oceanica, however when Wahyuni (1985) used streptomycin sulphate and penicillin to culture *S. serrata*, very high larval mortalities were reported, demonstrating that antibiotic use must be highly specific and is not a panacea for mud crab larviculture. A more process orientated approach to controlling bacteria in mud crab larviculture systems was recommended by Blackshaw (Blackshaw 2001). He stressed the need to maintain hygiene and to control pathogenic organisms, recommending microbially mature water and the use of probiotics, rather than use antibiotics which can lead to the development of resistant pathogens.

System design

Larviculture systems for crabs may need to respond to the biorhythms of the larvae as it has been demonstrated that there are rhythms of digestive enzyme activity in mud crab larvae (Li, Tang *et al*. 2000) These rhythms were shown to vary with light, larval stage and stage within the moult cycle.

The need to maximise water quality in mud crab larviculture through such methods as filtration and treatment with UV lights (Brick, 1974), re-circulation (Heasman and Fielder,1983) and more holistic system design (Mann *et al*. 1999) has been well documented. A range of parameters have been examined in attempts to improve larval production systems. Kasry (1986) found higher larval mortalities when using low salinities during the early stages of culture, whilst Baylon & Failaman (2001) suggested that salinity didn't affect the duration of larval development and that the highest survival of megalops was at a constant 32ppt for *S. serrata*. However, during the nursery stage of production Yu *et al*. (2001) found a shorter moult period at lower salinities.

Larviculture development in this project

An experimental approach was taken in this project to address the three parameters considered to have most influence over larval survival:

Water Quality,
Bacteriology,
Feeds and Feeding Regimes.

Trials carried out are grouped under one of these three headings. Along the way, the systems in which larvae were being cultured were progressively modified and improved. This work was undertaken at the Darwin Aquaculture Centre (DAC, Darwin, NT) and the Bribie Island Aquaculture Research Centre (BIARC, Moreton Bay, Qld). Each experiment is credited to the centre where the work was undertaken.

Figure 1. Broodstock holding tanks at DAC.

Standard Operating Procedures

To obviate the continual repetition of common methods used in the trials or experiments in this report, the standard operating procedures used at both the Darwin Aquaculture Centre (DAC) and the Bribie Island Aquaculture Research Centre (BIARC) for mud crab larviculture are summarised in the following sections. These are the methods as used unless stated otherwise.

Standard Operating Procedures for Larviculture at DAC.

Broodstock.

The broodstock crabs used in this section were identified as *Scylla serrata* according to the description of Keenan *et al*. (1998). Mature females were collected from the wild and held communally in a 5000 *L* temperature controlled, re-circulating system (Figure 1.) within a shed at the Darwin Aquaculture Centre.

When females were observed to be carrying eggs they were transferred to a separate re-circulating incubation system where they were held individually in a 100 L tank with reduced light levels and were not fed. When eggs were within 48 hours of hatching a small sample of eggs were removed for health assessment. The females were then be transferred to a 600 L hatching tank,

Following hatching (usually early morning), the positively phototactic first stage zoea larvae (Z1) were concentrated phototactivilly using a torch light and gently ladled by bowl to a 100 L plastic tub of iso-thermal seawater matched to the salinity and pH of the hatchery system. The larvae in the tub were then evenly dispersed in the water column by gentle hand movement. The total number was estimated by counting all the larvae in 20 random 3 mL samples and averaging and extrapolating these figures to the volume of the tub.

Standard design for multi-factorial, small-scale trials

The experimental scale rearing containers used were 5L hemispherical, acrylic, bowls containing 3L of culture water. The bowls used in this experiment were held on two floating “pontoons” (ten bowls each) within a 5000 L water bath (Figure 2) . The water to be used in the experimental bowls was made up each day with the salinity progressively lowered from 30ppt at the beginning of the experiment to approximately 25ppt at megalops stage. Gentle aeration was provided through a single glass 1 mL pipette to each bowl. Various treatments were imposed and five replicates of each were laid out in a randomised design across floating pontoons within the water bath.

Replacement culture water was maintained in a separate tank within the main water bath to maintain water temperature equilibrium with the culture water and to prevent temperature shock to the larvae during water exchange.

The larvae maintained in each bowl were individually counted and initially stocked at 10 larvae L^{-1} (i.e. 30 larvae per bowl). The larvae were slowly acclimated to the bowl by floating in a shallow tray with frequent small additions of the culture water. Each morning, the larvae were individually transferred into a new clean bowl containing new culture water from the replacement water tank using a 3 mL pipette. During this process they were counted, and any dead larvae removed. New additions of live food were made according to the experimental protocol. As the larvae grew pipettes with larger bores were used to minimise any physical damage to the larvae.

The standard feeding regime is shown in Figure 3.

The Temperature of several random bowls was measured each morning at 0800 hours. Ammonia and nitrite were also measured every other day from a single randomly chosen bowl from each treatment.

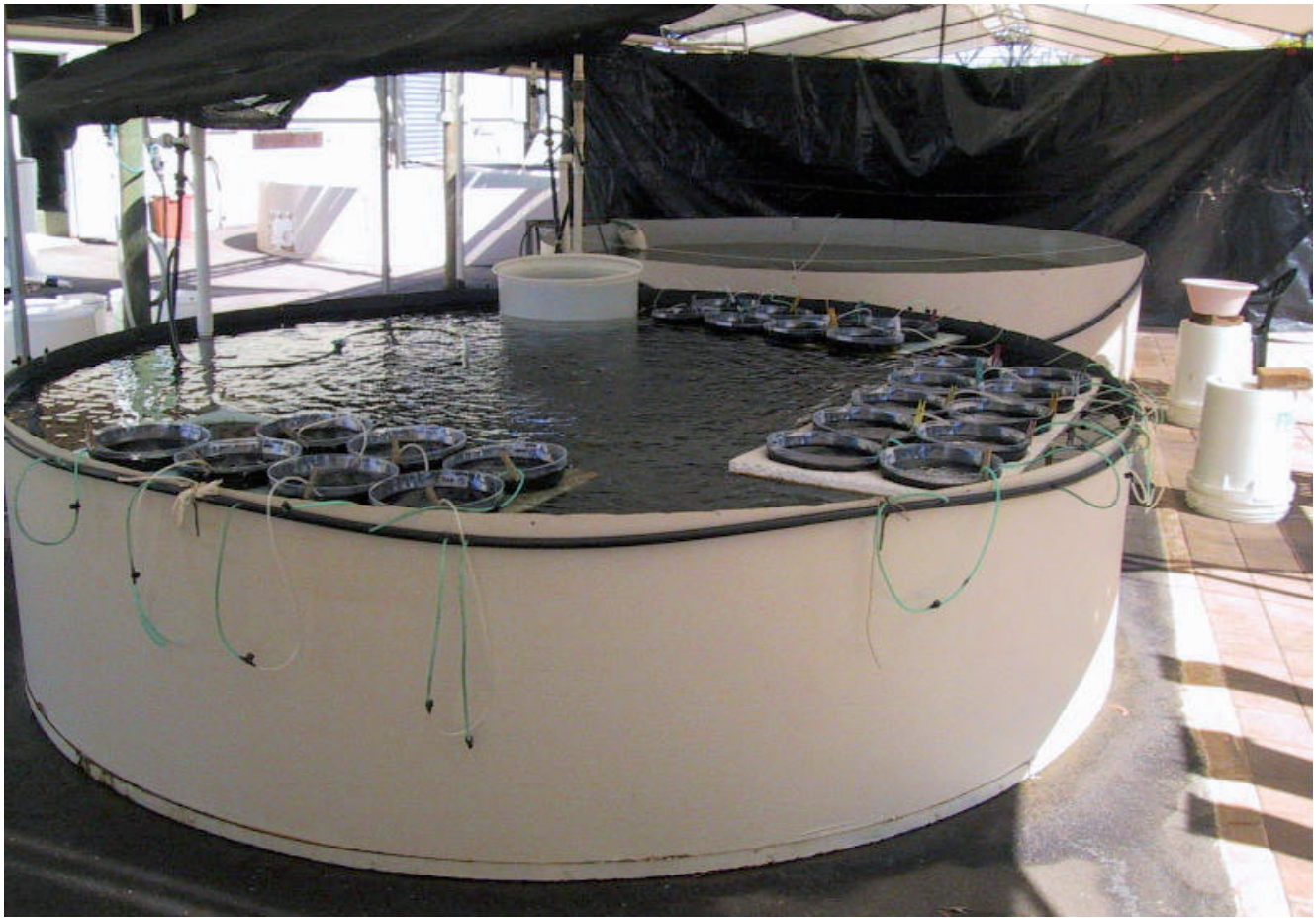

Figure 2. Experimental bowls in a water bath for small-scale trials at DAC.

Statistical analysis

Daily survival was expressed as the percentage of zoea alive at the time of counting, and duration to megalops was recorded in terms of days since hatching (termed Day 0). These variables were analysed by analysis of variance (Statistix V 4), and where significant differences were observed, they were separated using a least significant difference test. Residuals were examined to determine a requirement for data transformation.

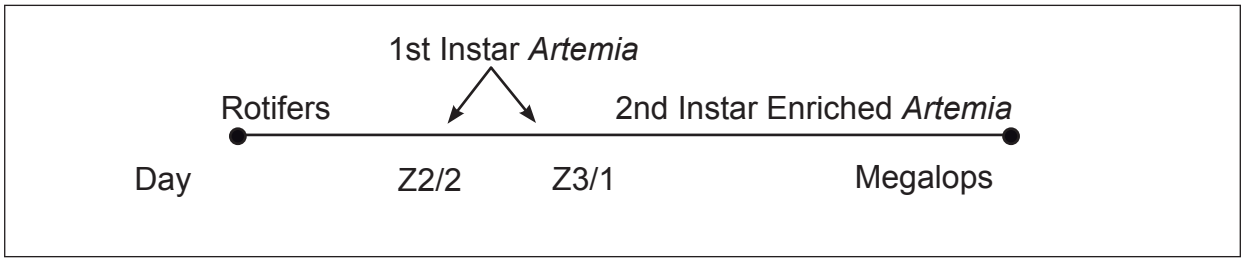

Figure 3. Generalised feeding regime.

Standard design for large-scale, replicated trials (1000 L).

Large-scale trials were carried out in 1000 L circular tanks in an effort to replicate commercial hatchery conditions. The fibreglass tanks were 150cm in diameter, with a gently sloping conical bottom beginning 60cm from the top of the tank. The internal walls were smooth and coated in a grey, food grade gel-coat.

The outside of the tanks were also insulated with an external wrapping of 40 mm thick polystyrene foam to minimise temperature fluctuations (Figure 4.). During cooler weather the top of the tank was covered with clear plastic sheeting to reduce temperature loss from the water's surface.

Numbers of crab larvae were estimated volumetrically as described for small scale trials, and stocked at the desired density. The sea water for all tanks had been settled and foam fractionated for at least 48 hours before use. Allocation of treatments to particular tanks was done in a randomised manner. Daily survival could not be quantified in these experiments as healthy larvae held their position in dense aggregations even with strong agitation of the water, however qualitative estimates were made. Surviving larvae were counted at the end of trials to obtain survival rates.

Seawater in the tanks was initially airlifted through a submerged, horizontally mounted 320μm screen and directed to a side mounted 700mm length of 90mm PVC pipe angled down at 45° (the "Bazooka"). A 45° elbow and 70–32 mm reducer attached to the lower discharge end of the "bazooka" was used to create a tangential current that in turn generated a continuous circular current that swirled solids back to the centre of the tank (Figure 29). On the end of this was a 45° elbow and reducers to 32mm. This pipe-work ensured a constant circular flow around the tank. A short sub-surface air-lift (an "AQUACLONE") was used to keep larvae up in the water column. At the zoea 2 larval stage (Z2) there was a switch from 320μm to 500μm screens and at Z3 a switch to 600μm screens. See Figure 4 for photograph of the set-up.

A number of 300-watt, glass immersion heaters were placed inside the "bazooka" to heat the passing water without directly contacting the larvae. Temperature of the culture water was maintained at 30°C by a thermostat with sensor in the tank.

All tanks received 30 000 larvae or 30/L at stocking. When megalops were observed in the larval tanks, selective harvesting was carried out using an appropriately sized screen (2000μm–2400μm).

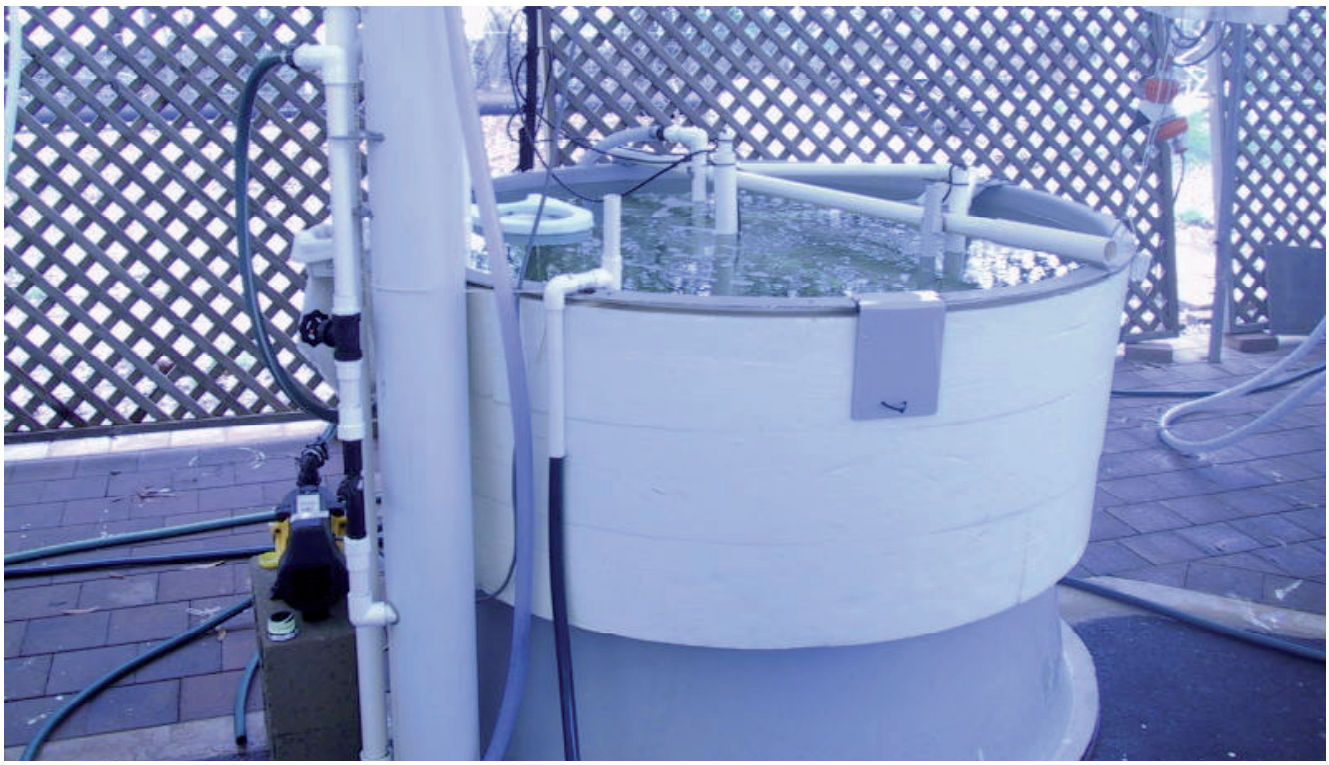

Figure 4. Standard set up of 1000 L tanks at DAC

McRoberts Larval Tanks

In some early larval trials, pairs of McRobert Aquaculture Systems® larval rearing tanks were used (Figure 5). These passive transfer tanks have a 1.5mm thick rubber liner under which low-pressure air can be pumped. The pumped air lifts the liner and displaces the water through an open chute to the identical “sister” tank. This facilitates the transfer of the culture water, including live feed and larvae, to a new clean tank. The old tank liner can then be cleaned and dried before reuse. Each morning the entire tank volume was displaced by inflating the liner to the clean sister tank.

Figure 5. McRobert Aquaculture Systems® larval rearing tank.

Feeds

Unless otherwise stated in the methods section for individual trials, live feeds used in experiments were as follows:

Algae

Each day, after counting the larvae and cleaning the small-scale bowls, a combined algal density of 5 x 104 cells mL^{-1} was added to the bowls. This comprised equal cell numbers of bag or carboy cultured *Nannochloropsis oculata*, *Chaetoceros mulleri* and a *T. Isochrysis*-like species called *PS11*.

In the large-scale trials, a concentration of 5x104 cells.mL^{-1} of mass outdoor cultured *N. oculata* was checked and maintained on a daily basis.

In many of the later trials, live algae were replaced with algal pastes (Instant Algae®). Methods of application are detailed in the individual trials where algal paste was used.

Rotifers

Two different rotifer culturing methods were used in the project.

In the first rotifers, to be used as live feed, were batch cultured primarily in *N. oculata*, and harvested each day. Rotifers were then enriched for at least 60 minutes in a combination of the three algal species before being concentrated in a 64*µ*m mesh bag and rinsed for five minutes in ultra-violet treated, filtered sea water. The species of rotifer used was *Synchaeta sp.*

In the second method, rotifers were cultured at high density (i.e. around 1000 mL^{-1}) in a re-circulating system using *Chlorella* paste (Pacific Trading Company Pty. Ltd.) as a feed. One day prior to crab hatching and stocking, rotifers were harvested by draining through a submerged 64*µ*m screen, were rinsed in 5*µ*m filtered UV treated seawater and were stocked to two 100 L tubs.

Rotifers were used to feed crab larvae during the first zoeal stage and the first day of zoea stage 2 (Z2/1). During the rotifer feeding phase, all larval tanks underwent a 70 per cent drain-down, top-up water exchange each day.

Residual rotifer counts were undertaken in the morning to assess feeding level and as an indicator of the status of rotifer health.

Artemia

Day 2, zoea 2 crab larvae (Z2/2) and first day zoea 3 crab larvae (Z3/1) were also fed newly hatched *Artemia nauplii* (AF grade INVE Brand : 430*µ*m length at hatch) at a rate of 0.5 *nauplii*. mL^{-1} in a single feed. Ultra-violet treated water was used for the hydrating, hatching, and rinsing stages. For larger larvae (Z3/2 to megalops) second instar *Artemia* were fed. These *nauplii* were enriched in Selco™ enrichment products as per the manufacturer's instructions for 24hrs.

The *Artemia* were hatched in static tanks of UV treated seawater along with 100ppm of INVE Hatch Controller, and were thoroughly aerated. The cysts were not sterilised with chlorine prior to incubation. The following morning the *nauplii* were harvested, thoroughly rinsed and restocked to a 20 L bucket of pre-filtered UV disinfected seawater for counting and distribution to the larval culture tanks.

From zoea 3 day 2 (Z3/2), GSL AAA *Artemia* offered were 48 hour (second instar) enriched. The same hatching method as above still applied, however after 24 hours the *nauplii* were harvested, rinsed and restocked at approximately 200/mL. These *Artemia* were then enriched DC DHA Selco™ (INVE) as per the manufacturer's instructions for approximately 24 hours then harvested and fed to the crab larvae. Larvae were fed *Artemia* in two feeds per day at 1200h and at1600h. Feed rates were increased gradually from 1.5/mL on day 4, to 2.0/mL on day 9 and further to 3.0/mL by day 12.

Residual *Artemia* counts were undertaken in the mornings to assess feeding level and as an indicator of the status of *Artemia* health.

Salinity control

Unless otherwise stated, salinity of the culture water was 30ppt at hatching and was then decreased gradually to 25ppm by the end of the Z5 stage.

Culture water pre-treatment

Sand filtered (5μm) seawater was passed through a 1μm filter bag and stored in two 100 000 L fibreglass storage tanks. This seawater was then foam fractionated for three days and allowed to settle, with aeration. It was then pumped to a series of shallow 9000 L tanks to be heated by immersion heaters or allowed to cool as required prior to use. The water for use each day was maintained at 30°C.

Routine operation of 1000 L culture tanks

The 1000 L larval culture tanks were arranged as two rows of three under a shade structure at the DAC. Temperature of the culture water was maintained at 30.0°±0.1°C by a thermostat with sensor in the tank. Water quality was measured in each tank, each morning at 0800h.

Larval culture tanks underwent a 70 per cent drain-down, top-up water exchange each day, at which time the walls of all tanks were wiped clean. Larvae were retained by submerged horizontal screens. Screens, airlifts and the Bazooka were removed and cleaned prior to drain-down. The tanks were partially drained each day before use by pumping the water out through the foam fractionators. This also served to flush the foam fractionators. The remaining 30 per cent of seawater holding the retained larvae was then recycled through the foam fractionators for 30 minutes. Additional foam fractionation & flow through with exchange storage seawater was applied for another 30 minutes. Tanks were then refilled with temperature adjusted storage seawater.

Experiments to Investigate the Effects of Water Quality on Larval Rearing Success

Large- and small-scale trials:

Effect of culture water pre-treatments and tank colour on survival of mud crab (*Scylla serrata*) larvae. (DAC–Batch 1)

Introduction

In order to identify the effect that various culture water pre-treatments or management regimes have on mud crab larval survival, two experiments were conducted concurrently. One experiment was carried out at small-scale in 5L bowls whist the other was a larger scale done in 1000 L tanks.

A secondary aim of comparing the effect of tank colour was included in the small-scale bowl experiment. The experiments were carried out in June 2001.

Methods

Standard Operating Procedures were employed as previously described.

Small-scale trial

Four rearing condition treatments used were as follows:

A: Seawater that had been sand filtered (<10μm) water which had been chlorinated at 10mg/l of active chlorine for 20hrs and de-chlorinated with sodium thiosulphate immediately before use. Transparent bowls were used.

B: Settled and foam fractionated water. This water was initially sand filtered to less than 10μm and pumped to a 20 000 L tank where the remaining solids were allowed to settle out before being vacuumed from the tank. The dissolved organic matter and remaining solids were actively removed by fractionation for at least 48 hours before use. Chlorination and de-chlorination was not carried out on the water used in this treatment. Transparent bowls were used.

C/D: Used the same water as “A”, but the outsides of the bowls were painted black and white, respectively.

Large-scale trial

Experimental treatments comprised the following rearing protocols:

A: The first treatment consisted of two replicates of the McRoberts passive transfer system. The experimental treatment imposed was an 80 per cent drain down, top-up, batch-style water exchange. A cylindrical 500μm screen over a 150mm diameter PVC perforated pipe prevented the accidental removal of the larvae during drain down. Algae, *Artemia* and rotifers were incidentally siphoned from the tank. Remaining live prey were not quantified or taken into consideration in daily funding regimes applied throughout the experiment.

B: The second treatment used three standard 1000 L insulated tanks. They were subjected to a daily 80 per cent drain down/top-up, batch style seawater exchanges, implemented in the same manner as discussed in treatment A.

C: The third treatment consisted of three replicate re-circulating systems. A standard 1000 L insulated tank was used as the culture vessel with a 100 L plastic tub filled with Bioballs™ as the biofilter. These bioballs were taken from an existing working system and were "fed" with ammonium chloride and sodium nitrite prior to the trial to encourage development of biofilms that convert ammonia to nitrate. An internal 500μm screen, similar to the other treatments, was plumbed into the central drainage point of the tank. This screen was removed for cleaning once each week. Water was allowed to overflow out an external standpipe, which regulated culture tank depth, into the submerged biofilter. The water was returned from the biofilter to the culture tank via a single 25mm diameter airlift. Live prey were allowed to circulate throughout the system. Each morning a 44μm screen was placed under the return flow for three hours to remove a proportion of the remaining live food. Remaining food was not quantified. These tanks were also not wiped clean at any stage throughout the trial.

Water quality parameters

Water quality parameters including dissolved oxygen (mg/l), pH, temperature (°C) and salinity (ppt) were measured every other day. Ammonia and nitrite from one replicate of each treatment was measured daily. The salinity was progressively reduced from 30ppt at the beginning of the experiment down to approximately 25ppt at megalops stage. Additionally, a single temperature logger was placed in a tank most evenings to measure the diurnal variation.

Bacteriology

At the end of the trial a single water sample from each one tonne tank was submitted for bacterial assay. Several live crab larvae were also submitted.

Algae

Each day after counting the surviving zoea and cleaning the bowls, microalgae at a combined algal density of 5 x 104 cells mL^{-1} was added to the bowls. These algae consisted of equal cell numbers of bag and carboy cultured *Nannochloropsis oculata*, *Chaetoceros mulleri* and *PS11*. In the 1000 L tanks, 5 x 104 cells per mL of mass outdoor cultured *N. oculata* were added.

Rotifers

Rotifers were cultured in *N. oculata*. Rotifers in both experiments were fed at a rate of 10 mL^{-1} in a single feed from day 0 (stocking) to day 9 when the majority of larvae were at the fifth Zoeal stage (Z5).

Artemia

From day 4 until day 10, the crab larvae were also fed newly hatched *Artemia nauplii*.

Results

Small-scale trial

Some of the crab larvae moulted to megalops stage as early as day 13, and were removed from the bowls to prevent cannibalism of the remaining zoea. Although these animals were no longer in the experimental units, they were considered to be survivors and are included in the subsequent counts.

Table 1 shows the daily percentage survival (±SE) of the crab larvae exposed to the four experimental treatments. Significant differences in survival were noted from day three onwards. All larvae in treatment A, C and D, the chlorinated water, had died by day 10, 9 and 9 respectively. However, almost a third of the larvae in treatment B, the settled and fractionated water, survived to the megalops stage. On day 18 they had all moulted to megalops. Error! Reference source not found..

Table 1. Daily percentage survival (±SE) of crab larvae from the bowl experiment (DAC Batch–1). See text for treatment explanations.

	Survival (%)			
Days	A	B	C	D
1	94.66 ± 2.71	98.66 ± 0.82	97.33 ± 1.25	96.00 ± 1.94
2	83.33 ± 2.11	89.33 ± 3.06	78.00 ± 4.29	73.33 ± 8.43
3	68.00 ± 6.29	78.67 ± 3.89	62.67 ± 4.52	60.67 ± 9.39
4	36.00 ± 13.31	73.33 ± 6.15	44.00 ± 11.66	15.33 ± 6.96
5	10.00 ± 4.71	70.67 ± 5.31	15.33 ± 7.27	8.67 ± 4.16
6	4.00 ± 2.45	68.00 ± 4.55	2.00 ± 1.33	2.67 ± 2.67
7	0.67 ± 0.67	63.33 ± 3.50	1.33 ± 0.82	1.33 ± 1.33
8	0.57 ± 0.67	58.00 ± 4.29	0.83 ± 0.75	0.57 ± 0.67
9	0.58 ± 0.67	55.33 ± 5.23		
10		53.33 ± 4.83		
11		53.33 ± 4.83		
12		51.33 ± 4.03		
13		48.67 ± 3.74		
14		48.67 ± 3.74		
15		48.67 ± 3.74		
16		37.33 ± 8.12		
17		32.67 ± 8.46		

Large-scale trial

Water quality

Water quality remained generally conducive to mud crab larvae survival and growth throughout the trial and there was little difference between tanks recorded from the daily water quality testing; dissolved oxygen ranged between 5.6 and 7.2 mg/l, pH ranged between 8.16 and 8.60, and temperature ranged between 28.1 and 30.5°C. The mean diurnal temperature variation of the three treatments was 0.8°C for the re-circulating tanks and the batch exchange tanks; however the McRoberts tanks had a mean variation of 1.25°C.

There were however quite obvious differences between treatments in terms of nitrogenous wastes. Mean morning total ammonia-nitrogen concentration was 0.275 mg L^{-1} for the batch exchange treatment, 0.389 mg L^{-1} for the re-circulating tanks, and 0.245 mg L^{-1} for the McRobert tanks. Daily concentrations are shown in Figure 6. Mean nitrite–nitrogen concentrations for the batch exchange treatments was 0.0025 mg L^{-1}, 0.22 mg L^{-1} for the re-circulating tanks, and 0.0085 mg L^{-1} for the McRobert tanks (Figure 7.) It can be clearly seen that the ammonia and nitrite concentrations were, in general, higher in the re-circulating systems. Nitrite concentrations steadily increased throughout the trial in this treatment.

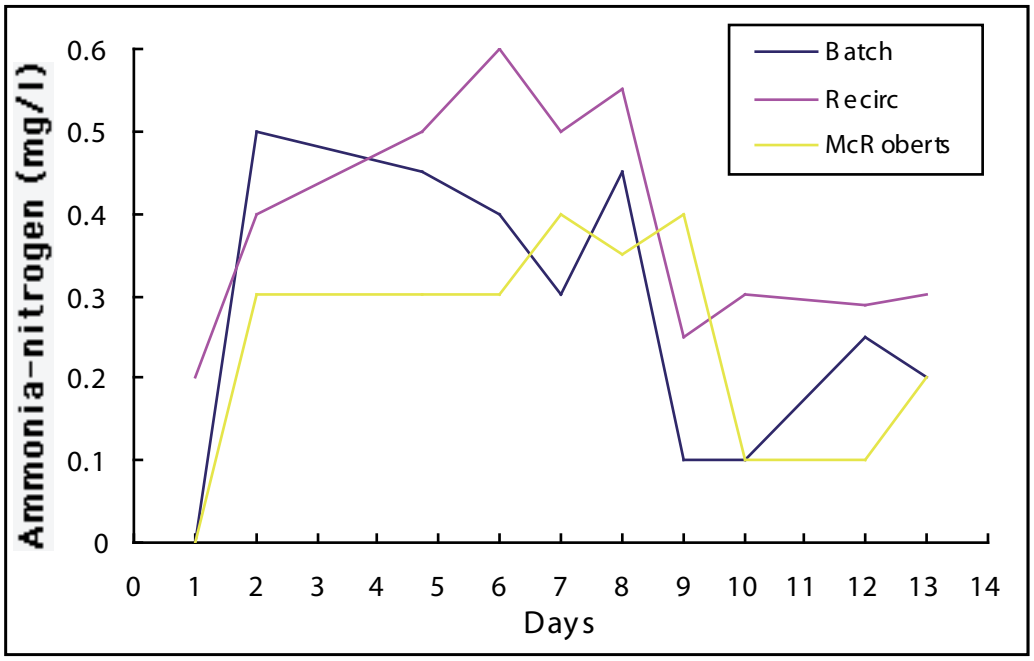

Figure 6. Daily ammonia–nitrogen concentration in the 1000 L tanks.

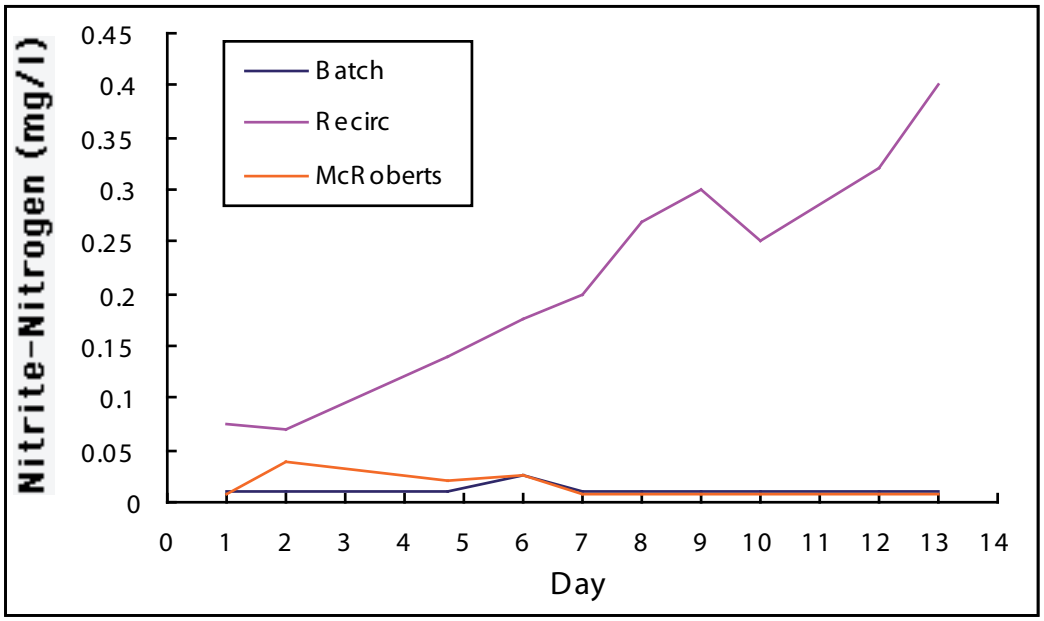

Figure 7. Daily nitrite–nitrogen concentration in the 1000 L tanks.

Survival

There appeared to be a significant level of mortality within the first three days of stocking leaving only an estimated 2000 to 3000 larvae per tank. There did not appear to be any difference between the batch exchange and re-circulating treatments at this stage, although the McRobert tanks experienced slightly higher mortalities. Survival appeared to be relatively stable in the batch and re-circulating tanks from day three onwards, however the McRobert tanks suffered significant mortalities throughout; such that by day 9 less than approximately 200 larvae per tank remained. On day 9, a few stage 5 zoea larvae were noticed in all tanks. On the morning of day 12 several animals which appeared to be moulting were seen swimming near the surface. This was later verified under a dissecting microscope.

The following morning (day 13) there were very low numbers, less than 20, of either megalops or zoea larvae in any of the tanks. These were treated and fed as usual. On day 14 only a few tanks had live animals remaining and the experiment was terminated.

Bacteriology

An unidentified strain of pink bacteria established small colonies in all tanks, however towards the end of the trial, the liners of the McRoberts tanks were almost completely covered by it.

A heavy bacterial load, including several species of *Vibrio*, was found in the live crab larvae. The water samples taken from the 1000 L tanks, all of which appeared cloudy on day 13 and 14, also yielded high *Vibrio* loads. Table 2 shows the mean *Vibrio sp.* counts from samples taken from the 1000 L tanks on day 14.

The McRobert tanks had significantly more *Vibrio* species per mL than the other treatments.

Table 2. Mean (± SE) *Vibrio sp.* per mL in the 1000 L experimental tanks (DAC Batch–2)

Treatment	*Vibrio sp.* counts. mL^{-1}
Batch exchange	4900 ± 1594a
Re-circulating tanks	5366 ± 1058a
McRoberts tanks	14500 ± 1500b

Discussion

The main aim of these experiments was to determine an appropriate water treatment regime for mud crab larval rearing. In the small-scale experiment over 30 per cent survival to megalops was obtained using settled and foam fractionated water. In contrast there was 100 per cent mortality by day 10 in the treatments receiving the chlorine/thiosulphate treated water. Previous experimentation carried out at several institutions, including our previous site and at the BIARC, yielded relatively good survival when using chlorinated water. Reasons for this mortality can only be speculated; however some likely explanations can be suggested. The active chlorine was supplied in a 125g L^{-1} hypochlorite solution where we had previously been using granular chlorine which is much more active (680g L^{-1}). It is possible an incorrect dose of chlorine or sodium thiosulphate may have been applied. An incorrect dose of thiosulphate may have been used, so that there would have been excess thiosulphate in the rearing water. Thiosulphate and its breakdown products are not known to be toxic to crustacean larvae, although it has not been tested on mud crab larvae.

Another possibility is the formation of toxic chemicals through the chlorination of high organic and sediment loads in the seawater. This seawater was passed through a series of sand filters however fine particles (< 5*µ*m) were observed to settle in the in the bowls. Chlorination of water containing high organic loads can lead to the formation of chloramines and bromoforms which are toxic to larvae of many species, but which can be removed by passing water through an activated charcoal filter. The fractionated water was not chlorinated. Effects of background colour (white, black or clear bowls) were not determinable due to the mortality of all larvae receiving the chlorinated water.

The water for the large-scale trial was also settled and fractionated, not chlorinated, and apparent high levels of survival were observed in all tanks excepting the McRobert tanks up to day 13. The lower levels of survival in these tanks may have been due to bacterial proliferation, possibly facilitated by the textured liner, providing a higher substrate surface area than the smooth-walled fibreglass tanks. The significantly higher levels of *Vibrio* species in the McRobert tank water on day 14, in part supports this conjecture. Larvae traversing the chute between the tanks were seen to undergo a rolling movement and were unable to maintain equilibrium. It is possible that this daily movement could have caused injury to the larvae, especially the pronounced dorsal spine, which may have led to bacterial infection. Altering methodologies such as the speed and depth of water during transfer may correct this perceived problem.

The floors of the fibreglass tanks were not cleaned during the experiment and a microbial community rapidly developed. A decision was made not to disturb this biofilm for fear of suspending organic matter and microbes. Up to day 12, this decision seemed vindicated as survival was high. It is not known if the biofilm contributed to the mass mortality on day 13, however recent microbial research supports a “critical mass” theory (Bruhn *et al* 2005). This theory suggests that bacteria can signal each other to release toxins when the bacterial numbers reach a point where a “quorum” of that particular bacterium exists. It is possibly a coincidence that the quorum was attained the same night as the final moult to megalops. However this seems unlikely as an explanation due to the survival of some larvae still at Z5, which subsequently died the following night, presumably at moult.

Moult death syndrome (MDS), death while moulting, has been known to occur in mud crab larvae as well as other crustacean species. Teshima (1997) attributed MDS to a lack of phospholipids in the diet. Second instar *Artemia nauplii* were fed to the larvae from day 11. These *nauplii* were enriched with Dry Selco™ to increase their nutritional value although the ultimate level of phospholipids, or ratios with other important lipids, is unknown. A by-product of enriching live food is the production of faecal and dissolved metabolic wastes by the live food in the culture tank, contributing to the nutrition of the microbial flora. Perhaps these metabolic wastes allowed the bacteria to reach a quorum on day 13.

Due to the mass mortality experienced in the 1000 L tanks it is difficult to determine whether there was any difference in larval survival between the re-circulating or batch-exchange water treatments. Both seemed equally effective at maintaining larvae and given the relatively high ammonia and nitrite levels in the re-circulating systems, the larvae seem tolerant of these waste loads. The daily disturbance of an 80 per cent water exchange also seemed to have little effect on the behaviour of the larvae.

There were also differences in developmental rates between the small-scale and large-scale trial. The larvae in the larger tanks seemed to be one or two days ahead of those in the bowls. The first megalops from the 1000 L tanks were noticed on day 12 while the first from

the bowls were found on day 14. Both experiments used larvae from the same female, but on average the 1000 L tanks were between 0.5 and 2.2°C warmer than the bowls at the morning measurement. The bowls were floating in a constantly exchanging seawater bath, while water for the 1000 L tanks was allowed to heat and cool slightly during the day. These tanks were also insulated.

The reason for the marked difference in survival rates between the foam fractionated water bowl treatment and the 1000 L tanks may be explained in several ways. Firstly the bowls were much cleaner than the 1000 L tanks. These bowls received a 100 per cent water exchange and were essentially transferred to a new tank each day. Also the live food in the bowls was added fresh each day, while in the 1000 L tanks there would have been some remaining live food whose nutritional value would have decreased. Additionally the relatively small number of rotifers required in the bowls was easily supplied and there was always an addition of10 rotifers/mL/day. A shortfall in rotifer supply for the 1000 L tanks meant that the remaining rotifers were frequently added at only 6–8 /mL/day. Other researchers have discontinued the use of rotifers much earlier at the Z2–Z3 stage with no apparent ill effect on survival (Li *et al*., 1999).

Overall, mortality of mud crab larvae in these experiments may be attributed to one or all of the following:

- residual thiosulphate,
- production of chloramines or other toxins,
- bacterial infection–possibly resulting from damage to dorsal spines, or
- poor nutrition resulting in MDS or other fatal syndromes.

None of the water treatment regimes used gave acceptable survivals in the large-scale trial whereas in the small-scale trial only the settled and foam fractionated water supported reasonable survival (32.67% ± 8.46) from Z1to megalops.

Investigation of the effect of culture water pre-treatments on survival of mud crab (*Scylla serrata*) larvae. (DAC–Batch 2)

Introduction

Since the early stages of research into larval crab rearing at DAC the standard water treatment has involved chlorination of the culture water to reduce bacterial loadings. This is a regular treatment in prawn hatcheries and in the past has provided reliably high larval survivals in small-scale crab experiments. However since moving from the previous site at Stokes Hill to the current Channel Island site the previously reliable methods have failed. This indicates that the methods developed may have been very site specific and perhaps explains why researchers at other locations have been unable to replicate the results achieved even when using similar methods. The sea water at Channel Island has very high sediment loading with a high percentage of organic matter (up to 15 per cent). The high organic load is likely to cause problems with larvae evolved to hatch and develop in off-shore waters. This may be particularly so when the water is chlorinated as toxic by-products can be formed when water high in dissolved organic matter is treated by chlorination. Additionally in the absence of a stable bacterial community after chlorination the high organic load present would favour the development of rapidly growing bacterial species in the culture water once it has been de-chlorinated . It is generally considered that various pathogenic species (e.g. *Vibrio sp.*) are common among these fast growing opportunistic species.

The previous two experiments carried out indicated that seawater settled for > three days produced better larval survival than chlorinated seawater.

Two further experiments to compare the effects of various water treatment methods on larval survival were conducted concurrently.

A small-scale, factorial design experiment carried out in 5L bowls and a large-scale experiment carried out in 1000 L tanks.

The treatments used in the bowl experiment were part of factorial design intended to clarify some of the confounding issues regarding the effects that various water treatments have on larval survival. A further two treatments additional to the factorial experiment were included to determine what effect the background colour of the rearing vessel had on larval survival.

Small-scale trial

Materials and methods

The female mud crabs spawned on the morning of the 13/3/2001 (Spawn Day 0) and hatching occurred on 23/3/01 (Spawn Day 10/Hatch Day 0) at 11.30 am.

Production of live food

Nannochloropsis oculata , *Chaetoceros mulleri* and *PS11* were cultured in the algal laboratory using F2 medium.

Rotifers were cultured in *N. oculata*, and harvested each day.

Artemia Inve (Brand 430 AF grade) were hydrated in fresh water then disinfected for 20 minutes with 200ppm of active chlorine. They were then incubated in a 40 L container with a fluorescent light above. First instar *nauplii* were harvested with a 105*µ*m net and then rinsed in 5*µ*m sand filtered seawater before use.

Small-scale trial

The rearing containers used in this experiment were forty-two 5L hemispherical clear acrylic bowls containing 3L of culture water.

All of the treatments were stocked at 10 larvae*/*litre with individually counted larvae and had the same feeding regime.

Feeding regime

Feeds were supplied in a single daily ration.

10 rotifers*/*mL*/*day from day one of zoea one (Z1/1) to megalops stage plus first instar *Artemia* (430 AF)at 0.5*/*mL*/*day from *Z2/*2 to megalops stage algae added each day to achieve a combined cell density of 5x104 (1:1:1 mix of *Nannochloropsis oculata*, *PS11*, Chaetoceros muelleri).

Experimental design-part 1

1) Factorial experiment to investigate various water treatments. There were three replicates of each treatment.

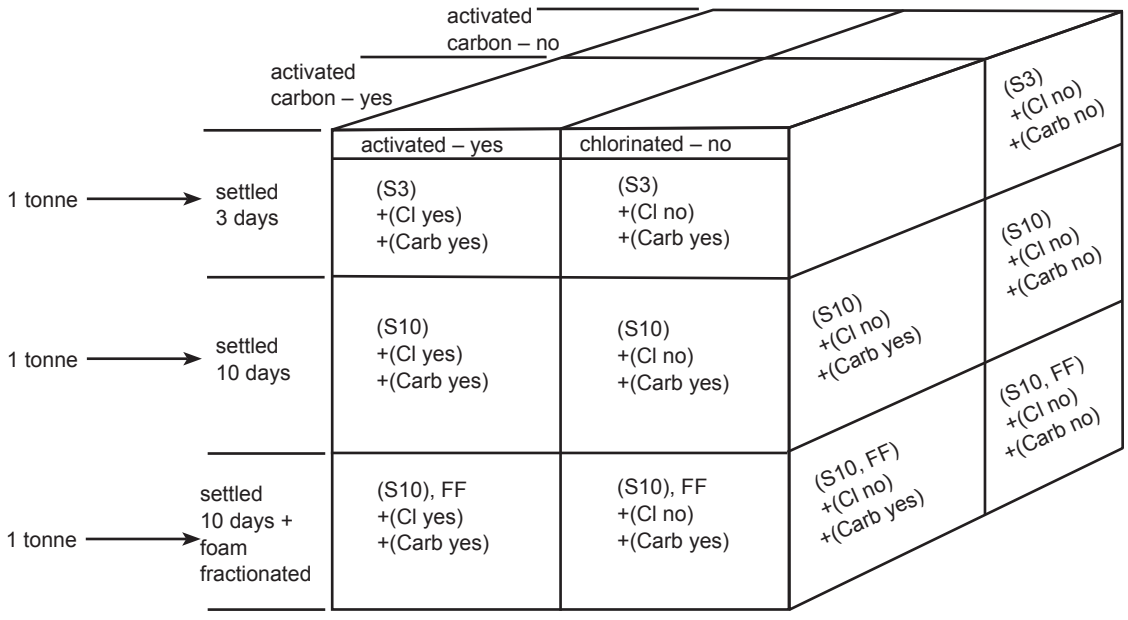

Figure 8. 3-D view of treatments in factorial experimental design.

Table 3. Treatments used in Factorial experimental design

Treatment Identification Number	Primary Water Treatment	Secondary Treatments	Tertiary Treatments
		Chlorinated	Activated Carbon Treated
1	Settled 3 day (S3)	+	+
2	Settled 3 day (S3)	+	–
3	Settled 3 day (S3)	–	+
4	Settled 3 day (S3)	–	–
5	Settled 10 days (S10)	+	+
6	Settled 10 days (S10)	+	–
7	Settled 10 days (S10)	–	+
8	Settled 10 days (S10)	–	–
9	Settled + Foam Fractionated for 10 days (S10, FF)	+	+
10	Settled + Foam Fractionated for 10 days (S10, FF)	+	–
11	Settled + Foam Fractionated for 10 days (S10, FF)	–	+
12	Settled + Foam Fractionated for 10 days (S10, FF)	–	–

For Table 3 (+) indicates that the water was treated as indicated and (–) indicates that the water was not treated as indicated.

Treatments 1 to 4, 5 to 8 and 9 to 12 utilised three different batches of sea water that had been treated as per the “Primary Water Treatments” in Table 3 prior to the first day of use (Day 0). These three original batches of water were used up by Day 6. From Day 7 a further three batches of water were used. The follow up batches of water had already been treated in the same manner as the original three batches prior to being used.

Description of primary water treatments

1. Settled three days (S3) = sand filtered (5μm) sea water stored indoors in a 1000 L fibreglass tank for three days prior to first use.
2. Settled 10 days (S10) = sand filtered (5μm) sea water stored indoors in a 1000 L fibreglass tank for ten days prior to first use.
3. Settled for 10 days + Foam Fractionated (S10, FF) = sand filtered (5μm) sea water stored indoors in a 1000 L fibreglass tank for ten days prior to first use. During this time the water was foam fractionated using a venturi driven foam fractionator.

Description of secondary water treatments

Chlorinated = chlorinated at 15ppm active chlorine for 16 hours before de-chlorination with sodium thiosulphate immediately prior to use.

Description of tertiary water treatment

Activated Carbon Treated = treated by being circulated through activated carbon (Reef Carbon™) by airlift for three hours prior to use. Where the two treatments were combined this was done immediately after de-chlorination.

All of the water to be used the next day for the twelve treatments was stored in 100 L tubs that were floated in a 7000 L water bath overnight to adjust the exchange water temperature to that of the culture water in the bowls.

The three replicates of each treatment were randomly allocated positions in the water

Small-scale trial part 2

Bowl background colour experiment

This trial was to determine if the background colour of the bowls had any effect on the survival of larvae through to megalops. Two different background colours, black and white were trialled. Treatment 2 from the factorial experiment (which was in a transparent bowl) was a control for this experiment. The two treatments with coloured backgrounds had the same water management as Treatment 12.

Large-scale trial

Two different types of tanks were used, “standard” tanks and McRobert tanks.

The “standard” tanks were 1000 L fibreglass tanks and the McRobert tanks were as described previously in SOP section.

The crab larvae were stocked at a rate of 10 perL (10 000 per tank). The water for all tanks came from one of two 20 000 L settlement tanks where the water had been settled and foam fractionated for three days before first use. Each 20 tonne tank was used for three days whilst the other one was settling and foam fractionating.

Eight 1000 L tanks were used across three different water management treatments (A,B,C), arranged in a completely randomised design.

Treatment “A”

Consisted of two replicates of the McRoberts passive transfer system as per Batch 1

Treatment “B”

Used three replicates of “standard” tanks as per Batch 1. Debris was siphoned from the bottom of these tanks daily and the sides wiped when the water level was low.

Treatment “C”

Consisted of three replicate re-circulating systems as per Batch 1. Debris was siphoned from the bottom of these tanks daily.

Feeding regime

The feeding regime was the same as in the bowls except for the algae used.

Insufficient *PS11* and *Chaetoceros muelleri* were available to provide the same algal mix as in the bowls so 5x104 cells per mL of mass outdoor cultured *N. oculata* only was added daily. *Nannochloropsis oculata* was mass cultured in outdoor 9000 L tanks using Aquasol ™ as fertilizer.

Water quality

Water quality parameters including dissolved oxygen (mg/l), pH, temperature and salinity (ppt) were measured daily. Ammonia and nitrite from one replicate of each treatment was measured daily. The salinity was adjusted from 30ppt at the beginning of the experiment down to approximately 25ppt at megalops stage.

Daily survival in the tanks was not quantified as it is extremely difficult to accurately estimate the number of larvae in a tank and attempts can be misleading.

Results – Small-scale trial

As shown in Figure 9 the survivals from Z1 to megalops ranged widely between the various treatments with the treatments utilising chlorinated water producing the lowest survivals overall.

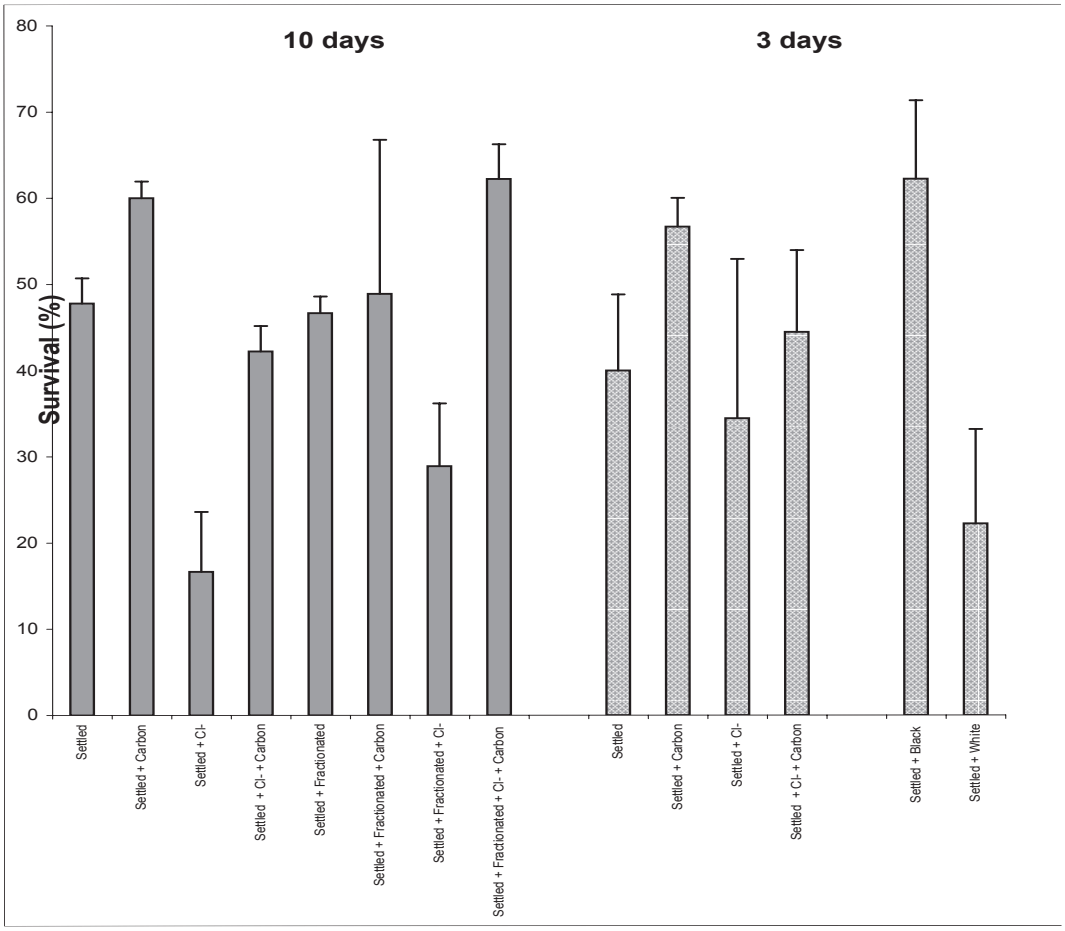

Figure 9. % survival (+ S.E.) from Z1 to Megalops for all treatments (DAC Batch–2).

There was no significant difference in survival between any of the primary or secondary treatments. However the treatments that received chlorinated water which was subsequently passed through activated carbon performed significantly better (95% sig. level = 0.05/6 = 0.0083, p-value = 0.005840) than those that were not treated with carbon after neutralisation of the chlorine (Figure 10.

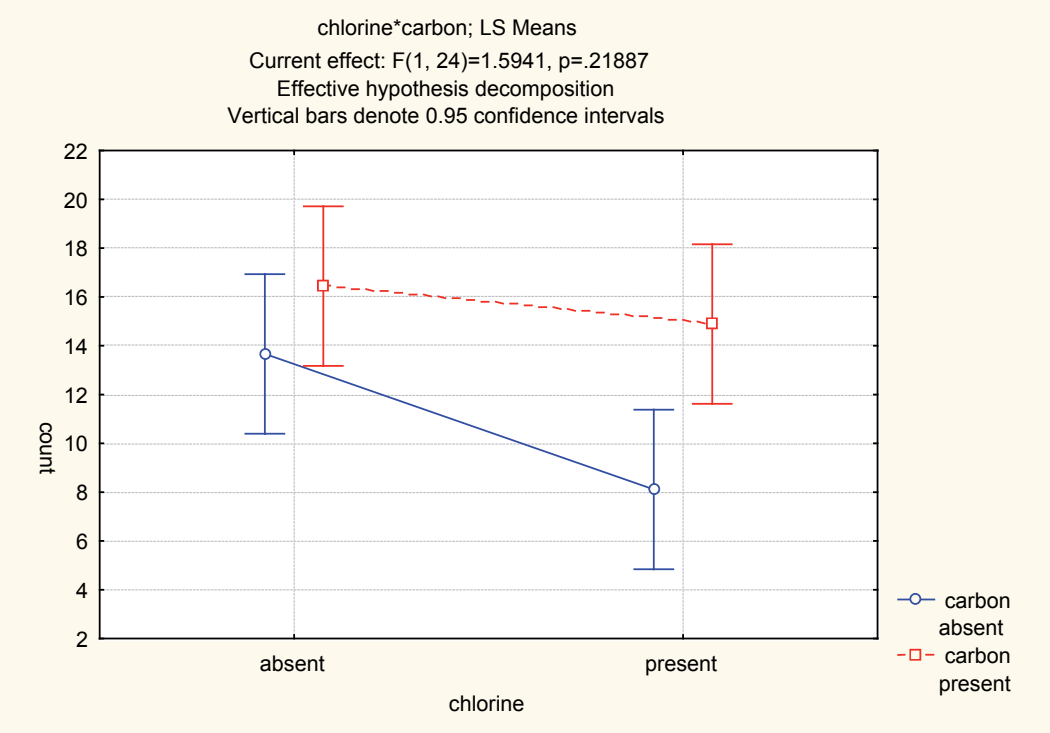

Figure 10. First order interaction for CHLORINE*CARBON.

Discussion – Small-scale trial

The results of this trial confirm those of Batch 1 in that the treatments that did not utilise chlorinated water performed significantly better than those that did. The detrimental effect on larval survival of the chlorination/neutralisation of culture water appears to have been reduced by further treating the water with activated carbon. This suggests that the chlorination process resulted in toxic substances developing in the water that were able to be removed by activated carbon.

There was no significant difference between the survivals produced by the different lengths of time that the water was settled or between the settlement and foam fractionation. These results would indicate that the most practical method of culture water pre treatment on a larger scale would be to settle the water for at least three days. Foam fractionation is not necessary but may be beneficial if water has particularly high organic loads.

Large-scale trial results

By Day 14, all of the tanks had a very low survival rate to megalops from the initial 10 000 zoea 1 stocked in each tank. Within all three treatments there was a wide variation in per cent survival. This has been a common outcome when culturing mud crab larvae in larger vessels.

Table 4. Table of results.

Treatment	Replicate	% survival to megalops	Pre-metamorphic survival in %	Comments MDS = moribund or died during metamorphosis Z5 = live zoea 5 at time of count
A (Mc Roberts)	1	5.2	7.6	Z5 =51, MDS = 292
A (Mc Roberts)	2	0.18	10.4	Z5 =28, MDS = 1013
B Batch	1	0	0	0
B Batch	2	2.1	3.0	Z5 =6, MDS = 85
B Batch	3	0.03	0.16	Z5 =0, MDS = 13
C Recirc	1	0.97	2.0	Z5 =4, MDS = 94
C Recirc	2	0.27	5.0	Z5 =65, MDS = 408
C Recirc	3	0.37	5.8	Z5 =21, MDS = 524

Discussion – Large-scale trial

The use of sand filtered seawater that has not been otherwise treated did not produce acceptable larval survivals at the DAC.

The use of chlorination was shown to not be as successful a method of water treatment as settlement and foam fractionation (See small-scale trial). However it is apparent that for large-scale larval rearing the pre-treatment of culture water by any of the methods used so far does not produce acceptable survival. The level of survival recorded in the small-scale trial was not repeated in the larger tanks, even when the same water pre-treatments were used. This would indicate that the poor survivals were not due to factors that could be overcome by pre-treatment of water using these methods.

Effects of altering tank water dynamics on survival of mud crab (*Scylla serrata*) larvae. (DAC–Batch 3)

Introduction

Previous trials showed the potential of the Aquaclone to modify tank water currents thereby maintaining larvae in the water column. Although this new technology appeared to aid in keeping crab larvae off the floor of the tank, initially it did not result in improved larval survival in large-scale trials. Previous poor results may have been due to damage of the larvae resulting from contact with the centrally mounted screens in the tanks.

The following larval rearing run (December 2001) was conducted in order to assess the efficacy of the AQUACLONES in keeping larvae in suspension when a central screen was not located in the upwelling current.

Methods

Two McRoberts tanks were used (see SOP DAC). A semi-recirculation system of water management was used whereby culture water was continually recalculated through the biofilter in an attempt to maintain a stable bacterial community, and every day an 80 per cent drain down, top up water change was carried out.

Water was transferred from one side to the other clean side once a day, before water exchange. The liner of the previously used side was cleaned using a soft broom and kitchen detergent and then thoroughly rinsed with fresh water, then allowed to dry prior to use the next day.

During this larval run the AQUACLONE was employed (Figure 11).

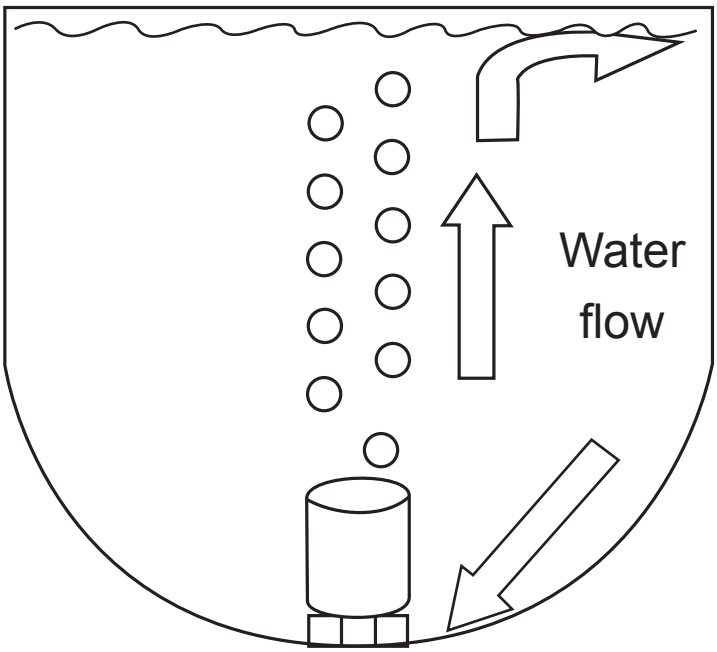

Figure 11. Aeration-powered up-weller ("Aquaclone") as used in the McRobert tanks.

Water was air lifted to biofilter (100 L tub filled with bioballs confined in a clear plastic tube to maximise contact between water and substrate), and was then gravity fed back to the tank. On return to the tank, water was first passed through activated carbon. A 500μm screen,

located midway between the wall of the tank and the centre, was used to retain larvae in the tank and prevented them from passing into the biofilter. Rotifers, *Artemia* and algae were however able to pass through the screen and circulate through biofilter. Each day for two hours, after the water exchange, 74*µ*m screens were used to remove “old” feed on return to tank.

Exchange water was settled in 20 tonne storage under black plastic to reduce light and to minimise salinity changes due to evaporation. It was also foam fractionated for at least four days prior to first use. Culture tanks were drained down daily (by 80 per cent) through the 500um screen and refilled.

Larvae were stocked at 30 L^{-1} in both tanks and were fed rotifers (*Synchaeta* sp) at a rate of 10 rotifers mL^{-1} in a single feed from stocking to day 2 of zoea 3 (Z3/2). *Artemia* (INVE 430 AF) were fed from Z2/2 to megalops at a rate of 0.5 *Artemia* mL^{-1} in a single feed. Microalgae (Live *Nannochloropsis oculata* @ 1.25 x 104 cells mL^{-1}; Live *Tetraselmis suecica* @ 3L (of approx 1.25 x 106 cells mL^{-1})/tank/day) were also added each day. Tahitian Isochrysis and *Thalassiosira weisfloggii* pastes (instant algae Reed Mariculture Pty. Ltd.) were added each day at a rate of 10 mL $tank^{-1}$ day^{-1}. Artificial feeds in the form of SP+ (INVE freeze dried Spirulina) and CD 2 ultra larval shrimp food (INVE) were also added to each tank at after drain down at 0.5g $tank^{-1}day^{-1}$.

Immersion heaters were placed in the biofilters to stabilise water temperature. Salinity started at 30ppt at hatching and then was decreased gradually to 25ppm by the end of the fifth Zoeal stage.

Results

The up-wellers maintained larvae in the water column and zoea moulted to stage 2 and 3 on time. Larvae appeared vigorous during the first four days; however after day 4 the larvae appeared weaker and seemed to spend more time on the floor of the tank. Water in both tanks appeared cloudy on the afternoon of day 6. The first significant drop-out subsequently appeared on day 7. Both tanks suffered massive mortalities and were terminated on day 9. There was no occurrence of moult death syndrome (MDS), in either tank. High numbers of *Vibrio harveyi* and *Photobacterium sp.* were isolated from both larvae and water samples.

Discussion

The AQUACLONE seemed to perform adequately in terms of keeping the larvae up in the water column, however it seemed to have no effect on the consistent problem of bacterial infection and subsequent mortality.

Effects of culture water management on survival of mud crab (*Scylla serrata*) larvae. (DAC–Batch 4)

Introduction

Previous failures in mass cultures of mud crab larvae have been attributed to bacterial infections. An attempt was therefore made to deliver the highest quality water our centre is capable of producing. Raw seawater was filtered through rapid flow sand filters to approximately 5μm. It was then slowly pumped through a secondary bank of sand filters before being passed through a 1μm filter bag. From here the water was passed through a UV light unit and was then foam fractionated and settled for approximately 4–10 days before use. In addition water was also passed through activated carbon as part of the re-circulating system.

The aim of the above filtration was to establish a stable non-pathogenic bacterial flora so that opportunistic, pathogenic bacteria are unable to establish. Furthermore, a seeding program was put forward as a means of further preventing the establishment of pathogenic bacteria. Benign bacteria could be introduced to the system to occupy the niche of potential pathogens. Thus far no specific probiotics have been developed for mud crab larval rearing in Australia, so a broad spectrum of bacteria was suggested as a source.

Biofilters are a known bacteria-rich environment and although pathogenic bacteria may also reside amongst the microbial flora, the risk of introducing these pathogens may be minimised by using biofilter material from a re-circulating system where the host has not been present. In our situation live bacteria from a finfish system was suggested as a source of non-pathogenic bacteria for our mud crab larval rearing system.

The following larval rearing run was conducted to assess the influence of incorporating established bacterial flora inn the larval rearing system.

Methods

Large-scale trial

Broodstock and hatching

The broodstock female, whose progeny were used in these experiments, was collected from the wild (Elizabeth River, Darwin Harbour).

After spawning and being held for eight days in the incubator system she was then transferred to a 1000 L hatching tank that was filled with 1μm filtered water, which has also passed through a UV light unit. Immediately prior to flowing into the hatching tank, the water was passed through bioballs that had been taken from a mature finfish (barramundi) re-circulating system and then a second 1μm filter bag. The water was supplied at approximately 3L minute^{-1}.

The following morning, the strongly phototactic and schooling first stage zoea (Z1) were concentrated with a torch light and collected.

Larval rearing

Two 1000 L McRoberts tanks were used. Water used to fill the tanks was taken from a 20 tonne storage that had previously been filled with 1μm filtered seawater. This water had also

been through a UV light unit and was settled and fractionated for 10 days. It was stored under black plastic to reduce light and to minimise salinity changes due to evaporation.

A semi-closed recirculation system of water management was used in the rearing tanks whereby culture water was continually recirculated through the biofilter. The biofilter was filled with the same bioballs that were used on the hatching tank. Each morning water was transferred from one side of the Mc Roberts tanks to the other clean side, before water exchange. The liner of the previously used side was cleaned using a soft broom and kitchen detergent and then thoroughly rinsed with fresh water, then allowed to dry prior to use the next day. Every day at approximately 0830h a 50 per cent drain down occurred, at which time the remaining water was foam fractionated for up to one hour. The return water from the fractionator was passed through a 1*µ*m filter bag in order to remove uneaten live food. New water from the storage was then flowed through for approximately 1hr and the rearing tanks were then refilled. From day 4 onwards, at 2400h a 100 per cent flow through water exchange was carried out using a timer controlled pump. At this time the water was also fractionated for approximately 1hr and uneaten food and microalgae was again removed from the tank with filter bags. At 0200h new *Artemia* and microalgae was added by means of a timer and pump.

A 500*µ*m screen located midway between the wall of the tank and the centre was used to retain larvae in the tank and prevented them from going through the biofilter. Rotifers, *Artemia* and algae were however able to pass through the screen and circulate through the biofilter. During this larval run the AQUACLONE was again employed.

Larvae were stocked at 30 per L in both tanks and were fed rotifers (*Synchaeta* sp) at a rate of 10 rotifers/mL in a single feed from stocking to day 2 of zoea 2 (Z2/2). Rotifers were boosted for two hours in a mixture of live *Nannochloropsis oculata*, live *Tetraselmis suecica*, Isochrysis paste (Reed Mariculture Pty Ltd.) and *Thalassiosira weisfloggii* paste (Reed Mariculture Pty Ltd.). First instar *Artemia* (INVE 430 AF) were fed from Z2/2 to megalops at a rate of 0.8 *Artemia* /mL split between the two feeding events at ~1030h and 0200h. Microalgae (live *Nannochloropsis oculata* @ 2.5 x 104 cells/mL; live *Tetraselmis suecica* @ 3L/tank/day) was also added each day. Tahitian Isochrysis and *Thalassiosira weisfloggii* pastes (instant algae Reed Mariculture Pty. Ltd.) were added each day at a rate of 10 mLs /tank/day split between the two feeding events. Artificial feeds in the form of 0.25g SP+ (INVE freeze dried Spirulina) and 0.25g CD 2 ultra larval shrimp food (INVE) were also added to each tank at drain down.

300-watt immersion heaters were placed in the biofilters in an attempt to keep water temperature stable. Salinity started at 30ppt at hatching and then was decreased gradually to 25ppm by the end of the fifth Zoeal stage.

Results and discussion

This trial was undertaken during the NT monsoon season and electrical storms shorted out several of the heaters and pumps on more than one occasion. This resulted in poor water quality conditions due to the non-occurrence of the 0200h foam fractionation and water exchange.

On day 6 it was noted that the overnight water exchange did not occur and the foam fractionator was run for one hour before anything else occurred. Larvae seemed to be active and vigorous. On the morning of the seventh day it was again noted that the overnight water

exchange did not occur and tank temperatures dropped to 28.5ºC. Numerous dead larvae were seen in the water column, and approximately 6000 were siphoned from each tank. The culture water appeared cloudy.

Mortalities steadily continued through to day 9 and both tanks were terminated. High bacterial loads were confirmed through bacterial plating. Larvae looked good up until equipment failures and poor water quality and high organic loads were considered likely factors in bacterial induced mortality.

Large-scale trial:

Comparison of mud crab larviculture in parabolic and circular, flat bottomed 5000 L tanks. (BIARC–Batch 1)

Introduction

A large-scale trial in commercial scale hatchery tanks was conducted to test the recent advances in mud crab larval culture knowledge, particularly relating to feed type and source, nutrition, water treatment and culture management protocols. Two standard tank types were used, the common flat bottomed circular tank and a parabolic tank that is typically used in prawn hatcheries in Australia. The parabolic tank is most efficient in maintaining particulate material and larvae in suspension in the water column, however it can promote very turbulent agitation of the culture.

Materials and methods

This production trial commenced on 4 November 2000. Larvae were produced and stocked into the culture tanks following BIARC SOP. Culture maintenance and monitoring followed the BIARC standard operating procedure. OTC was added to the cultures at 25ppm every second day.

Results

On day two the beginnings of a mucous mat were observed developing in patches on the sides and bottom of the parabolic tank but not in the flat- bottomed tank. The mucous mat in the parabolic further developed to cover a large part of the walls and on day 5 very few larvae remained and the culture was terminated. Between days 5 and 7 similar patches of mucous mat were observed on the bottom of the flat-bottomed tank. These disappeared by day 8 at which time larval survival was down to ~20 per cent. Larval survival showed a gradual decline from day 7 to day 16 when the culture was terminated. At this time there was a very small number of swimming megalops.

Discussion

This trial saw the return of the “mucous mat” syndrome that had occurred in the previous mass culture trial. This phenomenon has been observed at BIARC in previous years but has remained sporadic. Mass larval mortality typically occurs when the mat is observed. This trial provides further evidence that the development of a mucous mat in the culture is not affected by OTC. The mucous mat followed the typical pattern of disappearing three to four days after it was first observed. The larvae however continued to exhibit a continued decline in numbers which conforms to the typical mortality pattern reported by other hatcheries. The cause of this is generally attributed to more chronic bacterial influences. It has been observed in the BIARC hatchery and elsewhere that the application of an antibiotic does not necessarily stop all negative bacterial influences. Bacteriological plates revealed that when OTC at 25 to 50ppm was added to larval cultures the bacterial community appears very similar to untreated cultures.

Large-scale trial:

Comparison of the effect of settled versus chlorinated seawater treatment, with and without oxytetracycline treatment. (BIARC–Batch 2)

Introduction

It had been shown at BIARC and other research hatcheries that treatment of raw seawater prior to use in mud crab larviculture is critical to achieving successful outcomes. The type of treatment and its extent is may need to be varied with hatchery location as each site will have varying water quality issues. Experiments at BIARC determined that UV irradiation, chlorination/de-chlorination or maturation by settlement for more than nine days could all greatly improve the survival of mud crab larvae. Seawater maturation (settled seawater) and chlorination however promoted the highest larval survival, with matured water giving slightly better growth. Production of sufficient volumes of matured water can be difficult for some hatcheries because of the large volume of sea water required to be held in storage. On the other hand other hatcheries have reported reduced larval survival when chlorinated seawater is used, contrary to our previous results.

The BIARC hatchery had recently acquired eight new 1.3 tonne experimental larval rearing tanks. This experiment was the first opportunity to test them with mud crab larvae. The ultimate aim was to have all eight tanks set up as per the configuration developed at BIARC in the ACIAR project (FIS /1992/017), which incorporated the use of a smaller side tank and airlift generated water flow that circulated culture water between the culture tank and the side tank. At the time of conducting this experiment only two of the 1.3 tonne culture tanks had been set up this way providing a further opportunity to compare the circulating system with the basic static system.

The primary design of this experiment was to compare the survival and growth of larvae using settled or chlorinated seawater in mass culture and to apply OTC to discriminate between bacterial influences and chemical influences derived from chlorination.

Materials and methods

This production trial commenced on 1 January 2001 and was carried out in 8 x 1.3 tonne cylindro-conical fibre-glass tanks with a 10° angle on the base. Larvae were produced and stocked into the culture tanks following BIARC standard operating procedure. OTC was added to the appropriate treatments at 25ppm every day.

A basic 2 x 2 factorial was applied with system configuration added in an unbalanced design according to Table 5 of treatments below.

Table 5. Culture Water Treatment regimes (BIARC Batch–2)

Water	OTC	System
treatment		Design
Chlorinated	–	Circulating
Settled	–	Circulating
Chlorinated	–	Static
Settled	–	Static
Chlorinated	OTC	Static
Settled	OTC	Static
Chlorinated	OTC	Static
Settled	OTC	Static

Results

Survival results are shown in Figure 12.

In all the static larviculture systems, including with and without OTC, significant larval mortality commenced on day 2. At this time small patches of “mucous mat” occurred on the bottom of most tanks. This “mucous mat” did not develop further beyond a small number of patches, however larval survival rate of the static tanks declined at a rapid rate up to day 10 when the cultures were terminated. The two circulating tanks had excellent survival to day 10. On day 17, a 5 to 10 per cent incidence of moult death syndrome (MDS) of Z5 larvae occurred as megalops began to appear. MDS continued until day 20 when the tanks were harvested. The remaining tanks produced 2200 and 1000 vigorous megalops of normal morphology. This represented production rates of 1.7 and 0.75 megalops per litre.

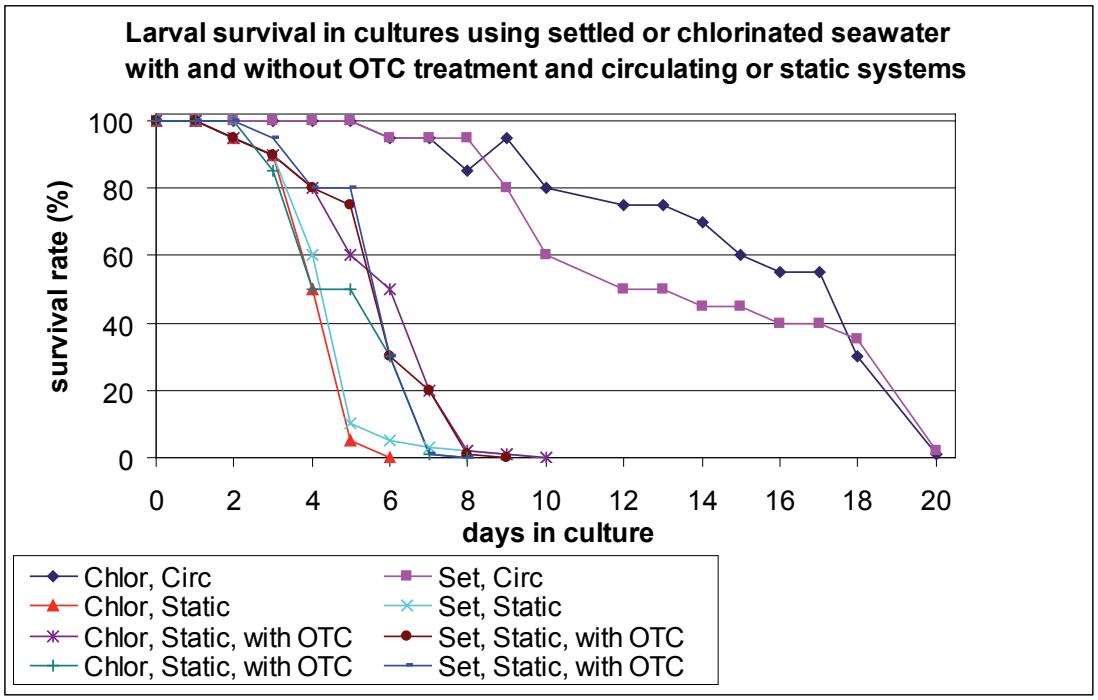

Figure 12. Larval survival (BIARC Batch–2).

Discussion

The difference between the system type, the static and circulating design, was dramatic but because of the un-replicated circulating system treatments no significant conclusions could be drawn from this trial. However, the results supported previous work, which although not as clearly defined, indicated that under conditions at BIARC the circulating systems performed better, warranting their use.

During this trial there was a small amount of “mucous mat” development in the first three days. On past experience this did not appear to fully explain the very high mortality of larvae in the static cultures. However the mortality was independent of the daily addition of OTC at 25ppm and was rapid within the first week of culture. These are both characteristics of the “mucous mat” syndrome experienced in previous large-scale trials at BIARC. Regardless of the aetiology it is interesting that the two culture systems performed so differently. In the past the circulating system has not completely inhibited the development of the “mucous mat” syndrome or other microbiological problems so effectively. It is also interesting that the circulated tanks maintained commercially acceptable survival and growth rates to the onset of megalops stage without the bacterial protection of OTC. This is further evidence that the typical high level of larval mortality observed, generally attributed to bacterial influences, is not a consistent phenomenon in large-scale cultures and sporadically larvae can do relatively well without antibiotic treatment. At this stage there is no indication as to the reason for variability in culture performance. Bacterial plating has not determined community structure patterns that correlate with culture performance.

In this experiment there was no difference between settled or chlorinated seawater. The survival and growth of larvae within the two static and circulated groups followed the same pattern regardless of initial water treatment method. This finding was consistent with previous work at BIARC.

Feeds and feeding regimes

Introduction

In an attempt to optimise larval survival (much research has focused on the larval feeding regimes for various mud crab species (DuPlessis, 1971; Heasman and Fielder, 1983; Jamari, 1992; Marichamy and Rajapakiam, 1992; Mann *et al*, 1999, Baylon and Failaman, 2001).

In Australia only one species *Scylla serrata* is currently under investigation. For this species, stocking densities of approximately 10 larvae mL^{-1} are commonly used and live food, including microalgae, is introduced at the time of stocking (Williams *et al*, 1999). Rotifers (*Synchaeta sp.* and *Brachionus sp.*) are fed throughout the Zoeal stages at approximately 10 mL^{-1} in conjunction with freshly hatched *Artemia nauplii* from the second day of the second zoeal stage (Z2/2). Researchers in Asian countries often utilise much higher larval stocking densities and incorporate much higher live food densities. Baylon and Failaman (2001) stocked larvae at up to 100 L^{-1} in a greenwater system where rotifers were included at 30 mL^{-1}, and *Artemia* were added at 0.5 to 5 mL^{-1} as the larvae grew from zoea 1 to zoea 5. Djunaidah *et al*. (2001) also used a greenwater rearing system with high larval densities of up to 100 L^{-1} and up to 60 rotifers mL^{-1}. Baylon *et al* (2001) also suggested a combined feeding regime of rotifers and *Artemia* to zoea stage 4 and then feeding of *Artemia* only from Zoea 5 on.

Nghia *et al* (2001) reviewed their larval rearing practises in Vietnam and have described what they considered to be their best larval rearing protocols. They have recommended the use of microalgae at cell densities of 0.1–3 million cells mL^{-1} along with (ICES) enriched rotifers at a density of 45 mL^{-1} for the first six days and an *Artemia* density of 20 mL^{-1} from day 4 onwards. Recently the nutritional value of *Artemia* as a live feed for mud crab larvae has also been investigated by Kobayashi *et al*. (2000) who suggested that the fatty acids eicosapentaenoic acid (EPA) and docosahexanenoic acid (DHA) should be present in appropriate levels and ratios in the *Artemia* to promote survival and growth. *Artemia* need to be enriched with HUFA's prior to being used as feed to achieve the desired levels.

While there seems to be a general agreement between research institutes in feeding rotifers initially and introducing *Artemia* as the larvae develop (Heasman and Fielder, 1983; Mann *et al*, 1999; Williams *et al*, 1999; Baylon and Failaman, 2001; Baylon *et al*., 2001; Djunaidah *et al*., 2001; Nghia *et al*., 2001) no agreement has yet been reached on the appropriate protocol and most institutions still use their own larval feeding regimes.

When the larvae are stocked at 10 individuals L^{-1} acceptable survivals have been achieved when rotifers were fed at 10/mL for the first Zoeal stage, which is followed by a switch to *Artemia* at the second Zoeal stage. Good survivals have been obtained using INVE AF grade (430*µ*m) Artemia. However, in many mass rearing experiments high levels of MDS are experienced. This syndrome has been attributed to insufficient dietary phospholipids and to poor health and vigour generally.

Nannochloropsis oculata, *Chaetoceros muelleri* and an Isochrysis-like prymnesiophyte titled *PS11* have routinely been used as live algae species for mud crab larval rearing at the DAC. These species complement each other in terms of essential fatty acid content, especially eicosapanetanoic acid (EPA) and docosohexanoic acid (DHA). *N. oculata* has very high levels of EPA (~37% of lipids) and no DHA, while *PS11* has relatively low levels of EPA (~2.5%) and high levels of DHA (~10%). C. muelleri is a diatom with higher levels of EPA (~15%) than of DHA (~4%). Another genus of microalgae that is often used in aquaculture hatcheries is *Tetraselmis*. This genus of algae produces only EPA (~9.5%).

Microalgae are expensive to produce owing to the necessity of axenic laboratory facilities. Master cultures are obtained from a reference collection, which are grown and scaled up to working cultures. Staff time and floor spaces are two of the main resources that are used in the production of microalgae

Observation of larvae feeding on de-capsulated *Artemia cysts* shed light on how early crab larvae actually take in some food items. The larvae will usually hold the abdomen curved under the cephalothorax while swimming. However when the *Artemia cysts* were offered and detected, the larvae lashed out with the abdomen to a hyper-extended position, sometimes scraping the dorsal spine with the uropods and telson. The uropods and telson would then be brought to the mouthparts. If the larvae had impaled a cyst with the spinous uropods and telson, it would then maintain the position and pierce the cyst with the mouthparts. Orange coloration could then be seen passing from the cyst to the gut of the larvae.

Currently the first live food organism, the rotifer is often enriched in commercial enrichment products prior to feeding e.g. INVE DC DHA Selco™ or DHA fortified *Chlorella*. Once in the larval rearing tanks, the rotifers maintain some of their nutritional value by feeding on the microalgae if this is supplied to the larval rearing system. If the use of microalgae can be restricted to the production and enrichment of rotifers then considerable savings can be made in not having to purchase or grow the algae to supply the culture tanks. After the rotifer stage,

the mud crab larvae are fed first instar AF 430 *Artemia nauplii* for two days prior to being fed artificially enriched) second instar GSL AAA *nauplii*.

The reason for the switch to different *Artemia* is primarily to do with cost, size and nutritional quality. The GSL *Artemia* are substantially larger, cheaper but contain lower levels of important nutrients than do the AF 430 *nauplii*. At Zoea stage 2 the crab larvae are only just able to feed successfully on the smaller first instar *nauplii*, so it is important that these have a higher nutritional quality without having to be enriched. As the larvae grow to Z3, they are able to successfully feed on a larger prey item, such as a second instar *nauplii*. The second instar *nauplii* is a feeding stage so artificial enrichment and improvements in nutritional quality of poorer and less expensive *nauplii* is possible. Once the larvae are being fed high quality or enriched Artemia, the supply of micro algae may be superfluous.

Even though rotifers do support larval development in the early stages they can be a source of pathogenic bacteria as they are often grown at relatively high densities and have high feed rate requirements. The high level of nutrients and particulates in rotifer cultures combined with the small size of screening required makes flushing of the cultures to minimise bacterial loads an inefficient process. In Vietnam “umbrella” stage *Artemia* fed to early stage larvae has been used with some success (Truong Trong Nghia *et al*, 2001).

Experiments to Investigate the Effects of Feeds and Feeding Regimes on Larval Rearing Success.

Preliminary optimisation of feeding regimes. (DAC–Batch 5)

Introduction

In a previous experiment (Batch 2) survival of mud crab larvae was compared using different water treatment methods, and different tank background colours. The best treatments from that small-scale experiment consisting of, at least three day settled, foam fractionated, and carbon filtered water, in a container with a black background, attained survival to megalops in excess of 70 per cent. These treatments were then adopted in this trial to compare the survival of mud crab larvae to megalops when exposed to different feeding regimes.

As noted in the introduction to this section there seems to be a wide consensus between research institutes in feeding rotifers initially, and introducing *Artemia nauplii* and sub-adults as the larvae grow. However no consensus has been reached on enrichment protocols, larval food densities, or the stages where the different species of live food are most beneficial or needed.

The following experiment was designed to assess at which stage, if at all, rotifers are required, the timing of *Artemia* introduction, and whether or not enrichment of live food is beneficial.

Methods

The standard operating procedures (DAC) were used for this small-scale trial, unless otherwise specified.

Five experimental treatments were imposed. Treatments differed in relation to the zooplankton feeding regimes and are summarised in Table 6 below. There were five replicates of each treatment arranged in a completely randomised design

Table 6. Feeding regime treatments for the small-scale experiment (DAC Batch–5).

Treat. No.	Treatment name	Rotifer feeding	*Artemia* feeding
1	*Artemia* only	Nil	Z1/1–megalops
2	Rotifers + *Artemia*	Z1/1–megalops	Z1/1–megalops
3	Rotifers from Z1/1 to Z3/2 + Artemia	Z1/1–Z3/2	Z1/1–megalops
4	Rotifers + *Artemia* from Z2/2	Z1/1–megalops	Z2/2–megalops
5	Enriched live food + artificial diets*	Z1/1–megalops enriched with INVE Dry Selco	Z2/2–megalops enriched with Frippak CD 2 Ultra larval shrimp food

*Spirulina (SP+™ INVE) and Frippak CD2 Ultra™ larval shrimp food (INVE) each to a final concentration of 1ppm.

Rotifers were produced as per standard operating procedure. Enriched rotifers for use in treatment five were held in a 20 L bucket containing 5g of Dry Selco™ (INVE) for between 60 and 120 minutes. In all the treatments the rotifers were fed at 10 mL^{-1}.

Artemia (INVE, AF grade: 430*μ*m) used throughout this experiment were newly hatched first instar *nauplii*, except for treatment 5. In this case, instar I *nauplii* were fed to the zoea from day 4 to day 9 and 24 hour on-grown *nauplii* were fed from day 10 onwards. These were enriched overnight in a 15L bucket containing 1g of Frippak CD2 Ultra larval shrimp food. *Artemia* were fed to all treatments at a rate of 0.5 mL^{-1} in a single feed. Treatment 5 also received artificial food as detailed in Table 6.

Statistical Analysis

Daily survival was expressed as the percentage of zoea alive at the time of counting. This data was analysed by analysis of variance, and where significant differences were observed, they were separated using Fisher's pair-wise least significant difference tests. Residuals were examined to determine a requirement for data transformation.

Results

At 0800 hours each day during the trial the temperature of water in the bowls varied between 20.8 and 27.4°C, averaging 22.4°C throughout the experiment. Ammonia-nitrogen ranged between < 0.05–0.3 mg L^{-1}.

Table 7 shows the daily survival data (±SE) for the five treatments in this experiment. At day two there was a highly significant difference ($P < 0.001$) in survival between treatments, with treatments 4 and 5 which were receiving rotifers only, at that point, having higher survival than those treatments receiving rotifers in combination with *Artemia* (treatments 2 and 3), or *Artemia* only (treatment 1). The relatively cold water temperatures experienced in this trial increased the average duration of the larval period to megalops to approximately 25 days. Crab larvae began moulting to megalops from day 18 onwards. These megalops were removed from the bowls to prevent cannibalism but were included in subsequent daily counts as they were considered to be survivors.

As well as having the poorest survival rates, treatment 1 also took longer to reach each larval stage. Megalops first appeared in most treatments on day 18, and on day 19 all treatments had megalops except the *Artemia* only treatment. Megalops did not appear in this treatment until day 21.

In treatments 2, 4 and 5, which were still receiving rotifers after day 10, there was a relatively small non-significant decrease in survival due to moult death syndrome (MDS) at the moult from Z5 to megalops. This condition was confirmed under a dissecting microscope by the appearance of the larvae dying while extricating themselves from old exoskeletons. Treatments 1 and 3, receiving only *Artemia* at this point, had seemingly lower levels of MDS.

Table 7. Daily survival of mud crab larvae to megalops stage under different feeding regimes (DAC Batch–5).

Day	Treatment 1 *Artemia* only	Treatment 2 Rotifers + Artemia	Treatment 3 Rotifers from Z1/1 to Z3/2 + Artemia	Treatment 4 Rotifers + *Artemia* from Z2/2	Treatment 5 Enriched live food + artificials*
1	96.67 ± 1.49	100.00	96.67 ± 3.33	100.00	96.33 ± 0.67
2	76.00 ± 3.56	87.33 ± 1.94	84.67 ± 2.91	98.67 ± 0.82	96.00 ± 1.94
3	62.00 ± 5.64	83.33 ± 2.79	84.67 ± 2.91	98.00 ± 0.82	91.33 ± 2.26
4	59.33 ± 5.10	81.33 ± 3.43	82.00 ± 3.09	98.00 ± 0.82	90.67 ± 1.94
5	48.67 ± 10.78	81.33 ± 3.43	82.00 ± 3.09	94.02 ± 1.26	89.33 ± 1.63
6	45.33 ± 11.48	78.67 ± 3.74	80.67 ± 3.56	91.35 ± 2.27	88.67 ± 1.70
7	40.67 ± 10.35	71.33 ± 3.89	78.00 ± 3.09	88.04 ± 2.73	86.67 ± 2.36
8	40.00 ± 4.55	68.00 ± 4.55	75.33 ± 2.71	84.04 ± 2.70	86.00 ± 2.45
9	38.00 ± 10.09	65.33 ± 4.55	73.33 ± 2.36	80.73 ± 2.72	82.00 ± 4.42
10	38.00 ± 10.09	63.33 ± 5.27	71.33 ± 2.26	79.40 ± 3.29	80.00 ± 3.80
11	36.67 ± 9.60	61.33 ± 6.72	68.00 ± 2.49	78.73 ± 3.64	76.67 ± 3.65
12	36.00 ± 9.15	60.00 ± 7.38	64.00 ± 4.64	76.13 ± 2.91	76.67 ± 3.65
13	35.33 ± 9.17	59.33 ± 7.92	63.33 ± 5.27	75.46 ± 3.14	76.00 ± 3.40
14	35.33 ± 9.17	58.67 ± 8.47	58.67 ± 9.81	75.46 ± 3.14	75.33 ± 3.43
15	35.33 ± 9.17	58.00 ± 8.47	56.00 ± 11.66	75.46 ± 3.14	74.67 ± 3.43
16	34.00 ± 8.33	54.67 ± 8.00	54.67 ± 11.33	70.84 ± 3.44	69.33 ± 5.31
17	33.33 ± 7.96	52.67 ± 8.06	53.33 ± 11.88	70.17 ± 3.69	69.33 ± 5.31
18	32.00 ± 7.35	52.00 ± 7.86	50.67 ± 11.52	67.57 ± 3.50	69.33 ± 5.31
19	29.33 ± 6.53	50.00 ± 7.07	50.67 ± 11.52	66.90 ± 3.76	69.33 ± 5.31
20	27.33 ± 6.09	43.33 ± 6.41	50.67 ± 11.52	64.26 ± 4.83	69.33 ± 5.31
21	27.33 ± 6.27	37.33 ± 6.09	48.00 ± 10.57	54.28 ± 8.55	61.33 ± 5.44
22	26.67 ± 6.15	36.00 ± 5.31	46.00 ± 10.13	54.28 ± 8.55	60.00 ± 6.75
23	26.67 ± 6.15	36.00 ± 5.31	44.67 ± 9.98	52.95 ± 8.63	59.33 ± 7.41
24	26.67 ± 6.15	36.00 ± 5.31	44.67 ± 9.98	52.95 ± 8.63	59.33 ± 7.41
25	26.00 ± 5.91				58.67 ± 7.35
26	26.00 ± 5.91				
27	26.00 ± 5.91				
28	26.00 ± 5.91				
29	26.00 ± 5.91				
30	26.00 ± 5.91				
31	25.33 ± 5.93				

Discussion

The aim of this experiment was to evaluate, the timing of introduction, and the cessation of feeding, of the two main live food organisms, rotifers and Artemia. The results of this experiment show that using rotifers as a feed significantly and directly improves survival of mud crab larvae in early stages up to Z3, and that when rotifers are removed from the feeding regime at the Z3 stage; there was very little effect on survival and minimal MDS at the moult from Z5 to megalops. This is apparent when comparing treatment 2 against treatment 3. When rotifers were removed from the feeding regime at Z3 survival was improved by almost 10 per cent.

The *Artemia* only treatment sustained losses of greater than 50 per cent before the Z3 stage. The lack of rotifers in the early stages of this treatment also slowed the growth process. This treatment was between three and seven days behind the other treatments, in terms of moult stages. The reason for the poor performance of mud crab larvae when fed *Artemia* alone can only be speculated, but may have been the result of *Artemia* being too difficult to catch and then to ingest, or they may have been lacking certain essential nutrients such as DHA, as was lacking from *Artemia* in the experiments of Quinito *et al*. (1999). Enriching with DHA-rich algal species or with artificial enrichment media from day one may overcome this problem, as later enrichment (treatment 5) proved superior overall. Whether the Z1 larvae could catch and consume an instar II *Artemia* may also be important, increasing *Artemia* density, in the early stages at least, may overcome this problem. It is also possible, but less likely, that the presence of virulent microbes associated with the *Artemia* contributed to the increased mortality.

Survival in early stages was significantly improved when rotifers were fed in combination with the Artemia. Furthermore when rotifers only were fed, survival in the early stages was again significantly improved. The reason for the poorer survival of the larvae when fed *Artemia* in combination with the rotifers, may have been due to visual confusion of the larvae resulting in a disturbance of the feeding process, or again due to microbes associated with the Artemia.

Interestingly, the above pattern was apparently reversed in the Z5 stage. All treatments still receiving rotifers at this stage suffered apparently greater levels of MDS than those receiving *Artemia* alone (treatments 2, 4 & 5 vs. 1 & 3). MDS has been attributed to insufficient levels of phospholipids in the diet (Teshima, 1997) and while rotifers may be ingested as part of the diet at this stage they may be deficient in these nutrients, or ratios with other important nutrients may be skewed. Alternately the stress of moulting when coupled with a dietary deficiency and added microbial loads from the rotifers may have caused the increased mortalities. It is also possible that the nutritional requirements of the larvae change as they develop so that while rotifers may have been nutritionally sound initially, they may not be suitable for later stages. This may be overcome by the use of artificial enriching media, and/or artificial food, which was provided in the suspension given to treatment 5. Survival in this treatment was similar to treatment 4 up to the Z5 stage, but suffered lower levels of MDS at the moult to megalops. Treatment 5 yielded the best survival to megalops overall, but the artificial enrichment media provided in the suspension seemed to have little effect in the earlier stages and in fact rotifers enriched with *PS11* yielded better survival of mud crab larvae up to day 5.

Optimising rotifer use in mud crab larval rearing. (DAC–Batch 6)

Introduction

In a previous experiment (Batch 5) it was found that the feeding of rotifers to zoea stage 3 significantly improved survival to megalops, as compared to the feeding of *Artemia* only. It was also found that there was no benefit in feeding rotifers past this zoea 3 stage, and if only rotifers were fed (i.e. without *Artemia* co-feeding) in the early stages then survival was also significantly improved.

These results prompted interest as to the most appropriate use of rotifers in the feeding regime for this species.

An appropriate feeding protocol should at least include:

- when the rotifers should be introduced, if at all.
- when the feeding of rotifers should be suspended.
- the most appropriate rotifer density.
- the most appropriate growth and enrichment protocol for the rotifers themselves.

While all these aspects are important and warrant investigation, the following experiment was designed to only assess at which stages rotifers are required in the feeding regime for mud crab larvae.

Materials and Methods

As per Darwin Aquaculture Centre SOP in addition to those described below.

Small-scale trial:

Water used to fill the bowls was settled and fractionated for 10 days before stocking. This water was stored overnight in a 100 L plastic tub submerged in the same water bath as the bowls. In addition, two 2 L capacity submerged carbon filters powered by a single 15mm diameter airlift in each were run for approximately 18 hours prior to use. New water for each day was taken from the large storage and treated as above.

There were five replicates of six treatments arranged in a completely randomised design. Each treatment received equal cell numbers of three species of microalgae to a final density of 5 x 104 cells per mL. The algae used were *Nannochloropsis oculata*, *Chaetoceros mulleri* and an un-named Isochrysis-like species titled *PS11* (Thinh, *et al*., 1999). Treatments differed in relation to the zooplankton feeding regimes and are summarised in Table 8.

Table 8. Feeding treatments, and codes, for the small-scale larval rearing experiment (DAC Batch–6).

Treatment Name	Rotifer feeding	*Artemia* feeding
NR	Nil	Z1/1–megalops
Z2	Stocking–Z2/2	Z2/2–megalops
Z3	Stocking–Z3/2	Z2/2–megalops
Z4	Stocking–Z4/2	Z2/2–megalops
Z5	Stocking–Z5/2	Z2/2–megalops
M	Stocking–megalops	Z2/2–megalops

Rotifers were mass cultured in a combination of *N. oculata* and *PS11*. Each day rotifers were harvested by draining through a submerged 64*μ*m screen, were rinsed in 5*μ*m filtered seawater and concentrated. They were then enriched in bag cultured *PS11* and *N. oculata* for 120 minutes prior to being fed at a rate of 10 rotifers mL^{-1} in a single daily feed.

Artemia (INVE, AF grade: 430*μ*m) used throughout this experiment were newly hatched first instar *nauplii*. *Artemia* were fed to all treatments at a rate of 0.5 mL^{-1} in a single feed.

Large-scale trial:

A comparison of larval rearing systems was also carried out in four 1000 L tanks. Two McRoberts tanks and two standard conical bottom tanks were used. Each tank was set up and operated as described in the SOP.

Each day the culture water and larvae were transferred to the new liner as previously described (SOP). After transfer of the Mc Roberts tanks, all tanks were drained to approximately 20 per cent and were refilled with 1*μ*m filtered, 4-day settled and foam fractionated seawater. This water was added to the reservoir so as to first pass through the carbon filter before entering the main tank. Each tank was furnished with a single airstone and was vacuumed when it was safe to do so.

Water quality

Each day, temperature and dissolved oxygen were measured using an Oxy-guard digital oxygen meter, pH was measured using a TPS WP-90 pH meter and salinity was measured using a refractometer. Total ammonia nitrogen and nitrite nitrogen were determined using the salicylate method and by diazotisation respectively. Hach reagents and colour wheels were used to measure concentrations.

Results

Small-scale trial:

Data relating to survival and duration to megalops for the various treatments is presented in Table 9. There were significant differences in survival ($P < 0.01$) and duration to megalops ($P < 0.001$) with the treatment receiving no rotifers having poorer survival and taking longer to reach the megalops stage.

Daily survival data for the various treatments is graphically presented in Figure 13.

Table 9. Mean survival (%) and duration to megalops (days) (±SE) for the various treatments in the small-scale larval rearing experiment.

Treatment	Survival to Megalops (%)	Duration to Megalops (days)
NR	32.00 ± 7.51b	22.28 ± 0.33b
Z2	78.00 ± 5.54a	17.32 ± 0.06a
Z3	70.67 ± 3.40 a	17.37 ± 0.11a
Z4	69.33 ± 3.23 a	17.22 ± 0.01a
Z5	62.67 ± 12.45 a	17.23 ± 0.08a
M	54.00 ± 12.4 ab	17.21 ± 0.08a

Values in columns with the same superscripts are not significantly different. For treatment codes refer to Table 8.

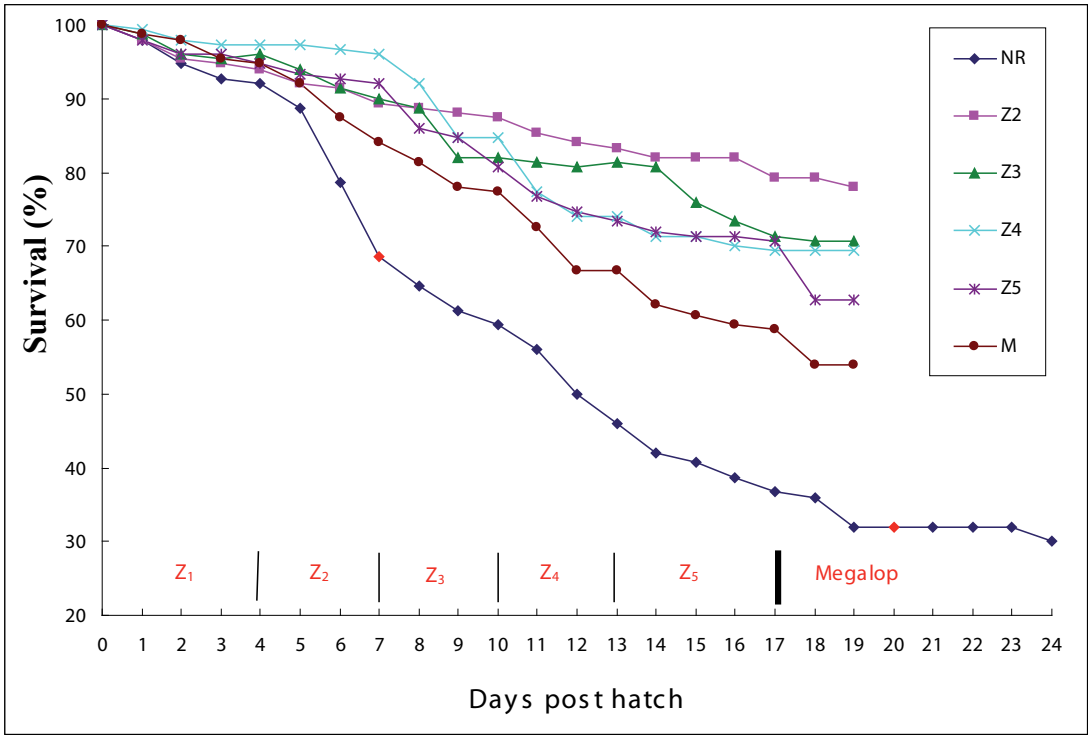

Figure 13. Daily survival for the various treatments in the small-scale larval rearing experiment. For treatment codes refer to Table 8 (DAC Batch–6).

For those treatments receiving rotifers, there was a weak yet significant ($P < 0.01$; $R2 = 0.18$) negative correlation between duration of rotifer feeding and survival to megalops (4).

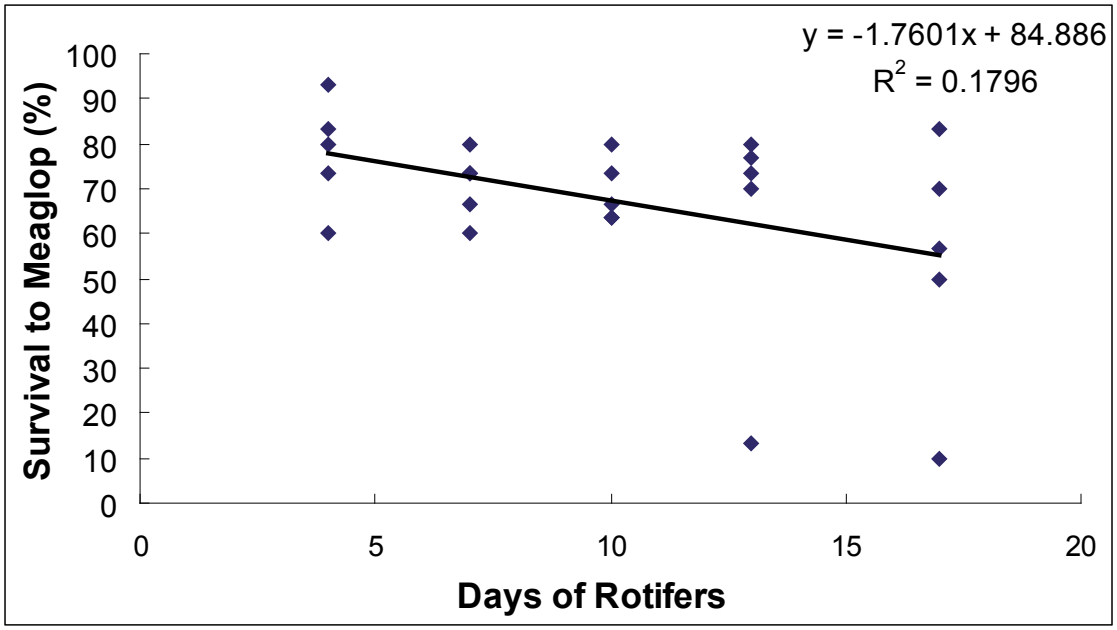

Figure 14. Regression of Survival to megalops on days of rotifer feeding for those treatments receiving rotifers.

Results

Large-scale trial:

Water Quality

Water quality remained generally conducive to mud crab larval growth and development. Temperature ranged from 26–29.2°C, dissolved oxygen was between 5.2 and 6.3 mg/l, pH was between 7.74 and 8.08. Total ammonia nitrogen reached a maximum of 0.5 mg/l in one tank on one day and no nitrite was detected in any of the tanks at any time.

Survival

All tanks seemed to have a progressive drop in numbers throughout the trial; however one of the Mc Roberts tanks suffered a major crash on day 10 and was terminated. Megalops appeared in the remaining tanks on day 12. These megalops remained in the tanks overnight and the trial was terminated on day 13. Larvae were then individually counted. The results are presented in 10

Table 10. Number of Megalops and Zoea 5 larvae and overall % survival (Z1-megalops) from each tank.

Tank	Number of Megalops	Number of Zoea 5	Total	Overall survival %
Std 1	438	61	499	4.99
Std 2	1093	35	1128	11.28
McRoberts 1	204	181	385	3.85

Discussion

Small-scale trial

The results of the small-scale trial unequivocally show that when rotifers are not fed in the first zoeal stage, moulting to the second zoeal stage is delayed, and survival is much reduced. This result is the same as for experiment Batch 5. After that experiment it was established that feeding rotifers past the Z3 stage was not necessary, and that co-feeding of *Artemia* from stocking was detrimental to survival. In the present work it was shown that there was no advantage in continuing the feeding of rotifers past the Z1 stage, and in fact there was a trend of decreasing survival, the longer the rotifers were part of the feeding regime. The significant regression of survival to megalops on duration of rotifer feeding provides further evidence that rotifers should not be fed after the first zoeal stage. It may be possible to feed rotifers for the first few days and then switch to *Artemia* while the larvae are still at the Z1 stage although this was not tested and further experimentation is required.

Other factors to consider are the density of rotifers fed, and the relationship to larval density, enrichment of rotifers prior to feeding, and whether co-feeding with *Artemia* is necessary or beneficial. In this experiment there was a sharp change over from feeding rotifers to feeding *Artemia* with no apparent negative effect, although it was not expressly tested for.

Discussion

Large-scale trial

Larvae spent a substantial amount of time on the floor of the tanks. This was especially evident immediately after water changes, and was termed a “shocking” behaviour. Large numbers of larvae often grouped together on the floor of the tanks and this behaviour seemed to be worse on moult days. This was perceived as a negative occurrence, as hard-shelled animals were in close contact with soft-shelled, newly moulted, animals and there is potential for physical damage to the larvae from being impaled on the terminal abdominal spinous processes on the hard shelled larvae.

There seemed to be a steady decline in survival from stocking although there was a crash in one of the Mc Roberts tanks on day 10. There was also moult death syndrome in some tanks. As noted previously this problem has been linked to a nutritional deficiency/ imbalance.

The problem of larvae lying on the floor of the tanks may be overcome by having a more spherical or conical bottom with a centralised aeration device to induce mixing currents. This may keep larvae in the water column especially when moulting.

Small-scale trial:

Effects of microalgae on growth and survival of mud crab larvae. (DAC–Batch 7)

Introduction

Previous experiments have focussed on the effects of zooplankton live food organisms on survival of Mud Crab (*Scylla serrata*) larvae. We now have a reliable zooplankton feeding protocol for the rearing of larvae of this species.

When the larvae are stocked at 10 individuals per litre, rotifers should be fed at 10/mL for the first Zoeal stage, which is followed by a switch to *Artemia* at the second Zoeal stage. Good survivals have been obtained using INVE AF grade (430*μ*m) Artemia. However, in the previous mass rearing experiment high levels of MDS were experienced. This syndrome has been attributed to insufficient dietary phospholipids and to poor health and vigour generally.

Nannochloropsis oculata, *Chaetoceros muelleri* and an Isochrysis-like prymnesiophyte titled *PS11* have routinely been used as live algae species for mud crab larval rearing at the DAC. These species complement each other in terms of essential fatty acid content, especially eicosapentanoic acid (EPA) and docosohexanoic acid (DHA). *N. oculata* has very high levels of EPA (~37% of lipids) and no DHA, while *PS11* has relatively low levels of EPA (~2.5%) and high levels of DHA (~10%). C. muelleri is a diatom with higher levels of EPA (~15%) than of DHA (~4%). Another genus of microalgae that is often used in aquaculture hatcheries is *Tetraselmis*. This genus of algae produces only EPA (~9.5%).

Microalgae is expensive to produce owing to the capital and human resources required. Hatchery efficiency and profitability would be markedly improved if crab larvae could be reared without microalgae. For this reason an experiment was designed and conducted to determine if microalgae was necessary for survival and growth of *S. serrata* larvae; and if necessary which species of microalgae, or combination of species, delivers the highest levels of survival and growth.

Methods

The trial utilised the standard operating procedures for small-scale trials with only minor changes. *Artemia* were fed to all treatments in a single feed at a rate of 0.5 mL-1 from day 4 to day 6, at 0.75 mL^{-1} from day 7 to day 9 and from 1 mL from day 10 to day 14 when the trial ended.

Treatments differed by the species or mixture of microalgae provided. There were three replicates of 12 treatments arranged in a completely randomised design. Each treatment received equal cell numbers of microalgae to a final density of 5 x 104 cells mL^{-1} eg if three species of microalgae were added in combination then 5 x 104/ 3 = 1.66 x 104 cells of each species were added from each species. The microalgae species used were *N. oculata*, C. mulleri, *Tetraselmis suecica*, and an endemic unnamed Isochrysis-like species titled *PS11* (Thinh, *et al*., 1999). The algae was grown at DAC us F@ nutrient medium. Treatment descriptions and codes are presented in Table 8.

Table 11. Feeding treatments, and codes, for the small-scale microalgae larviculture trial (DAC Batch–7).

Treatments	Codes
No Algae	No Algae
Chaetoceros	C
Nannochloropsis (Nanno)	N
PS11	P
Tetraselmis	T
Chaetoceros + Nanno	CN
Chaetoceros + *PS11*	CP
Chaetoceros + *Tetraselmis*	CT
Nanno + *PS11*	NP
Nanno + *Tetraselmis*	NT
PS11 + *Tetraselmis*	PT
Chaetoceros + Nanno + *PS11*	CNP

Megalops sizing

At the end of the trial, megalops were fixed in 10 per cent seawater formalin for storage. Later, to determine if the presence, or species, of microalgae had an effect on the growth of megalops, at least 10 individuals from each treatment were measured across the rostrum for comparison of rostral width after Gardner and Northam (1997) (Figure 15). An eyepiece graticule was used to determine size to within 10 microns.

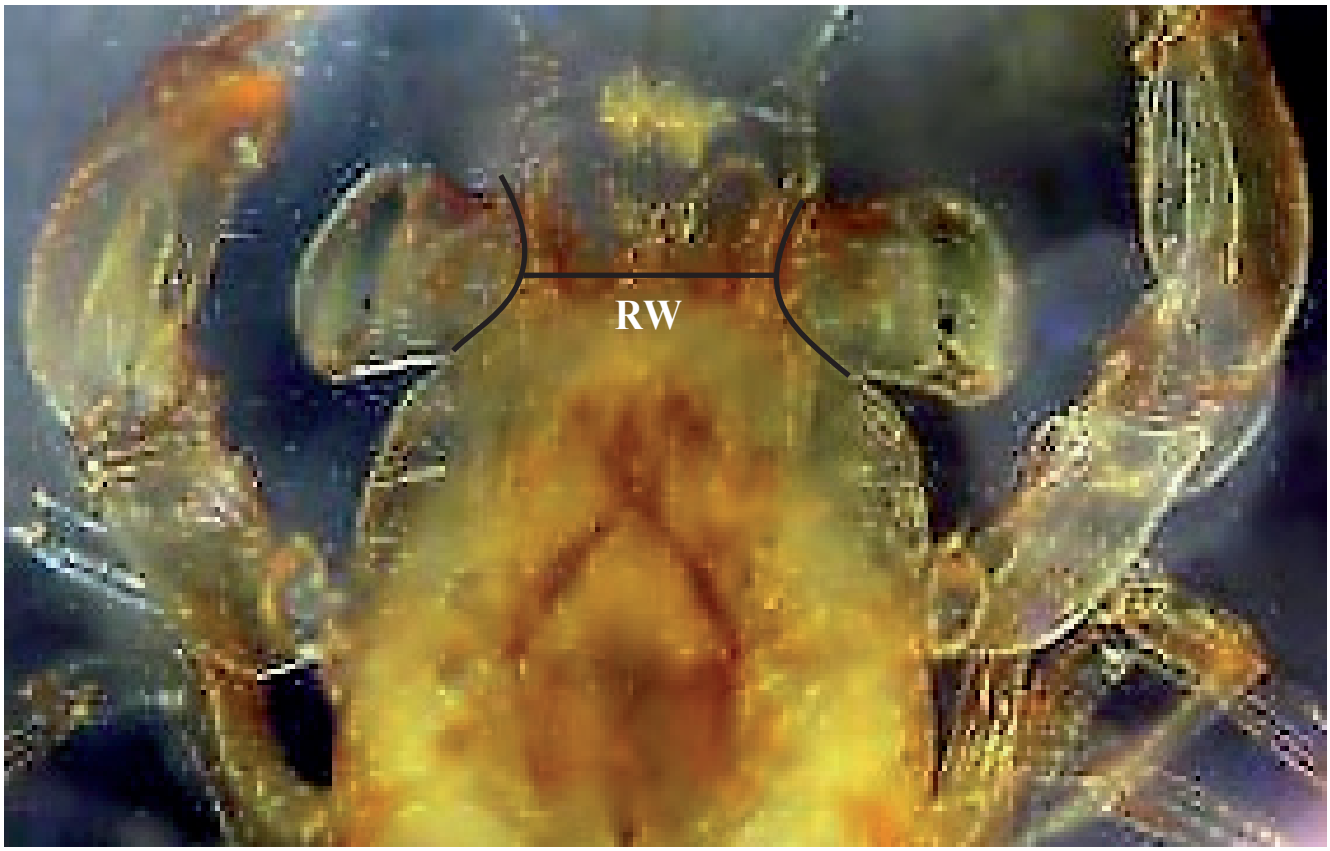

Figure 15. Rostral width measurement recorded on megalops.

Statistical Analysis

Daily survival was expressed as the percentage of larvae alive at the time of counting. Mean rostral width for each replicate was determined on completion of the trial. This data was analysed by analysis of variance (SYSTAT V7, SPSS), and where significant differences were observed, they were separated using a least significant difference test. Residuals were examined to determine a requirement for data transformation.

Results

Data relating to survival of the larvae to the megalop stage is presented in Table 12. There was no significant difference (P = 0.75) in survival to megalop between treatments, however the high variability of the no algae treatment was noticeable.

Table 12. Survival to megalop (%) (±SE) for the various treatments.

Treatment*	Survival to megalop (%)
No Algae	51.11 ± 25.63a
C	73.33 ± 5.09a
N	73.33 ± 5.09a
P	73.33 ± 7.70a
T	74.44 ± 2.94a
CN	74.44 ± 5.88a
CP	70.00 ± 6.67a
CT	70.00 ± 5.77a
NP	75.56 ± 2.22a
NT	72.22 ± 5.56a
PT	67.78 ± 5.56a
CNP	82.22 ± 2.94a

*Treatment codes from Table 8.

Daily survival throughout the experiment is depicted in Figure 16. The combination of *Nannochloropsis*, Chaetoceros and *PS11* tended to yield better survival, although it was not significantly better than the other treatments. The no algae treatment suffered a steady decline from day 4 onwards.

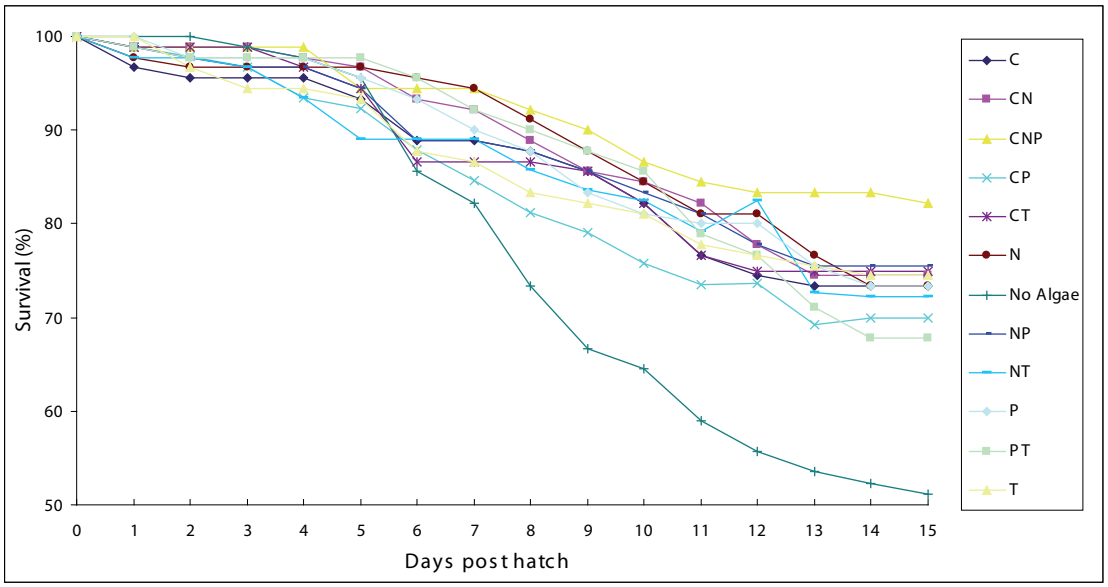

Figure 16. Daily survival throughout the larval rearing trial. (DAC Batch–7).
Error bars have been omitted for clarity

Data relating to rostral width for the various treatments is presented in Table 13 and Figure 17. There was a highly significant difference ($P < 0.001$) in rostral width between treatments with the “No Algae” treatment having a significantly smaller mean rostral width.

Table 13. Mean rostral width for the megalops in the various treatments. Measurements with the same superscripts are not significantly different ($P > 0.05$).

Treatment	Rostral width (microns)
No algae	756.55 ± 3.60a
C	850.56 ± 10.32 cd
N	775.83 ± 26.94ab
P	847.50 ± 13.92 cd
T	846.73 ± 22.47cd
CN	851.43 ± 7.95cd
CP	863.22 ± 11.48d
CT	833.33 ± 17.74c
NP	863.95 ± 8.55d
NT	833.74 ± 17.55c
PT	851.60 ± 8.44cd
CNP	794.15 ± 15.48b

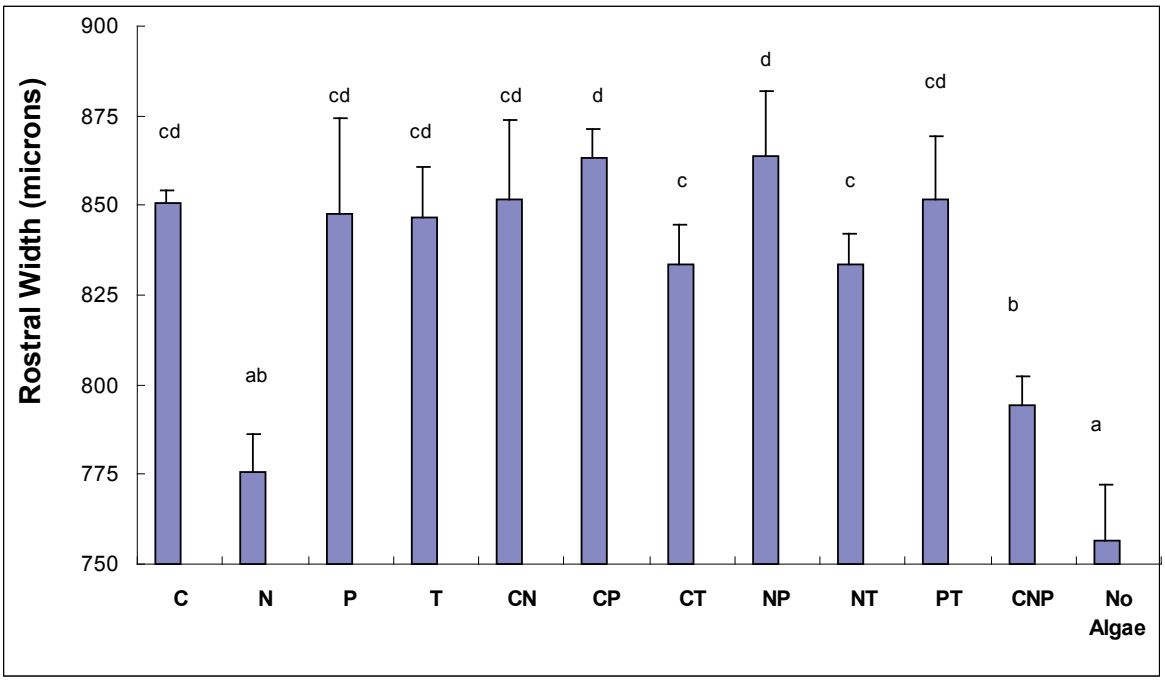

Figure 17. Mean megalops rostral width for the various treatments.
Bars with the same superscripts are not significantly different (P > 0.05) .

The combinations of Chaetoceros and *PS11*, and *PS11* and *Nannochloropsis*, produced megalops with the widest rostral width. It was interesting to note that the CNP treatment which had the highest larval survival in this trial, produced megalops with the most narrow rostrum.

Discussion

This trial showed that the presence of microalgae at concentrations of 5 x 104 cells mL^{-1} improved survival of larval mud crab larvae, compared to no algae being provided, and resulted in larger megalops, as measured by rostral width.

The treatment receiving three species gave numerically the best survival although it was not significantly better than the other treatments.

Interpreting the growth data has many constraints. Firstly biochemistry of the microalgae was not tested, and biochemistry may change under different environmental growth conditions. Secondly, as treatments were fed a specific number of cells mL^{-1}, actual weight of nutrients between algal species will differ with cell volumes.

These results indicate value in providing microalgae to mud crab larviculture systems. In addition to their assumed nutritional value, the microalgae may also assist in controlling water quality by stripping some nutrients from the water, such as ammonia and nitrite, and some microalgae have been reported to have bactericidal properties (Irianto, A., Austin B., 2002).

Effects of various feeding regimes on growth and survival of mud crab larvae. (DAC–Batch 8)

Introduction

Observation of larvae feeding on partially de-capsulated *Artemia cysts* shed light on how early crab larvae actually take in some food items. The larvae will usually hold the abdomen curved under the cephalothorax while swimming. However when the *Artemia cysts* were offered and detected, the larvae lashed out with the abdomen to a hyper extended position, sometimes curving the abdomen back and upwards to scrape the dorsal spine with the uropods and telson. The uropods and telson would then be brought to the mouthparts. If the larvae had impaled a cyst, with the spinous uropods and telson, it would then bring the cyst to the mouthparts and commence eating the cyst. Orange coloration would then be seen passing from the cyst to the gut of the larvae.

Often in mass culture experiments larvae congregate on the floor of the tanks. These events are usually associated with mortality and are suspected moulting events. Given that the uropod spines so easily penetrate a partially de-capsulated *Artemia* cyst, it was hypothesised that pre-moult larvae may be causing substantial damage to the newly post-moult or moulting larvae. This damage would probably result in mortality due to the poorly developed immune response of the crab larvae, and the relatively high bacterial numbers commonly occurring in the rearing systems, particularly on the tank bottom.

Bacteriology indicated that a lot of *Vibrio* spp. were in the biofilm and the dead bodies of live feeds and larvae. Removal of the biofilm, the detritus and old feed is still a major difference between the small-scale larval rearing in the bowls and the 1000 L tanks. To reduce accumulation of detritus and tank biofilm an attempt was to be made to keep all larvae and feeds constantly in suspension. The aim of the mass culture experiment is to prevent or minimise contact between larvae and the tank bottom and between larvae while moulting by keeping them suspended in the water column. We aim to achieve this by using an aeration-powered up-weller, the “Aquaclone”.

In previous small-scale larval rearing experiments live food protocols have been investigated, and a standardised feeding regime has been adopted. This includes a mixture of live microalgae at 5 x 104 cells mL^{-1} fed throughout, rotifers fed at 10 mL^{-1} for the first four days, and first instar *Artemia* (INVE AF 430’s) from day 5 until megalop at 0.5-2 *Artemia* mL^{-1}. This regime has tended to yield the best survival in previous experiments at approximately 70 per cent. It was also found in a previous experiment that the feeding of different species, and combinations of microalgae, effects size of megalops as indicated by rostral width (RW).
In order to improve survival and to increase size and perhaps health of megalops artificial enrichment media and artificial diets will be fed to larvae in small-scale experiments.

Methods

Small-scale trial:

As per Darwin Aquaculture Centre SOP in addition to those described below six experimental treatments with five replicates of each were imposed and are tabulated in Table 14.

Table 14. Treatments* imposed for DAC–Batch 8.

No.	Treatment
1	No microalgae provided, standard rotifers, and standard Artemia
2	Standard algae, standard rotes, and standard *Artemia*
3	*N. oculata* @ 2.5 x 104 + *Tetraselmis suecica* @ 1.25 x 104 cells mL^{-1} , standard rotifers and standard Artemia
4	Standard algae, Rotifers boosted with Frippak CD 2, standard Artemia
5	Standard algae, standard rotifers, standard Artemia, freeze dried krill from Z2/2
6	Standard algae, Rotifers boosted with Frippak CD 2, standard Artemia, freeze dried krill from Z2/2

*See notes below for explanation of “standard” treatments.

Standard Algae

Equal cell numbers of three species of microalgae to a final density of 5 x 104 cells mL^{-1}. The algae used were *Nannochloropsis oculata*, *Chaetoceros mulleri* and an endemic unnamed Isochrysis-like species titled *PS11*.

Standard Rotifers

Rotifers (*Synchaeta sp.*) were mass cultured in microalgae, usually a combination of *N. oculata* and *PS11*. Each day rotifers were harvested by draining through a submerged 64*µ*m screen, were rinsed in 5*µ*m filtered seawater and concentrated to a 100 L tank. These were then enriched in a combination of *PS11* and *N. oculata* for 60 minutes prior to being fed at a rate of 10 rotifers mL^{-1} in a single daily feed.

Artificially enriched rotifers

Artificially enriched for rotifers treatments 4 and 6 were taken from the concentrated standard rotifers. They were held in a 20 L bucket containing 5g of Dry Selco™ (INVE product) for 60 minutes.

Standard Artemia

Artemia (INVE, AF grade : 430*µ*m) used throughout this experiment were newly hatched first instar *nauplii*. These were disinfected in 200-ppm hypochlorite solution for 20 minutes and were hatched in a custom-made flow through hatcher. *Artemia* were fed to all treatments in a single feed at a rate of, 0.5 mL^{-1} from day 4 to megalop.

Large-scale trial:

Methods

Two standard 1000 L fibreglass conical tanks, and two McRobert tank systems were used. All tanks operated as re-circulating systems although no specific biofiltration media was included. Water in the tanks overflowed through a central vertically mounted 500*µ*m screen to a 100 L sump, which contained a thermostat sensor (± 0.1ºC), a 300-watt bar heater, and a carbon filter. Water was airlifted from the reservoir back to the main tank. The Mc Roberts tanks had a central screen and water was airlifted from inside the screen to the reservoir then overflowed back to the tank. Other equipment was also included as per the SOP.

Water used in the tanks was settled and foam fractionated for a minimum of three days prior to use. Each morning the entire McRoberts culture was transferred to the clean liner. All tanks then received an 80 per cent drain-down top-up water exchange, at which time the walls of the standard tanks were wiped clean. Provided the great majority of larvae remained in the water column, the slow larvae that have congregated on the bottom will be vacuumed from the system. An AQUACLONE, was be used to keep larvae up in the water column. The AQUACLONE for the McRoberts tanks were manufactured from 300mm lengths of 150mm diameter PVC pipe, held approximately 15 mm from the bottom of the tank on three equally spaced legs. A ceramic airstone delivering fine bubbles was centrally mounted inside the AQUACLONE causing water to be displaced upwards thereby drawing water and moulting larvae from the bottom of the tank and keeping them in suspension. For the standard tanks, a ring of perforated trickle irrigation pipe was bound to the bottom margin of the screened central standpipe.

Top-up water was pumped from the 20 tonne storage tank through a 1μm bag filter, to a five tonne tank for overnight storage. In the morning a 3KW immersion heater was used to heat the water to the required temperature before filling the culture tanks.

Larvae were stocked at 30 L^{-1}, which is three times higher than normal to allow for unhealthy larvae to be removed while still allowing several thousand megalops to be produced. Three species of microalgae were used. *Nannochloropsis oculata*, *Chaetoceros mulleri* and an endemic Isochrysis-like species titled *PS11* were added in equal cell numbers to a combined density of 5 x 104 cells mL^{-1}. The rotifer *Synchaeta sp.* was supplied at 10 mL^{-1} from stocking until day one of zoea stage 2. From day 2 of zoea 2, first instar *Artemia* were initially added at a rate of 0.5 mL^{-1}.

Tanks were continuously monitored for detritus or mortalities and these were removed as soon as they were noticed. Screens, airlines and airstones were wiped down to remove attached biofilm in tanks at least once daily.

Results

Small-scale trial:

Temperature in the bowls ranged between 28.1 and 29.5ºC and salinity was adjusted from 30‰ at stocking down to 25‰ at the end of the trial. Ammonia peaked at 0.2 mg/L and no nitrite was detected.

Data relating to survival of the various treatments is presented in Table 15. Survival in this experiment was very low overall, with only four of the treatments having larvae that reached the megalop stage.

Table 15. Mean survival to megalop for the various treatments in the small-scale larval rearing trial.

	Treatment Numbers					
	1	2	3	4	5	6
Survival to Megalop (%)	4.66 10.4	± 12.00 26.83	± 11.33 18.79	± 0.0 0	18.00 31.76	± 0.0 0

Daily survival for the treatments is graphically presented in Figure 18.

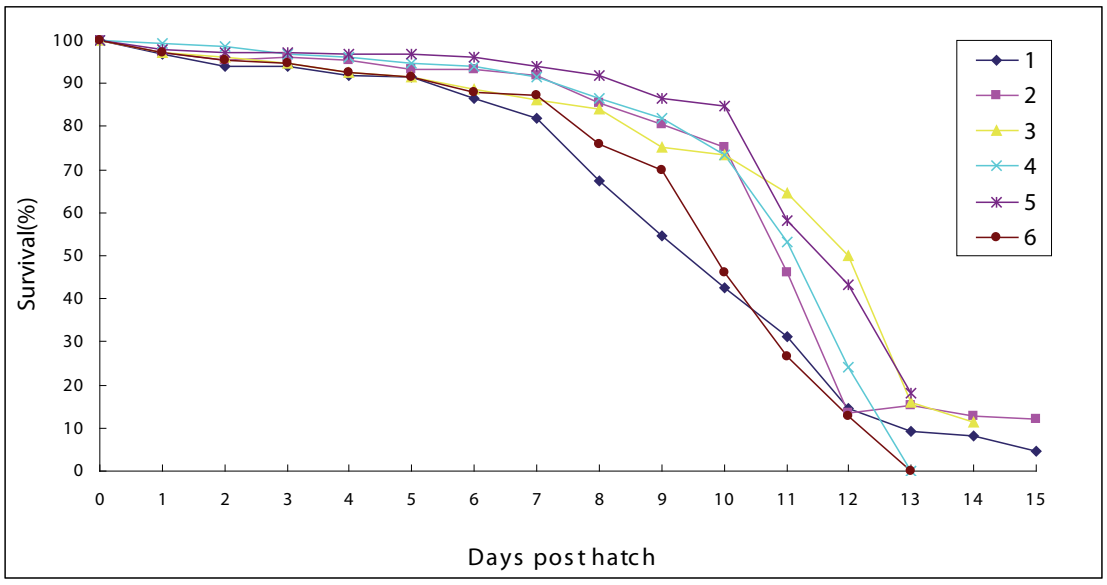

Figure 18. Daily survival for the various treatments in the small-scale larval rearing experiment (DAC Batch–8).

At approximately day 10 there was a mass mortality event in all treatments due to a *Vibrio sp.* bacterial infection. Prior to this, on day 8, there was a trend (P = 0.053) of poor survival by treatment 1, which was not receiving microalgae.

Results

Large-scale trial:

Temperature ranged between 28.8 and 30.1ºC, pH ranged between 7.96 and 8.29, ammonia peaked at 0.3 mg/L and nitrite reached a maximum of 0.1 mg/L.

There was a substantial drop out in larval numbers on day one in all tanks. Any larvae not vigorous enough to remain in the water column were vacuumed from the tanks along with any mortalities. On day 2 survival was estimated (by direct estimation) to be between 30 and 50 per cent in all tanks.

The AQUACLONE performed well in keeping larvae up in the water column although many larvae sustained damage to spines thought to be the result of collisions with the centrally mounted vertical screens.

On day 6 Standard tank 2 suffered a large drop in numbers with many of the carcasses examined having an injury to either the lateral or dorsal spines. This dropout continued until day 9 when this tank was terminated. *Vibrio harveyi* was identified in tank water of all tanks and also in the gut of some larvae. All tanks were terminated with fewer than 100 larvae alive by day12.

Discussion

Very little in the way of conclusions can be taken from this experiment due to the very poor survival in all treatments in both large and small experimental systems. The krill used in the small-scale larval rearing seemed to precede the *Vibrio* infection and subsequent mortality event, although the freeze-dried krill holds very few viable bacteria. The krill may have been an ideal substrate for the endemic *Vibrio* sp bacteria. Cross infection between treatments could have been facilitated by the communal use of counting, monitoring equipment or by aerosols.

In the large-scale trial damage to the larvae may have been attributable to contact with the screen. This damage could have facilitated entry of pathogenic bacteria and subsequent disease. Moving of the screen to a more lateral position in the tank may reduce contact and damage.

The centrally mounted screens should be placed laterally and water could then be moved by airlifts. AQUACLONES can then be used, to good effect, to keep larvae and live food in suspension.

Small-scale trial:

Influence of microalgae on survival of mud crab larvae. (DAC–Batch 9)

Introduction/Aim

To assess at which stages, if at all, the addition of microalgae is beneficial to the growth and survival of mud crab larvae.

Methods

The standard operating procedures for small-scale trials were used, exceptions as follows.

The “lawn” or bacterial growth from a TCBS plate growing a probiotic (Pro-A) was added to the heating tank prior to filling the hatch tank into which the broodstock for this trial was held. Also, the lawn from a second TCBS plate was added to a 20 L bucket held in a thermostatically controlled (15C ± 2C) refrigerator and was slowly pumped into the heating tank over the next 12 hours so that water flowing into the hatching tank was inoculated with Pro-A.

The water used in the experimental bowls was made up each day with the salinity adjusted to 30ppt at stocking. All bowls received OTC throughout the experiment at a concentration of 50ppm.

To the tubs which the concentrated, harvested rotifers were added, which had been previously filled with UV treated 5*µ*m filtered water, approximately 1 x 106 cells of a previously isolated OTC resistant probiotic contender (PRO-A), as well as 5g of OTC (50ppm) was added. Fifty mL of *Chlorella* paste was then added to the tub. Rotifers for subsequent days were treated in the same way.

First instar (GSL AAA INVE Thailand) *Artemia* were fed when the larvae reached day 2 of Z2 (Day 6) up until day one of Z3 (Day 8). These were hatched in static tanks of UV treated seawater. *Artemia cysts* were added to the hatcher along with 100ppm of INVE Hatch Controller and 1g L^{-1} of sodium bicarbonate as a pH controller, and were thoroughly aerated. The cysts were not sterilised with chlorine. The following morning the *nauplii* were harvested and restocked to a 20 L bucket for counting and distribution to the culture tanks. *Artemia* were fed at a rate of 1.5 *nauplii* mL^{-1} in two feeds, rather than the usual 0.5 *nauplii* mL^{-1}.

Five experimental microalgae feeding regimes were used as detailed in 16. There were five replicates of each treatment arranged in a completely randomised design.

Table 16. The duration of microalgal treatment.

Treatment Code	Stages Z1	Z2	Z3	Z4	Z5
No algae					
Z1 only	→				
Z1–Z2	→	→			
Z1–Z3	→	→	→		
Z1–Z4	→	→	→	→	
Z1–Z5	→	→	→	→	→

The micro algal treatment consisted of three algal paste species (Instant-algae, Reed Mariculture Pty Ltd, USA) mixed together as detailed in Table 17.

Table 17. Algal densities used.

Species	Final density per day (cells mL^{-1})
Nannochloropsis oculata	3.32 x 104
Isochrysis galbana–Tahitian strain (T. iso)	1.66 x 104
Tetraselmis sp.	1.66 x 104

Results

Water quality in the small-scale trial was as follows; dissolved oxygen always above 6.3, pH between 7.84 and 8.02,temperature between 27.2 and 29.6°C, and salinity was maintained between 28 and 30ppt.

Substantial mortalities occurred in all treatments from day three onwards. Survival data for the small-scale experiment is presented in Table 18 and Figure 19. By day 6 survival was as low as 1 per cent in some bowls and the trial was terminated. Some bowls reached Z2 on day 5 although it was less than 50 per cent.

Table 18. Survival of mud crab larvae on day 6 of the trial.

No algae	Z1 only	Z1-Z2	Z1-Z3	Z1-Z4	Z1-Z5
32.67± 6.09	23.33± 6.24	25.33± 5.54	33.33± 2.98	34.67± 8.92	26.00 ± 2.87

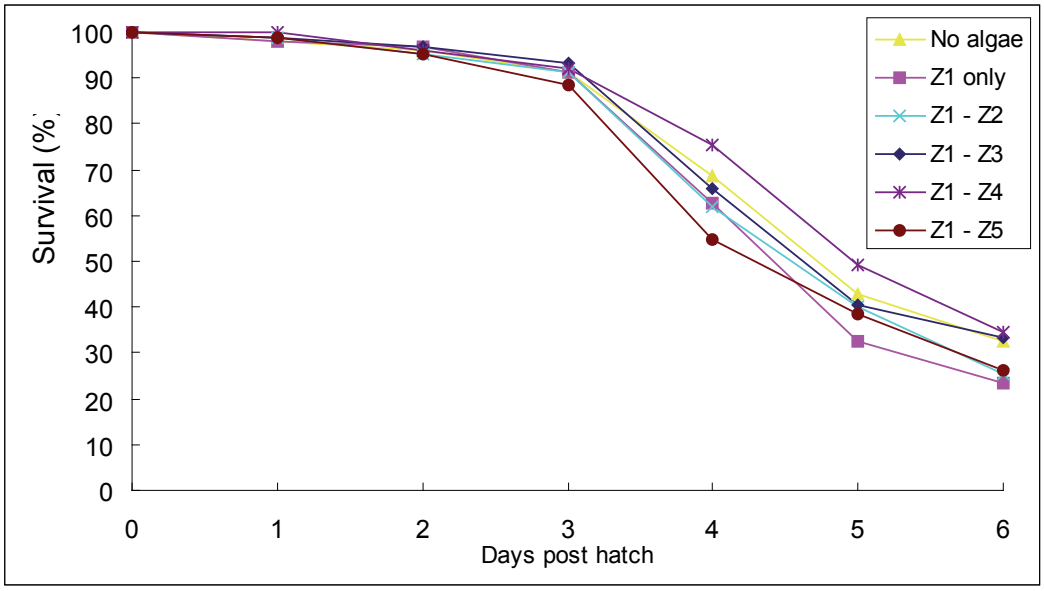

Figure 19. Daily survival for the various treatments in the small-scale experiment (DAC Batch–9). Error bars omitted for clarity.

Discussion

The survival of the larvae during this trial was so poor as to disallow any meaningful elucidation of results, although the treatment not receiving any microalgae seemed to be performing as well as the other treatments which is contrary to previously obtained results.

The recommendation was that this trial should be repeated with better quality larvae.

Small-scale trial:

Examination of the duration of rotifer feeding required in mud crab larviculture and of the value of de-capsulated *Artemia cysts* as a feed for Z1 larvae. (DAC-Batch 10)

Introduction

Previously in this project (Batch 5) it was found that use of newly hatched *Artemia nauplii*, did not support good growth or survival of Z1 larvae in comparison to using rotifers. This might be because of the swimming ability of the Artemia, which may assist them in avoiding predation, or at least being more difficult to catch, and the relatively large size of the *Artemia* relative to a Z1 larvae . A potential solution to this was to feed de-capsulated cysts to the larvae. These were a non-swimming feed, with very similar nutrition to newly hatched first instar Artemia.

This trial was undertaken to re-assess the usefulness of extending the duration of rotifer feeding to the Z2 and Z3 stages, with and without OTC, and to determine the potential of de-capsulated *Artemia cysts* as a feed to the Z1 stage.

Methods

The small-scale trial was set up using SOP, with the exception of methods mentioned in the following section.

Six experimental treatments were examined as outlined in Table 19.

Table 19. Feeding regimes investigated. (DAC Batch–10)

Treatment	50ppm OTC
Rotifers to Z2/1	Yes
Rotifers to Z2/1	No
Rotifers to Z3/1	Yes
Rotifers to Z3/1	No
De-capsulated *Artemia cysts* to Z2/1	Yes
De-capsulated *Artemia cysts* to Z2/1	No

When rotifers or de-capsulated cysts were no longer offered, the larvae were fed live, routinely hatched *Artemia* until Z3/1 and OTC was no longer administered. After this the larvae were fed *Artemia* which had been enriched for 24 hours in DC DHA Selco™ (INVE).

Approximately 1 x 106 cells of a previously isolated OTC resistant probiotic contender (Pro A) were added to the tubs, in which rotifers had been harvested and then concentrated into, following SOP,. Seventy mL of *Chlorella* paste and 5g of OTC (50ppm) were also added during an18-hour enrichment. Rotifers for subsequent days were treated in the same way.

For the treatments being fed *Artemia cysts*, AF grade 430*µ*m (INVE Thailand) cysts were used. These cysts were partially de-capsulated by placing hydrated (one hour in freshwater)

cysts in a 12.5 per cent liquid sodium hypochlorite solution. The cysts were monitored for de-capsulation grossly by eye for colour change, and confirmed with the aid of a dissecting microscope. When the chorion of a small percentage had been sufficiently degraded to expose the orange embryo, sodium thiosulphate was added and the de-capsulation process was halted. The great majority of cysts still had some residual chorion membrane. The cysts were then thoroughly rinsed in UV treated ultra-filtered seawater. A count of cysts per mL was completed, and they were then frozen into a solid block of the same water. Appropriate quantities of the ice block, containing the partially de-capsulated cysts, was rapidly thawed using UV treated water each day to provide feed.

First instar (AF 430 INVE Thailand) *Artemia* were fed the day that > 50 per cent of the treatment reached the appropriate stage according to the experimental protocol, using SOP. When 50 per cent of a treatment reached the Z3 stage, then the *Artemia* offered were 48 hour old (second instar +) enriched. The same hatching method as above still applied, however after 24 hours the *nauplii* were harvested, rinsed and restocked at approximately 200 mL^{-1}. At the same time as the *Artemia nauplii* were stocked into the enrichment tub 10 per cent of a Pro A Petrie dish lawn was added. These *Artemia* were then enriched with 250ppm (INVE) DC DHA Selco™ for six hours, prior to another 100ppm addition of the enrichment media. After 20 hours a further 150ppm was added, and the culture was harvested and fed after approximately 24 hours. Half the daily feed was fed at 1200, and the remaining half was further enriched in 250ppm DC DHA Selco™ until 1600 hours.

Artemia were fed at rates that were progressively increased from 1 mL^{-1} to 3.0 mL^{-1} as the larvae developed to Z5

Three algal paste species (Instant-algae, Reed Mariculture Pty Ltd, USA) were mixed and added to bowls as tabulated below.

Table 20. Algal species and densities used.

Species	Final density per day (cells mL^{-1})
Nannochloropsis oculata	3.32 x 104
Isochrysis galbana–Tahitian strain (T. iso)	1.66 x 104
Tetraselmis sp.	1.66 x 104
Statistical analysis	

Per cent survival from Z1 to megalop in the small-scale larval rearing experiment was analysed by one-way ANOVA.

Results

Water quality in the trial was reported as follows; dissolved oxygen was maintained above 5.5 mg L^{-1}, pH between 7.89 and 8.12, temperature between 28.1 and 29.5°C, and salinity ranged between 32 and 30ppt.

Daily survival data for the small-scale experiment is presented in Figure 20. Survival when OTC is used in presented in Figure 21 and survival at day 3, 6 and 8, and final survival to megalop is shown in Table 21.

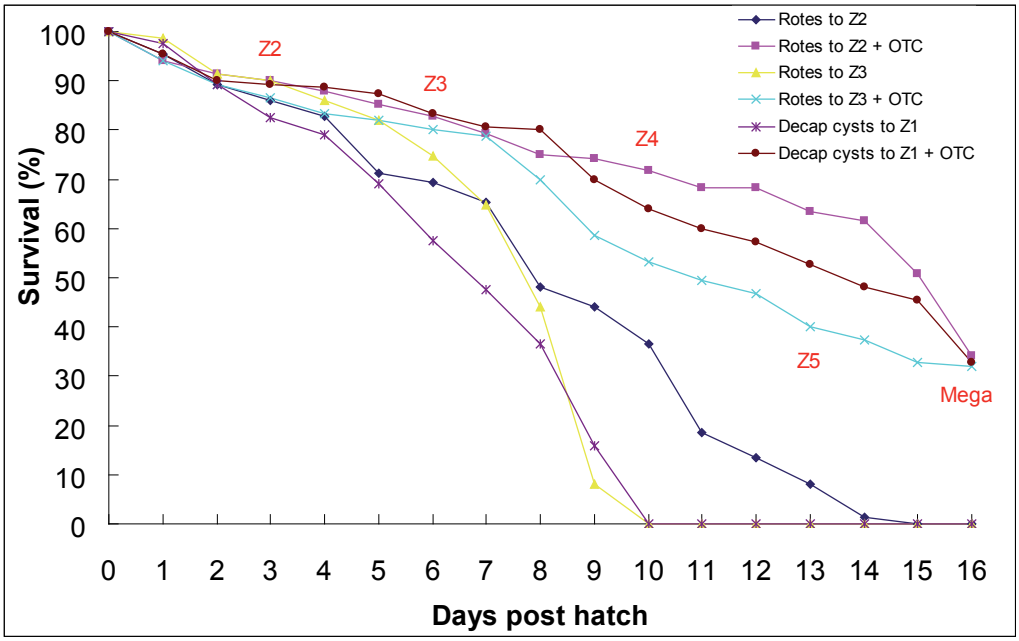

Figure 20. Daily survival of mud crab larvae in the various treatments throughout the experiment. (N.B. Rotes refers to rotifers) (DAC Batch–10).

Table 21. Survival (± se) of mud crab larvae on day 6 and final survival from Z1 to megalop for the various treatments in this trial.

Treatment	Survival day 3 (%)	Survival day 6 (%)	Survival day 8 (%)	Survival (%) to megalop
Rotifers to Z2	86.00 ± 3.40 a	65.33 ± 6.80 a	48.00 ± 11.38 ab	0.00 a
Rotifers to Z2 + OTC	90.00 ± 4.34 a	79.33 ± 4.52 a	75.00 ± 5.18 b	34.17 ± 9.85 b
Rotifers to Z3	90.00 ± 2.58 a	64.67 ± 8.47 a	40.83 ± 14.99 a	0.00 a
Rotifers to Z3 + OTC	86.67 ± 1.49 a	78.67 ± 1.70 a	70.00 ± 2.36 b	32.00 ± 11.28 b
De-capsulated cysts to Z1	82.50 ± 7.86 a	47.50 ± 18.02 a	36.66 ± 13.81 a	1.33 ± 1.33 a
De-capsulated cysts to Z1 + OTC	89.33 ± 3.71 a	80.67 ± 2.21 a	80.00 ± 1.82 b	32.67 ± 1.63 b

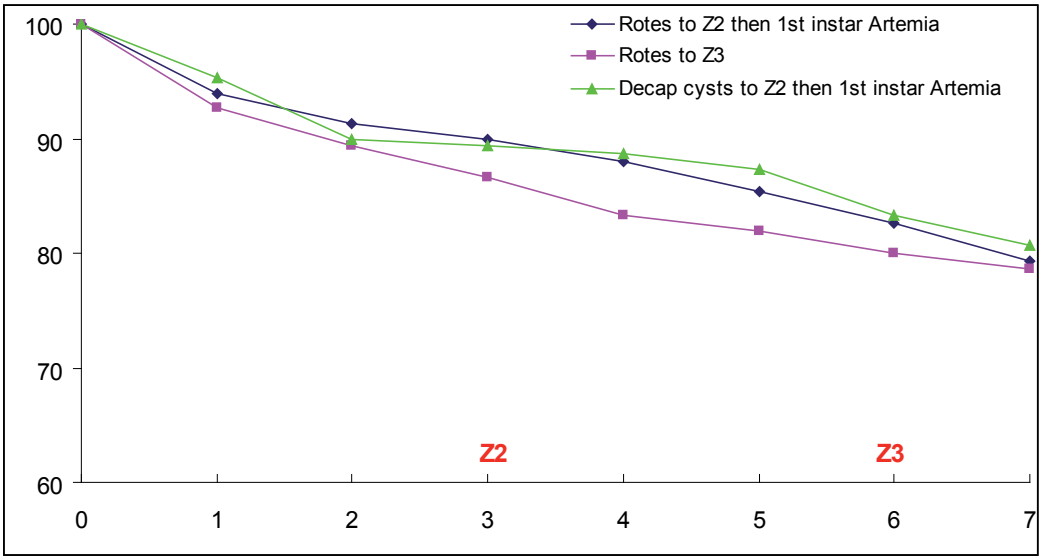

Figure 21. Survival to day 7 (Z3/1) when OTC was used.

Discussion

There was no significant difference (P > 0.05) in survival of mud crab larvae between the treatments up to day 7. By day 8, however significant differences were apparent between treatments receiving OTC and those not. There was no significant effect of the various feeding methods which indicated that the feeding of partially de-capsulated cysts is adequate for Z1 larvae.

The trial was terminated on day 18 when all remaining larvae had moulted to the megalops stage. There was a highly significant difference (P = 0.008) in survival to megalops between treatments, with those treatments receiving OTC having higher survival. The feeding of de-capsulated cysts without OTC did not prevent the larvae from experiencing high levels of mortality, presumably related to bacterial infection which had previously been linked to the rotifer culture. It can be hypothesised that there was some cross contamination between non-OTC treated rotifer-fed experimental units and other treatments given their close proximity and shared counting equipment. The OTC treatment, where administered, prevented the mass mortality.

Large-scale trial:

Preliminary trial, the effects of replacing rotifers with de-capsulated *Artemia cysts* in mud crab larviculture. (DAC–Batch 11)

Introduction

In a previous trial (Batch 9), no significant difference in mud crab larval survival at Z3 stage was found between larvae fed, either de-capsulated *Artemia cysts* or rotifers, to Z2 stage when grown in 3L volumes.

This trial was undertaken to assess the growth and survival of early stage mud crab larvae fed de-capsulated *Artemia cysts* during Z1, in semi-commercial conditions.

Methods

A lawn from one plate of a newly isolated potential probiotic, isolated from a healthy larval tank in a previous run, was added to the hatcher.

Upon hatching at 0915, the larvae were given 1g of INVE PL 150 larval shrimp food to ensure they were not too long without food. Within 15 minutes most of the larvae sank to the floor of the tank and were re-suspended with vigorous aeration. An attempt was made to concentrate the larvae with a torch light, however this was unsuccessful, and therefore a large volume of the hatch water was required to gain the quantity of larvae needed for the experiment. Whether the artificial food or another exogenous factor contributed to the poor schooling, or an endogenous factor (generally poor larval quality) was responsible remains unknown. All tanks were stocked with 30 000 larvae or 30 L^{-1} at stocking.

Filtered (5μm) water was passed through a 1μm filter bag into two 100 tonne fibreglass storage tanks. This water was then foam fractionated for three days and allowed to settle, with gentle aeration. The water was then pumped through a UV steriliser prior to being used for filling, or replacing water in, the larval rearing tanks. The water used to fill the larval rearing tanks was the same as the water in the hatch tank, so no acclimation was necessary prior to stocking the larvae.

Trial specific protocols

Six standard 1000 L cylindro-conical fibreglass tanks were used. These tanks were arranged as two parallel rows inside the hatchery at the DAC. This protocol contrasted with the more usual practice of conducting trials outside of the main hatchery building under a water-proof shade structure.

All six tanks had a novel set-up consisting of a 25mm (axis) x 15 mm “T” on a rotating hub (Aquasonic, Pty Ltd, Australia) located in the tank’s central drain hole (Figure 22A). A 19mm plastic garden hose was connected to the end of the drain which delivered water to the rotating hub. On the inside of the tank, two 15 mm diameter arms were connected to the “T” and extended close to the wall of the tank. These were angled slightly upwards to follow the profile of the conical base of the tank (Figure 22B). At the outer end of the arms, a 4mm micro-irrigation valve was inserted horizontally. (Figure 22C). Water under pressure squirting

through these 4 mm valves effectively provided forward propulsion for the arms and caused the arms to rotate on the hub. Fifteen 1mm holes were drilled along the bottom edge of the arms, directing water to the floor of the tank in the manner of a spray bar, with the aim of re-suspending any settled food.

Water used to drive the rotating arms was delivered by a submerged power head pump (Rio 1700; Taipei, Taiwan) drawing water from inside a horizontally mounted 320μm screen. Some of the water drawn from inside the screen was also directed to the side mounted 700mm length of 90mm PVC pipe angled down at 45° (the Bazooka). The bazooka was fitted with a 45° elbow and reducers to 32mm. This pipe-work ensured a constant circular flow around the tank. An “AQUACLONE “(80 mm diameter) was mounted over the rotating arms hub to resuspend any larvae (Figure 22A), that settled at the bottom of the cone.

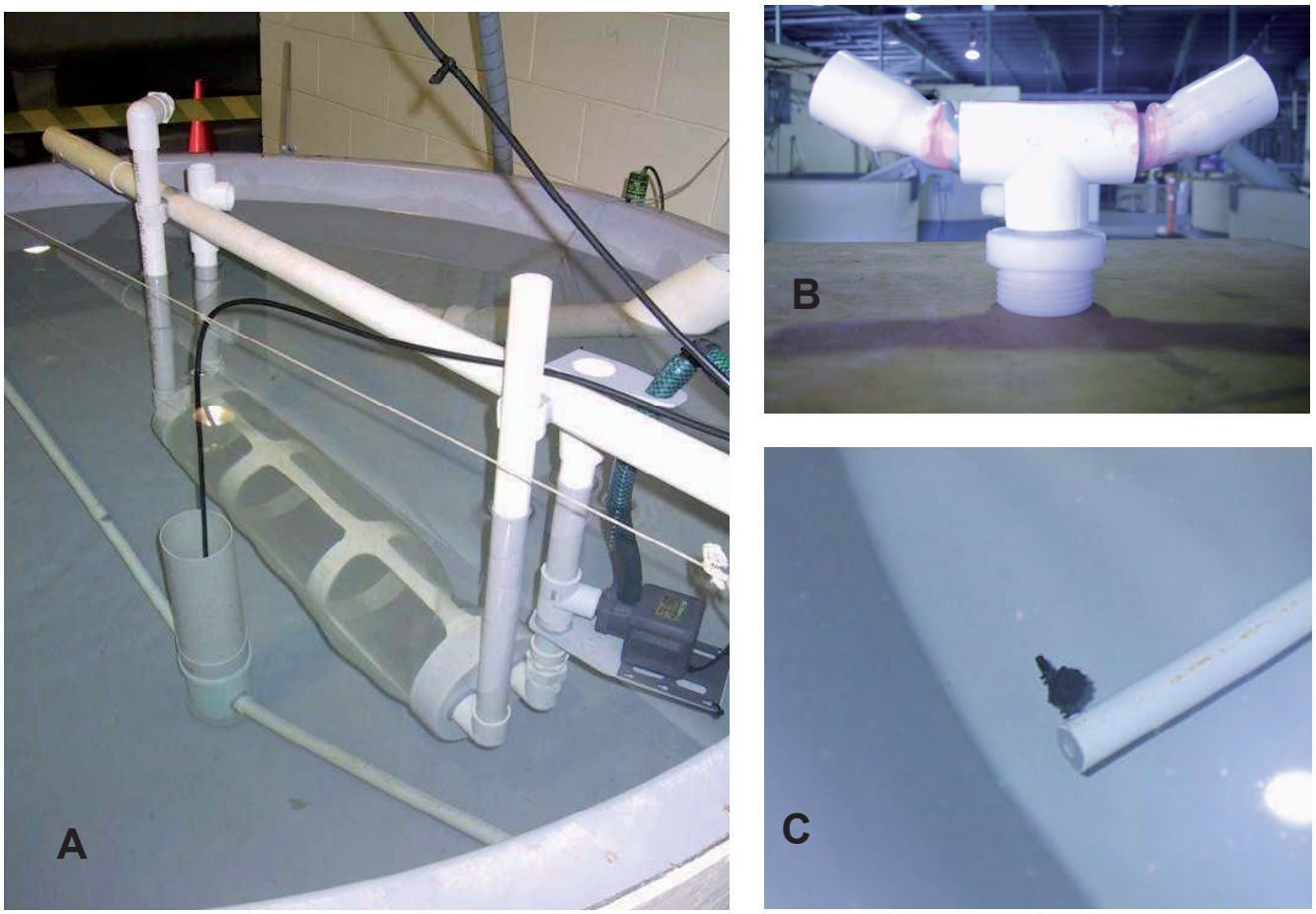

Figure 22. Diagram of 1000 L tank set up with rotating spray bar arm. **A:** Complete rotating arm system including centrally located hub and spray bar arms with aquaclone, horizontally mounted screen, submersible aquarium pump connected to garden hose pumping water to rotating hub, and bazooka. **B:** Rotating hub and “T” with inflected joiners to accommodate spinner arms. **C:** 4mm micro-irrigation valve used as director on distal end of spinner arms.

At Z2 there was a switch from 320μm to 500μm screens, and at Z3 a switch to 600μm screens.

Two 300-watt immersion heaters were placed inside the Bazooka in order to heat the water without directly contacting the larvae. Temperature of the culture water was maintained at 30.0 ± 0.1°C by a thermostat with sensor in the tank. Water quality was measured in each tank, each morning at 0800h.

All tanks underwent a 30 per cent drain-down, top-up water exchange for the first four days, at which time the walls of all tanks were wiped with a rubber “windscreen wiper “ type blade.

This tended to dislodge any settled particles. Screens, airlifts, aquarium pumps and the Bazooka were removed and hosed with freshwater prior to drain-down. The foam fractionator was filled while draining was taking place and the remaining 70 per cent of water was then fractionated for 120 minutes. Tanks were then re-filled with storage water.

From Z2/2 the hub system, rotating arms, aquaclone and power head were replaced by the standard screen and aquaclone system. A flow-through water exchange system was initiated after cleaning. No further drain downs were applied over the remainder of the experiment. Instead the tanks were fractionated for up to three hours each morning to remove the old food and improve water quantity. Salinity was maintained at 29ppt throughout the experiment.

Rotifers grown under SOP were provided as required.

AF grade 430*µ*m (INVE Thailand) were used for treatments in which *Artemia cysts* were fed up to Z2/1,. These were partially de-capsulated by placing hydrated (1 hour in freshwater) cysts in a 12.5 per cent liquid Sodium hypochlorite solution. The cysts were again monitored for de-capsulation by colour change, and with the aid of a dissecting microscope. When the chorion of a small percentage had been sufficiently degraded to expose the orange embryo, sodium thiosulphate was added and the de-capsulation process halted. The great majority of cysts still had some intact chorionic memmbrane. These cysts were then thoroughly rinsed in UV treated ultra-filtered seawater. A count of the number of cysts mL^{-1} was made after after which the entire suspension of cysts was frozen into a solid block. This ice block, containing the partially de-capsulated cysts, was rapidly thawed using UV treated water and refrozen each day. 1 cyst mL^{-1} was provided, once per day.

Feeding of first instar (AF 430 INVE Thailand) *Artemia* was commenced the day after Z2's first appeared. These were grown and harvested using SOP.

From Z3, enriched 48 hour old (second instar +) *Artemia* were fed to the larvae. The same hatching method as above still applied, however after 24 hours the *nauplii* were harvested, rinsed and restocked into enrichment tubs at approximately 200 mL^{-1}. These *Artemia* were enriched with 250ppm suspension of (INVE) DC DHA Selco for six hours, when another 100ppm of the enrichment media was added. After 20 hours a further 150ppm was added, and the culture was harvested and fed after approximately 24 hours. Half the daily feed was fed at 1200, and the remaining half was fed at 1600 hours. Feed rates were 1 mL^{-1} day^{-1}.

The generalised feeding regime is presented in Table 22.

Table 22. Generalised feeding regime for the current experiment (DAC Batch–11).

Treatment	Larval stage	Feed type	Feed rate
Cysts at Z1	Z1	De-capsulated Cysts	1 mL^{-1} day^{-1}
Cysts at Z1	Z2	First instar *Artemia nauplii*	1 mL^{-1} day^{-1}
Cysts at Z1	Z3, Z4	Enriched second instar + Artemia	1 mL^{-1} day^{-1}
Rotifers at Z1	Z1	Rotifers	1 mL^{-1} day^{-1}
Rotifers at Z1	Z2	First instar *Artemia nauplii*	1 mL^{-1} day^{-1}
Rotifers at Z1	Z3	Enriched second instar + Artemia	1 mL^{-1} day^{-1}

Algae

Four algal paste species (Instant-algae, Reed Mariculture Pty Ltd, USA) were mixed and added to the larviculture tanks as tabulated below in Table 23.

Table 23. Algal pastes used.

Species	Final density per day (cells⁄mL)
Nannochloropsis oculata	1.0 x 104
Tetraselmis sp.	1.0 x 104
Isochrysis galbana–Tahitian strain (T. iso)	2.0 x 104
Thalassiosira weisfloggii	2.84 x 103

Statistical analysis

The percentage mud crab larval survival under the different treatments was examined at day 4 and at harvest on day 9. Data was analysed by one-way ANOVA. Counts of larvae were undertaken by paddling the tanks to suspend the larvae evenly as possible and then counting ten randomly taken 250 mL samples.

Results

Seawater quality in the trial was maintained within the following limits; dissolved oxygen above 5.1 mg L^{-1}, temperature 29.8–31.0^{o}C, salinity 29–30ppt and pH 7.95–8.16.

Table 24 shows the survival data at various stages throughout the nine day experiment. Difference in survival between treatments at day 4 were not significant (the day that Z2 larvae usually appear). At this point however, there was a highly significant difference ($P < 0.001$) in moult stage, with almost 90 per cent of zoea in the cyst treatment at Z2 (74 out of 84 sampled), compared to none in the rotifer feeding treatment (0 out of 71 sampled).

Table 24. Treatments used and survivals (DAC Batch–11)

Treatment	Day 4 Mean Survival (%)	Z2 (%)	Day 9 Mean Survival (%)
Cysts at Z1	32.89 ± 6.35 a	87.21 ± 6.71 a	18.89 ± 2.40 a
Rotifers at Z1	22.01 ± 6.86 a	0 b	2.48 ± 1.49 b

There was also a significant difference ($P < 0.01$) in survival at harvest on day 9 between treatments, although the percentages of survival were considered low. Progression through successive moult stages was protracted in both treaments, but particularly so in the rotifer treatment in which three zoeal stages were present on day 8.

Discussion

The reason for the poor growth may have been linked to batch quality or to the larviculture system being operated inside the hatchery, whereas usually it is operated outside of the main buildings. This was the first attempt at growing the larvae inside. Larvae grown in this system would have experienced far lower light levels than those maintained outdoors under shade structures.

The system was not entirely successful in maintaining cysts in the water column, although very little fouling was found on the floor of the tank. Usually the cysts were found stuck to the sides of the tanks. Having said this, zoea are commonly observed feeding off the walls of tanks and hence this aspect of feeding strategy, involving use of *Artemia cysts*, may warrant further investigation. Certainly the improved survival of larvae fed cysts rather than rotifers was encouraging, even though overall survival rates in this trial were low.

Large-scale trial:

Examination of the use of de-capsulated *Artemia cysts* and artificial feeds in mud crab larviculture. (Batch 12–DAC)

Introduction

Following an encouraging result from the previous trial (Batch 10) where de-capsulated *Artemia cysts* were used as a first feed for Z1 larvae, further understanding of this feed's value was required.

As a result the team assessed the growth and survival of mud crab larvae fed de-capsulated *Artemia cysts* during Z1 in combination with artificial diets, with and without OTC treatment.

Methods

Up until the hatching of the larvae, SOP had been followed. Upon hatching, the larvae were given 0.2g of Gemma TM micro 150 (Skretting) feed in the 100 L counting tub.

Sand filtered (5*µ*m) water was passed through a 1*µ*m filter bag into two 100 000 L fibreglass storage tanks. This water was then fractionated for three days and allowed to settle, with aeration. This water was then pumped through a UV steriliser prior to filling, or replacing water in, the larval rearing tanks. The water used to fill the larval rearing tanks was the same as the water in the hatch tank, so no acclimation was necessary prior to stocking the larvae. From day 1, a 100 per cent/day flow through was carried out.

Six standard 1000 L tanks were used. They were arranged in two parallel rows of 3, inside the hatchery at the DAC.

All six tanks were set-up as described in the previous trial.

No rotifers were fed during this experiment. De-capsulated cysts were prepared as in the previous trial (Batch 10). These cysts were then thoroughly rinsed in UV treated ultra-filtered seawater. These cysts were then frozen into a solid block of the same water. Each one ton tank was fed the product from 5g of dry cysts, spread over at least two feeds per day.

Each tank was fed 0.33g of Gemma TM Micro 150, and 0.66g of INVE CD2 larval shrimp food each day, spread over two feeding events.

No algal pastes were fed during this experiment.

Every second day the tanks received the lawn from a plate of the previously isolated probiotic Pro A.

Results

Water quality in the trial was maintained as follows; dissolved oxygen above 5.6 mg L^{-1}, temperature 30.0–31.7°C, salinity consistent at 30ppt and pH 7.87–8.17.

Tank 6 overflowed on day 1, although many larvae remained. Whilst there was also a large

drop off in larval numbers in tank 4 on day 3, evidence of mass mortality in the form of large numbers of dead larvae were absent.

Tanks 1, 2 and 4 were terminated on day 4 due to mass mortality. Likewise tanks 5 and 6 were terminated on day 5, and tank 3 on day 6 following mass mortalities.

A verification plate, innoculated from a 24 hour old probiotic plate, was made on day 4, in an attempt to verify the viability of the lawns that were being added. This verification plate failed to grow any bacteria, and it was hypothesised that the probiotic plates were growing vigorously (as large lawn-type colonies did establish on the plates), but were exhausting all available resources and then dying. This meant that the probiotic lawns that were being added, were of no use and in fact may have promoted the growth of harmful bacteria in the cultures.

Discussion

The poor survival of larvae in this trial may have been due to poor batch quality or perhaps to having been cultured inside the hatchery. This was the second failed attempt, at growing the larvae inside the hatchery building using de-capsulated cysts.

Large-scale trial:

Comparison of growth and survival of mud crab larvae fed either dead frozen *Artemia* (first instar) or live rotifers during Z1. (Batch 13-DAC)

Introduction/aim

A trial was undertaken to compare the growth and survival of mud crab larvae fed either euthanased *Artemia nauplii* or rotifers during Z1.

Methods

This trial was carried out using SOP for large-scale trials with a few variations. The hatch tank and larval rearing tank were maintained at similar temperatures of 31.5ºC, and 31.6ºC respectively.

Six standard 1000 L tanks were used. These tanks were set up as described in SOP for AQUACLONE trials.

All tanks received OTC at 50ppm during the first four days of this 17 day experiment.

Rotifers produced using SOP, were enriched with 70 mL of *Chlorella* paste and 5g of OTC (50ppm) over an 18-hour period. Bacteria were harvested from a plate of the probiotic Pro A and added to the rotifers. Rotifers fed to crab larvae subsequently were treated in the same way.

Rotifers were added at a rate of 15–20 rotifers mL^{-1} in a single daily feed.

Treatments

A: Frozen, first instar (AF 430 INVE Thailand) *Artemia* were fed in the place of rotifers from Z1 to Z2/2. (These were hatched in static tanks of UV treated seawater. *Artemia cysts* were added to the hatcher along with 100ppm of INVE Hatch Controller and 1g/L of Sodium Bicarbonate, and thoroughly aerated. The cysts were not sterilised with chlorine. The following morning the *nauplii* were harvested and frozen at a known concentration. These were fed at 0.5 mL^{-1}

B: Fed rotifers from Z1 to Z2/2.

First instar AF 430 *Artemia nauplii* produced by the same hatchery method described above were fed to Z2 larvae. Feeding commenced one day after 50 per cent of zoea had attained Z2. These were fed live at 1.0 mL^{-1}

When the larvae reached the Z3 stage (Z3/2) the *Artemia* fed were GSL AAA 24 hour enriched (INVE, DC DHA Selco) *nauplii*. Half the daily feed was fed at 1200, and the remaining half was fed at 1600 hours. These were fed at 2.0 *Artemia* mL^{-1}.

Residual rotifer and *Artemia* counts were undertaken each day to assess feeding level.

Four microalgal pastes (Instant-algae, Reed Mariculture Pty Ltd, USA) were mixed and added to the tanks, from stocking to day 12, as tabulated below.

Table 25. Table of algal species used.

Species	Final density per day (cells mL^{-1})
Nannochloropsis oculata	1.0 x 104
Tetraselmis sp.	1.0 x 104
Isochrysis galbana–Tahitian strain (T. iso)	2.0 x 104
Thalassiosira weisfloggii	2.84 x 103

From day 13 until day 15, only three algal paste species (Instant-algae, Reed Mariculture Pty Ltd, USA) were used as per Table 26. below.

Table 26. Table of algal species used.

Species	Final density per day (cells⁄mL)
Nannochloropsis oculata	3.32 x 104
Isochrysis galbana–Tahitian strain (T. iso)	1.66 x 104
Tetraselmis sp.	1.66 x 104

Every day, bacteria were harvested from a plate of the previously isolated probiotic Pro A and added to each tank.

Results

Water quality during the trial was recorded as follows;. dissolved oxygen always above 6.0 mg L^{-1} , temperature 29.4–30.0ºC, salinity steady at 30ppt and the pH between 7.88 and 8.40.

All tanks had some Z2 larvae by day three (our usual standard for 30ºC) although there were only a few. The rotifer treatment averaged 25 per cent Z2 on day 3, while the frozen *Artemia* fed treatment averaged 22 per cent.

Larvae in all tanks remained healthy until day 12, when those in tanks three and 6, that had been fed frozen *Artemia* at Z1, were found to have sustained considerable mortality. The following day (Day 13), tanks across both treatments (2, 3, 4 and 6) suffered mass mortality and were terminated. Tanks 1 and 5 still appeared healthy but were given a 50ppm OTC treatment over the next two days in an effort to produce some crablets.

Both tanks yielded viable megalops, from days 15 to 17 with progressive harvesting on successive days.

As detailed in Table 27 only megalops harvested on days 16 and 17 were retained and on-grown.

Table 27. Survivals to megalops (DAC Batch–13).

Day	Tank Treatment	Total No.	Survival (%)
15	T5–Rotes	630	
15	T1–Rotes	269	
16	T5–Rotes	6,181	
16	T1–Rotes	3,472	
17	T5–Rotes	5,278	
17	T1–Rotes	6,458	
	T5–Rotes	12 089	40.30
	T1–Rotes	10,199	34.00
	Total	22,288	

Some of the megalops harvested on day 16 first became benthic on day 19, and crablets appeared on day 21. Some C2's appeared on day 24. Many crablets started using refuges on day 26 and the first C3 appeared on day 27.

The two tanks of crablets were harvested on Day 36 post hatch. The crablets were at stage C2 and C3. A total of 9580 crablets were split into four tanks furnished with mussel rope and mesh hides originally developed for red claw crayfish (Cherax quadricarinatus), and were on-grown for another fortnight. These were again harvested and a total of 4274 (44%) were recovered. These were then graded and stockpiled for a further three days before being stocked into into ponds at the Golden Prawn farm, in two batches comprising C5–C7 and C3–C5 stages . In all 3243 crablets were stocked to ponds.

Discussion

As a result of the mass mortalities in larval populations late in the experiment, and the fact that OTC was used at this point to ensure some crablet production, very little can be gleaned from the results of this experiment. Never the less, it would appear that frozen *Artemia* may be a useful substitute feed during Z1–Z2 larval stages as the high larval mortalities did not occur until the larvae were at Z5 and one of the tanks fed rotifers also suffered a high mortality. This suggests that the mortalities were a result of bacterial infection rather than a nutritional factor . Further experiments are needed to assess the utility of substituting rotifers with frozen artemia, with and without the use of OTC.

Large-scale trial:

Attempted large-scale production of megalops for crab growth experiments incorporating particulate krill (*Euphausia pacifica*) as a dietary supplement. (BIARC–Batch 3)

Introduction

Earlier research (ACIAR FIS/1992/017) identified that the most commonly used type of *Artemia* (Great Salt Lakes [GSL]), did not support satisfactory growth and development of mud crab larvae when used as the sole dietary component (Mann *et al*, 2001). The hatchery methods used by BIARC included a dietary supplement to make up for the assumed nutritional deficiency of GSL Artemia. Typically commercially available marine prawn larval and post-larval diets were used to rear mud crab larvae as they had previously been shown to be successful in significantly reducing the incidence of abnormal larval morphology and completion of the larval cycle.

Freeze dried krill meal is a rich source of marine fatty acids, proteins and carotenoids and as such was considered a potential supplement to Artemia. Moreover, previous small-scale trials at BIARC had demonstrated that krill could be effective as a dietary supplement. Additionally, a large fraction of freeze dried krill meal particles are buoyant in seawater than similarly sized prawn diets. This is an advantage in large-scale cultures where sedimentation of feed and waste on the tank bottom is a problem.

This large-scale trial was undertaken in an attempt to produce commercial quantities of megalops for grow-out trials.

Materials and methods

This production trial started on 27 September 2000. Mass culture was conducted in 8 x 500 L cylindro-conical tanks following the standard BIARC operating procedure, except that water exchange was dictated by results of water quality testing, rather than in accordance with the prescribed routine. Freeze dried krill meal was dry blended and sieved to produce particles sizes similar to the prawn diets typically used (100–150μm for Z1–Z2 and 200–250μm for Z3). The particulate krill supplement was applied in the standard way.

Results

Mass mortality in the cultures started on day 6 and by day 10 most cultures were terminated. By day 13 the remaining two cultures with very low survival were terminated. No megalops were produced.

Discussion

There were no clues as to the cause of the sudden mass mortality. The larvae had uniformly progressed to the second instar with high survival, estimated to be from 70–90 per cent, and water quality parameters and tank appearance were acceptable. Larvae also maintained a good appearance and vigour prior to the onset of mass mortality. This sudden mortality phenomenon was one that was regularly experienced.

Large-scale trial:

Comparison of DHA/EPA enriched and non-enriched *Artemia* (BIARC–Batch 4).

Introduction

Larval nutrition experiments conducted by BIARC during the previous ACIAR (FIS/1992/017) found that enrichment of *Artemia* with DHA and EPA rich emulsions did not improve megalops production rates or affect growth. Recent evidence provided by Nhia *et al* (2003), indicated that small but significant larval survival improvement occurred following the use of high DHA and EPA level lipid emulsions to enrich Artemia. Coupled with this, one of the leading manufacturers of hatchery products, INVE, have recently improved their lipid enrichment product. This trial compared the use of the new *Artemia* enrichment product with the standard *Artemia nauplii* diet.

Materials and methods

This experiment commenced 22 September 2004.

Larvae were produced, stocked and maintained in 8 x 1.2 tonne culture tanks following BIARC standard operating procedures. *Artemia nauplii* were enriched as per the manufacturer's instructions.

Treatments were applied to the eight culture tanks as outlined in Table 28.

Table 28. Experimental treatments (BIARC Batch–4).

Oxytetracycline treatment	Enriched Artemia	Name	No. tanks
Yes	Yes	OTC+Enr	3
Yes	No	OTC—Enr	3
No	Yes	Con+Enr	1
No	No	Con—Enr	1

Oxytetracycline was added prophylactically every second day at 50ppm.

Results

The larvae in the cultures without OTC suffered severe mortality in the first four days and were discontinued before the *Artemia* enrichment treatment was applied. During this same period the cultures receiving OTC treatment also experienced high mortality but stabilised at a moderate survival rate for the following 10 days. From day 14 to harvest on day 22 mortality occurred primarily due to moult death syndrome (MDS) as larvae prepared to moult to the megalops stage. There was no apparent difference in the pattern of mortality or the incidence of MDS between the cultures fed *Artemia* with or without enrichment. Each replicate of these treatments produced megalops, but at very low rates. The mean production rates were 0.14 and 0.44 megalops L^{-1} for the, with and without, enriched *Artemia* treatments respectively.

Discussion

Enrichment of the *Artemia* did not affect the performance of the larvae, either in survival or growth. The INVE enrichment product is used in fish hatcheries where it has been shown to significantly enhance the DHA and EPA levels of the fish larvae as well as growth and survival. The lack of response by mud crab larvae indicates that this form of DHA and EPA is unlikely to be a limiting factor for the larvae and supports previous data which used a similar enrichment product. It has also been suggested that MDS may occur due to DHA and EPA deficiency in the larvae however in this experiment the incidence of MDS was similar in both experimental treatments.

Small-scale trial:

Effects of two feeds (and potential immuno-stimulants) on mud crab larval survival. (DAC–Batch 14)

Introduction

As an alternative to the use of antibiotics, immuno-stimulants including glucans and lipo-polysaccharides can be used to stimulate the non-specific immune response in some animals. These organic compounds invoke a response from the non-specific immune system making it more ready to combat infectious agents such as bacteria and viruses.

Two feeds including one that had claimed immuno-stimulant properties for fish were investigated. Skretting Pty Ltd has created a range of artificial diets, Gemma™ for use in larval finfish rearing that include glucans (which are reported to have immuno-stimulant capabilities). Another "artificial" feed Cyclopeeze™, was also tested. Cyclopeeze consists of a cyclopoid crustacean harvested from the Artic sea, which is freeze-dried whole. The manufacturers claim the animals contain very high levels of HUFAs, which may be able to stimulate increased survival due to increased overall health.

Methods

SOP for small-scale trials with the exceptions set out below.

Salinity of hatch water and during larviculture was 29ppt.

All bowls received OTC treatment at 50ppm throughout the trial.

Five experimental feeding treatments, were imposed and are presented in Table 29. There were five replicates of each treatment arranged in a completely randomised design.

Table 29. Feeding regime for immuno-stimulant small-scale experiment (DAC Batch–14).

	Gemma Micro (1ppm)	Rotifers ($10ml^{-1}$)		Artemia ($1–3ml^{-1}$)		Cyclopeeze (1ppm)
A: Control, standard method (Std)		Z1/1 Z2/1	–	Z2/2 Mega	–	
B: Std + Gemma	Z1/1–Mega	Z1/1 Z2/1	–	Z2/2 Mega	–	
C: Std +Cyclopeeze		Z1/1 Z2/1	–	Z2/2 Mega	–	Z4/1–Mega
D: Std + Gemma + Cyclopeeze	Z1/1–Mega	Z1/1 Z2/1	–	Z2/2 Mega	–	Z4/1–Mega
E: Gemma only	Z1/1–Mega	Nil		Nil		Nil

Rotifers were produced and harvested, and concentrated as per standard operating procedures. After which approximately 1 x 106 cells of a previously isolated probiotic contender (Pro A) were added. Fifty mL of the *Chlorella* paste and 5g of OTC (50ppm) were

then added to both during an18-hour enrichment. Rotifers for subsequent days were treated in the same way.

Inert feeds were fed in two feeds daily

Statistical Analysis

The percentage survival in this small-scale larval rearing experiment was analysed by one-way ANOVA.

Results and discussion

Water quality was monitored daily. Dissolved oxygen was always 100 per cent saturated, pH varied between 7.95 and 8.13, temperature varied between 27.8 and 29.9C, and salinity was maintained at 30ppt.

Survival data for is presented in Table 30 and Figure 23. There was a highly significant difference (P = 0.003) in survival of mud crab larvae to day 16, with survival being significantly higher in treatments with Gemma™, than those without.. Some bowls reached Z2 on day 5 although it was usually less that 50 per cent. The trial was terminated on day 16 when the majority of larvae had failed to thrive and were still in the Z3 and Z4 stages. The larvae would normally be Z5 or megalops after this culture period.

Table 30. % Survival of mud crab larvae at Day 16.

Treatment	% Survival
Standard (Std)	6.67 ± 2.79 b
Std + Gemma™	48.67 ± 14.05 a
Std +Cyclopeeze	1.33 ± 1.33 b
Std + Gemma™ + Cyclopeeze	52.67 ± 16.61 a
Gemma only	11.33 ± 7.11 b

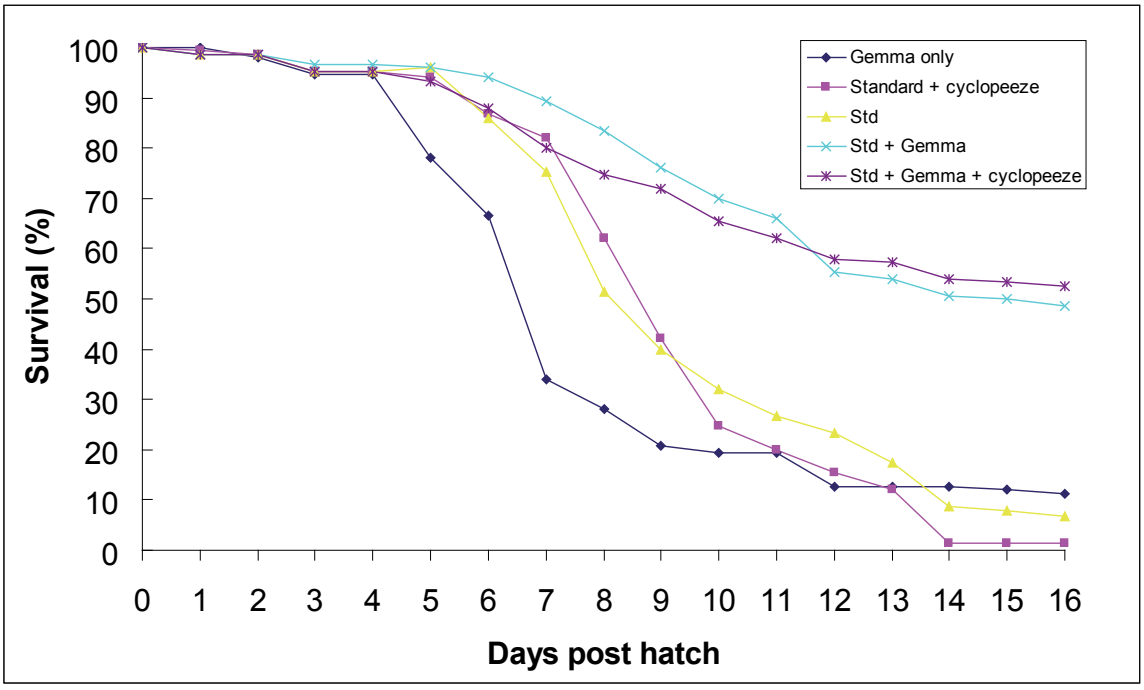

Figure 23. Daily survival of larvae subject to the different feeding regimes. Error bars omitted for clarity. (DAC Batch–14).

The Gemma™ diet alone did not support good growth or survival of the larvae, although when used in conjunction with the standard feeding regime survival and growth of the larvae was significantly enhanced, compared to the standard feeding regime.

The cyclopeeze used in this experiment was not tested prior to its use in this trial and proved to be difficult to screen to smaller sizes suitable for early stage larvae . The feed itself is probably more suited to later stage zoea and/or megalops and as this trial was terminated prematurely, it was only offered for two days ie. from Z4, as the larvae developed at a much slower rate than normal.

The Gemma™ diet deserves further investigation as a potential supplement in crab larviculture. Whether it has immuno-stimulant properties for mud crab larvae or was just a useful feed will need to be determined through further work.

Large-scale trial:

Preliminary examination of batch and dosed feeding regimes, combined with two rotifer enrichment regimes on mud crab larval survival. (DAC–Batch 15)

Introduction

In previous semi-commercial (large-scale) larval rearing runs, high levels of survival to megalops were achieved with a previously developed standard live food feeding protocol. This protocol consisted of rotifers grown and enriched on fresh water *Chlorella* paste and fed at 10 rotifers mL^{-1} fed to Z2, followed by first instar *Artemia nauplii* initially at 1 *nauplii* mL^{-1}, and finally, enriched *nauplii* up to $3ml^{-1}$ from Z3 to megalops. Water in larviculture tanks was maintained as a green-water culture through daily addition of a mixture of three or 4 algal pastes to provide a final concentration of 6.6 x104 cells mL^{-1}. All live feeds were offered in two equal feeding events, four hours apart during a standard working day.

Even though this protocol consistently delivered reasonably good survival rates, further improvements were sought.

This large-scale experiment was to determine if artificially enriched rotifers and periodic dosing of live feeds to maintain their nutritional profile (particularly DHA) is effective in promoting improved survival in mud crab larval rearing systems.

Methods

Six standard 1000 L tanks were used.

Rotifers were grown, harvested and fed as per standard operating procedures.

During the *Artemia* feeding phase, a flow through water exchange regime was implemented in all tanks. The flow through water was passed through a 1*µ*m filter bag, two in-line five tonne tanks to settle and cool water before overflowing into a 1 tonne tank where the water was heated to the operating temperature of 30ºC. From here the water was pumped through a UV steriliser to an elevated 100 L reservoir which supplied the experimental tanks via gravity siphoning through 13 mm hoses. Water exchange was at a rate of 1.4 l min^{-1} initially equating to two full exchanges per day, and was doubled at zoea stage 4 for the rest of the trial.

All tanks received OTC at 50ppm from stocking to Z2/1. All tanks received 30 000 larvae or $30l^{-1}$ at stocking. When megalops were observed in the larval tanks, selective harvesting using a 2000*µ*m screen was carried out.

Treatments in the experiment related to the feeding regime and are described below as treatment 1 (T1) and treatment 2 (T2).

Rotifers

Approximately 1 x 106 cells of a previously isolated probiotic contender (PRO-A) were added into the two tubs into which rotifers were routinely harvested.

To one of these tubs, 50ml of the *Chlorella* paste and 5g of OTC (50ppm) were then added during an18-hour enrichment (T1). To the other tub, 7.5g of (INVE) DHA Protein Selco and 50ppm OTC were added at stocking, and a further 7.5g of DHA Protein Selco was added via a dosing pump overnight (T2). The rotifers were allowed four hours in the enrichment tub after the total volume of enrichment media was added. Rotifers were treated in the same way for subsequent days.

Rotifers were harvested, and thoroughly rinsed in UV treated, 5*µ*m filtered sea water (30ppt). The *Chlorella* enriched rotifers (T1) were offered over two equal, discrete feeding events at 1200 h and 1600 h. The artificially enriched rotifers (T2) were given a half ration at 1200 h, with the remaining half added via dosing pump between 1600 h and 2000 h, and 2400 h and 0400 h. Rotifers were fed at a rate of $15ml^{-1}day^{-1}$.

Artemia

First instar (AF 430 INVE) *Artemia* were fed to both treatments during day 2 of zoea 2 and day 1 of zoea 3.

Artemia for both treatments were hatched using SOP. The following morning the *nauplii* were harvested and restocked to a 20 L bucket for counting and distribution to the culture tanks, or stocking to the enrichment tank. First instar *Artemia* were fed at a rate of 1.5 *nauplii* mL^{-1} in two feeds for T1. For T2 the *Artemia* were added at a rate of $0.75ml^{-1}$ at 1200 h with another $0.75ml^{-1}$ being dosed in overnight between 1600 h and 2000 h, and 2400 h and 0400 h.

From day 2 of zoea 3, GSL AAA (INVE Thailand) 24 hour enriched *Artemia* were fed. The *Artemia* were generally harvested from the hatch tank around 1400 h. These were rinsed in UV sterilised ultra filtered water and restocked to a 100 L tub which had previously been chlorinated (5ppm for 60 mins), neutralised, and inoculated with the previously mentioned probiotic contender (PRO-A). 250ppm of DC DHA (INVE) was added prior to the Artemia, with a further 100ppm going in at 1600 h. At 0800 h the following morning another 150ppm of DC DHA was added. Both treatments were fed these Artemia, although T1 received two feedings per day at 1200 h and 1600 h, whereas T2 received a half ration at 1200 h with the remainder dosed in overnight as per the first instar *nauplii*. Feed rate increased to $2ml^{-1}$ at this stage (Z3 & Z4), and was further increased to $3ml^{-1}$ at zoea stage 5.

Table 31. Generalised feeding regime for trial (DAC Batch–15).

	Treatment 1 (T1)	Treatment 2 (T2)
Rotifers	*Chlorella* enriched 7.5ml^{-1} at 1200 7.5ml^{-1} at 1600	Artificially enriched 7.5ml^{-1} at 1200 7.5ml^{-1} dosed in overnight
First instar Artemia	1ml^{-1} at 1200 1ml^{-1} at 1600	1ml^{-1} at 1200 1ml^{-1} dosed in overnight
Enriched Artemia	DC DHA Selco enriched	DC DHA Selco enriched Half ration at 1200
	Half ration at 1200 Half ration at 1600	Half ration dosed in overnight

Statistical analysis

Per cent survival in the current experiment was analysed by one-way ANOVA without transformation.

Results

Dissolved oxygen was maintained above 5.5 mg L^{-1}, pH was between 7.53 and 7.91, temperature between 29.1 and 30.5°C, and initial salinity was 30ppt and dropped to 27ppt by the megalops stage.

Survival to megalops, and number of megalops produced during each day of harvesting, is presented in Table 32 and graphically in Figure 24. A total of 74123 megalops were produced over the two days of harvesting. There was no significant difference (P = 0.08) in survival of mud crab larvae to megalops between the two treatments. Overall survival (± se) for T1 was 51.41 ± 8.28 compared to 30.95 ± 2.67 for T2.

Table 32. Megalop harvest and survival for the two treatments.

Date	Tank/treatment	Total Number of megalops.	Overall Survival (%)
19/03/2003	T1/T1	6360	
19/03/2003	T3/T1	12061	
19/03/2003	T5/T1	6871	
19/03/2003	T2/T2 dosed	5263	
19/03/2003	T4/T2 dosed	3947	
19/03/2003	T6/T2 dosed	3801	
20/03/2003	T1/T1	12500	62.87
20/03/2003	T3/T1	4751	56.04
20/03/2003	T5/T1	3728	35.33
20/03/2003	T2/T2 dosed	5190	34.84
20/03/2003	T4/T2 dosed	5702	32.16
20/03/2003	T6/T2 dosed	3497	25.83
	Grand Total	**74123**	

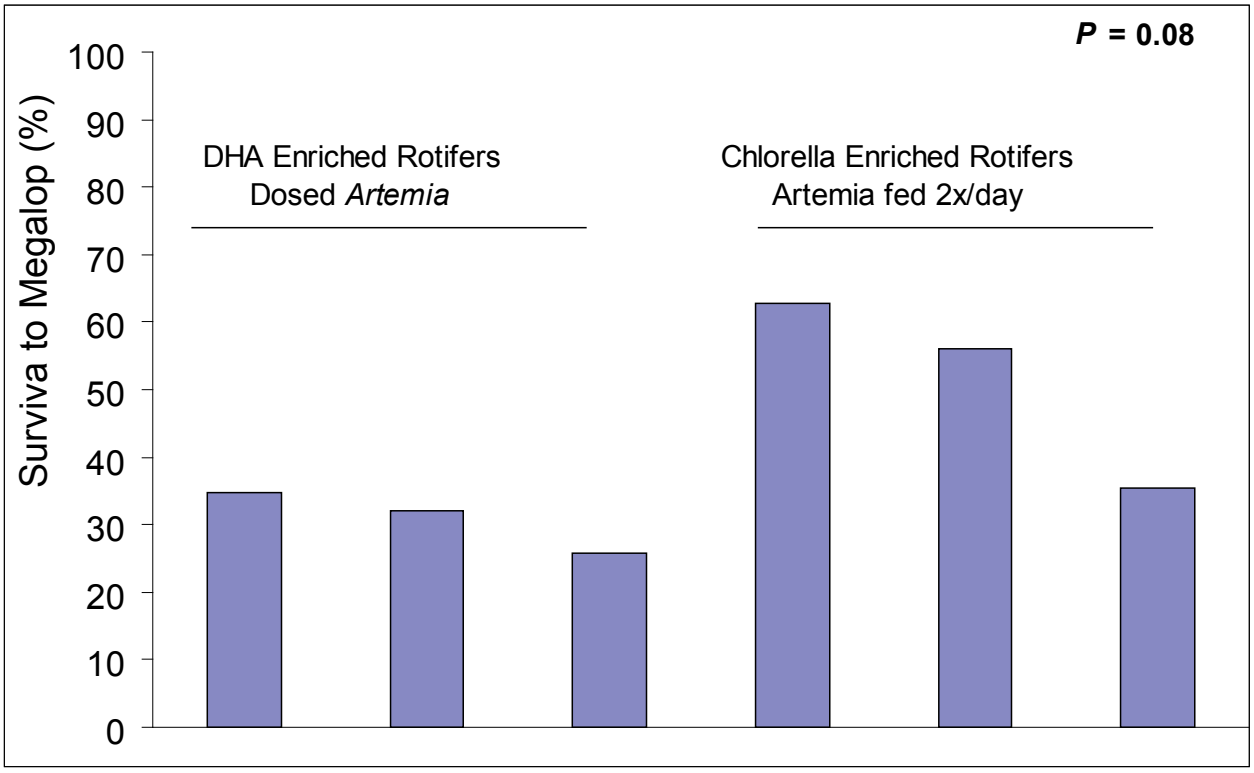

Figure 24. Survival in individual tanks in the semi commercial experiment. (DAC Batch–15).

Discussion

Treatment 1 (*Chlorella* enriched rotifers in combination with two feeds of *Artemia* per day) produced the higher survivals, though not significantly higher than Treatment 2 (DHA enriched rotifers with dosed Artemia). These results have shown that the nutritional requirements of mud crab larvae are not overly specific and provided the basic requirements are met a range of diets may be suitable. This trial involved the comparison of two different factors, enrichment medium and method of application in an attempt to find gross differences. Had there been a significant different between treatments further trials would have been required to separate the role of the individual factors.

Bacterial control

Introduction

The results from the ACIAR mud crab program indicated that control of bacteria was a significant factor impacting upon mud crab larval survival. In particular high levels of *Vibrio* spp. were associated with poor larval survival. However, at the start of this FRDC project our collective understanding of the bacteriology of mud crab systems was poor.

In this project two strategies were used to gain a better understanding of the bacteriology of mud crab larval systems. First, a detailed study of bacteria in mud crab larval systems was commissioned through a PhD scholarship. Secondly, a systems approach was taken to gaining a practical understanding of the direct effects of bacteria in larval production systems, and how they might be better managed and controlled, to obtain more consistent results.

This section of the report details the work and findings of the systems approach.

One of the key needs of this work was to determine which effects in mud crab larval rearing systems were likely to be bacteriological in nature, and which were not. Appropriate tools to experimentally control bacteria had to be found. Preliminary tests showed that the strains of bacteria commonly associated with mortality in mud crab larvae were susceptible to treatment with oxytetracycline (OTC), a drug well known to the animal production industry. In consultation with the Department's Manager of Aquatic Animal Health, it was decided that a relatively high dose (50ppm) should be used to determine if limiting bacteria with anti-microbial agents would have beneficial effects on larval survival and growth.

The anti-biotic oxytetracyline was the primary tool used, together with other disinfectants and water treatments. This antibiotic, which is registered for use in prawn culture in the US, is widely used in many forms of food production. The potential value of probiotics, some developed within the project and other commercially available products, were superficially examined as a potential solution to the bacterial problem.

Once the interactions at a gross level, between bacteria and successful mud crab larviculture were better understood, efforts were then made to come up with commercially feasible solutions to mitigate bacterial contamination by pathogenic strains. Practical solutions to water pre-treatment and quality management, filtration, nutrition, tank shape and hydraulics were found during this study and reported elsewhere, however it was found that successful, consistent, reliable larviculture of mud crabs was possible only once pathogenic bacteria in the systems could be controlled.

Experiments to Investigate the Effect of Bacterial Control on Larval Rearing Success

The effect of the antibiotic oxytetracycline on growth and survival of mud crab larvae (Part 1). (DAC–Batch 16)

Introduction/aim

The following experiment, carried out in June 2002, was designed to assess the effects of OTC on the growth and survival of mud crab larvae in mass cultures and in small-scale rearing.

Water quality

Water for the experiment was stored in two, 20 000 L tanks with black plastic covers (Figure 16). Tanks were connected by a 50mm PVC pipe which allowed water movement between tanks (connector). Filling the tanks was achieved by filtering the water through a 1μm filter bag and an ultra-violet steriliser. Water was introduced to tank 1 only. Water was then drawn into an electric pump that forced the water through a venturi-operated foam fractionator. Water from the fractionator flowed into tank 2. When full, the water re-circulated through the fractionator continuously. Therefore, all storage water in tank 2 had been through the fractionator. Water used to fill the experimental tanks was always taken from tank 2.

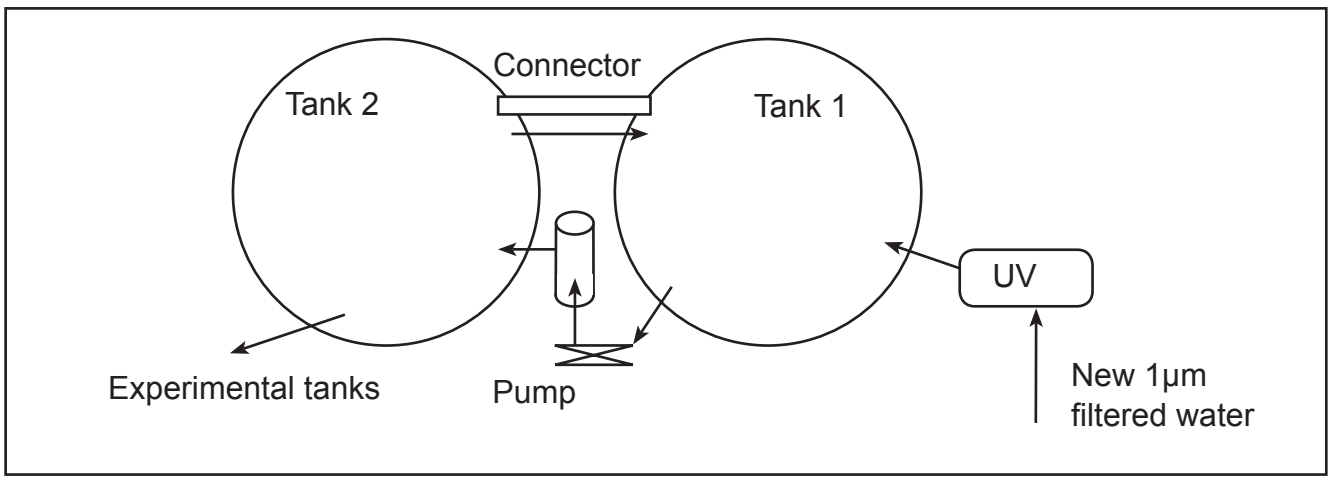

Figure 25. Storage water system for the experiment

Methods–Small-Scale trial

Clear acrylic bowls used to hold cultures (see General Methods) were placed inside an identical bowl which had the outer surface painted black, providing a darkened opaque background.

Water used to fill the bowls was stored overnight in a 100 L plastic tub submerged in the same water bath as the bowls. In addition, a 2L capacity submerged carbon filter powered by a single 15mm diameter airlift, was run for approximately 18 hours prior to use. New water for each day was taken from storage tank 2 and treated as above.

Two experimental treatments with five replicates of each were imposed:

A. A daily addition of 50ppm OTC to the culture water
B. No antibiotics.

The SOP for small-scale trials was followed with 50ppm of OTC added on a daily basis to those cultures receiving that treatment. The five replicates of each treatment were arranged in a completely randomised design.

From stocking until day eight, 1.25 x 104 cells per mL of bag cultured live *Nannochloropsis oculata*, was added to each bowl. In addition, a solution of "Instant algae™" pastes, consisting of *Thalassiosira weisfloggii*, Isochrysis *sp.* (T.ISO) and *Tetraselmis suecica*, was added. The solution was made by combining 20mls of each algal paste in a measuring cylinder. Clean saltwater (30ppt) was then added to double the existing volume. This was then mixed by shaking for not less than 30 seconds before the volume was again doubled with clean saltwater, and again thoroughly mixed. The total volume was then made up to 2L, and 1.5ml of this solution was added to each bowl each day. From day 9 onwards *N. oculata* paste was also added to the above solution but only 20ml was used.

Artemia nauplii were fed at a rate of $3ml^{-1}$ daily from day 4 in a single feed. There was no overlap between feeding of rotifers and Artemia.

The temperature of random bowls was measured each morning at 0800.

Methods–Large-scale trial

Two standard 1000 L fibreglass conical tanks, and two McRobert Aquaculture Systems® tank systems were used. All tanks were designed to operate as re-circulating systems although no biofiltration was included. Water in the tanks was airlifted through a submerged, horizontally mounted 500*µ*m screen to a 100 L reservoir, which contained, a thermostat sensor, a 300-watt bar heater, and a carbon filter. After which the water will overflowed from the reservoir back to the main tank.

Water used in the tanks came from storage tank 2. Each morning the experimental tanks underwent a 70 per cent drain-down top-up water exchange, at which time the walls of all tanks were wiped clean. An AQUACLONE was used to keep larvae in the water column. Before water exchange in the McRobert Aquaculture Systems® tank, the water and larvae were moved to the new sister tank.

Top-up water came from a five tonne tank adjacent to the experimental area. Each day this tank was filled, through a 1*µ*m filter bag, from storage tank 2. A 1KW immersion heater with thermostatic control was used to heat the water to the required temperature before filling the culture tanks.

Tanks were continuously monitored for detritus or mortalities and these were removed as soon as they are noticed. Screens, airlines and airstones in tanks were removed and cleaned in freshwater to remove attached biofilm in tanks at least once daily.

One of each tank type, McRoberts Aquaculture Systems™ and the standard 1000 L tanks, were used in each treatment. In one treatment OTC at 50ppm was administered daily to 1 standard tank and 1 McRoberts tank system, no OTC was added to the two tanks in the other treatment.

Results–Small-scale trial

Temperature of the larval rearing units ranged between 26.9 and 28.8ºC, which is considered appropriate for this species.

No larvae from the control treatment, without OTC reached the megalop stage. Mean survival to megalop in the OTC treated bowls was 26.00 ±3.86 per cent, which was significantly higher than the untreated control ($P < 0.01$). In fact, significant differences were apparent as early as Day four ($P = 0.01$) and this difference remained throughout the experiment. Daily survival is presented in Figure 26.

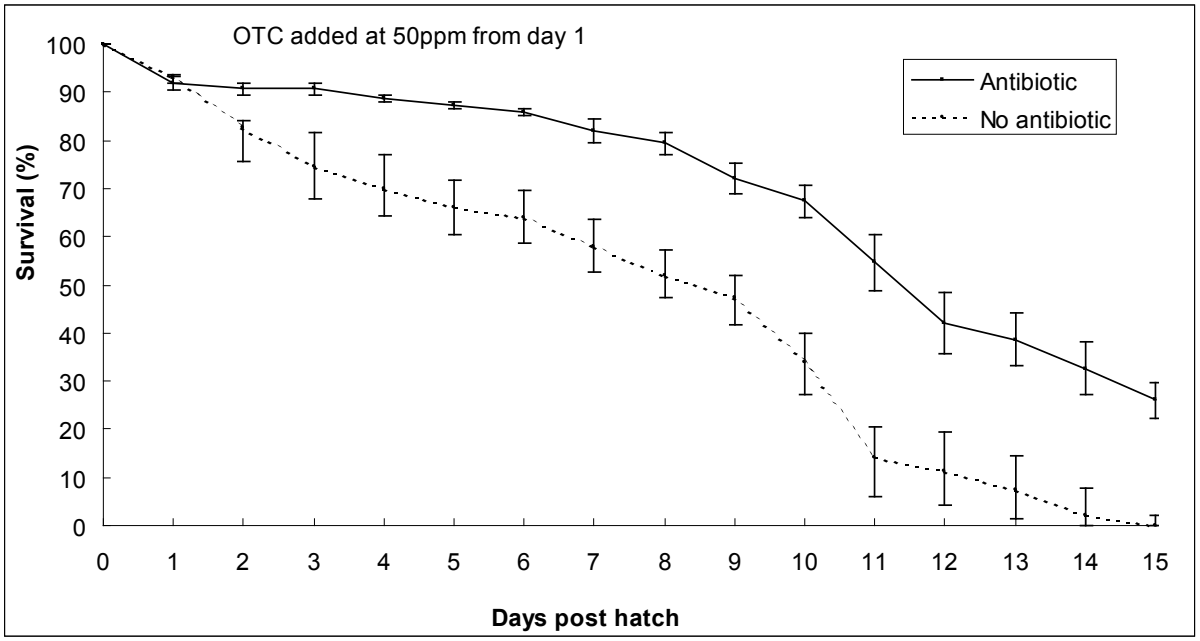

Figure 26. Survival (± S.E.) of *S. serrata* larvae Z1 to megalops with and without daily antibiotic treatment. Significant differences were apparent from day 4. (DAC Batch–16).

Results–Large-scale trial

Crab vigour seemed generally better in the tanks receiving OTC. It was difficult to estimate survival during the larval rearing, as the larvae receiving OTC were able to maintain their position in the tank when aeration was increased in an attempt to evenly distribute the larvae.

On day 9 one of the tanks not receiving OTC suffered substantial mortality and was terminated. The same pattern of mortality occurred in the other un-treated tank and it was terminated on day 12.

Approximately half of the Z5 larvae in the McRoberts Aquaculture Systems™ tank that was receiving OTC, moulted to megalop on day 14. As there was a large number of Z5 larvae remaining, the megalops were left in the tank and it was harvested the following day. Many of the remaining Z5's were dead the following morning and had suffered physical damage probably resulting from megalopa predation. Data for the number of megalops harvested and percentage survival is presented in Table 33.

Table 33. Survival Z1 to megalop for the 4 tanks during the mass culture experiment (DAC Batch–16). McR = McRobert Aquaculture Systems™ tank, Std = standard 1000 L tank.

Day	Tank	Treatment	Number	Survival (%)	Comments
Day 14	McR	OTC	6385	21.28	Many Zoea 5 deaths
Day 10	McR	No–OTC	0		Terminated
Day 13	Std 1	No–OTC	0		Terminated
Day 15	Std 2	OTC	2111	7.04	

Discussion

The results of the small-scale larval trial demonstrated that addition of oxytetracycline (OTC) at 50ppm significantly improved larval survival. It can be hypothesised that OTC may impact upon part of the microbial flora in the larval rearing units, and in doing so improves survival to megalop. There was still a high level of mortality in the treatment receiving OTC, however this may be due to factors other than the microbial community. Alternately a microbe or microbes, which may cause some mortality in mud crab larvae, may be inherently resistant to OTC, and this may have caused the relatively poor survival.

The improved survival with OTC treatment was duplicated in the large-scale experiment. Moulting to megalop was somewhat protracted in both tanks that received OTC and as such survival was reduced by an estimated 50 per cent. The remaining Z5 larvae that did not moult up were preyed upon by those megalops which moulted first. It is estimated that if all larvae had moulted to megalop on the same day, survival may have been as high as 40 per cent in the McRoberts tank and 15 per cent in the standard tank.

A method of screening the larger megalops from the tank whilst leaving the remaining Z5 larvae was suggested as a strategy for minimising mortality due to predation of megalops on Z5 larvae.

The effect of the antibiotic oxytetracycline on growth and survival of mud crab larvae (Part 2). (DAC–Batch17).

Introduction/aims

All materials, methods and protocols are the same as for the preceding trial (Batch 15). The trial was carried out in April, 2002.

The aim of the small-scale trial was to determine the minimum levels of OTC needed to be effective so as to minimise use of OTC.

The aim of the large-scale trial was to determine if removing the larvae at Z4 and thoroughly cleaning the tank would overcome problems thought to be associated with build up of organic matter in the tank.

Methods

Small-scale trial

For the small-scale larval rearing experiment, four experimental treatments were imposed:

A. Daily addition of 50ppm OTC
B. Daily addition of; 25ppm OTC;
C. Daily addition of 10ppm OTC
D. No OTC.

Large-scale trial

On day 9 onwards when most larvae are Z4, larvae from the non-OTC treated tanks were screened from their tank using a 1.4 mm (fly screen) mesh. The larvae from the McRobert Aquaculture Systems™ tank went straight into the newly filled alternate tank in the twin-tank system. Larvae from the standard tank were put into a 100 L tub with new water. The tank was cleaned with detergent and refilled. Larvae were then transferred in water to the new , clean tank. When megalops were found in the tanks, they were screened out with a 1900μm nylon mesh (Australian Filter Specialists), leaving behind the Z5 larvae.

Results

Small-scale trial

Temperature of the experimental units measured at 0800 h remained between 26.6 and 28.7°C and salinity ranged from 34ppt at stocking, down to 27ppt at the end of the experiment.

Table 34. Mean survival (%) (±se) Z1 to megalop for the various treatments.

Treatment	No OTC	10ppm OTC	25ppm OTC	50ppm OTC
Final survival (%)	45.00 ± 6.16	90.48 ± 2.04	92.67 ± 0.66	87.33 ± 5.20

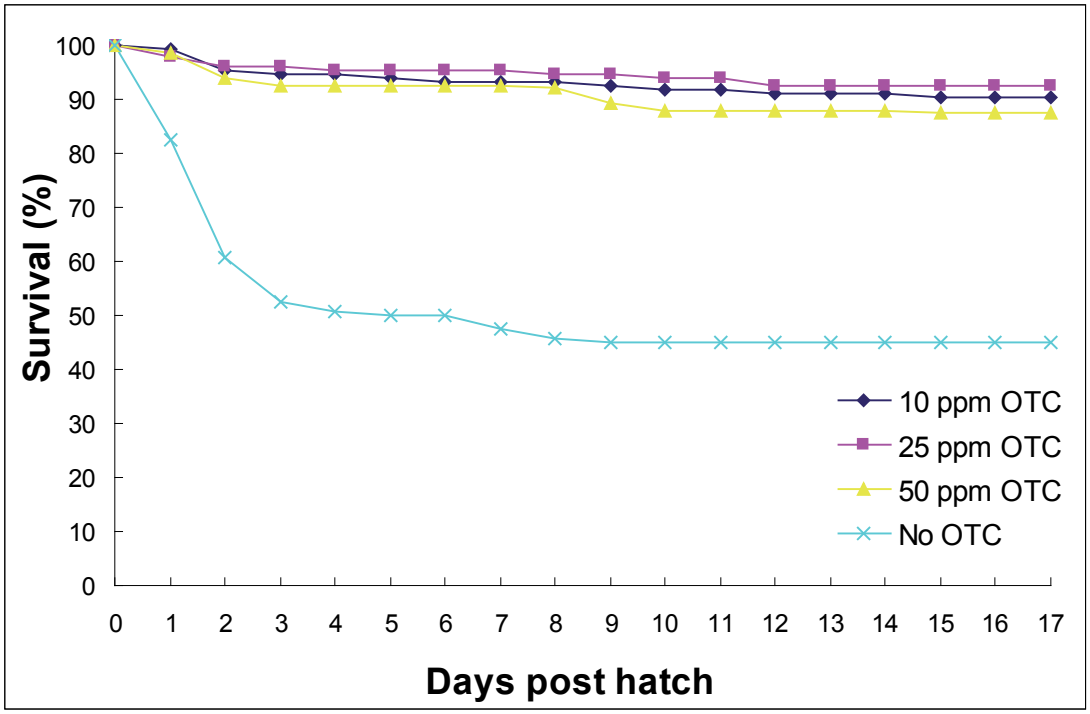

Figure 27. Daily survival Z1 to megalops for the various treatments in the small-scale larval rearing experiment. (DAC Batch–17).

There was a highly significant difference ($P < 0.001$) in survival between treatments with the no OTC treatment having significantly poorer survival than the other treatments (Table 34). There was no significant difference in survival between the any of the treatments receiving OTC. Daily survival data is presented in Figure 27. Ninety-four per cent of all mortalities in the no OTC treatment occurred within the first three days, while rotifers were being fed.

Large-scale trial

Larval vigour seemed generally better in the tanks receiving OTC. It was difficult to estimate survival during the larval rearing, as the larvae receiving OTC were able to maintain their position in the tank when aeration was increased in an attempt to evenly distribute the larvae.

There was a large dropout in larval numbers in the tanks not receiving OTC in the early stages which was then followed by a steady daily decline.

Data relating to the number of megalops harvested from the tanks is displayed in Table 35. Megalops were first harvested on the 14 of May which was day 13.

Overall survival to megalops in one of the OTC treated tanks was almost 45 per cent which was approximately double the survival of the best tank in Batch 15.

Table 35. Number of megalops harvested and zoea 5 larvae remaining at the end of the trial on day 15 (DAC Batch–17).

Date	Tank	Treatment	Number of Megalops	Number of Zoea 5's	Survival to Megalop (%)	Overall survival (%)
14/05/02	Standard tank 1	OTC	4222			
14/05/02	McRoberts tank 2	OTC	600			
15/05/02	Standard tank 1	OTC	5542			
15/05/02	McRoberts tank 2	OTC	5021			
16/05/02	Standard tank 1	OTC	3611	763	44.58	47.13
16/05/02	McRoberts tank 2	OTC	3200	625	29.40	31.49
16/05/02	Standard tank 2	No OTC	379	269	1.26	2.16
16/05/02	McRoberts tank 1	No OTC	77	385	0.26	1.54

Discussion

The excellent survival of the larvae in the small-scale rearing experiment would suggest that the poor survival usually encountered is in fact primarily attributable to bacteria. The results also suggest that the bacterial species responsible for or aiding mortality is susceptible to OTC. Even at 10ppm mud crab larval survival was excellent. A very great proportion of all mortalities in the no-OTC treatment occurred in the first three days, while rotifers were being fed. This would suggest that bacteria associated with the rotifers might be responsible for these mortalities. Once rotifers were removed from the system and the water was fully exchanged, mortalities all but ceased. In fact there were no mortalities from day 9 until the end of the trial even in the no OTC treatment.

The results obtained in the mass rearing experiment were very similar to the previous experiment (Batch 15). Final survival to megalop in Batch 15 was approximately 20 per cent in the best tank. Cannibalism was blamed for a substantial drop in survival over the last night of culture in that batch. The move to a strategy of screening out the megalops from the tank while leaving the Zoea 5 larvae behind, was highly successful and led to greatly improved survival as the Z5 were not preyed on by the Megalops. This procedure will now be adopted for all future batches.

Effects of feeding antibiotic (Oxytetracycline) treated live feeds to mud crab larvae. (DAC–Batch 18)

Introduction

One of the main concerns for antibiotic use is the development of resistant strains of bacteria. In an effort to eliminate the use of antibiotics in mud crab larval culture, and to gain an understanding of where the bacteria are found in the production system, treatment of the live zooplankton feed organisms with Oxytetracycline (OTC) was recommended, along with the preliminary evaluation of probiotics.

It was previously suggested that virulent bacteria might have been introduced to the culture unit with the rotifers. In the previous small-scale larval rearing experiment (Batch 16) there was a marked increase in larval mortalities during the rotifer feeding stage in the no-OTC treatment. In the antibiotic treated bowls survival was not affected.

Numerous strains of bacteria have been isolated from various cultures at the DAC, and two that have been found in OTC treated larvae have been cultured for use as potential probiotics. Some probiotics have been used because they produce bacteriostatic or even antibiotic properties (eg *Bacillus sp.*) while others are used because they reproduce very quickly and can out compete more virulent species or strains (eg *Vibrio alginolyticus*).

The following experiments were conducted to assess the effects on larval survival of treating the live food organisms with OTC, compared to a full time exposure to 50-ppm. They also aimed to assess the effects of potential probiotics.

Methods–Small-scale trial

Comparison of mud crab larval survival rates in larviculture systems using OTC treated feeds, two potential probiotics and dosing with OTC.

The five experimental treatments used were:

A: Daily addition of 50ppm OTC;
B: OTC-treated feeds;
C: No OTC (control);
D: Addition of Probiotic A
E: Addition of Probiotic B.

Using the SOP for small scale trials, OTC and probiotics were added daily to the appropriate bowls, as indicated in the treatments above. There were five replicates of each treatment arranged in a completely randomised design.

Live feeds

Each day, 1.25 x 104 cells per mL of bag cultured live *Nannochloropsis oculata*, was added to each bowl. In addition, a solution of “Instant algae™” pastes, consisting of Isochrysis *sp.* (T.ISO) and *Tetraselmis suecica* was added. The solution was made by combining 40mls of T. *suecica* and 20ml of Isochrysis *sp.* algal paste in a measuring cylinder. Clean saltwater

(30ppt) was then added to double the existing volume. This was then mixed by shaking for not less than 30 seconds before the volume was again doubled with clean saltwater, and again thoroughly mixed. The total volume was then made up to 2.1Ls, and 1.5ml of this solution was added to each bowl each day.

Rotifers were cultured and harvested (SOP) and concentrated into approximately 2000ml^{-1} in two 50 L plastic tubs. To these tubs, 20mls of the T.ISO, and 20mls of the T. *suecica* “Instant algae” pastes were added. To one of these tubs, 2.5g of OTC was also added making a solution of 50ppm. For day 0, the rotifers had been enriched in this way for 24 hours prior to use. On days 1–3 however, only a 3hr enrichment was possible. Rotifers were fed at a rate of 10 rotifers mL^{-1} in a single daily feed. A bacterial suspension was prepared by harvesting the lawn of Pro A or Pro B from a standard petrie dish grown overnight on TCBS media. The bacteria were re-suspended in 10ml of sterile saline and the contents then added directly to the rotifer enrichment tank. This gave a concentration of about 106 cells.mL^{-1}.

The rotifers were then harvested and rinsed as per standard procedures.

Each day, harvested *Artemia* were enriched in two batches at a density of 300ml^{-1} in 300ppm DC DHA Selco (INVE). To one batch 50ppm of OTC was added, while the other was not treated.

Artemia were hatched using standard procedures with the commercial product Hatch Controller, INVE providing almost bacteria free *nauplii*. Boosting was carried out using standard procedures and the commercial product DC DHA Selco, INVE. This resulted in boosted *Artemia* with a relatively low bacterial load.

To try and influence the strains of bacteria growing in the boosting tank, selected bacteria were added at the start of boosting. A bacterial suspension was prepared by harvesting the lawn of probiotic A or B isolates grown overnight on TCBS media. The bacteria were re-suspended in 10ml of sterile saline and the contents added directly to the *Artemia* boosting tank (within 1 hour of setting). This gave a concentration of about 106 cells.mL^{-1}.

After boosting and inoculation with either Pro A or Pro B, the *Artemia* were harvested and rinsed as per standard procedures for feeding to the larvae. A sample of the concentrated *Artemia* was taken for bacteriology prior to feeding the *Artemia* to the larvae.

Water quality

Temperature and salinity of random bowls was measured each morning at 0800.

Statistical analysis

Survival data was analysed by one-way ANOVA followed by an LSD comparison of means test.

Methods–Large-scale trial

Comparison of mud crab larval survival rates in larviculture systems using OTC treated feeds with systems using 50ppm of OTC in the culture water.

Two standard 1000 L fibreglass conical tanks, and two McRobert tank systems were used although the McRobert tanks were used as static tanks, with no daily exchange to a cleaned lined tank as in some other trials. All tanks were operated as re-circulating systems, although

no bio-filtration was included. Larvae were stocked at 30 L^{-1} on day 0. Algae was added to the culture system at the same density as the preceding small-scale trial.

Tanks were managed in the same way as Batch 16, including daily removal of larvae for tank cleaning after Z4 and megalops harvesting.

One of each tank type, McRobert and standard fibreglass1000 L tanks, were used in each treatment. OTC was added at a rate of 50ppm to one 1000 L tank and one McRobert tank each day after water exchange. The other treatment received OTC treated feeds as outlined above in the previous small-scale trial (with the exception that no probiotic was added).

Results–Small-scale trial

Water temperatures ranged from 21.7ºC to 28.9ºC at 0800 h and averaged 25.8ºC throughput the experiment.

Data relating to survival to megalops for the various treatments is presented in Table 36 and Figure 28. There was a highly significant difference ($P < 0.001$) between survival in the 50ppm OTC treatment and the other treatments, which were not significantly different from one another.

Table 36. Survival to megalop (%) (±se) for the various treatments in the small- scale larval rearing experiment. Values with different superscripts are significantly different ($P < 0.05$).

Treatment	No OTC	50ppm OTC	OTC treated feeds	Probiotic A	Probiotic B
	13.91 ± 2.22b	82.50 ± 3.31a	29.33 ± 12.2b	25.25 ± 9.49b	27.33 ± 9.04b

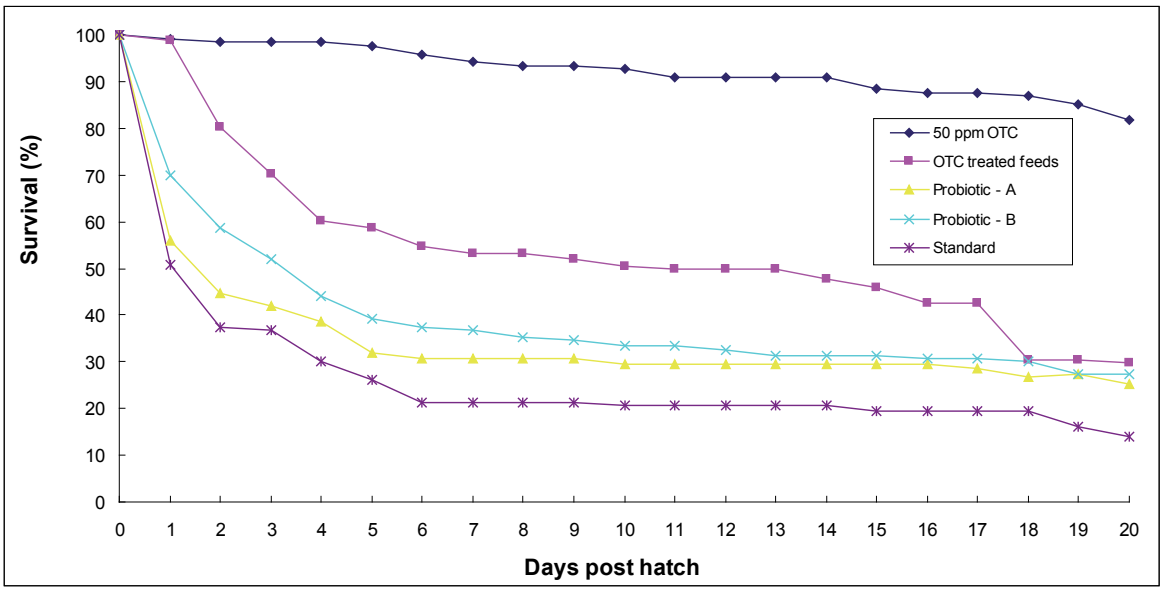

Figure 28. Daily survival for the treatments in the small-scale larval rearing experiment. (DAC Batch–18).

Results–Large-scale trial

On day 3, all tanks developed a very viscous mucous which trapped algae, rotifers and even some larvae. Some of the larvae had died while attempting to moult.

On day 14, small numbers of dead larvae were seen for the first time in the OTC treated feeds tanks. These were found not to be suffering from MDS; damage to the dorsal spine and “cloudy” haemolymph suggested bacterial infection may have been the cause. Daily screening to remove megalops may have caused the damage. There was subsequently a large drop-out on day 15 in this treatment and almost a 100 per cent crash in both of the tanks on day 16 when the larvae would be expected to moult to megalops.

On day 17 the 50ppm OTC-treated culture water tanks were harvested. Almost 11000 megalops were harvested from the McRobert tank and 5100 from the 1000 L fibreglass tank.

Discussion

The OTC treated feeds did not provide any significant improvement in larval survival to megalops compared to No OTC live feeds treatment(control) in the small-scale trial. So even though much of the bacterial contamination may be associated with the live feeds, the treatments utilised to treat the feeds in this trial were not successful in reducing mortalities associated with bacteria. Whilst this trial alone does not indicate a causal link, between bacteria associated with the feeds and mortalities, the improved larval survival from the OTC treated tanks would indicate a likely bacterial link to the mortalities. The probiotics treated feeds also did not give significantly improved survival over the control

The large-scale trial comparing OTC treated tanks with OTC feeds indicated that similar results could be expected at a semi-commercial scale, with significant numbers of larvae only being produced from the OTC treated tanks.

Observations of damage to larval crabs in the latter phases of the large-scale cultures may have been attributable to the screening of tanks to move larvae after day 8. This result suggests that the less screening of larvae the better, although this has to be balanced against improved survivals obtained from screening of megalops from mixed cultures with Z5 larvae and the value of larvae being moved into clean tanks.

A preliminary trial of a potential probiotic on survival of mud crab larvae in large-scale culture. (DAC–Batch 19)

Introduction

Several bacterial isolates, potential probiotics, were identified by Morris Pizzutto, a Ph.D. student working on this project. These isolates were taken from previously successful larval rearing experiments and were subsequently cultured.

Aim

To determine if a potential probiotic bacteria, (Pro A), added to the live feeds would increase mud crab larval survival in a larger scale trial

Methods

The methods used were as per standard operating procedures for large-scale culture.

Water treatment

Water used in this trial had been stored in a covered tank for 10 days prior to its use. Ten days prior to hatch, water storage commenced. Ultra-filtered (5μm) water was passed through a 1μm filter bag and an Ultra Violet treatment unit. This water was then pumped through a venturi operated foam fractionator and was diverted to various storage tanks so that culture water throughout the experiment was stored for at least 10 days. The day prior to its use, the water was pumped to a tank adjacent to the experimental tanks and water quality was adjusted to approximate the salinity and temperature of the culture units.

Six 1000 L tanks were used. The two treatments (with and without Pro A) had three replicates each. These tanks were arranged as two parallel rows of three under an outside shade structure at the DAC. All tanks operated as re-circulating systems, although no biofiltration was included. Water in the tanks was airlifted through a submerged, horizontally mounted 500μm screen and directed to a side mounted 700mm length of 90mm PVC pipe angled down at 45° (the “Bazooka”, Figure 29). Previously water was lifted several centimetres higher into a 100 L bio filter tub. On the end of this bazooka was a 45° elbow and reducers to a 32mm terminal piece of pipe. This pipe-work generated a constant circular flow around the tank. An AQUACLONE was used to keep larvae up in the water column. At Z3 there was a switch to 600μm screens.

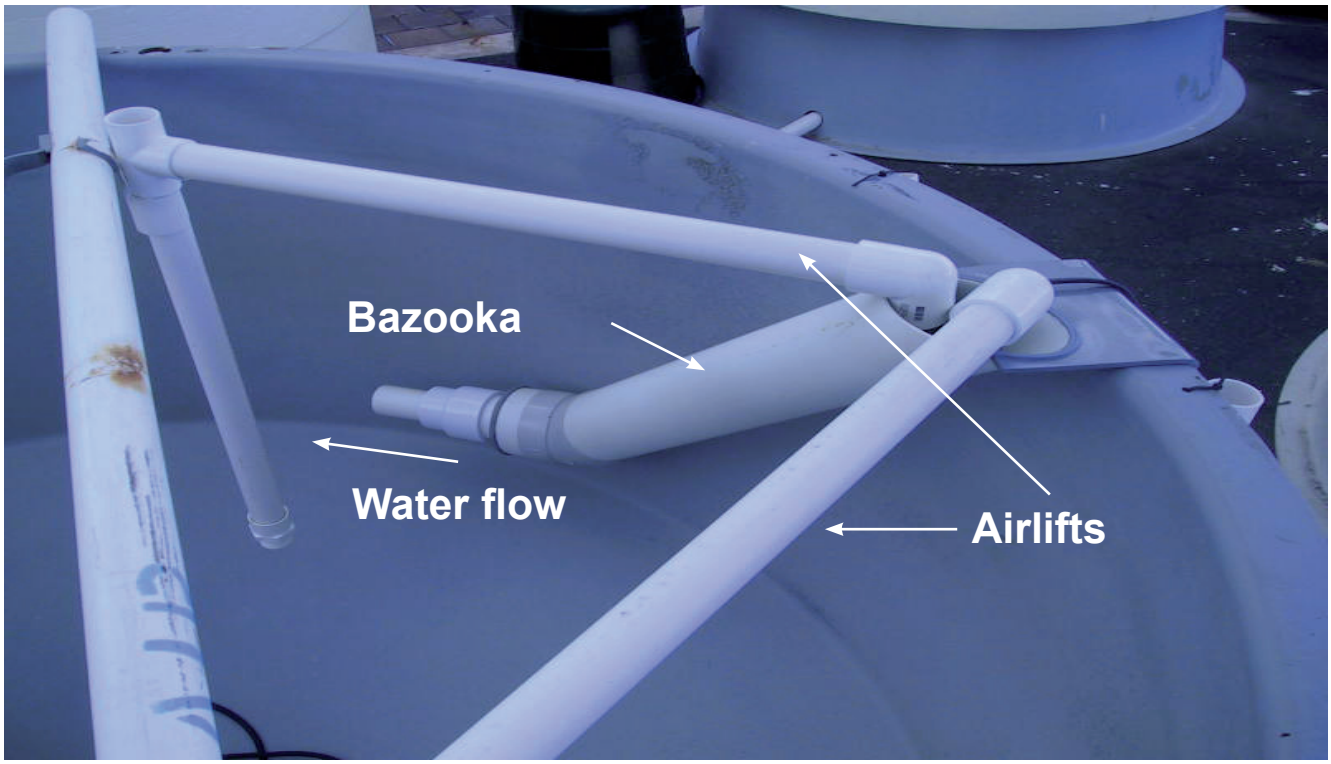

Figure 29. The “Bazooka” in situ.

Two 300-watt immersion heaters were placed inside the Bazooka in order to heat the water without directly contacting the larvae. Temperature of the culture water ranged between 26.0°C and 28°C from Day 0 to Day 9. From Day 10 on temperature was maintained at 29°C (± 0.1) by a thermostatically controlled immersion heater.

From day 0 to Z3, experimental tanks underwent a daily 70 per cent drain-down, top-up water exchange, at which time the walls of all tanks were wiped clean. Screens, airlifts and the Bazooka were removed prior to drain down. The remaining water was then foam fractionated for 30 minutes, followed by foam fractionation & flow through with storage water for another 30 minutes. Tanks were then refilled with storage water. One fractionator was shared between two tanks. After fractionation of one tank had finished, the fractionator was hooked up to the second tank and was used to drain it (effectively flushing the unit). This tank was then fractionated as above. The fractionators were flushed with freshwater each day after use.

At Z4 all larvae were screened from the tanks into 100 L tubs using 1410μm and 850μm nylon mesh screens. The tanks were fully drained, cleaned with detergent, rinsed, and refilled with storage water i.e. 100 per cent water exchange. Larvae were then restocked to their original tanks

Survival was assessed on day three and day 6 by increasing aeration in the culture units in an attempt to evenly distribute the larvae. Ten 800ml samples were then taken from each tank and all larvae were counted and returned to the tanks.

Live feeds

Rotifers produced using the standard procedure were treated overnight in two 70 L tubs prior to feeding to the larvae. These tubs were previously filled with UV treated 5μm filtered water. To one of these tubs, 2.5 x 106 cells mL^{-1} of Pro-A was then added. From 1 day prior to hatching, to Day 2 of larval rearing, 90 million rotifers were harvested by draining through

a submerged 64*µ*m screen. The rotifers were then rinsed in 5*µ*m filtered seawater and equally divided between each tub. Then 50ml of the *Chlorella* V 12 paste and 3.5g of OTC (50ppm) was then added to each. The following morning (ie. the day of feeding) 5ml each of *Thalassiosira weisfloggii*, *Tetraselmis sp.* and T.ISO "Instant algae" pastes were added, and the rotifers were enriched. Rotifers were then harvested, rinsed and fed at a rate of 10 rotifers mL^{-1} in a single daily feed.

First instar AF 430 *Artemia* were fed on day 2 of Z2 and day 1 of Z3. These were hatched in static tanks of sterilised seawater. Water was sterilised with 200ppm of active chlorine for 60 minutes and de-chlorinated with a sodium thiosulphate solution. 100ppm of INVE Hatch controller was added to the hatcher. The following morning the *nauplii* were harvested and restocked to a 20 L bucket. The Pro-A treatment *Artemia* were bathed in the probiotic (2.5 x 106 cells mL^{-1}) for 1 hour, and the standard treatment were held in UV treated seawater.

From Z3 day 2, *Artemia* fed to the tanks were 48 hour (second instar) enriched. The same hatching method as above still applied, however after 24 hours the *nauplii* were harvested, rinsed and restocked to 20 L buckets of sterilised seawater. Both of these received 6ml of (INVE) DC DHA Selco and 50ppm of OTC. The Pro-A treatment also received 2.25 x 106 cells mL^{-1} of Pro-A.

The generalised feeding regime is presented in Figure 30.

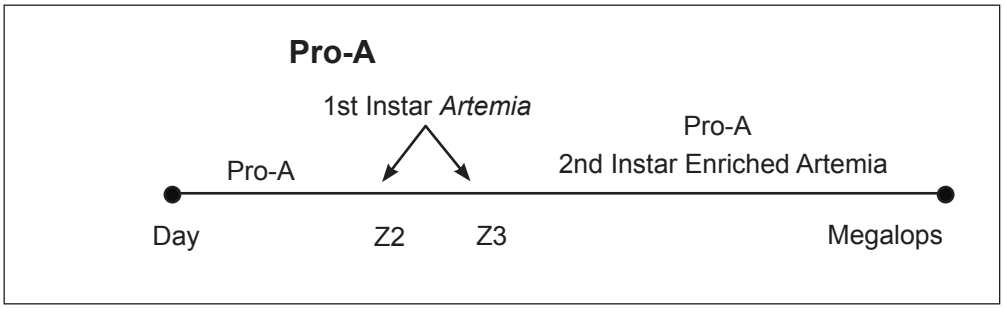

Figure 30. Generalised feeding regime for the current experiment.

Results

Larvae seemed healthy and vigorous after hatching and during the first days of culture. However several thousand larvae were seen to be sticking to the submerged horizontal screens. This may have resulted from increased water flow through the screens from using the original air pressure and volumes, but with a decreased lift height or from the larvae being too weak to move away.

Survival at days three and 6 is presented in Figure 31. There was no significant difference in survival between treatments at either sampling. All tanks suffered heavy mortalities on day 10, one day after screening, and many of the carcasses showed damage to the dorsal and lateral spines and a cloudy haemolymph. All tanks were subsequently terminated on day 11 due to very low numbers.

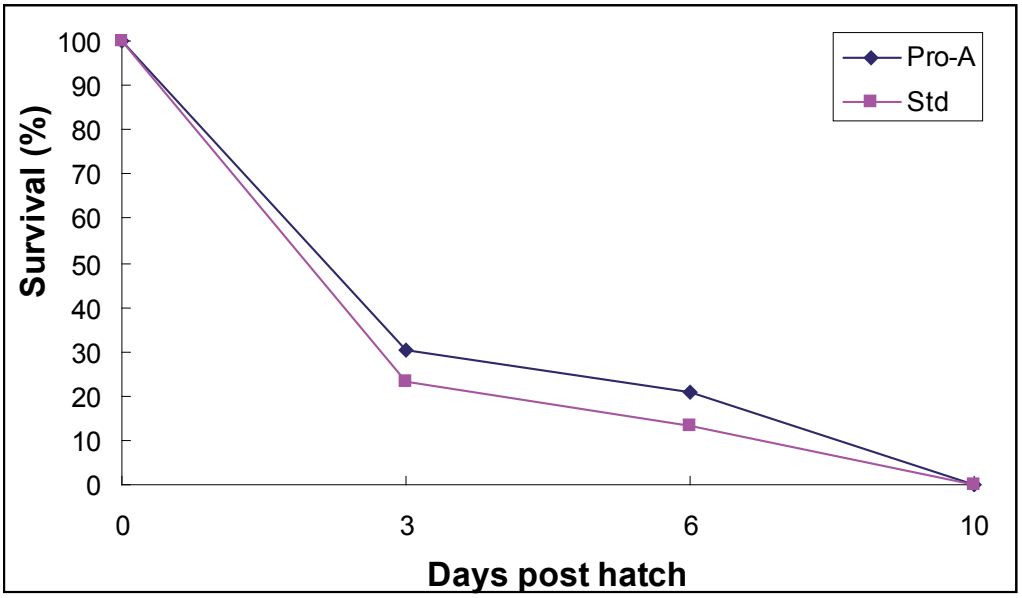

Figure 31. Survival (%) of larvae in the two treatments of this experiment. (DAC Batch–19).

Discussion

Further development and assessment of putative probiotics on a small-scale, including bowl trials, should precede any further large-scale rearing trial. In addition wherever possible the control treatment should be one which achieves routinely good larviculture results for comparison, unlike this trial where the un-treated control performed poorly as well.

This trial indicated that tank design and flow-rates through tanks are critical when dealing with mud crab larvae. The problem experienced in this trial with larvae being stuck to screens may be overcome through a combination of reducing mesh size so that larvae do not become lodged in holes and reducing flow through rate.

Influence of OTC, Betadine, chlorinated water on larval survival in bowls. (DAC–Batch 20)

Introduction

The use of other anti-microbial products including antiseptics such as povidone–iodine (Betadine) was suggested as a means of controlling bacterial communities in mud crab larviculture. Also the selective use of antibiotics for treatment rather than prophylactic use was put forward as a an alternative method of use of these chemicals.

Methods

The methods used were primarily as listed in SOP for small-scale trials. Water used to fill the bowls was stored overnight in either a 40 L plastic bucket or a 100 L plastic tub submerged in the same water bath as the bowls. New water for each day was taken from the storage tank adjacent to the mass rearing area. The 40 Lbucket was treated with 15ppm active chlorine for approximately 20 h before use.

Six experimental treatments (5 reps of each) were used. Continuous baths of povidone-iodine at 0.5ppm, 1.0ppm and 2.0ppm, and an untreated control, as well as a chlorinated water treatment (without iodine) and a “Dip” treatment. The dip treatment involved a daily dip in 300ppm formalin for 30 seconds, immediately followed by a 30 second dip in 50ppm povidone-iodine. There were five replicates of each treatment arranged in a completely randomised design.

Rotifers cultured and harvested using the standard method. Forty mls of the *Chlorella* V12 paste and 3.5g of OTC (50ppm) were then added to both during a four hour enrichment process. For the following three days rotifers were enriched with *Chlorella* V12 and OTC overnight.

Prior to feeding, rotifers were harvested, thoroughly rinsed and fed to the OTC tanks at a rate of 10 rotifers mL^{-1} in a single daily feed.

Artemia for the trial were produced, harvested and enriched as described in the standard protocol.

The generalised feeding regime is presented in Figure 32.

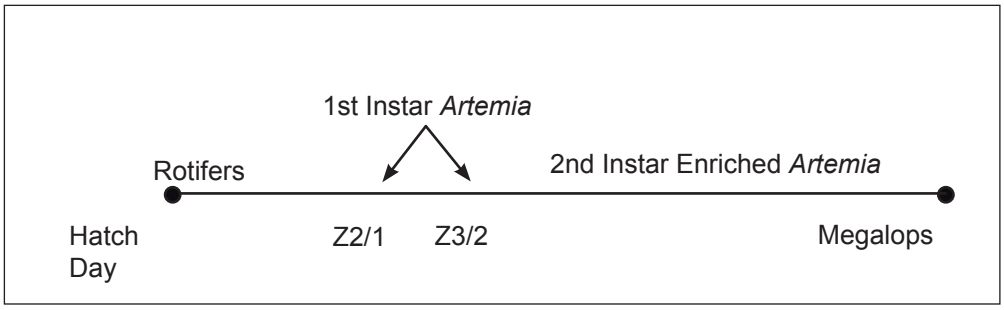

Figure 32. Generalised feeding regime.

Results

Temperature throughout the small-scale trial ranged between 26.3 and 31.1°C, and averaged 29.4°C at 0800hrs. Data relating to survival throughout the experiment is graphically presented in Figure 33, while mean survival to megalop for the various treatments is presented in Table 37.

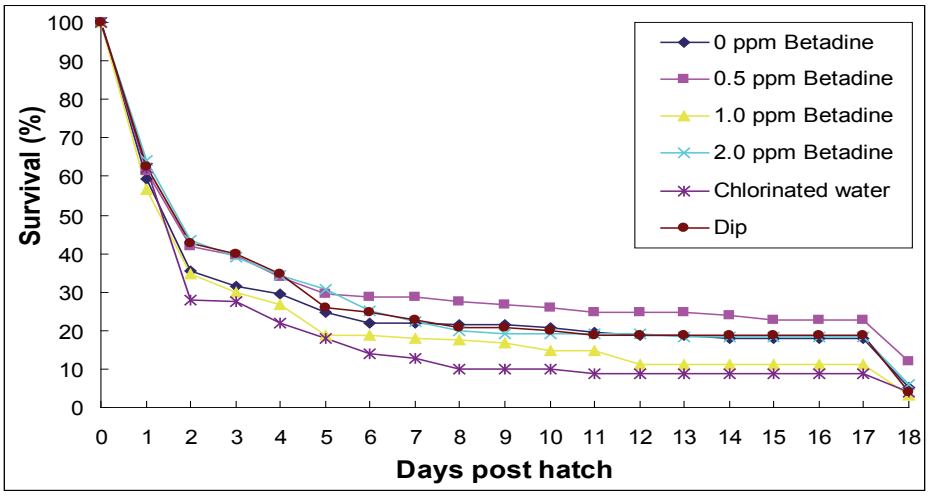

Figure 33. Daily survival of the mud crab larvae throughout the experiment. (DAC Batch–20).

The majority of the mortalities occurred within the first two days, however there was a significant difference ($P < 0.01$) in survival to megalops for the various treatments, with the 0.5ppm betadine treatment out performing the others.

Table 37. Survival (%) (±SE) to megalops for the various treatments in the small-scale larval rearing experiment.

Treatment	0ppm Betadine	0.5ppm Betadine	1.0ppm Betadine	2.0ppm Betadine	Chlorinated water	Dip
Survival (%) (± SE)	5.33 ± 2.00[a]	12.00 ± 0.82[b]	3.33 ± 1.05[a]	5.83 ± 0.83[a]	4.00 ± 0.67[a]	4.00 ± 0.67[a]

Discussion

Given the less than optimal conditions the newly hatched larvae were subjected to in this trial (as a result of the larvae hatching earlier than expected) including a lack of food for a considerable duration, long term crowding, possible poor water quality, high organic loads and probable microbial proliferation, the results of the small-scale experiment should be viewed with caution. Had the larvae been removed from the hatching tank immediately after hatching and provided with food and better water quality, the initial poor larval survival may not have occurred. There may also have been a long term effect on larval vigour and general quality as a result of poor conditions for a period after hatching. This in turn may have affected the treatment effects and as such the results yielded may be erroneous. Also the larvae in the bowls were not counted on days 16 and 17 and any Z5 larvae which moulted to megalops in the bowls during this period would have preyed on the remaining zoea.

The significantly higher survival of the 0.5ppm iodine bath over the other treatments is difficult to explain. Perhaps the 0.5ppm concentration was enough to inhibit potential bacterial pathogens without having a direct effect on larval health. The two higher concentrations, 1ppm and 2ppm, may have directly effected the larvae as a toxin. It is unlikely that 0.5ppm of iodine is enough to inhibit bacterial growth and the low overall survival in the experiment makes the result doubtful at best, especially as there was no significant difference in survival up to day 15. The experiment should be repeated with better quality larvae.

Large-scale trial:

Comparison of continuous dosing of mud crab larvae with OTC, with a system where its use is limited up to the Z2 stage, after which flow-through of settled water is used (part 1). (DAC–Batch 21)

Introduction

The use of OTC in mud crab larviculture trials significantly improved survival rates, indicating that bacteria were likely a major contributor to larval mortality. As bacterial growth is highly dependent on organic matter in larviculture systems, one strategy for minimising bacterial growth is to reduce organic loads in the systems. One method of achieving this is through water exchange.

Methods

In the following large-scale experiment, survival of mud crab larvae was compared between a constant oxytetracycline (OTC) bath treatment and a treatment that involved a similar bath but only to Z2, followed by constant water exchange without OTC addition.

Six standard 1000 L tanks were used. These tanks were arranged as two parallel rows of three under an outside shade structure at the DAC. All tanks operated as re-circulating systems, although no bio-filtration was included. Tanks were run as per the standard method for large-scale trials.

From stocking until Z2/2, both treatments underwent a 70 per cent drain-down, top-up water exchange, at which time the walls of all tanks were wiped clean. Screens, airlifts and the Bazooka were removed and cleaned prior to drain-down. Tanks were drained through the foam fractionator, which served to flush the units each day before use. The remaining water was then foam fractionated for 30 minutes, followed by foam fractionation & flow through with storage water for another 30 minutes. Tanks were then filled with storage water. OTC was added at 50ppm to the OTC treatment every day at 15:00. From Z2, day 2, the flow through water treatment began at 1.4 $lmin^{-1}$ equivalent to two full exchanges per day, and the OTC was not added to these tanks (Figure 34)

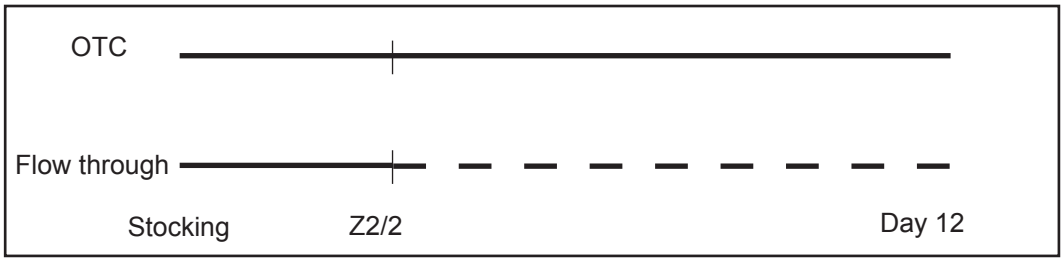

Figure 34. Treatments in the current experiment. Solid line denotes OTC addition at 50ppm, Dotted line denotes flow through without OTC addition.

All tanks received 30 000 larvae or 30 L^{-1} at stocking. Selective harvesting using a 2000μm screen was initiated when megalops were first observed in the larval tanks.

Results

Data relating to the megalops production and survival from the experimental tanks is presented in Tables 38 and 39. A total of 83 079 megalopa were produced from all tanks, however there was no significant difference (P > 0.05) between treatments for the number of megalops produced, or for survival. That was consistently high averaging 40 per cent in flow–through treatment and 53 per cent with continuous exposure to 50ppm OTC.

Table 38. Number of megalopa produced and survival (%) from the experimental tanks (DAC Batch–21).

Tank/Treatment	24 Oct 02	25 Oct 02	Total produced	Survival (%)
T1–OTC	6929	10929	17858	59.5
T2–OTC	6143	12143	18286	61.0
T4–OTC	1700	9429	11129	37.1
T3–Flow through	5027	4101	9128	30.4
T5–Flow through	8786	3861	12647	42.2
T6–Flow through	7941	6090	14031	46.8

Table 39. Summary of results for OTC versus flow-through.

Treatment	Total number	Mean Survival (%) to Megalops
Flow through	35807 a	39.78 ± 4.86 a
OTC	47271 a	52.52 ± 7.73 a

On average, the flow through tanks yielded 60.37 per cent of the total megalops harvest on the 24 of October (first day of megalops), whereas the OTC yielded 29.2 per cent on the same day. These percentages were significantly different (P = 0.021), and indicated that the average developmental stage of the larvae in the OTC treated tanks was marginally behind that of those in the flow-through tanks.

Discussion

This result indicates that OTC usage can be limited to the second day of Z2, without significant impact on the production of mud crab megalops in large-scale larviculture systems. It was also interesting to note that the results indicated that treatment with OTC may slow or limit growth and development of larvae, compared to those grown without it.

The use of a more sophisticated larviculture system (as used in this trial), that incorporates removal of organics using a foam fractionator, temperature control, improved circulation and keeping larvae in suspension, is considered critical in maximising survival rates. especially during the early z1 to z2 rotifer feeding stages, and hence maximising yields of vialble megalops.

Large-scale trial:

Comparison of continuous dosing of mud crab larvae with OTC, with a system where its use is limited up to the Z2 stage, after which flow-through of settled water is used (part 2). (DAC–Batch 22)

Introduction

This trial was a repeat of DAC Batch 21, designed to compare survival of mud crab larvae between a constant oxytetracycline (OTC) treatment and a treatment that involved OTC treatment to Z2, followed by constant flow-through without OTC addition.

Methods

All materials and methods are the same as for the previous trial, but repeated with a different batch of mud crab larvae during December 2002.

Results

Temperatures recorded at 08:30 daily varied between 29.4 and 30.6°C, pH ranged between 7.42 and 7.96, dissolved oxygen was always over 5ppm and salinity was maintained between 28 and 30ppt throughout the experiment. On day three and on day 7, afternoon tank temperatures reached over 32°C due to high ambient temperatures although these were reduced to within normal ranges by the following morning.

Survival data relating to yields of megalops are presented in Table 40. A total of 73 819 megalops were produced. As was noted in Batch 21 larvae in the OTC treatment were slower growing and moulting to megalops, compared to the flow- through treatment.

Table 41 shows the survival to megalops for individual replicates from batches 21 and 22. This pooled data demonstrates that there was no significant difference ($P = 0.97$) in survival when OTC was used only in the rotifer feeding stage followed by flow through water exchange (flow-through treatment), compared to its use throughout the larval rearing period (OTC treatment) in combination with batch water exchange.

There was however a highly significant difference ($P < 0.001$) in the proportion of all megalops harvested on the first day of appearance of megalops. On average (combined data from Batches 21 and 22) the flow-through treatment yielded 71.0 ± 5.79 per cent on the first day compared to just 27.33 ± 4.05 per cent for the OTC treatment.

Table 40. Megalops harvest numbers and overall survival. (FT = flow-through treatment, OTC = continuous OTC treatment) (DAC Batch–22).

Date	Tank	Total No.	Survival (%)
12 Dec. 02	T2–FT	13681	
	T3–FT	11111	
	T6–FT	10208	
	T1–OTC	2847	
	T4–OTC	1458	
	T5–OTC	3750	
13 Dec. 02	T2–FT	1597	50.93
	T3–FT	1944	43.52
	T6–FT	4306	48.38
	T1–OTC	8056	36.34
	T4–OTC	7639	30.32
	T5–OTC	7222	36.57
Overall	FT	42847	47.61 ± 2.17
	OTC	30972	34.41 ± 2.05
Total		73 819	

Table 41. Percentage survival to megalops for the individual replicates and overall survival (± se) for the two treatments using combined data from two batches of larvae.

Batch	OTC	Flow Through
21	59.52	30.43
21	60.95	42.16
21	37.10	46.77
22	36.34	50.93
22	30.32	43.52
22	36.57	48.38
Overall	43.47 ± 5.40 a	43.70 ± 2.96 a

Note: Same superscript indicates that values are not significantly different.

Discussion

The results of the large-scale trial were very similar to the previous experiment (Batch 21) and again demonstrated that mud crab larvae can be reared with high survival through to megalops if OTC treatment is used only during the rotifer stage, and is then followed by a constant flow-through water exchange. Whether constant water exchange is necessary is yet to be determined. As this technology has now been applied to several batches of larvae

yielding similar results, it can be described as a reliable method of producing mud crab megalops. Further it demonstrates that bacterial control is most critical during the rotifer feeding stage of larviculture, highlighting the outstanding need to develop a production technique that is not antibiotic dependent during the first few days of culture.

Small-scale trial:

The effect of different temporal OTC treatments on mud crab larval survival (part 1). (DAC-Batch 23)

Introduction

This trial was run to further investigate possible ways to further investigate possible ways of reducing or eliminating the use of OTC in the production of mud crab larvae.

Methods

This experiment was conducted in three parts.

Six experimental treatments were imposed. The treatment dosage of OTC was 50ppm. In treatments in which it was used, OTC was provided in a single daily dose.

A: Control, to which no OTC was added
B: OTC treatment ceased at Z2
C: OTC treatment ceased at Z3
D: OTC treatment ceased at Z4
E: OTC treatment ceased at Z5
F: OTC treatment ceased at megalops

There were five replicates of each treatment arranged in a completely randomised design.

Live feeds

Four algal paste species (Instant-algae, Reed Mariculture Pty Ltd, USA) were provided to the larval rearing tanks as tabulated below on a daily basis.

Table 42. Algal species and densities used

Species	Final density per day (cells mL^{-1})
Thalassiosira weisfloggii	1.33 x 104
Nannochloropsis oculata	1.66 x 104
Isochrysis galbana–Tahitian strain (T. iso)	1.66 x 104
Tetraselmis sp.	1.66 x 104

Rotifers and *Artemia* produced under SOP were provided at usual densities, although the rotifers were treated with 50ppm OTC.

Results

Temperature ranged between 28.6 and 30.3°C; salinity remained between 29 and 31ppt; and pH ranged from 8.01–8.08.

Data relating to survival on days two, three and twelve is presented in Table 43. There was a highly significant difference in survival ($P < 0.001$) as early as day 2 with the treatment not receiving OTC having significantly lower survival than all the other treatments. The remaining

treatments were not significantly different from one another. These results are repeated on day three ($P < 0.001$) and on day 12 ($P < 0.001$), although the treatments receiving OTC did sustain substantial mortality as the trial progressed. Daily survival throughout the experiment is graphically presented in Figure 35.

Table 43. Per cent survivals of larvae with different OTC treatments. Data in columns with same superscript are not significantly different ($P > 0.05$).

Survival (%)			
Treatment	Day 2	Day 3	Day 12
No OTC	86.00 ± 1.94 a	72.67 ± 4.40 a	6.00 ± 2.21 a
OTC to Z2	96.66 ± 2.58 b	96.00 ± 2.67 b	46.00 ± 11.42 b
OTC to Z3	95.33 ± 1.70 b	95.33 ± 1.70 b	56.00 ± 9.79 b
OTC to Z4	92.66 ± 3.40 b	90.67 ± 3.56 b	69.33 ± 7.70 b
OTC to Z5	97.33 ± 1.25 b	97.33 ± 1.25 b	61.33 ± 6.96 b
OTC to Meg	95.33 ± 2.26 b	94.67 ± 2.26 b	58.67 ± 7.71 b

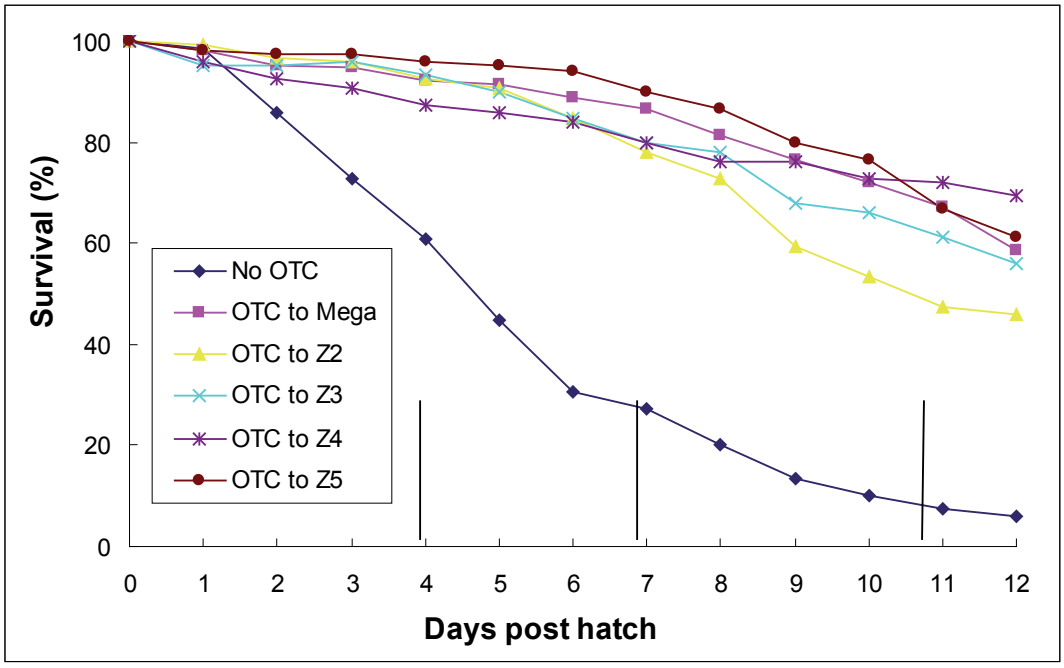

Figure 35. Daily survival (%) of mud crab larvae after different temporal treatments of OTC at 50ppm. Vertical lines adjacent to the X-axis signify the various larval stages. (DAC Batch–23).

As this experiment was prematurely terminated on day 12 of what should have been a longer trial, then three treatments, OTC to Z4, OTC to Z5 and OTC to megalops are actually replicates of one treatment, OTC to Z4.

Other than the “No OTC” treatment whose survival was reduced from day 2 onwards, the remaining treatments showed the same general pattern of low level daily mortality regardless of whether OTC was being supplied or not. The “OTC to Z2” treatment was not significantly different to the remaining treatments at day 12, even though it had not received OTC since day 4, while the “OTC to Z5” and “OTC to megalop” were still receiving the antibiotic.

Discussion

The significant differences in larval survival in the early stages of this experiment again indicate that it is likely that larvae are exposed to highly virulent bacteria within the system soon after stocking. It can be seen from Figure 35 that there was minimal mortality in those treatments receiving the OTC treatment, whilst the “No OTC” treatment suffered losses in the order of 10 per cent day^{-1} up until day 4.

A likely source of potentially harmfully bacteria is rotifers, being the first live food organism offered. Rotifers are typically included in the feeding regime up until day 2 of Z2 (see Batch Trials 5, 6 & 9), as was the case in this experiment. When rotifers were removed from the feeding regime, which coincided with the discontinuation of the OTC in the “OTC to Z2” treatment, mortalities did not significantly increase compared to the other treatments still receiving OTC. It would appear likely that the rotifers were the source of the virulent bacteria responsible for the mortality. The two treatments that reached the point of OTC discontinuation “OTC to Z2” and “OTC to Z3” performed numerically poorest, although not significantly so, up until the end of the experiment. This may indicate that even though 100 per cent water exchange was being carried out during the trial, virulent bacteria were being sustained in the cultures. The most obvious continuing source of the bacteria is on the larvae themselves.

As the results of this trial again support the conclusion there is no significant value in continuing to treat with OTC beyond Z2, it confirms earlier results (DAC Batchs 21 & 22) linking significant mortalities to the first few days of culture, notably when rotifers are being fed. This has two major implications. Firstly, there is no reason to utilise OTC treatment beyond the Z2 stage, and secondly that more effort needs to be targeted on controlling bacterial infection associated with feeding rotifers and the first two larval stages.

Small-scale trial:

The effect of different temporal OTC treatments on mud crab larval survival (part 3). (DAC–Batch 26)

Introduction

This trial was run to further ensure that the apparent need for OTC treatment in relation to the rotifer feeding period (Z1–Z2) is not batch dependent.

Methods

All protocols were the same as for (Batches 23 & 24) except only three treatments were imposed:

A: Control, no OTC treatment

B: OTC treatment to Z2

C: OTC treatment from Z1 to megalops (* experiment terminated at Z4),

OTC was added at 50ppm. There were five replicates of each treatment arranged in a completely randomised design.

Statistical analysis

Per cent survival in the small-scale larval rearing experiment was analysed by one-way ANOVA.

Results

Dissolved oxygen was always saturated, pH was between 7.84 and 8.04, temperature was between 29.1 and 31.2°C and salinity was kept between 28 and 31ppt.

Survival data for the small-scale experiment is presented in Table 44 and Figure 36. There was a highly significant difference ($P < 0.001$) in survival of mud crab larvae apparent as early as one day after stocking with the “no-OTC” treatment suffering in excess of 45 per cent mortality. Similar to the previous experiments, the treatments receiving OTC suffered low levels of daily mortality. The larvae reached Z2 on day 5 so the last OTC treatment for the “OTC to Z2” treatment occurred on day 4. The trial was terminated on day 12 when the majority of larvae were in the Z3 and Z4 stages. The larvae would normally (without OTC treatment) have been at Z5 by this time at the temperature used. There was no significant difference ($P > 0.05$) between the OTC treatments at any point during this experiment. The experiment was terminated on day 12 due to low survival and slow development of the larvae.

Table 44. Survival of mud crab larvae on day 1 and at the end of the trial (day 12).

	Day 1	Day 12
No OTC		
	54.00 ± 11.52 [a]	0.00 ± 0.00 [a]
OTC to Z2	98.67 ± 0.82 [b]	28.00 ± 8.79 [b]
OTC to mega (*Z4)	100.00 ± 0.00 [b]	30.67 ± 10.4 [b]

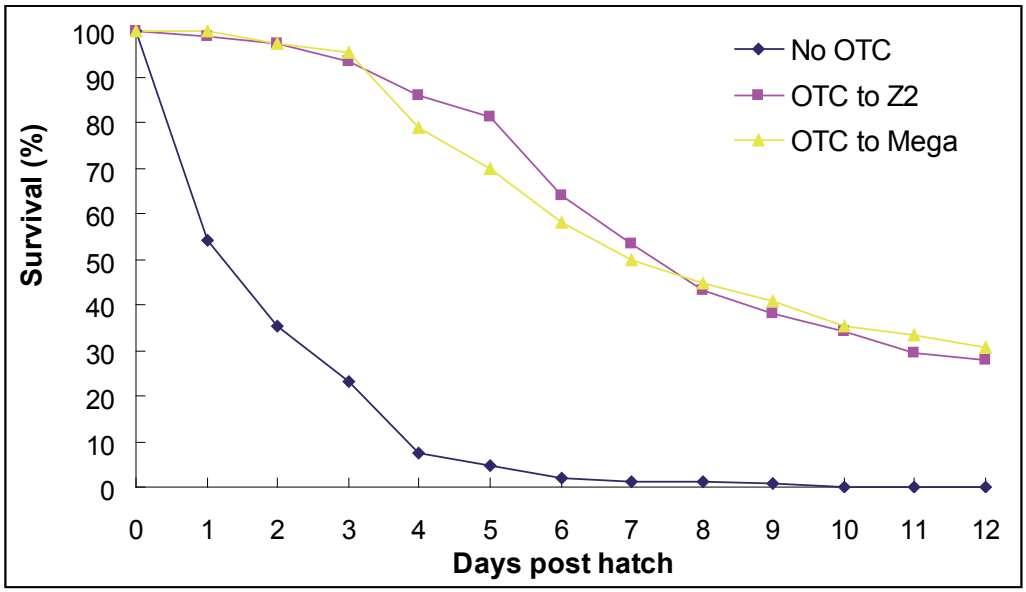

Figure 36. Daily survival for the various treatments in the small-scale experiment. Error bars omitted for clarity. (DAC Batch–26).

Discussion

The small-scale experiment again demonstrated the usefulness of oxytetracycline (OTC) in mud crab larviculture systems. It has also confirmed that the OTC can be limited to use during the rotifer feeding phase only (Z1–Z2), pointing to the rotifers as a source of the virulent bacteria associated with routine mortality. The very high mortality experienced in the bowls during the first night in the treatment not receiving OTC might also suggest that this particular batch of larvae were generally poor in comparison to other treatments. The fact that they did not respond to light as zoea 1 stage larvae usually do, may support this contention. After day 5 when the OTC was discontinued the mortality remained similar to the treatment still receiving OTC. This then lead us towards a new line of inquiry with regards to larval rearing protocols, namely if a clean rotifer replacement diet or organism can be found then the use of OTC may not be necessary.

Large-scale trial:

Influence of a conditioned biofilm, OTC and flow-through settled water on survival of mud crab larvae to megalops. (DAC–Batch 25)

Introduction

In the previous two large-scale experiments (see DAC Batches 21 and 22), flowing through settled water after Z2 enabled good survival of larvae to megalops, equivalent to those larvae receiving daily OTC treatment. This may have been due to the establishment of a beneficial biofilm growing in the tanks. An established and actively growing biofilm may out-compete any introduced virulent bacterial strains.

In order to reduce or eliminate the use of OTC, the small-scale experiment from Batch 21 was run again (Batch 22) to determine if the use of OTC was necessary throughout the larval period or if it could be eliminated after a certain stage. The majority of mortality usually occurred in the first few days of stocking and if OTC use can be reduced to the first few days then large benefits, both economically and environmentally can be achieved.

Methods

SOP were used, with the exception that one day prior to hatching the berried female was given a 0.2ppm Benzalkonium chloride bath for five minutes.

Treatments

After the DAC Batch 22 trial, two tanks were not cleaned or sterilised, but were left with a flow through water supply and the established biofilm. These two tanks made up one treatment in the current experiment. The remaining two treatments included a flow through treatment from stocking to megalops with sterilised tanks (ie. without an established biofilm); and a 50ppm daily OTC bath from stocking to Z2 followed by a constant flow through regime.

Results

There was a noticeable dropout in all tanks after 24 hours. Residual rotifer counts were varied between treatments. Those treatments receiving OTC had the highest counts (~13ml^{-1}) whereas the flow through treatments were down to 1–2.5ml^{-1}. The larvae in the flow through treatment appeared weak on days three and 4. On day 5 larvae in all treatments appeared weak and on day 6 both the flow through and no OTC followed by flow-through treatments, were terminated. Only the OTC followed by flow through treatment was continued.

On day 13, both tanks in the remaining treatment sustained almost a 100 per cent mortality due to moult death syndrome at the moult from Z5-megalops.

Discussion

The only treatment which was to survive as far as Z5 t was the “OTC to Z2 followed by flow through” treatment. All other treatments appeared weak in the first few days followed by a significant drop out and termination of the treatments on day 6. There was a problem with

a small pump which was used to control the flow-through water supply to the tanks and subsequently the flow was stopped sometime during the night of the fifth day. Whether this was responsible for the mass mortality or was coincident with it remains unknown.

It would appear that an established bio-film was not able to out compete the virulent bacteria presumptively associated with the rotifers.

Small-scale trial:

Influence of varying the temporal use of oxytetracycline (OTC) on mud crab larval survival. (DAC–Batch 27)

Introduction

At one stage during the project, larval survivals in small-scale trials appeared to be low compared to earlier trials, even when using OTC to control bacteria, and in combination with previously successful feeding and water quality strategies.

It was decided to re-visit the timing of OTC treatments in an attempt to further limit its use and to trial some new experimental rearing bowls in case a toxic chemical had been leaching from the old ones..

Methods

Standard small-scale trial operating procedures were used except the that “the lawn” or bacterial growth from one Petrie dish of Pro-A (a potential probiotic) was dosed into the hatch tank overnight. The potential probiotic, Pro-A was being trialled at this time. Salinity of hatch water applied to all larval stages was 29ppt.

Seven experimental treatments were imposed.

A: New bowls, no OTC treatment,
B: Old bowls and no OTC treatment
C: 3 hour OTC treatment
D: 1 day OTC treatment
E: 2 day OTC treatment
F: 3 day OTC treatment
G: 4 day OTC treatment

New bowls were used in treatments A and C–G. There were five replicates of each treatment arranged in a completely randomised design.

Rotifers

Rotifers were produced using standard operating procedures. Approximately 1 x 106 cells of a previously isolated probiotic contender (Pro-A) was added to the harvested and concentrated rotifers. They were then enriched with *Chlorella* paste and OTC (50ppm) for 18 hours. Rotifers for subsequent days were treated in the same way.

Rotifers were fed to all bowls at a concentration of 20 rotifers mL^{-1}, in a single daily feed.

Artemia

Artemia were produced using the standard operating procedure, except that from zoea 3 day 2 (Day 8), *Artemia* offered were 48 hour old (second instar +) enriched. The same hatching method as above still applied, however after 24 hours the *nauplii* were harvested, rinsed

and restocked at approximately 200ml^{-1}. These *Artemia* were enriched with 250ppm (INVE) DC DHA Selco for six hours, prior to another 100ppm addition of the enrichment media. After 20 hours a further 150ppm was added, and the culture was harvested and fed after approximately 24 hours. Half the daily feed was fed at 1200, and the remaining half was further enriched in 250ppm DC DHA Selco until 1600 hours.

Feed rates increased gradually from 1.5ml^{-1} on day 4, to 2.0ml^{-1} on day 9, and further to 3.0ml^{-1} by day 12.

Three algal paste species (Instant-algae, Reed Mariculture Pty Ltd, USA) were used in the bowls as tabulated below.

Table 45. Algal species and densities used

Species	Final density per day (cells mL^{-1})
Nannochloropsis oculata	3.32 x 104
Isochrysis galbana–Tahitian strain (T. iso)	1.66 x 104
Tetraselmis sp. 1.66 x 104	

Statistical analysis

Percentage survival in the small-scale larval rearing experiment was analysed by one-way ANOVA.

Results

Dissolved oxygen remained > 5 mg L^{-1}, pH was between 7.91 and 8.24, temperature was between 28 and 29.2°C, and salinity ranged between 32 and 35ppt.

Daily survival data for the small-scale experiment is presented in Figure 37 and final survival to megalops is shown in Table 46.

There was no significant difference between any of the treatments in survival of mud crab larvae up to day 5 after which differences started to appear. The trial was terminated on day 18 when all remaining larvae had moulted to the megalops stage. There was a highly significant difference (P = 0.008) in survival to megalops between treatments with those receiving OTC for more than 1 day having higher survival than short term or no OTC treatment.

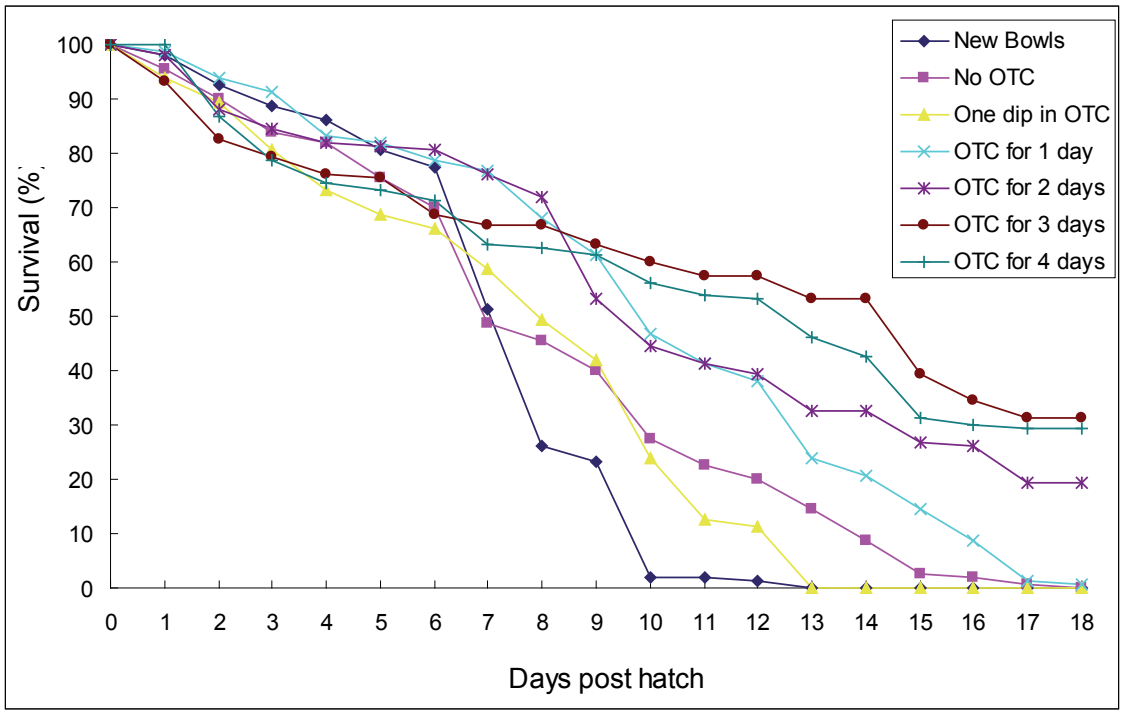

Figure 37. Daily survival of mud crab larvae in the various treatments throughout the experiment. (DAC Batch–27).

Table 46. Survival (± se) of mud crab larvae on day 18.

Treatment	Survival (%)
A: New Bowls–no OTC	0 a
B: Old Bowls–No OTC	0 a
C: One dip in OTC	0 a
D: OTC for 1 day	1.49 ± 0.67 a
E: OTC for 2 days	19.33 ± 11.56 ab
F: OTC for 3 days	31.33 ± 10.52 b
G: OTC for 4 days	29.33 ± 12.75 b

Discussion

This trial demonstrated that utilising standard operating procedures for small-scale trials including OTC treatments still resulted in reasonable survival of larvae to the megalops stage. Whilst other trials using similar treatments have had higher final survival rates, the reasons for this are unknown but could include between batch quality of larvae, pathogenic bacteria not susceptible to OTC, and larval handling abilities of individual technicians.

At this point it would appear that the standard protocol is the best one developed thus far.

Large-scale trial:

Influence of OTC followed by static rearing in combination with a probiotic addition to rotifers, on survival of mud crab larvae to megalop under semi-commercial conditions. (DAC–Batch 28)

Introduction

In previous large-scale larval runs (DAC Batches 21 & 22) relatively high levels of survival to megalops were achieved. Moreover, no significant differences in survival occured, when OTC use from zoea stage 2 was replaced by a flow-through water exchange protocol using settled seawater. This experiment was aimed at testing whether flow-through water exchange from day 2 can eliminate the need for more extensive use of OTC from the rearing protocol.

Methods

Six 1000 L tanks were set up as per SOP and larvae were cultured in them under two protocols.

Treatments

A: Water in the tanks underwent a 70 per cent drain-down, top-up water exchange for the first four days, at which time the walls of all tanks were wiped clean. Screens, airlifts and the Bazooka were removed and cleaned prior to drain-down. The tanks were drained through the foam-fractionator, which served to flush the units each day before use. The remaining water was then foam-fractionated for 30 minutes, followed by fractionation and flow through with storage water for another 30 minutes. Tanks were then re-filled with storage water. These tanks then received OTC at 50ppm during the first four days of larval production. After this time a flow-through water exchange system was established and the tanks were fractionated for up to four hours each morning to remove old food and reduce organic wastes in the water.

B: Water in the tanks was drained down and topped up the day after stocking, but thereafter put on a flow-through water exchange protocol.. No OTC was administered to these tanks throughout the experiment, and a similar tank cleaning procedure was undertaken–including fractionation for up to four hours each morning.

Rotifers (at a rate of 20 rotifers mL^{-1}) and *Artemia* were both produced and fed according to standard operating procedures.

Results

One of the no-OTC tanks was allowed to cool overnight to 27.9°C due to a faulty thermostat and this tank suffered a high degree of mortality that morning. Otherwise, water quality in the trial was generally as required. Dissolved oxygen was maintained above 5.2 mg L^{-1}, temperature between 29.1 and 30.8°C, and salinity ranged between 31 and 35ppt, but did not change more than 1ppt per day.

Substantial mortality began to occur in the untreated tanks (no OTC and flow-through) from day 5 onwards, and these tanks were terminated on day 11 during the Z5 stage. Mortality also began to occur from day 6 in the tanks that were treated with OTC during the first four days. It seems most likely that a disease outbreak began in the untreated tanks that quickly spread to the previously treated tanks. There was then a gradual decrease in zoea numbers punctuating in mass mortality due to moult death syndrome (MDS) at the moult from Z5 to megalop over days 12, 13 and 14. No live megalops were found in any tanks.

Examination of the remaining Z5 larvae on day 12, when MDS was first noticed, revealed high levels of a syndrome which is manifested by the precocious development of chelae in a position of close proximity to the posterior margin of the cephalothorax, where the abdomen later develops. This syndrome appears to precede MDS.

Discussion

This trial demonstrated that it is unlikely that flow-through water exchange from the start of larval rearing is an adequate replacement for OTC treatment for the first few days of larviculture. The subsequent poor survival even of larvae that had been treated with OTC, demonstrated that there was still problems to be overcome in consistently producing megalops without OTC treatment.

The cause of MDS is still unknown. Death occurs as the weakened larvae undergo ecdysis. This is known to be an energetically expensive process, and weak or unhealthy larvae are unable to completely extricate themselves from the old moult. Crustaceans lay down a thin cuticle on the gills that is shed at moulting. One theory is that a slow moult, perhaps resulting from a weakened larval state, results in death as the larvae must undergo gas transfer across a double layer of cuticle. This then results in hypoxia and death.

Research from Japan (Suprayudi 2002) suggests that excess feeding can result in precocious and rapid growth of morphological characteristics such as the chela, which hinders clean and rapid moulting. More recent research (Suprayudi 2004) suggests that DHA is important for the somatic growth of mud crab larvae, as DHA fed larvae are often larger than normally fed larvae. We can speculate that we may be over supplying the larvae in terms of DHA and feed density generally. A reduction in feed density and a change away from specifically DHA enriched *Artemia* may overcome problems such as the syndrome experienced in this trial and MDS.

Large-scale trial:

Influence of Oxytetracycline (OTC) use during zoea stage 1 on mud crab larval survival to megalops in a batch water exchange system. (DAC–Batch 29)

Introduction

This trial was carried out to further investigate the influence of OTC during Z1 in a batch water exchange system. Previous trials involving a flow through water exchange protocol after the OTC use (see DAC Batches 21 and 22), proved highly successful. However, it is possible that the good survival achieved may have been due to the water exchange protocol used and not solely the result of OTC treatment.

The following trial tested the effect of OTC on larval survival in a run where the water exchange regime was a batch exchange system from Z1 through to megalops.

Methods

Two treatments with three replicates of each were tested using large-scale SOP:

Treatment A: OTC at 50ppm from Z1 to Z2 day 1 with batch water exchange regime for all stages.

Treatment B: No OTC with batch water exchange regime for all stages.

Artemia were fed at the following rates:

Z2/2 = 1.5 *Artemia* mL^{-1} day^{-1} of 430 AF

Z3/1 = 2.0 *Artemia* mL^{-1} day^{-1} of 430 AF

Z3/2–Z4 = 2.0 *Artemia* mL^{-1} day^{-1} of enriched *Artemia* GSL AAA

Z5/1 = 3.0 *Artemia* mL^{-1} day^{-1} of enriched GSL AAA Artemia

Three algal paste species (Instant-algae, Reed Mariculture Pty Ltd, USA) were used as tabulated below. Algal paste was added daily to the tanks in two feeds to give a total daily addition to achieve the cell densities shown in Table 47.

Table 47. Algal species and densities used

Species	Final density per day (cells⁄mL)
Nannochloropsis oculata	1.66 x 104
Isochrysis galbana–Tahitian strain (T. iso)	1.66 x 104
Tetraselmis sp. 1.66 x 104	

Results and Discussion

All tanks were observed to have high survival of larvae until Day 7 at which stage the tanks that had not received OTC (Treatment B) started to appear weaker than the larvae that had received OTC to Z2 day 1 (Treatment A). The Treatment B larvae dropped out during the water change and were slow to return to the water column and when they did their swimming action was slow and they did not form schools as did the Treatment A larvae. By the afternoon of Day 8 the larvae in the Treatment B tanks were dying and they were terminated on Day 9 as less than 100 larvae remained in each.

There was still observed to be high survival in the remaining tanks (Treatment A) until Day 13. On Day 14 a great majority of the Z5 larvae were trapped by their exuviae as they moulted up to megalops (symptomatic of MDS) and the tanks were terminated. The results for Treatment A are shown below in Table 48.

Table 48. Survival for Treatment A (OTC till Z2/1) (DAC Batch–29).

Tank number	Live Z5	Megalops
1	2666	55
4	1888	0
5	<100	0

These results strengthen the view that most of the improvement in performance of mud crab larval cultures is a result of the effect of OTC and not as a result of the water management regimes. Although OTC used for the first four days did improve survivorship to Z5 (at least) it did not prevent MDS.

In large-scale trials in particular there is a significant risk of cross-infection between tanks, which may confound the results of trials from such things as aerosol drift. If tanks were better quarantined, this risk could be reduced.

Small-scale trial:

The effect of low-salinity rotifers on early stage mud crab larval survival. (BIARC–Batch 5)

Introduction

For several months prior to the commencement of this project unexplained mass mortality of crab larvae was occurring in the first three days of culture. This was a new phenomenon and required investigation to determine the cause. Results of laboratory trials involving the prophylactic use of oxytetracycline (OTC), to control culture bacteriology, had implicated bacteria as the major causative agent responsible for mass mortalities, however plating had not revealed any indication as to the specific causative agent.

Culturing rotifers at low salinity, 10ppt, was tested as an acceptable way of manipulating rotifer cultures so that they may have a more “friendly” bacterial microflora, which removed the need for antibiotic treatment. This laboratory trial was a bioassay of the rotifers cultured under standard operating procedures (35ppt) and the low salinity rotifers to quantify the effectiveness of the method. OTC was also applied to provide further evidence of bacterial involvement in the early mortality event.

Materials and methods

This experiment commenced on the 18July 2000. Small-scale culture followed the BIARC standard operating procedure except for the first 5 days where rotifers were harvested from two rotifer production units, one maintained at ambient salinity (35ppt) and one at 10ppt. The experimental design was a 2 x 2 factorial, 35 and 10ppt rotifers with or without oxytetracycline (OTC) addition. Treatments were duplicated. OTC was added to the larviculture bowls at 25ppm every day at the time of water exchange.

Results

Results are presented graphically in Figure 38. Culture bowls fed rotifers grown at 35ppt, without the protection of OTC in their medium, rapidly succumbed to disease with 100 per cent mortality within two days. Bowls continuously treated with OTC did not show a distinct mortality event, regardless of the rotifer source, however exhibited gradual losses. Bowls fed low salinity (10ppt) rotifers, but not treated with OTC did not exhibit the abrupt initial mortality of the high salinity (35ppt) rotifer fed treatment, but by day 6 had significant mortality. This treatment continued with low numbers to produce megalops.

Discussion

Reduction of the rotifer production salinity from the normal level of 35ppt to 10ppt significantly improved early larval survival. As a result low salinity cultured rotifers were recommended for hatchery operations at BIARC. The suspected bacterial pathogen could not be readily isolated and identified as it did not appear to grow on the standard bacteriological plating media so in the absence of more detailed knowledge of the causative organism this broad brush approach was required.

The pattern of mortality of the better performing treatments, particularly those with OTC added to the culture medium, were indicative of other factors continuing to adversely impact on larval survival. It should be noted that OTC did not strongly inhibit bacterial growth in the larval cultures, Similar numbers of bacteria were measured in cultures with and without OTC. It could therefore be hypothesised that OTC was differentially suppressing bacteria of high virulence to crab larvae. app. The more gradual pattern of mortality as observed in this experiment was a common phenomenon but did not occur consistently.

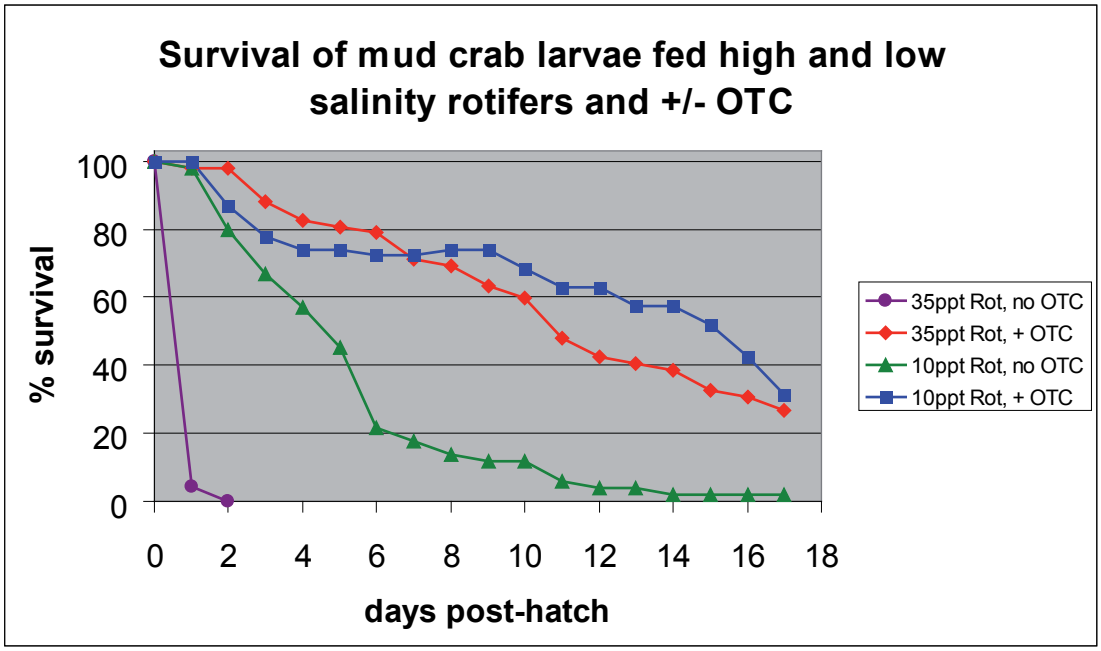

Figure 38. Per cent survival of larvae fed high and low salinity rotifers. (BIARC Batch–5).

Large-scale trial:

The use of low salinity rotifers in mass cultures. (BIARC–Batch 6)

Introduction

Evidence from the previous small-scale trials indicated that the early mass mortality syndrome experienced in mud crab larviculture may be partially mitigated by the use of rotifers cultured in low salinity seawater (10ppt). The use of low salinity cultured rotifers was a change in hatchery protocol and there was little information on their use for culture of mud crab larvae. This trial was the first to examine the influence of low salinity rotifers on large-scale culture mud crab larval culture.

In this trial the opportunity was also taken to investigate two alternative feeding regimens relating to the rotifers. Evidence from the literature and reports from other research institutions was not conclusive regarding the optimum feeding regimen for rotifers. The use of different regimes may have indicated an influence of local factors.

The two feeding regimens tested were the “standard feeding regimen” where rotifers are phased out to *Artemia* mid-way through the Z2 and the “extended rotifer regimen” where rotifer addition was continued for the duration of the culture, in addition to *Artemia* applied from mid-Z2.

Methods

This production trial commenced on the14 September 2000. The large-scale culture was conducted in 8 x 500 L cylindro-conical tanks following the BIARC SOP, although there was no water exchange in the first week of culture following a protocol which had been found to be successful at RIA3, Vietnam. The live feed was applied as per Table 49. A particulate diet, Lansy (INVE) was applied in the standard way.

Table 49. Feeding regimens used in the low salinity rotifer culture trial (BIARC Batch–6).

		Regimen 1	Regimen 2		
larval	approx.	rotifers	Artemia	rotifers	Artemia
stage	day	mL^{-1}	mL^{-1}	mL^{-1}	mL^{-1}
Z1 to Z2	1 to 3 (4)	10	0	10	0
Mid toZ2	4 (5)	10	0.5	10	0.5
late Z2 to Z3	5 (6)	5–10	0.5	5–10	0.5
Z3 onwards	6 →	residual	0.5	3–5	0.5

Results

To day 5, the last day that all tank treatments were identical, survival was variable, from 40 to 90 per cent. By day 7 the experiment was terminated due to mass mortality in all tanks.

The bacteriological plating of the cultures indicated what is considered to be a “healthy” bacterial community with no sucrose negative *Vibrio* or luminous *Vibrio*.

Discussion

There was no indication as to the cause of the mass mortality. The pattern of mortality was not consistent with that caused by the presumptive bacterial pathogen previously present in the high salinity rotifers. There was potential for the low salinity rotifers to have contributed to the mortality, however this pattern of mortality had previously been regularly observed in mass cultures. While there were no good clues as to the cause, from experience we anticipated that bacteria are implicated in the mortality.

Small-scale trial:

Effect of live food culture salinity, diet supplementation and OTC treatment on mud crab larval growth and survival. (BIARC–Batch 7)

Introduction

Previous work in an ACIAR project identified that the commonly used *Artemia* type, from the Great Salt Lakes (GSL) in USA, does not support full growth and development of mud crab larvae when used as the sole dietary component. Other *Artemia* types, such as that from Vietnam, can support normal growth and survival when used as a sole dietary source. The Vietnamese and similar *Artemia cysts* can be difficult to obtain and expensive. It has previously been determined that the addition of a dietary supplement can rectify the deficiency of GSL *Artemia* and promotes larval performance similar to that when using the expensive Artemia.

Commercially available marine prawn larval and post-larval diets have been used as they have been shown to significantly reduce the incidence of abnormal larval morphology and completion of the mud crab larval cycle. Krill, processed to form micro-particulates, has also been demonstrated to provide the same benefits.

Preliminary evidence from BIARC indicates that the early mass mortality syndrome recently experienced in the hatchery may be mitigated by using low salinity reared rotifers. The basis for the use of low salinity (10ppt) to produce rotifers is that at these low salinities the presumptive bacterial pathogen(s) causing early mass mortality are excluded. The use of low salinity cultured rotifers is new to hatchery protocols and it was required to accumulate data on their use for the culture of mud crab larvae.

Materials and methods

This experiment commenced 23 October 2000. In this experiment four treatments were applied in a factorial manner:

1. Rotifers–from either high or low salinity culture
2. *Artemia* type–Vietnamese or GSL
3. Diet–with and without krill supplement
4. OTC–treatment or not.

There was no replication of individual treatments. In this multi-factorial design, replication was embedded in the treatment combinations. OTC treatment followed the BIARC standard operating procedure. Freeze dried krill was dry blended and sieved to produce particles sizes similar to the prawn diets typically used (150–300μm). The particulate supplement was applied in the standard manner.

It was anticipated that each of the treatments would affect culture bacteriology and therefore it was likely that significant interactions would occur among the treatments.

Larvae were produced and cultured in the laboratory used the BIARC standard operating procedure. The 2 x 2 x 2 x 2 factorial design was set up as per Table 50.

Table 50. Feed and treatment regimen for trial (BIARC Batch–7)

	Rotifers	Artemia	Suppl't	Antibiotic
1	low sal	GSL	—	+
2	low sal	GSL	—	—
3	low sal	GSL	+	—
4	low sal	GSL	+	+
5	low sal	Viet	—	—
6	low sal	Viet	—	+
7	low sal	Viet	+	+
8	low sal	Viet	+s	—
9	high sal	GSL	—	+
10	high sal	GSL	—	—
11	high sal	GSL	+	—
12	high sal	GSL	+	+
13	high sal	Viet	—	+
14	high sal	Viet	—	—
15	high sal	Viet	+	—
16	high sal	Viet	+	+

Results

Megalops production started on day 17 and continued to the end of the experiment on day 22. A wide range of culture performance occurred across all treatments with larval survival rates to megalops ranging from 0–64 per cent. Per cent megalops production rate, that is the proportion of larvae surviving to megalops stage of initial stocked number, varied significantly within the main treatments of antibiotic and rotifers (Table 51A). When comparing mortality associated with moulting from Z5 to megalops, ie. that attributed to moult death syndrome (MDS), significant differences within the main treatments also occurred in rotifer, antibiotic and supplement treatments (Table 51B).

The three best performing treatments all used low salinity rotifers in combination with OTC and had production rates of megalops from 56 to 66 per cent. All four treatments using high salinity rotifers without OTC failed early in the culture cycle and did not produce any megalops while those using low salinity rotifers without OTC had production rates ranging from 8 to 40 per cent (mean 31%). When grouped by rotifer salinity and application of OTC the low salinity rotifers with OTC had a significantly higher survival rate (see Figure 39).

In the diet treatments, mean megalops production was the same at 32 per cent for the Vietnam *Artemia* treatments, with and without the krill supplement, and GSL *Artemia* with supplement (Table 51A. Mean % megalops production [of initial Z1 stocked] across all treatments.). The GSL *Artemia* without supplement performed significantly poorer ($p<0.05$) with a mean survival rate to megalops of 11 per cent (Figure 40).

The incidence of moult death syndrome (MDS) at the Z5–megalops moult was significantly higher in the GSL without supplement treatment (p=0.02) with 67 per cent of Z5 attempting to moult succumbing to MDS. The least MDS occurred in the Vietnamese *Artemia* with supplement with 4 per cent of larvae dying due to MDS.

Table 51A. Mean % megalops production (of initial Z1 stocked) across all treatments. The estimated standard error for all treatments is 3.52.

Supplement		Antibiotic**		Rotifers**		Artemia	
+ suppl	– suppl	+ anti	– anti	high sal	low sal	GSL	Viet
32.0	21.8	38.2	15.5	12.5	41.2	21.5	32.2

**=p<0.001

Table 51B. Mean Z5–megalops MDS (% of total Z5 number) across all treatments. The estimated standard error for all treatments is 3.52.

Supplement*		Antibiotic*		Rotifers*		Artemia	
+ suppl	–suppl	+ anti	– anti	high sal	low sal	GSL	Viet
4.5	14.5	10.5	8.5	4.2	14.8	15.8	3.2

*=p<0.05

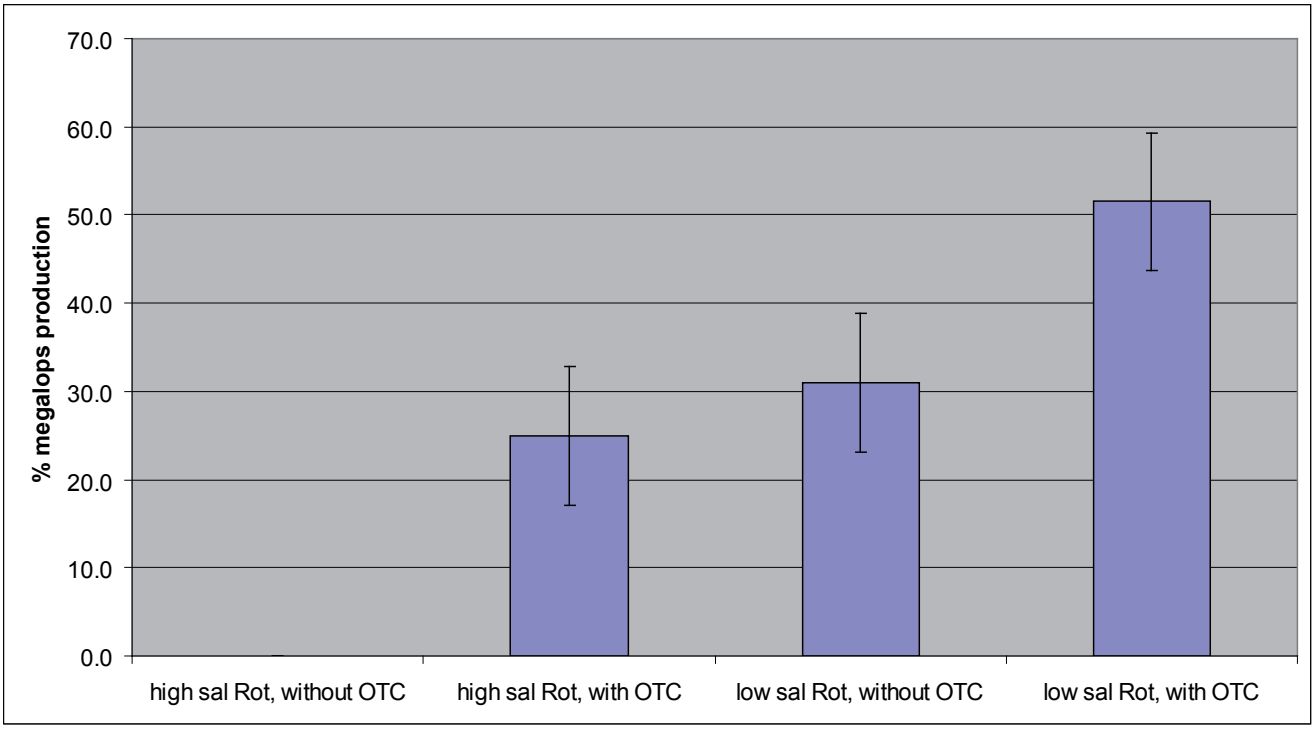

Figure 39. Megalops production when fed high and low salinity produced rotifers with and without OTC treatment. Error bars indicate least significant difference at the 5% level. (BIARC Batch–7).

Discussion

The survival of larvae fed the rotifers produced at different salinities, 35 and 10ppt, further supports the potential for utilising low salinity production of rotifers to combat the bacterial problem for mud crab larvae culture. Use of low salinity is a relatively simple tool that has

now been shown to be effective in reducing of rotifer borne presumptive bacterial disease. The significantly enhanced survival rates when OTC is used to treat the larval culture supports that the difference between the rotifer types is due to bacteriology, however bacteriological plating did not reveal differences in community structure. It is apparent that culturing rotifers in low salinity does not significantly compromise their nutrition value to the larvae since growth and development were as expected. Additionally it does not appear to change rotifer productivity, however a detailed examination of this aspect was not conducted. The rotifer *Brachionus plicatilus* is known to tolerate a wide salinity range from several ppt to greater than 40ppt.

This experiment also confirmed the necessity of using a diet supplement when using *Artemia* derived from the Great Salt Lakes. Supplementation with particulate freeze-dried krill enhanced larval performance to be equal to that of larvae fed Vietnamese Artemia. The hypothesised deficient component in the GSL *Artemia* appears to be fully satisfied in the Vietnamese *Artemia* as supplementation with krill did not further enhance megalops production rates. However, examination of the survival rate alone does not take into account the incidence of MDS. Those cultures receiving Vietnamese *Artemia* supplemented with krill had a significantly lower incidence of MDS compared with those that did not get the supplement. This result further supports results of previous work in which the occurrence of MDS was linked to nutrition.

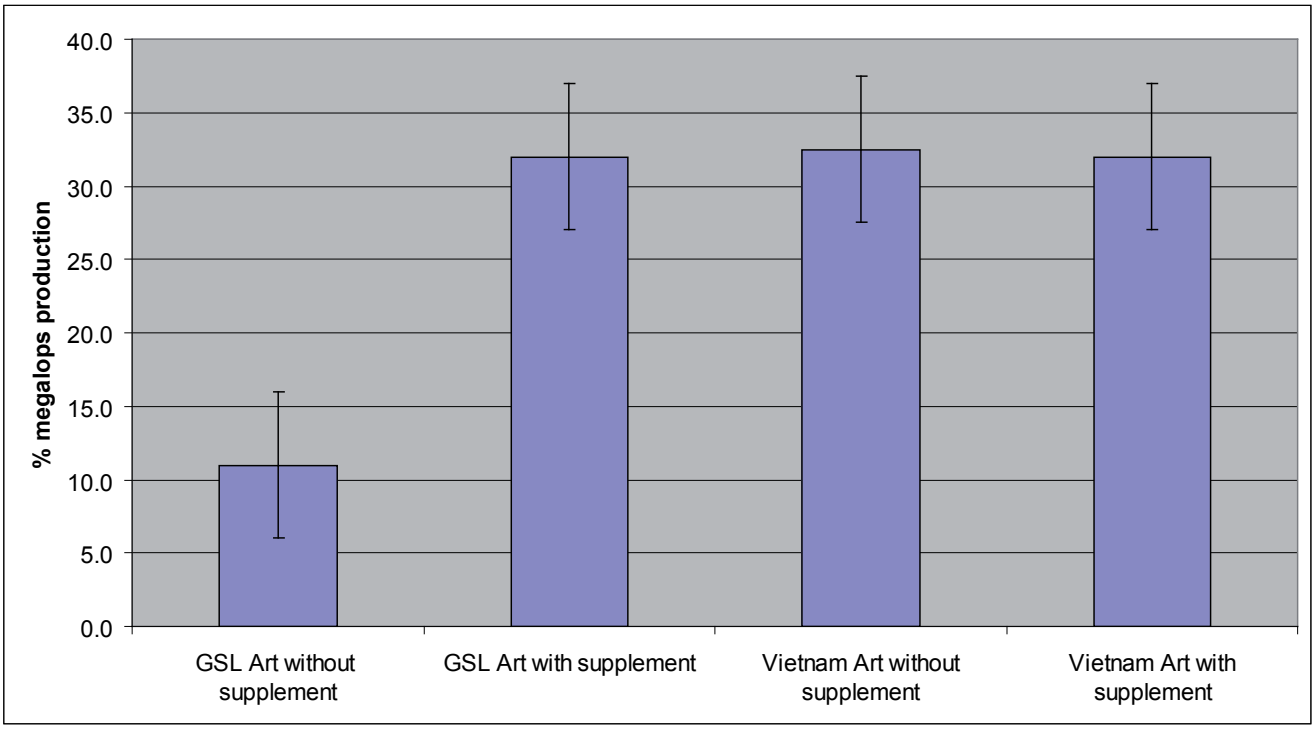

Figure 40. Megalops production when fed Great Salt Lake or Vietnam *Artemia* with and without krill dietary supplement. Error bars indicate least significant difference at the 5% level.

Small-scale trial:

Comparison of larviculture using artificial seawater or settled seawater, combined with OTC treatment of water and rotifers. (BIARC–Batch 8)

Introduction

Seawater sourced from the inshore waters has been continually used for larval cultures at BIARC. Potential exists for an infectious agent to be present in our local inshore waters that negatively impacts on larval survival and development, despite various treatments being applied to it prior to use. Whilst in laboratory cultures there have been periods of consistent high larval survival, there remain episodes of mass mortality or low survival rates of unknown causes. Some mortality events have been correlated with a high incidence of luminescent or potentially pathogenic *Vibrio*, such as V. *harveyi* or V. alginolyticus. Seawater pre-treatment at BIARC has consistently produced nil viable *Vibrio* cells in plating checks so there is confidence in the effectiveness of treatments used. However it is hypothesised that the organic or inorganic chemistry of the local waters may promote rapid development of potential pathogens that are introduced into larval cultures by other vectors, such as rotifers.

Rotifers are the least microbially controlled input into larval cultures and as such are a highly suspected vector of pathogens into the larval cultures. A number of trials at DAC and BIARC have demonstrated that rotifers are a likely source of presumptive bacterial pathogen(s) that have been responsible for mass larval mortality in the first three days of culture. It has been shown that the application of oxytetracycline can greatly improve larval survival if used to treat larvae fed rotifers. A more sustainable modification to the rotifer production system, incorporating low salinity medium to grow the rotifers, was subsequently developed to reduce this contamination risk. However bacterial plating still confirms that there are potentially pathogenic *Vibrio* and other bacterial species present in the rotifer production system.

An experiment was conducted to investigate the use of artificial seawater to explore the impact of seawater source on larval culture dynamics. Additionally pre-treatment of rotifers before being fed to the larvae was examined to identify any need to further improve the rotifer production system.

Materials and methods

This experiment commenced 8 July 2001. The experiment design was a 2 (settled or artificial seawater) x 4 (OTC treatment regimens) factorial with duplicate treatments (Table 52). The artificial seawater used was “Aquarium salts” from Aquasonic.

Larvae were produced and cultured in the laboratory following the BIARC SOP. For the OTC treatments, larval cultures were treated with 25ppm OTC either continuously, ie added daily following water exchange, or for the first day only. Rotifers were treated with 100ppm OTC for one hour following harvest and concentration. They were then flushed well prior to feeding to the larvae .

Table 52. Summary of treatment culture regimens (BIARC Batch–8).

Treatment	Seawater type	OTC treatment
1	artificial	initial 24h
2	artificial	continuous
3	artificial	none
4	artificial	rotifers only
5	settled	initial 24h
6	settled	continuous
7	settled	none
8	settled	rotifers only

Results

Larval growth and survival was similar among all treatments until day four . At this point two treatments, artificial seawater with no OTC treatment and settled seawater with rotifers only treated with OTC, sustained higher mortality than the other treatments over the next five days (Figure 41). Of the remaining treatments, only the settled seawater with continuous OTC treatment showed consistently high survival through to day 21 when the experiment was terminated. The survival rate of megalops ranged from 0–78 per cent among the treatments with the settled seawater and continuous OTC treatment far out-producing the other treatments . The most significant pattern in megalops production that emerged was the consistently high moult death syndrome rate among the artificial seawater treatments. In these treatments most of the larvae that survived to the end of the Z5 stage did not successfully complete the megalops moult (Figure 42). This is compared with the settled seawater treatments which only suffered minor incidence of MDS. The brief treatment of harvested rotifers with OTC did not have a positive effect on the survival to zoea to megalops.

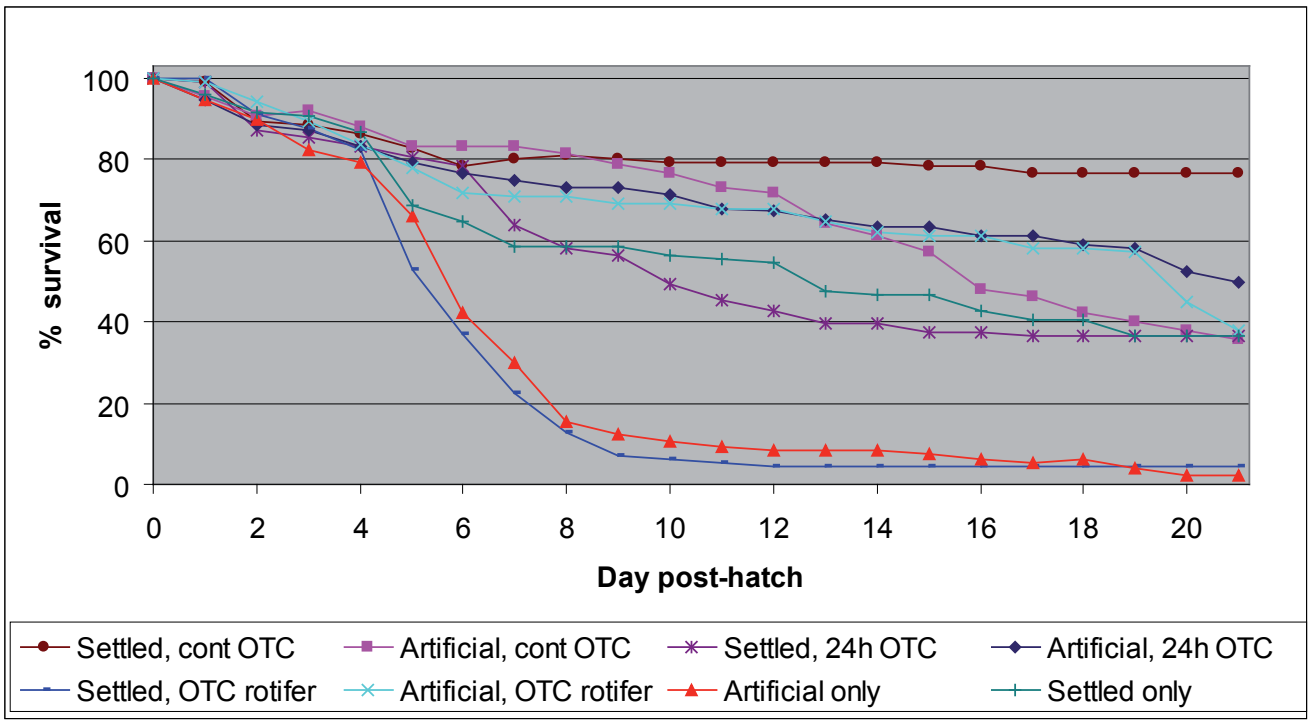

Figure 41. Larval survival in artificial and settled seawater under different oxytetracycline treatment regimens. (BIARC Batch–8).

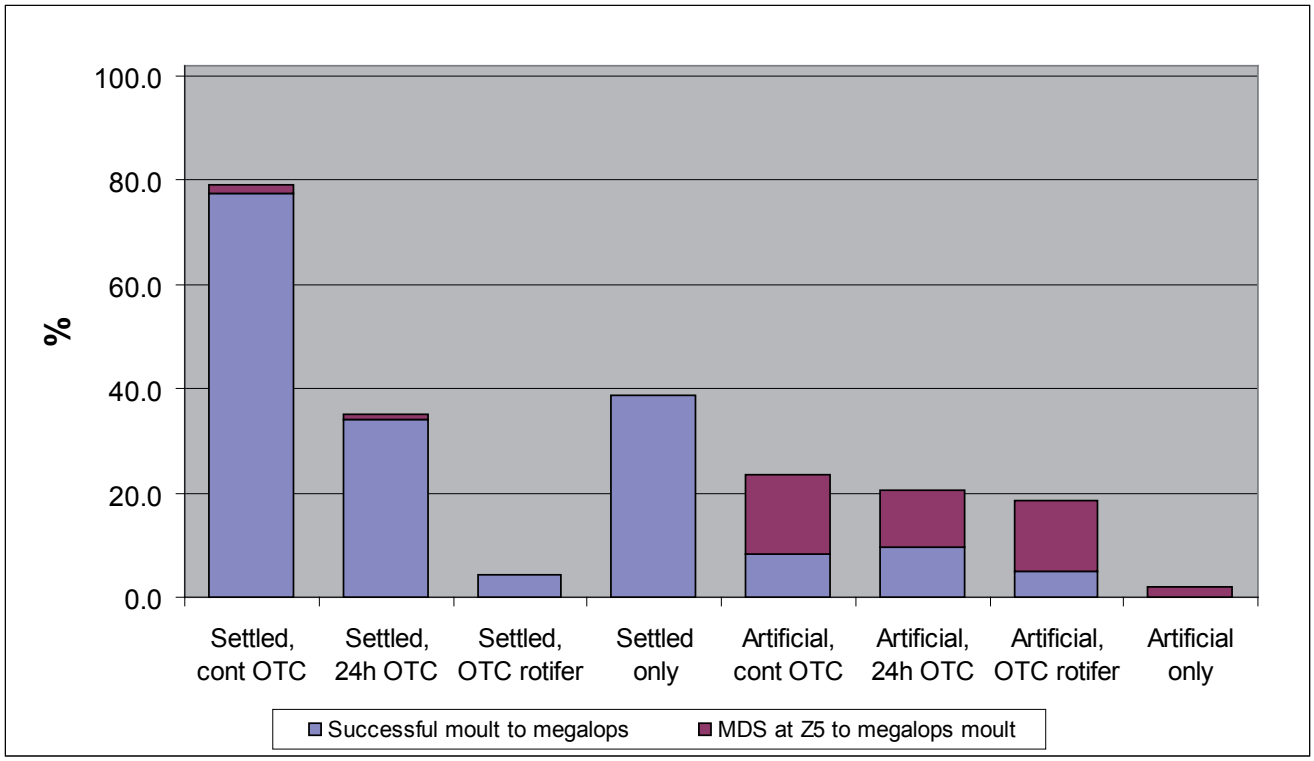

Figure 42. Per cent successful and unsuccessful Z5 moulting to megalops under different water treatments.

Discussion

Whilst artificial seawater supported acceptable larval survival to the end of the zoeal stages, it did not support development to the megalops stage. It is not clear why this should be since these seawater salts are used to maintain sensitive invertebrates and fish in marine aquariums. Previous experimental work at BIARC had determined that larval nutrition was strongly implicated in MDS and diet variation was used to predictably manipulate its incidence. The occurrence of a high level of MDS in the artificial seawater only may however provide evidence of the complexity of MDS aetiology. It is known that rehydrated seawater salts can result in slightly different chemistry compared with normal seawater however the influence of this on chemical and biological dynamics in this larval culture application is not known. The result does however indicate that artificial seawater is not an effective tool that can be used for controlled experiments of mud crab larval culture.

In this trial only continuous exposure to OTC consistently protected larvae from high mortality. The variability of response to OTC indicates that seemingly negative influences can affect larval cultures randomly, leading to high within treatment variability. This unpredictability (that may be related to delayed cross-infection amongst treatments not continuously protected by OTC) has strong repercussions on the ability of experiments to elucidate the impact of applied treatments even under relatively controlled, but perhaps inadequately quarantined conditions.

Large-scale trial:

Larval culture using oxytetracycline and local bacterial isolate as a potential probiotics. (BIARC–Batch 9)

Introduction

At the time of this trial (Feb–March 2002), both BIARC and DAC had determined that there were significant and consistent differences in the success rate of small-scale and large-scale mud crab larviculture. It was apparent that the dynamics in the small systems were very different to large tanks. It was therefore important to extensively test culture protocols determined to be promising in small-scale trials at the large-scale. The large-scale hatchery at BIARC consisted of four 6000 L parabolic tanks. These were very similar to the large parabolic tanks used commonly by Australian commercial prawn hatcheries.

Mud crab larviculture at BIARC and other hatcheries have experienced high mortality of apparent bacterial aetiology. BIARC had recently experienced four patterns of mortality in large-scale larval cultures:

1. Rapid mass mortality in the first three days;
2. Mass mortality between days 2 to 6;
3. Gradual mortality between day 5 and 18; and
4. Accelerated mortality starting at day 14.

The first pattern was characteristic of a virulent agent that was derived from the rotifer culture. Changing the rotifer production system to low salinity, 10ppt, rectified this problem. The second pattern was linked to the development of a mucous mat on the bottom of the tank, where live larvae were physically trapped in the mat, but there may have been other causes of mortality linked to the mat's development. The third pattern was that which is typically experienced by mud crab hatcheries and is generally ascribed to "bacterial influences". The fourth pattern was characteristic of moult death syndrome (MDS) as the larvae prepared to undergo the first metamorphosis moult from Z5 to megalops.

This large-scale trial aimed to compare two methods of manipulating the bacterial community within the culture and as such is particularly addressing mortality patterns 2 and 3 as described above.

Methods

This experiment commenced 17 February 2002. Larvae were produced and stocked into the culture tanks following BIARC standard operating procedure except for the application of the two test treatments as detailed below.

Each treatment was applied to two parabolic 6000 L tanks.

In the antibiotic, OTC, treatment a daily 10ppm dose rate is applied. While 10ppm is lower than what is considered the minimum bacteriostatic dose, unreported evidence indicated that 10ppm reduces larval mortality. It should be noted that even at 50ppm OTC "normal" quantities of *Vibrio* and other heterotrophic bacteria were present in the cultures.

OTC treated. Prior to stocking the cultures newly hatched larvae were bathed in 150ppm OTC for two hours. Thereafter OTC was added to the cultures at 10ppm every day.

Probiotic treated. Prior to stocking the cultures newly hatched larvae were bathed in seawater contained a high density of a potential probiotic isolate for two hours. Thereafter the isolate was added to the cultures every day.

The bacterial isolate with potential probiotic properties was sourced from previous larval cultures that experienced high survival rates. On bacteriological plates this citrate positive isolate had anti-*Vibrio* activity. The isolate was cultured in bacteriological nutrient broth for 48h and 1L of this was added to the larval cultures daily. This rate of application equated to an inoculum of approximately 1x106 cells mL^{-1}.

Results

The day following addition, density of the probiotic bacterium were relatively low at between 400 and 9200 CFU mL^{-1}. On day three the mucous mat developed strongly in all tanks and mass mortality of larvae was observed. Additionally in one of the probiotic tanks a high level, 38 000 CFU mL^{-1}, of luminous *Vibrio* occurred. The cultures were terminated on day 9 due to very low survival.

Discussion

The two treatments applied to the culture tanks, OTC and probiotic isolate did not appear to exert sufficient influence over the bacterial community structure that developed within the cultures. The mucous mat developed as per its typical pattern. Evidence, now drawn from several culture trials, is that OTC applied daily at concentrations between 10 and 25ppm does not inhibit the development of the mucous mat. It should be noted that when OTC is added daily and there is a less than 100 per cent full exchange per day, 25 per cent daily in this trial, OTC accumulates to higher than the applied concentration.

The probiotic isolate used in this trial was one that had characteristics considered necessary to be effective. It grew well under typical rearing conditions at BIARC, it had anti-*Vibrio* properties on bacteriological plates and its prevalence in larval cultures had some correlation with larval survival. This correlation was however not consistent which may indicate a more complex situation than the simple effect hypothesised. There was no indication that in the large-scale cultures that the potentially probiotic isolate had any significant impact on the bacterial community that developed in the culture. In particular, luminescent *Vibrio*, a risk group in terms of potential pathogens, became prevalent in one of the probiotic treated cultures on day 3.

Factors influencing mucous mat development in mud crab larval cultures and potential for chemical inhibition of mucous mat development. (BIARC–Batches 10–13)

General Introduction

Development of a mucoid matrix of apparent bacterial origin on the bottom of mud crab larval culture tanks at BIARC has been consistently linked to mass mortality of larvae in the early stages of their culture. This matrix, referred to as a mucous mat, physically traps live larvae however there may also be additional causes of larval mortality linked to the mat's development. In the opinion of an experienced veterinarian, the mat was most likely of bacterial origin due to the absence of definable structures. However attempts to plate the causative organisms on bacteriological media have been unsuccessful and it has not been possible to maintain the mat outside of the brief period of its appearance in the larval cultures. Additionally its occurrence remains unpredictable and sporadic.

Chemical inhibition of mucous mat development with oxytetracycline and formalin has not been successful so other treatment regimens to prevent this cause of mass mortality were considered.

The mucous mat followed a typical pattern of emergence, development and disappearance. The first signs of mucous mat appeared on the tank bottom between days 2 and 4 as dark patches of particulate debris, larvae and rotifers, both live and dead. The patches had a defined border and were relatively thick, rising from the bottom of the tank by 2 to 4 mm and were typically round in shape in early development but can expand to cover a large portion of the tank bottom. These characteristics distinguish mucous mat from normal sedimentation of debris on the bottom of larval culture tanks.

No report in the literature clearly defines this type of problem in marine larval culture systems for other species and few other mud crab culture researchers have reported such a phenomenon. One prawn hatchery operator in Australia commented that he had once seen something similar to that described but others had never seen it. One report of a shrimp hatchery in Malaysia seemed to indicate the occurrence of a similar "sticky" mass causing high mortality.

The primary way of combating mucous mat at BIARC has been to siphon all bottom debris from the tank daily and wipe the bottom. The mat however can quickly reform and become extensive over-night. It is also suspected that the observable bottom formation may not be the sole cause of mortality during the mucous mat episode as larval mortality is far greater than those larvae trapped within the matrix.

A series of experiments were conducted that investigated culture management practises that may influence the development of mucous mat, including larval stocking density, addition of rotifers, water exchange regimen, water treatment and the potential for chemical intervention.

It is considered that parameters that affect the rate of water quality deterioration such and stocking density may contribute to the conditions which promote mucous mat development. Additionally chemical treatments, both antibacterial and anti-microbial agents were tested

alone and, in one experiment, in combination to identify potential means of rapidly treating a culture in the event of an identified outbreak. Both oxytetracycline and erythromycin are broad spectrum anti-bacterials that have been used effectively in prawn hatcheries. These were both trialled, as well as two commonly used anti-microbials, to determine a treatment that could potentially be applied to cultures as a last resort in the event that the mucous mat development was observed in cultures. It was hoped that this would reduce its catastrophic impact on mud crab larval cultures until other less invasive methods were developed.

The following are reports of experiments conducted in the BIARC hatchery that were undertaken to gain some understanding of mucous mat development and methods of inhibiting its development. It should be noted that while each of these trials had a strong experimental agenda they were also designed to produce post-larvae for nursery experiments.

Large-scale trial:

Influence of continuous water exchange on mucous mat development. (BIARC–Batch 10)

Introduction

Standard larval culture method at BIARC involved static cultures with daily, batch water exchange. This trial was designed to provide information on the potential of semi-continuous flow through of new seawater to inhibit mucous mat development. Additionally the effect of tank loading was tested by comparing the standard stocking rate and feeding rate with increased levels.

Materials and methods

This experiment commenced 28 February 2002. The experimental design was a 2 x 2 factorial of 2 x seawater treatments, settled/UV, and culture loading rate, standard/high. Larvae were produced and stocked into the 4 x 1.2 tonne culture tanks following BIARC standard operating procedure. Cultures were maintained as per BIARC SOP except to the application of water exchange treatments and stocking feeding treatments as outlined. The high loading treatments were stocked at 300 $Z1L^{-1}$ and fed rotifers at 1.5 times the standard rate, 13–15 rotifers mL^{-1}. The two sources of seawater were used for flow through in each of two tanks, settled (matured as per BIARC standard operating procedure) and UV treated seawater. Flow through occurred for 15 to 16 hours overnight at a consistent rate of 3L min^{-1}. This flow rate equates to 225–240 per cent water exchange per day.

Results

Mucous mat developed in all tanks in the typical pattern between day 2 and day 3 of culture. Larval survival declined from approximately 90 per cent on day 2 to less than 10 per cent of day 3. All cultures were terminated on day 4.

Discussion

There was no indication that mucous mat development was influenced by exchange source water or loading rate of larvae and feed. Additionally the pattern of mucous mat appearance was the same as that previously occurred so that there is no apparent benefit to implementing a high rate of semi-continuous water exchange regimen.

The incidence of mucous mat development within the spring/summer season of 2001–02 was the greatest on record for the BIARC hatchery. In the past there had been spontaneous disappearance of the phenomenon that have remained unexplained. There is no evidence that mucous mat development poses a real threat to commercial crab hatcheries as there is no record of its persistence elsewhere in crab or other species hatcheries with only anecdotal comments of rare occurrences. The problem appears to be specific to BIARC. Detailed research on the phenomenon is constrained by the sporadic nature of its occurrence, the inability to isolate the causative organism(s) on bacterial plates and the inability to maintain a sample of the mat beyond its several day period of existence in the larval cultures.

Large-scale trial:

Chemical inhibition of mucous mat development using formalin, trifluralin and erythromycin. (BIARC–Batch 11)

Introduction

Formalin is a widely used anti-microbial particularly effective against fungus and protozoans. It also has weak antibacterial properties. Trifluralin is used widely in overseas hatcheries to combat fungal contamination in hatcheries. Both of these chemicals were trialled for effectiveness against the mucous mat upon the recommendation of colleagues in crab hatcheries in the Philippines and Indonesia.

This trial tests the potential for the anti-microbials formalin and trifluralin as well as the antibacterial, erythromycin, to inhibit mucous mat in large-scale cultures.

Materials and methods

This experiment commenced 16 March 2002. There were four treatments, three chemicals applied as per the Table 53 and a control with no chemical addition. Each treatment was duplicated. Larvae were produced and stocked into the 8 x 1.2 tonne culture tanks following BIARC standard operating procedure. Cultures were maintained as per BIARC standard operating procedure except for the application of chemical treatments as per the experimental design. Treatments were applied daily after water exchange.

Table 53. Application rates for chemicals applied to culture tanks (BIARC Batch–11).

Treatment	Concentration	
	day 0–1	day 2 onwards
Formalin	5ppm	5ppm
Trifluralin	0.1ppm	0.5ppm
Erythromycin	2ppm	4ppm
Control	–	–

Results

The typical mucous mat did not develop in any of the eight tanks including the control tanks with no chemical treatment. However minor patches of similar appearance to the mucous mat occurred in one control tank and both erythromycin tanks on days 2 and 3. The erythromycin treatment showed the highest mean survival to day three but there was a large discrepancy between the duplicate cultures, 6–44 per cent. All other cultures showed consistently low survival. Cultures were terminated on day 3.

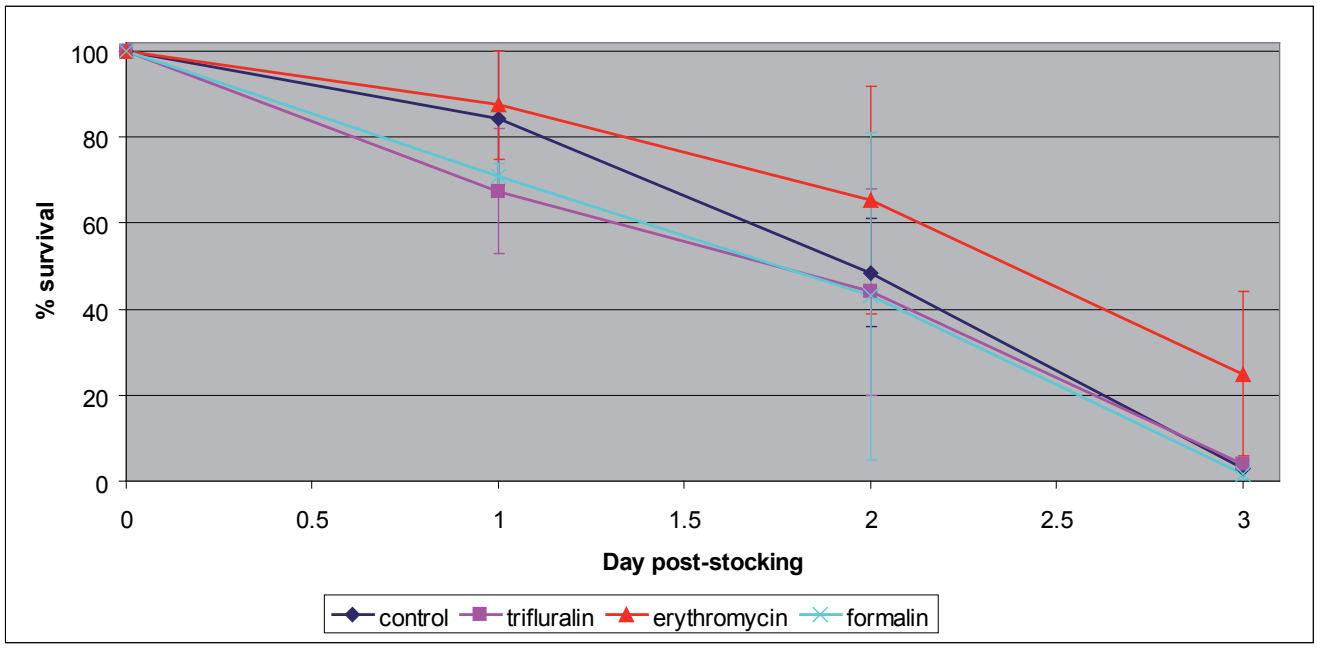

Figure 43. Larval survival in large-scale cultures under different chemical treatments. Error bars indicate range of duplicates. (BIARC Batch–11).

Discussion

It is difficult to draw anything meaningful regarding mucous mat development from this trial. Typical mucous mat did not develop however even in its absence larval mortality was rapid within the first three days. The occurrence of small patches in some cultures may indicate that the causative organism(s) were present but did not proliferate to form the typical morphology but still adversely affected the larvae. Application of a broad spectrum antibiotic, erythromycin, did not protect the larvae from mortality to a significant extent however there was some indication that survival was slightly enhanced in this treatment. These results do not indicate any impact on the larvae by formalin or trifluralin either positive or negative. Application rates for these two chemicals were as advised by hatchery operators however the maximum non-toxic dose is not known for continuous application as used in this trial.
A subsequent toxicity trial of erythromycin in laboratory cultures indicated that long term exposure to this chemical did not compromise larval survival or development up to 14 days continuous exposure at 50ppm. In this trial survival rate was enhanced by erythromycin at 10, 30 and 50ppm compared with untreated control cultures.

Large-scale trial:

Chemical inhibition of mucous mat development using erythromycin and combinations of erythromycin and formalin and trifluralin. (BIARC–Batch 12)

Introduction

The previous experiment examined the ability of formalin, trifluralin and erythromycin to inhibit mucous mat. These chemicals have differing modes of anti-microbial and antibacterial activity. Therefore there is potential for synergistic inhibitory activity of these chemicals against mucous mat. This trial examined the combination of erythromycin, an antibacterial, and formalin and trifluralin, both chemicals with known anti-microbial and anti-fungal properties. Formalin also has some antibacterial activity though relatively weak. It has been suspected that the rotifers could be the vector for the mucous mat organisms as the mat development always occurs during the rotifer feeding phase. There is however no causative link yet established. This experiment compared cultures fed rotifers in the standard manner with cultures fed *Artemia nauplii* only.

Materials and methods

This experiment commenced 23 March 2002. There were eight treatments, three chemicals were applied as per Table 54 and a control with no chemical addition in each of two feeding treatments–the BIARC standard feeding method which feeds with rotifers for the first five days and without rotifers where *Artemia nauplii* only were fed from day 0. Chemical addition at the concentrations listed in Table 54 occurred every second day from day 0 (day of stocking) after water exchange. Larvae were produced and stocked into the 8 x 1.2 tonne culture tanks following BIARC standard operating procedure. Cultures were maintained as per BIARC standard operating procedure.

Table 54. Chemical treatments applied to larval cultures every second day. Each chemical treatment was applied to cultures with and without rotifers (BIARC Batch–12).

Treatment regimen	Erythromycin	Trifluralin	Formalin
E	10ppm	–	–
E & T	10ppm	1.0ppm	–
E & F	10ppm	–	10ppm
Con	–	–	–

Results

The first indications of mucous mat development occurred on day three in all tanks with small patches of material with the typical mucous mat morphology on the tank bottom. The small patches persisted through to day 6 in all tanks and did not proliferate beyond this extent. Sparsely spaced mucoid accretions occurred in tanks after day 6 some with the typical circular patch morphology and some showing a “streaked” pattern of development.

Larval survival dramatically decreased in all tanks over the first three days, even before the first indication of mucous mat development was observed. The mortality was least pronounced in two treatments, *Artemia* with erythromycin and *Artemia* with erythromycin and formalin . In the treatments receiving no chemical addition, the rotifer treatment was terminated on day 5 and the *Artemia* treatment on day 7 due to almost complete mortality . By day 12 both the *Artemia* and rotifer trifluralin treatments survival was below 1 per cent and were also discontinued. The remaining four treatments *Artemia* and rotifers with erythromycin and with erythromycin and formalin continued through to day 22 when megalops were harvested (Figure 44). A total of 9350 megalops were produced with the highest production of 5000, representing a production rate of 5 per cent, occurring in the *Artemia* with erythromycin and formalin treatment (Table 55).

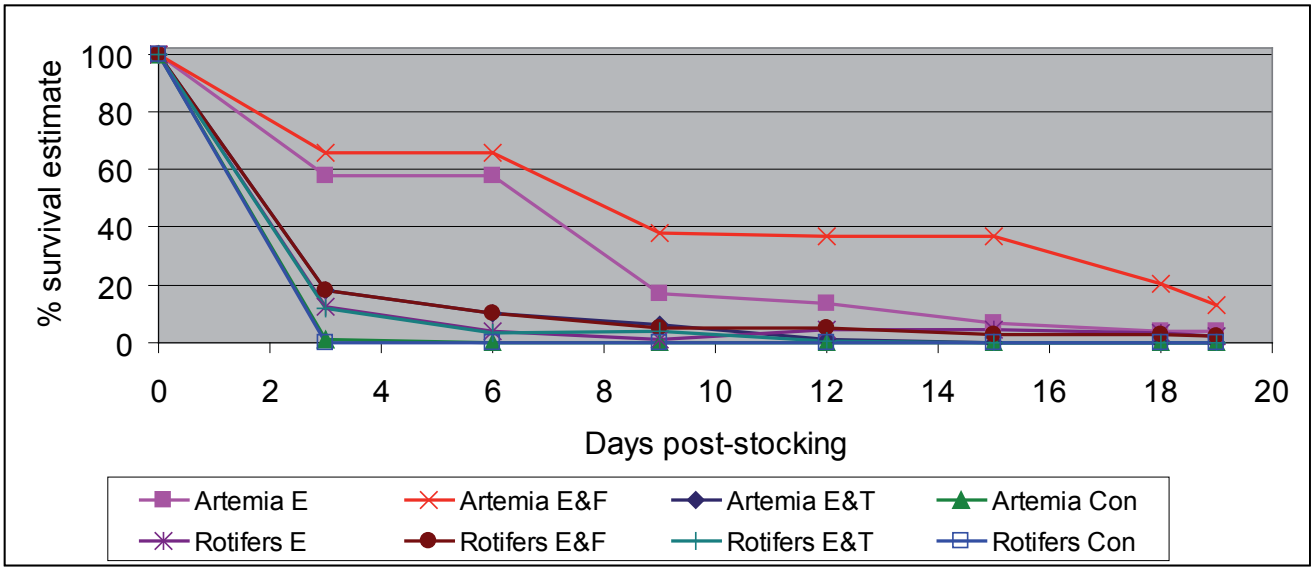

Figure 44. Larval survival in large-scale cultures under different chemical treatments. Values are estimates derived from volumetric sampling. Where values of a treatment showed increasing survival (due to sampling error) the average survival estimate over the relevant period is presented. (BIARC Batch–12).

Table 55. Estimated number of megalops produced and per cent production rate (of initial number stocked) in cultures under different chemical treatments.

Treatment	Artemia	Artemia	Rotifers	Rotifers
regimen	E	E&F	E	E&F
No. megalops	1700	5000	950	1700
% production	1.70	5.00	0.95	1.70

Discussion

The purpose of the chemical treatments was to demonstrate whether they could control the development of the mucous mat. There was no indication that any of the chemical treatments reduced mucous mat development compared with the control tanks receiving no chemicals. Even though the mucous mat did not proliferate to the extent of covering a large proportion to the tank bottom, as has been previously observed, it developed similarly in all tanks including the control tanks. Similarly there was no indication that the use of rotifers promoted the development of the mucous mat syndrome, occurring to the same extent in both rotifer and *Artemia* only cultures.

Each treatment was not replicated so defining “practical” differences among treatments was reliant on a consistent pattern of treatment effects occurring within the experimental regimes. Several patterns emerge however would require subsequent experimentation to define whether there is real significance to the occurrence. One pattern is that the two treatments receiving trifluralin did not survive past day 12. There are potential toxicity issues with prolonged exposure to this chemical, however there is no apparent benefit to continuing this line of investigation. A second pattern is that both feed treatments suffered rapid early mortality regardless of chemical treatment and was most catastrophic in the treatments that did not receive the antibacterial chemicals. This pattern of survival has been observed previously both with and without the occurrence of mucous mat. Bacteriological influences are implicated in the mortality as typically application of oxytetracycline mitigates although does not stop the severity of the mortality.

In this experiment feeding with *Artemia* only in the early larval phase did not appear to compromise survival, but neither did it enhance survival relative to rotifers in the absence of antibiotic. Therefore rotifers may not necessarily be the source of bacteria in this trial. In previous laboratory and mass culture trials the use of rotifers has consistently promoted faster growth and higher survival. It is interesting that the two best performing cultures for the first week of culture were only fed Artemia, however caution needs to be exercised in interpreting this result due to the limited nature of the experimental design and the typically wide within-treatment variability that occurs in this line of research. The relative number of megalops produced in each of the four successful treatments was consistent with the pattern of survival through the early part of the cycle. Again, in hind sight, this trial may possibly be compromised by cross-contamination resulting from inadequate quarantine arrangements.

Large-scale trial:

Chemical inhibition of mucous mat and impact on culture bacteriology using erythromycin and oxytetracycline at low and standard larval stocking densities. (BIARC–Batch 13)

Introduction

Both oxytetracycline and erythromycin are broad spectrum anti-bacterials that have been used effectively in prawn hatcheries. The previous trial indicated that erythromycin (ETM) at 10ppm may not affect mucous mat development, however it may enhance larval survival and megalops similarly to oxytetracycline (OTC). This trial investigated ETM and an elevated dose rate. This is compared with OTC applied at 50ppm in the standard manner. Both anti-bacterials are applied to cultures with the normal larval stocking rate (100 larvae L^{-1}) and with a low stocking rate (30 larvae L^{-1}) to assess impact of stocking rate, and therefore culture loading, on mucous mat prevalence and general culture bacteriology.

Materials and methods

This experiment commenced 6 June 2002. A 3x2 factorial design of chemical treatment and stocking density produced six treatment combinations as listed in Table 56.

Larvae were produced and stocked into the 6 x 1.2 tonne culture tanks following BIARC standard operating procedure. Cultures were maintained as per BIARC standard operating procedure with antibacterial treatments added every second day.

Table 56. Chemical treatments applied to larval cultures every second day (BIARC Batch–13).

Antibacterial (Z1 L^{-1})	Dose rate (ppm)	Stocking density
Erythromycin (ETM)	30	100
Erythromycin (ETM)	30	30
Oxytetracycline (OTC)	50	100
Oxytetracycline (OTC)	50	30
None (Con)	–	100
None (Con)	–	30

Results

The first mucous mat patches of debris on the tank bottom occurred on day three of culture as five small patches of debris in the control tank stocked with 100 larvae. Subsequently on day four all tanks had a similar prevalence of patches on the bottom. None of the tanks developed larger patches during the trial. Both control tanks with no antibacterial treatment suffered heavy mortality in the first four days of culture and were both terminated by day 7. All other cultures except the OTC stocked with 30 larvae treatment showed relatively high early

mortality with an estimated survival rate of less than 50 per cent on day 7. On day 22, both ETM tanks were terminated due to almost complete mortality (Figure 45). To this day there had been no moulting to the megalops stage in any tanks. Both OTC tanks were continued to day 40 during which time the larvae remained vigorous with only gradual daily mortality. The larvae did not grow however and by day 40 were still almost entirely Z4 and Z5 stage. There had been only a small number of megalops occur between days 26 and 40. Cultures were terminated on day 40.

Bacteriological plating indicates that the OTC treatments had less total *Vibrio* as well as lower sucrose negative and luminescent *Vibrio* numbers compared with the ETM treatment.

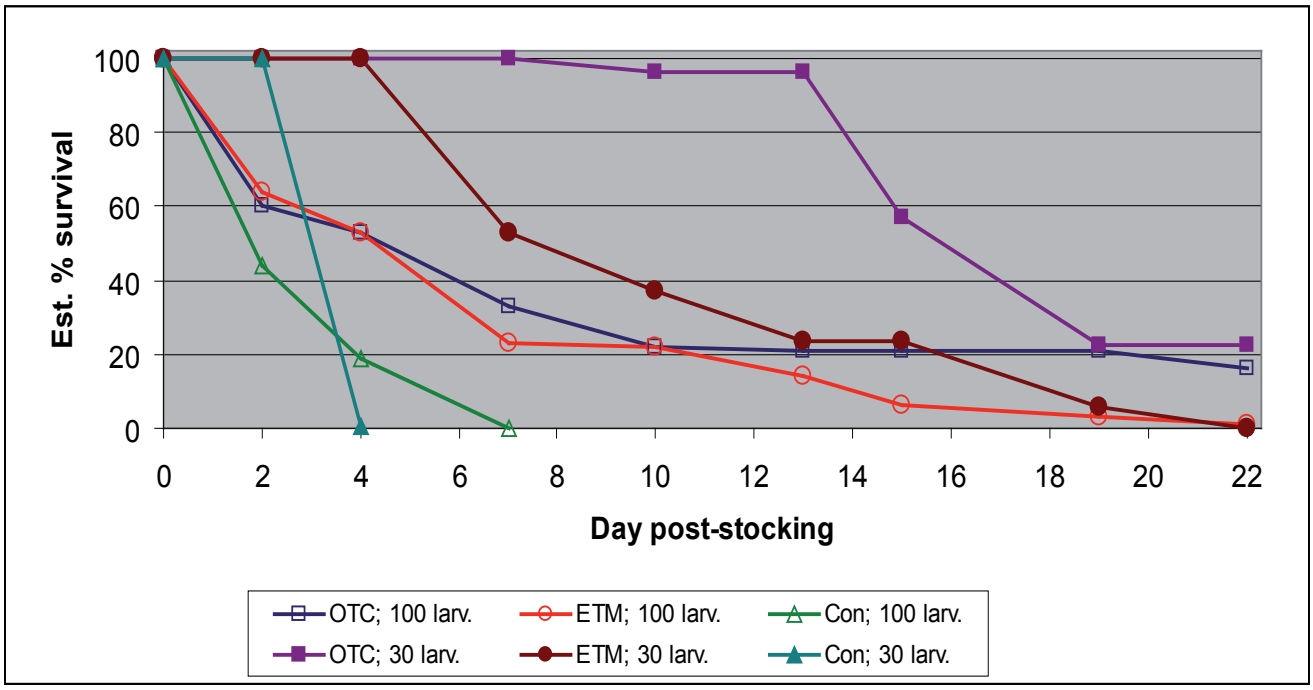

Figure 45. Larval survival in large-scale cultures with and without antibacterial treatment under two stocking density regimens. Values are estimates derived from volumetric sampling. Where values of a treatment showed increasing survival (due to sampling error) the average survival estimate over the relevant period is presented. (BIARC Batch–13).

Discussion

The pattern of apparent mucous mat development followed that of the previous trial where it did not develop further than a small number of small patches. Also similar to the previous trial its failure to continue development to cover a large proportion of the bottom cannot be attributed to the inhibition by the anti-bacterials as the same pattern occurred in the untreated tanks. It is therefore apparent that even a relatively high dose of erythromycin did not control the bacteriology of the cultures to a very high degree. The bacteriological plating indicated that *Vibrio* may be less inhibited by erythromycin at 30ppm than oxytetracycline at 50ppm. This may explain why the oxytetracycline treatment larvae persisted longer than those of the erythromycin treatment. Erythromycin is an antibacterial previously used in prawn hatcheries as it was found to be effective against *Vibrio*sis. In this trial however oxytetracycline appeared to be more effective. This may be explained by OTC acting against bacteria not associated with the mucous mat, as has been found elsewhere in this report.

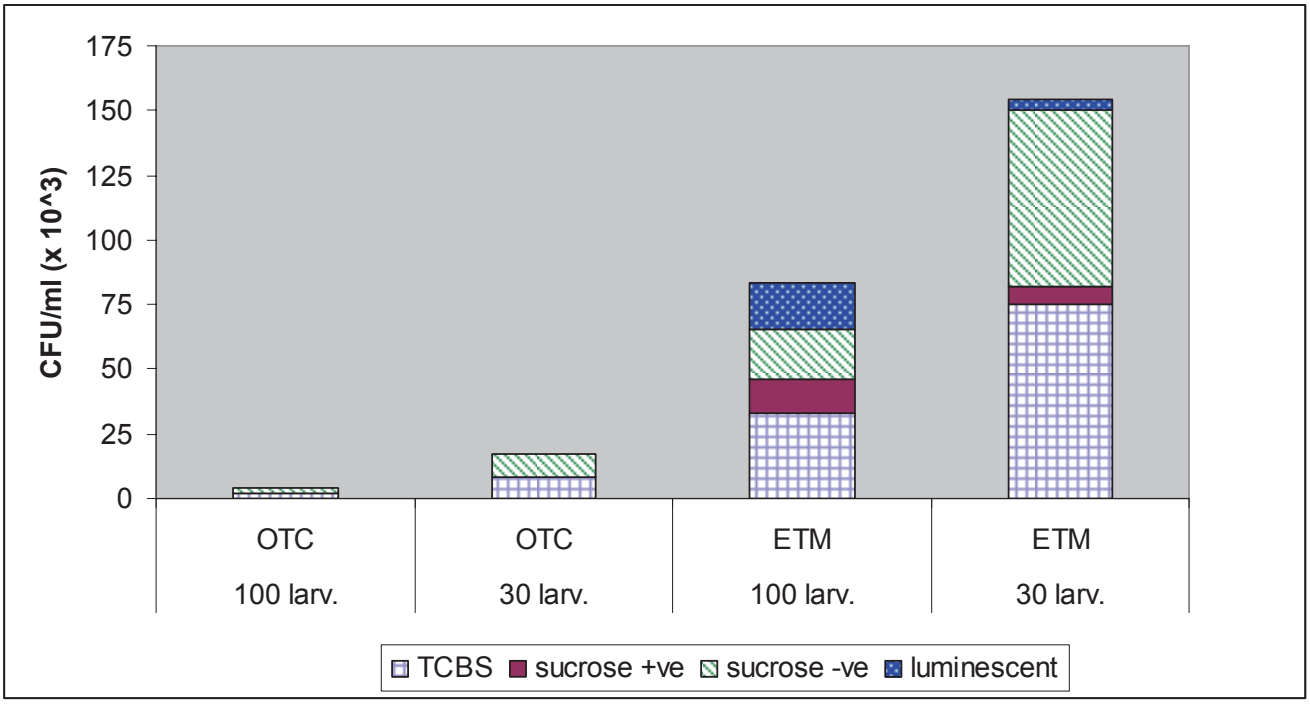

Figure 46. *Vibrio* colony forming units (CFU) mL^{-1} of water from each of the antibacterial treated cultures, plated on TCBS medium. Samples taken on day 14 of culture.

Stocking density did not appear to affect the appearance of mucous mat patches or their persistence in the cultures. Further there is no clear trend as to whether larval survival was influenced by stocking density in the early larval stages. For valid statistical analysis of this experimental data a further one or two replicates of this experiment would need to be conducted. However this line of investigation is not worth pursuing further as the result gained, although not statistically analysable, provided sufficient information on the potential for erythromycin to be used as a last resort chemical for the control of mucous mat and other bacteriological problems encountered in the hatchery.

The almost complete cessation of larval growth at the Z4 and Z5 stage has not been observed before. Under adverse conditions larvae that stop growing typically die within a short time. It is an indication of the plasticity of larval development patterns that the final zoeal stages can persist for more than 20 days. The cause is unknown but is unlikely to be related to the exposure of the larvae to oxytetracycline as previous trials have not encountered a similar phenomenon.

Large-scale trial:

Reducing oxytetracycline usage rates. (BIARC–Batch 14)

Introduction

In recent years large-scale production trials have consistently indicated that at the BIARC hatchery application of an antibacterial to the larval cultures is required to ensure an acceptable level of productivity. Oxytetracycline is routinely added at intervals throughout the larval cycle. Using this method megalops production has become much more predictable with catastrophic larval mortality events occurring less frequently. The only exception to this is the occurrence of the mucous mat, which does not appear to be inhibited by conventional antibacterial and anti-microbial treatments. Use of oxytetracycline, or any other antibiotic, is considered to be undesirable so measures are sought to initially at least greatly reduce its usage rate and ultimately to not use it at all.

This experiment investigated the potential to restrict the period of OTC application during the larval cycle.

Materials and methods

This experiment commenced 23 March 2003. Larvae were produced and stocked into 6 x 1.2 tonne culture tanks following BIARC standard operating procedure. Cultures were maintained as per BIARC standard operating procedure. OTC was added to the tanks at a rate of 25ppm per day. Three OTC addition treatments in duplicate were applied to the tanks as follows:

1. OTC application stopped at the end of Z2 stage on day 6 (OTC-Z2)
2. OTC application stopped at the end of Z4 stage on day 15 (OTC-Z4)
3. OTC application stopped at the end of Z5 stage on day 19 (OTC-Z5)

Results

Up until day 7 all six tanks had similar survival and growth rates, however by day 11 the OTC-Z2 treatment tanks with OTC addition stopped on day 6 showed comparatively high mortality rates. This treatment was discontinued on day 17 due to negligible survival. The OTC-Z4 and OTC-Z5 treatment tanks were harvested on day 20 and 21 when approximately 40 per cent of the population was megalops stage and the rest zoea 5. In total 36,800 megalops were produced from the four remaining tanks and used to fully stock the nursery system for production of crablets for subsequent crablet experiments. There was no discernable difference in the megalops production rates between the OTC-Z4 and OTC-Z5 treatments (Figure 47).

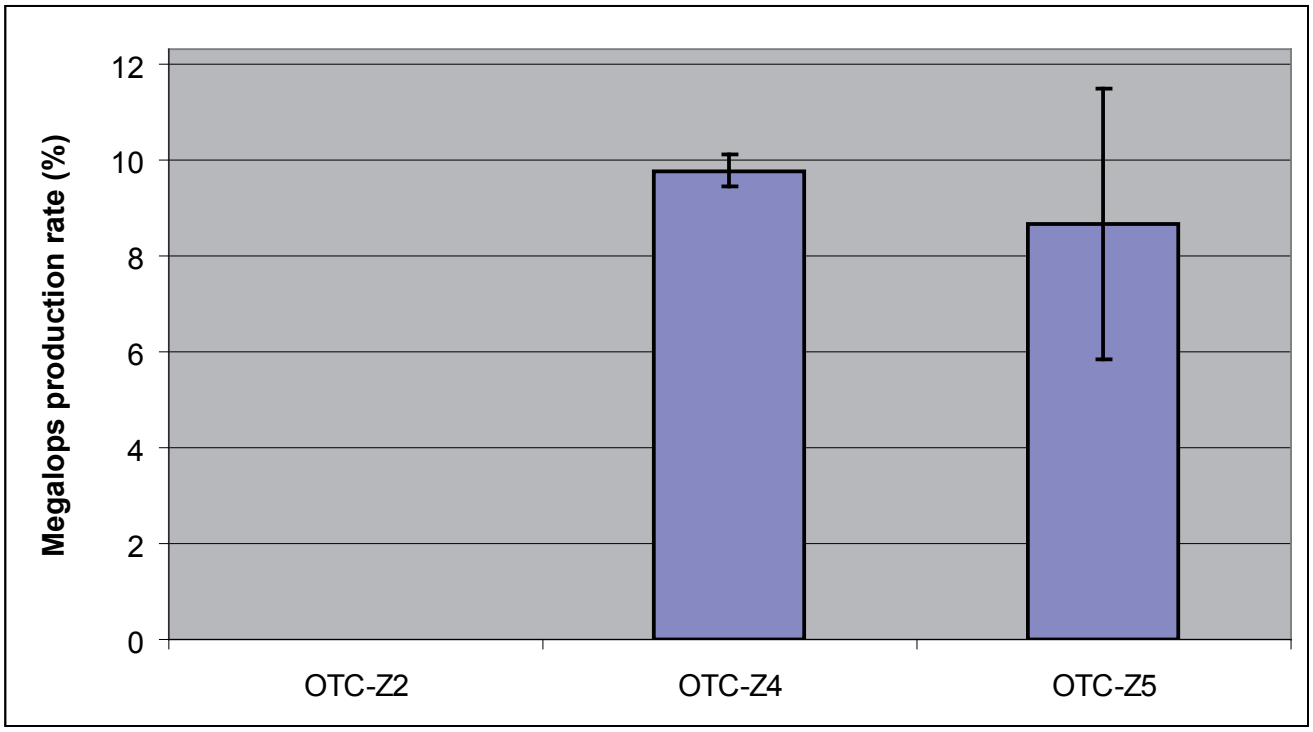

Figure 47. Mean per cent production rate of megalops when treatment with OTC was discontinued at different instars. Error bars indicate range of duplicate tanks. (BIARC Batch–14).

Discussion

This limited trial indicated that the larval cultures benefited from the added level of bacteriological control possible by oxytetracycline addition through the later stages of development. However it indicated that it may be possible to stop OTC addition at Z4 rather than continuing treatment through to the end of the larval cycle. It was apparent that stopping OTC treatment after completing only approximately one third of the larval cycle duration left the larvae susceptible to deleterious bacteriological influences.

Work at the Darwin Aquaculture Centre found differing effects in relation to the time at which OTC treatment was discontinued. These differences may be a reflection of different systems and feeding protocols used at the two centres.

Large-scale trial

Examination of the benefit of high density stocking of mud crab larvae. (DAC–Batch 30)

Introduction

In earlier large-scale trials it was noted that the majority of mortalities occurred during the Z1 stage. From these results it was theorised that if significant numbers of Z2 larvae could be produced, (eg 30 000 Z2 per tank), it should then be possible to get the majority of the remaining larvae through to megalops and then crablet, once the major period of mortality (during Z1) was over. The most obvious way to ensure that sufficient numbers of Z2 are available for stocking, would be to stock Z1 at very high density, assuming the usual significant losses.

The aim of undertaking larval rearing on a large-scale was to work at a scale approximating commercially production. The ultimate goal of the large-scale work was to produce an acceptable number of crablets per larval run, regardless of the starting number of larvae. In the case of mud crabs, with extremely high fecundity, it was considered this could be a feasible option.

This trial was undertaken to compare the larviculture strategy of initial high density larviculture and subsequent redistribution of larvae at zoea 2, with standard operating procedures developed.

Methods

Six standard 1000 L tanks were used. These tanks were arranged as two parallel rows of three under an outside shade structure at the DAC. All tanks operated as re-circulating systems although no biofiltration was included. An AQUACLONE was used to keep larvae up in the water column.

Two 300-watt immersion heaters were placed inside the Bazooka in order to heat the water without directly contacting the larvae. Temperature of the culture water was maintained at 30°C (± 0.1) by thermostatically controlled immersion heaters.

Throughout the experiment, tanks underwent a 70 per cent drain-down, top-up water exchange regime. After drain-down the walls of all tanks were wiped clean. Screens, airlifts and the Bazooka were removed and cleaned prior to top-up. The 70 per cent of the water drained-down each day served to flush the foam fractionation units each day before use. The 30 per cent of the water remaining in the tank was then foam fractionated for 30 minutes, followed by fractionation & flow through with storage water for another 30 minutes. Tanks were then filled with storage water. Each tank had its own foam fractionator.

Experimental Treatments

Treatment A: Tanks stocked at 30 Z1 mud crab larvae L^{-1} and received a daily treatment of 50ppm OTC

Treatment B: Tanks stocked at 250 Z1 mud crab larvae L^{-1}, survivors of which were redistributed into separate culture tanks at Z2. No OTC treatment.

There were three replicates of each treatment.

Only enough larvae were available to stock two tanks at 28.75 larvae L^{-1} following the Z1 stage of treatment B.

When megalops were observed in the larval tanks, selective harvesting using a 2000μm screen was carried out.

To the rotifers produced, harvested and concentrated using SOP, 40ml of *Chlorella* V12 paste and 3.5g of OTC (50ppm) were then added to both during a four hour enrichment process on day 1 of the trial. For the following three days rotifers were enriched with *Chlorella* V12 and OTC (50ppm) overnight. Rotifers were then fed to larvae in treatment A at 10 rotifers mL^{-1} in a single daily feed, whilst those in treatment B received 20 rotifers mL^{-1} in a single daily feed

Results

The larvae did not hatch on Spawn Day 10 (ie 10 days since spawning) as was expected, although there was a significant pre-hatch release. It is probable that the eggs hatched soon after dark on SD10 and would therefore not have been transferred to the culture tanks for up to 15 hours or fed for up to 17 hours after hatching.

Temperature of the cultures ranged from 28.1– 30.2°C, averaging 29.3°C during the trial.

There appeared to be substantial mortality during Z1 stage in all tanks, but especially in the high density tank where of 250 000 larvae stocked, only 57,500 (~23%) were harvested on day 4 at Z2 stage.

Data relating to final survival to megalops is presented in Table 57. Megalops were first noticed on day 12 in the “high density at Z1” tanks. Significant numbers of megalops were seen in all tanks on day 13 and selective harvesting began on this day. Treatment A yielded survival rates to megalops of 29 per cent to 38 per cent, whilst treatment B had <1 per cent to 15 per cent, although all these died within three days of moulting to megalop.

Discussion

Given the less than optimal conditions the newly hatched larvae were subjected to, including no food for a considerable duration, crowding, possible poor water quality, high organic loads and probable microbial proliferation, the results of this trial could only be considered to be preliminary at best. Had the larvae been removed from the hatching tank immediately after hatching and provided with food and better water quality, the initial poor larval survival may not have occurred and better overall survivals may have been obtained.

The survival in the mass cultures was reasonably good after the initial dropout, even in the treatment B. Treatment B tanks were given 50ppm OTC on days 8 and 9 as a precautionary measure, after several bodies were seen on the bottom and floating in the currents on day 8 as it had already been established that the treatment was not as productive as the OTC treatment. There were less mortalities on day 9, and by day 10 the mortalities had apparently all but ceased demonstrating that OTC could be applied in response to a disease outbreak. However on day 13 there were again substantial numbers (100’s) of bodies in the treatment B tanks and this time they were not treated with OTC. There were again substantial mortalities on day 14 and both tanks were terminated on day 15. Four thousand seven hundred megalops were screened from one treatment B tank over days 13 and 14, but fewer than 20 were removed from the other tank. Those that were removed were stocked to a new tank and were monitored as usual

but they crashed after a further two days. The cause was probably bacterial from the appearance of the water in the tanks although no samples were taken for bacteriology.

The 50ppm OTC treatment again proved successful in supporting the successful culture of mud crab larvae to megalops. To further investigate the value of initial high density stocking of Z1 larvae, this trial would need to be repeated with better quality larvae.

Table 57. Daily megalops production (DAC Batch–30).

Day	Tank No./ Treatment	Number sampled	Number of samples	Sample volume (mL)	Total volume (l)	No. of Megas
13	T1–A	46	10	300	100	1533
	T2–A	93	20	300	100	1550
	T3–A					33
	T5–B					20
	T6–B	101	10	300	100	3367
14	T1–A	54	10	72	100	7500
	T2–A	66	10	72	100	9167
	T3–A	57	15	72	100	5278
	T5–B					
	T6–B	40	10	300	100	1333
15	T1–A	26	15	72	100	2407
	T2–A	8	1	300	20	533
	T3–A	25	10	72	100	3472
	T5–B		Tank terminated			
	T6–B		Tank terminated			

	Totals	Megalops	Zoea	Survival %
	T1 –A	11441	500	39.8
	T2–A	11250	200	38.1
	T3–A	8783	300	30.3
	T5–B	20	12500	sick/dying
	T6 –B	4700	3400	sick/ dying

Large-scale trial:

Comparison of tank size and design. (BIARC–Batch 16)

Introduction

Two types of larviculture tanks were used at BIARC for large-scale production trials. Experimental scale tanks of 1.2t capacity were used for trials requiring replication of treatments and commercial scale tanks of 6t capacity were used for "proving" treatments in a system with potential application for commercial hatchery operators.

With the primary aim of producing sufficient numbers of megalops for nursery and grow-out experiments at BIARC this trial compared the performance of the experimental and commercial scale larviculture systems.

Materials and methods

This experiment commenced 2 September 2003. Larvae were produced, stocked and maintained in 2 x 1.2 tonne and 2 x 6 tonne culture tanks following BIARC standard operating procedures. Oxytetracycline was added every second day at 50ppm. The standard water exchange regimen was changed to alternating 40 per cent and 80 per cent on successive days for tanks.

Results

Survival in all tanks, both 1.2 and 6 tonne, was similarly high up to day 10, however following this all tanks showed consistent mortality up to the day of harvest (Figure 48). There was no apparent difference in the pattern of survival between the two groups of tanks. Towards the end of the larval cycle developmental rate diverged between the 1.2t and 6t tanks with larvae in both 6t tanks taking an extended period of time to complete the Z4 and Z5 stage compared with larvae in the 1.2t tanks (Figure 48). Megalops were harvested on day 23 from the 1.2t tanks and days 29 and 31 from the 6t tanks. In total 22 300 megalops were harvested from all four tanks representing production rates per tank volume of 1.2 to 3.9 megalops L^{-1} (Table 58).

Discussion

The large and small tanks showed similar patterns of larval survival indicating that tanks conditions were not significantly different between the two sizes. However from the Z4 stage, larval developmental rate was much slower in the larger tanks. It is speculated that this could be due to the relative inefficiency of removal of uneaten, old live feeds from the larger tanks. The feed collection system used at BIARC was driven by 50 mm diameter airlifts and due to the limited flow the water filtration rate was relatively higher in the smaller tanks. Therefore less old, nutrient depleted food was left in the 1.2t tanks. More efficient feed removal systems will need to be developed for the larger tanks. This should simply be a matter of increasing the diameter of the "within tank" larval screen and increasing the capacity of the airlift by using 100 mm pipe and greater airflow. Sufficient megalops were produced to supply the subsequent crablet experiments however the rates of production were uniformly low at 3.9 and less megalops L^{-1}. The trial indicated the risk management value of using culture systems that can potentially produce in excess of the post-larvae required.

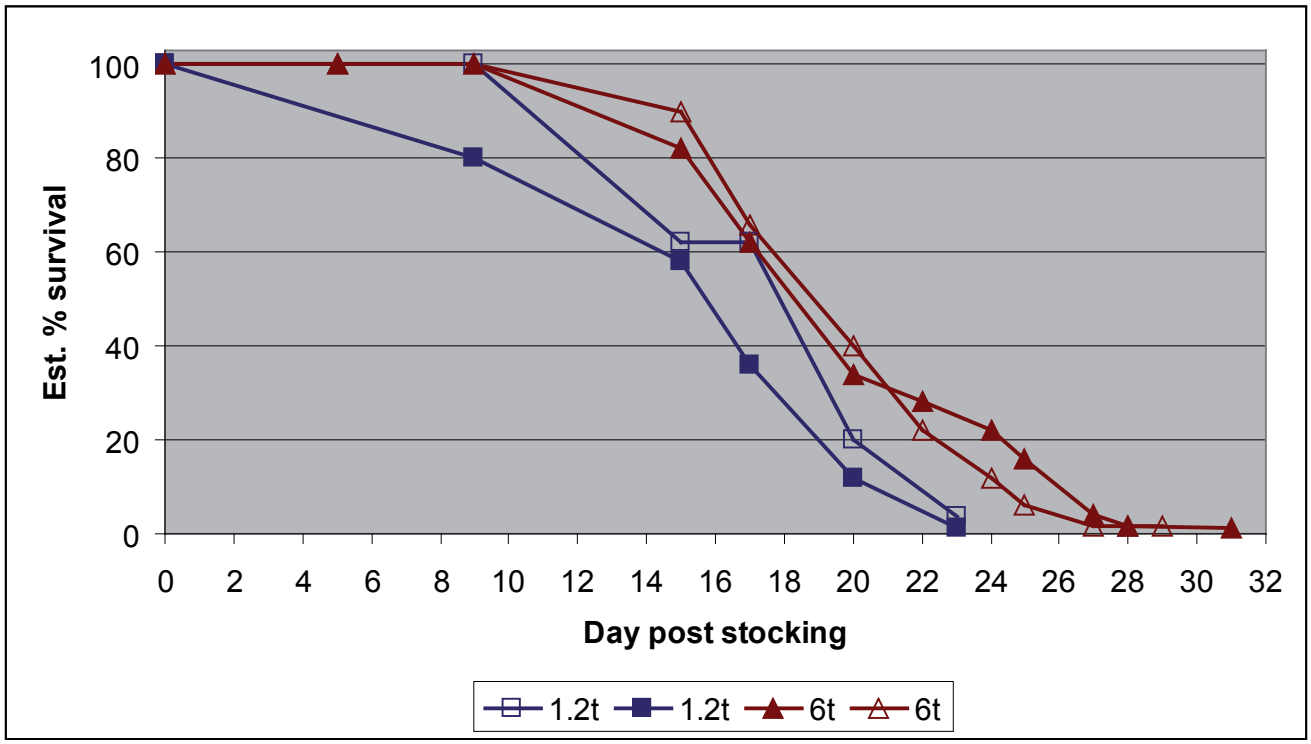

Figure 48. Per cent larval survival in large-scale cultures.
Values are estimates derived from volumetric sampling. (BIARC Batch–16).

Table 58. Megalops harvest results of experimental and near-commercial scale hatchery tanks.

Tank volume	Harvest day	No. megs	Production rate
(tonne)		harvested	(megalops L^{-1})
1.2	23	4200	3.9
1.2	23	1320	1.2
6	31	7600	1.3
6	29	8800	1.5

Larviculture Discussion

Standard Operating Procedures

The two aquaculture research centres involved in this trial (DAC and BIARC) used similar, but significantly different standard operating procedures. These systems were based on their respective experiences, the equipment available to them and staff to operate them. The DAC based in the Northern Territory takes its water from Darwin Harbour, a mangrove fringed harbour which experiences 8m tides and can have a very heavy sediment load, whilst the Queensland based BIARC collects its seawater from a surf beach area. Darwin has two distinct seasons, wet and dry, whilst Brisbane has four recognisable seasons. This makes between site comparisons difficult at best. What could be said about the two sets of standard operating procedures by the end of the project, is that juvenile crabs could be produced from both centres, with much more reliability and consistency than at the start of the project.

The standard operating procedures are reflected in the CD attached to this report, in addition to their description earlier in this chapter. These procedures shouldn't be considered to represent an optimal system for production, but can be considered advanced enough to support commercial hatchery production.

Bacterial Control

The decision to use oxytetracycline (OTC) as a tool to better understand bacterial control in mud crab larval production systems was one which rapidly pinpointed key periods of risk in the production cycle. It led to refinement of feeding regimes, water treatment and the design of the larval rearing system.

Having experimentally identified mud crab larval survival could be improved by the addition of OTC at 50ppm at both a small and large-scale, the project rapidly moved to examine its effect at different stages of the production trial. A number of trials clearly demonstrated that restricting the use of OTC from hatch to day 2 of zoeal stage 2, resulted in no significant differences in larval survival compared to the use of OTC throughout the larval production cycle. Whilst there was no significant difference between the use of OTC at concentrations ranging from 10–50ppm, it was considered that 50ppm should be used. The rationale for this was that it was a recommended dosage for a range of fish and shellfish treatments, and that a higher concentration, short duration treatment regime minimised the risk of the development of resistant bacteria.

The period when OTC appeared to be most needed, from hatch to day 2 of zoeal stage 2, corresponded with the period of addition of rotifers as feed for the mud crab larvae. More detailed analysis of bacteriology of the systems is detailed in the Bacteriology section of this report. This apparent relationship between larval mortality and rotifer feeding led to exploration of the value of treating the rotifers themselves with OTC, but a number of trials (DAC Batches 18 and 28) found this not to be effective.

In several trials it was noted that whilst larval survival was enhanced by the use of OTC to day 2, zoeal 2 stage, it did appear to slow their growth and inter-moult time.

A preliminary attempt was made to examine the effect of iodine at low concentrations (0.5ppm) as an alternate to OTC, however the result was uncertain and no further work undertaken.

Substitution of rotifers with decapsulated cysts in trials at the DAC (Batches 9,11, 12 and 13) showed some promise, however this was not fully investigated during the study, and remains an outstanding issue to investigate.

Whilst OTC was found to be effective generally in minimising mortalities, it was not found to be a cure-all. Significant mortalities apparently caused by the moult death syndrome (between Z5 and megalops) still occurred on an irregular basis.

System Design

In recognition that bacteria appeared to be a major contributor to mud crab larval mortalities, the system was modified to reduce bacterial numbers. As bacteria require an organic base on which to multiply, protein skimmers (foam fractionators) were introduced at the DAC (but not at BIARC) to both incoming water and to operational larval tanks to reduce the build-up of organic wastes in tanks. Pre-filtration, filtration, and the time that water was settled for prior to trials varied between DAC and BIARC. As mud crab larvae appear very sensitive to good water quality and bacterial conditions during culture, every effort should be made to ensure that system design is optimised to produce high quality seawater, with stable parameters eg temperature and salinity, and minimise bacterial proliferation.

As biofilms, presumably with a significant bacterial content build up on larval tank walls a combination of innovations were introduced to minimise larval contact with the biofilm and to minimise its influence. At the DAC, a small device locally referred to as an aquaclone was put in larval production tanks. This device assisted in lifting water from the bottom to the top of the tank and in swirling water around the tank. This device led to crab larvae spending less time in contact with tank surfaces and its biofilm. Its introduction meant that larvae were better kept in suspension and were less likely to congregate on the bottom of the tank as they are prone to do without such a device.

The second direct approach biofilm control was to routinely remove it using a soft sponge or cloth on a regular basis during drain down of tanks during daily or routine water change or cleaning events. In both DAC and BIARC the development of a mucous mat on the tank walls or bottom was reported. At the DAC this appeared to be controlled by physical removal and water quality management; however it appeared to be more of a problem at BIARC where it was believed to have been, at least in part, a cause of numerous mortality events amongst the culture larvae. This may have been linked to BIARC's decision not to incorporate foam fractionators into their system as was the case at DAC. The fact that numerous anti-microbials had no effect on suppressing mucous mat formation may strengthen the case for the "mucous mat" to be considered to be constructed from non-living materials.

A number of results led to changes in mud crab larval system designs, particularly at DAC where numerous designs were tried. If flow-through rates in tanks were too high, larvae could be stuck to screens. Flow rates and screen sizes were adjusted to alleviate this problem.

The use of flow-through of water in larval rearing tanks from day 1 to overcome mortalities during the first few days of larval culture, as an alternative to the use of OTC was examined, but was not found to be useful.

Concerns over cannibalism, in particular of the Z5 stage by megalops, led DAC to use a mesh screen to separate megalops out of tanks with Z5 larvae as soon as they moulted. This was found to significantly enhance survival.

A number of studies outside of this project, demonstrated that maintaining a stable temperature in production systems improved mud crab larval survival. This experience is also recognised in commercial prawn hatcheries in Australia. As a result heaters with very precise thermostats (±0.5°C) were used to maintain a stable temperature in larval production tanks. To assist in reducing heating costs and to improve thermal stability, larval production tanks were also covered in expanded polystyrene insulating panels at the DAC. It was found at both the DAC and BIARC that particular care is needed to make sure that larvae do not come into direct contact with heater elements. In addition as stable temperatures appear to minimise crab larval mortalities, it is important that any water changes are made with water at the same temperature as that already being experienced by the larvae.

As cross-contamination between tanks in large-scale rearing systems may be an issue, appropriate system design needs to ensure adequate quarantine between tanks to prevent contamination.

Bacteriology of mud crab larval systems (DAC)

Introduction

It has been understood for some time that successful rearing of mud crab larvae requires control of the bacteriology of larviculture systems. Brick (Brick 1974) found that antibiotics (penicillin-G and polymyxin-B) were necessary to rear larvae of *S. serrata* to the megalops stage, however survival from megalops to crablet was not affected by the their use. Kasry (Kasry 1986) used the same mixture of antibiotics with some success on the same species. A variety of antibiotics or anti-bacterial chemicals have been used as experimental tools in mud crab larviculture. Using prefuran Anil and Susellan (Anil and Suseelan 1999) obtained a larval survival rate of 23 per cent culturing S. oceanica, however when Wahyuni (Wahyuni 1985) used streptomycin sulphate and penicillin to culture *S. serrata* very high larval mortalities were reported, demonstrating that antibiotic use is not a panacea for mud crab larviculture. A more process orientated approach to controlling mud crab larviculture systems was recommended by Blackshaw (2001). He stressed the need to maintain hygiene and to control pathogenic organisms, recommending microbially mature water and the use of probiotics, rather than use antibiotics which can lead to the development of resistant pathogens.

Background

Work on the bacteriology of mud crab larval systems was undertaken by Morris Pizzutto, as a PhD student funded by this project. Unfortunately at the time of this report going to press no thesis draft or comprehensive report of this work has been completed. As a result this section is an overview of the work undertaken and a summary of findings with little detail. Abstracts from two oral papers are attached as appendices.

Bacteria–a presumptive issue in mud crab larval culture

Bacteria were identified as a presumptive agent causing significant mortalities in mud crab larval systems. This is because where a range of disinfection techniques (e.g. uv, chlorine) or microbials (e.g. OTC) are applied to mud crab larval production systems, increased survival is seen. In addition when larvae are grown at low concentrations in small containers and subject to daily water change, similar high survival is seen.

In an attempt to more clearly demonstrate that bacteria were a causative agent of mortality in mud crab larval systems, moribund larvae were sectioned and stained with haematoxylin and eosin. Necrotic mid-gut epithelial cells and hepatopancreas cells were seen. It was likely that this necrosis was most likely as a result of bacterial action.

OTC–a useful tool

In numerous trials judicial use of OTC at 50ppm to Z1–Z2 larval culture systems, during the rotifer feeding period, vastly improved survival of larvae through to the megalops stage.

As identified in the bacterial control section of this report, OTC was found to be a useful experimental tool to better understand the production of mud crab larvae and the role of bacteria in mud crab larval culture systems. If we assume that OTC acts to control bacteria proliferation in larval systems, by deduction it is then also evident that in general the feeding regime and control of water quality in the production in this project were adequate to support reasonably high larval survival.

However the mechanism by which OTC controls the effects of the various bacteria found in the mud crab larval systems remains unknown.

Bacteriology to test improvements in system design

After some small-scale trials identified that improved larval survival could be achieved using water that had been stored for some time, the bacteriology of this water was examined. If filtered seawater was stored for a week it was found that *Vibrio* levels in it fell to undetectable levels, although a range of other marine bacteria were found.

During the project INVE released a new product, Hatch Controller, to control bacteria associated with the hatching of Artemia. It was found that *Artemia* hatched using Hatch Controller were virtually free of *Vibrio* and that other bacterial levels were extremely low. In addition the product DC-DHA Selco when used to enrich *Artemia* also resulted in a reduced bacterial load, compared to enrichment with other products. These two products were as a result considered to be important components of any mud crab larval production system during the project. Having said that the overall bacterial loading in enriched *Artemia* was reduced, it should also be reported that a range of bacteria were found associated with them. These included *Vibrio* alginolyticus, V. proteolyticus, V. icthyoenteri and V. *harveyi*. Of these V. *harveyi* and V. icthyoenteri were tested and found to be potentially virulent.

Sampling mud crab larval production trials

Seven large-scale mud crab larval production trials were sampled bacteriologically. In each trial larvae were sampled at each stage eg zoea 1, zoea 2, water was sampled every second day and biofilm on tank walls every third day. The samples were grown on TCBS plates. Over 2000 bacterial isolates were collected.

Identification of bacteria

Bacteria were grouped using protein profiling, and separated used the SDS-PAGE system. Further to this DNA amplification profiling was undertaken to further identify and differentiate bacteriological isolates.

Virulence trials

The major groups of bacteria found from the isolates were tested for their virulence. In these trials some containers with just 10 recently hatched mud crab zoea were exposed to the isolates at a concentration of 105 c.f.u. mL^{-1} and compared against a control of the mud crab zoea without any bacteria. Table 59 summarises that work.

Table 59. Summary of assessment for virulence of various isolates.

Isolate	Colony morphology	MicroSys ID	Concensus (4 trials)
425	minute sucrose neg	*Photobacterium sp.*	virulent
720	minute sucrose neg	*Photobacterium sp.*	virulent
798	minute sucrose neg	*Photobacterium sp.*	virulent
519	minute sucrose neg	*Photobacterium sp.*	ns
422	spread sucrose pos	Poor ID	marginal
719	spread sucrose pos	Poor ID	marginal
871	spread sucrose pos	Poor ID	marginal
1030	spread sucrose pos	Poor ID	ns
486	spread sucrose pos	Poor ID	marginal
423	3mm cream	*V. alginolyticus*	ns
511	3mm cream	*V. alginolyticus*	ns
865	3mm cream	*V. alginolyticus*	ns
1059	3mm cream	*V. alginolyticus*	ns
743	flat sucrose pos	*V. harveyi*	ns
1009	flat sucrose pos	*V. harveyi*	marginal
507	sucrose pos	*V. harveyi*	marginal
585	sucrose pos	*V. harveyi*	ns
926	luminous sucrose neg	*V. harveyi*	ns
1119	luminous sucrose neg	*V. harveyi*	ns
1023	luminous sucrose neg	*V. harveyi*	marginal
723	luminous sucrose neg	*V. harveyi*	virulent
868	luminous sucrose neg	*V. harveyi*	virulent
864	luminous sucrose neg	*V. harveyi*	virulent
584	sucrose neg	*V. harveyi*	ns
722	sucrose neg	*V. harveyi*	ns
822	sucrose neg	*V. harveyi*	marginal
1094	sucrose neg	*V. harveyi*	ns
447	sucrose pos	Poor ID	virulent
965	1mm cream	*V. icthyoenteri*	virulent
474	flat sucrose pos	*V. meditteranei*	ns
747	flat sucrose pos	*V. navarrensis*	ns
435	sucrose pos	*V. navarrensis*	ns
1054	flat sucrose pos	*V. tubiashii*	ns
1109	sucrose pos	*V. tubiashii*	ns
1206	flat sucrose pos	*V. tubiashii*	ns

Epidemiology

In numerous trials in the larviculture section of this report, the feeding of rotifers has been related to a period of heavy mortalities amongst the mud crab larvae.

Nursery Phase

Experiments to investigate culture of mud crabs during the nursery phase

Preliminary assessment of settlement substrates for mud crab (*Scylla serrata*) megalops. (DAC–Batches 29 and 30)

Introduction

One of the key needs of the megalops stage of mud crabs prior to metamorphosing to a crablet is to find a suitable substrate for settlement. Two experiments were conducted to assess the effect on growth and survival of settlement substrates for mud crab megalops and crablets.

Materials and methods

Mud crab megalops used for this experiment were produced at the DAC from Batches 15 and 16.

Two temporally separated replicates were conducted, and these took place when the megalops became available after each larval rearing experiment. The megalops from each larval rearing experiment were pooled, counted and evenly distributed to the experimental tanks. Treatments were assigned to individual tanks randomly.

Each replicate was conducted in 3.8 m diameter round, fibreglass, flat- bottomed tanks. Water depth was kept at around 70cm. This gave a tank floor base surface area of 11.3 m^2 and a volume of 7.9m^3. A total of 2860 megalops were stocked to each tank over two days giving a density of 253 megalops m^{-2} of tank bottom area or 0.36 megalops L^{-1} of total tank volume .

Three experimental treatments were examined. .

A: The control treatment consisted of a tank without substrate.

B: Conditioned Aquamats™ (Meridian Applied Technology Systems, USA).

C: Unconditioned shade cloth.

The Aquamats were conditioned in a flow-through raw seawater tank for several months prior to use in this experiment. They were of the floating type and were made to sink to the bottom of the tank by inserting a sand-filled 32 mm PVC capped pipe into the hem. As the finger-like strands of the Aquamats were longer than the water depth, the hem section was rolled around the “fingers” until the tips were just below the surface. The Aquamats were approximately 1.8 m in length and were arranged somewhat radially within the tank (Figure 49).

Figure 49. Arrangement of Aquamats in the experimental tank for Treatment B.

The shade cloth treatment (Figure 50) consisted of four sheets of 70 per cent shade cloth the same length as the Aquamats arranged in a cross, but the centre of the tank was unobstructed. The material was folded over a supporting string line and made a “double surface”. The material was spray washed in freshwater before addition to the tanks.

Figure 50. Arrangement of shade cloth in the experimental tank for Treatment C.

The control contained no added settlement substrate, other than tank peripherals that were supplied to all tanks such as, the airstone, airline and outlet screen. Moderate aeration was continuously supplied throughout the experiment to all tanks.

Salinity adjusted seawater (30ppt) to be used in the experiment was introduced 1 day prior to stocking and was filtered to 1*µ*m before being foam fractionated for approximately 12 h. Megalops were acclimated to the new water over a period of 30 minutes. After stocking a very slow (~1L min^{-1}) flow through was initiated in all tanks with full salinity (~36‰) 1*µ*m filtered seawater.

From day 0 (stocking) until day 2, megalops were fed first instar INVE AF 430 *Artemia* at approximately 0.5ml^{-1}. Some of these *Artemia* persisted in the tanks for several days. Also, from stocking to day 7 the megalops were fed daily, 2g of an artificial prawn starter diet T1 (750*µ*m–1000*µ*m) (Grobest Pty Ltd. Jakarta Indonesia) per tank. From day 8 to harvest they were fed up to 4g of T2 (1000*µ*m–1700*µ*m, Grobest Pty Ltd. Jakarta Indonesia) per tank. The T1 diet would float at the surface for some time, which allowed the megalops to feed on it. The T2 diet was a sinking crumble diet that was better suited to the benthic phase of megalops stage or crablets.

Statistical analysis

Survival is expressed as the percentage of animals alive at harvest relative to the initial number stocked. Measurements of crablet carapace width at harvest were also taken for comparison. Survival and size data were analysed by one-way ANCOVA with days of culture as the covariate using SYSTAT v7.0 for windows (SPSS). All data was assessed for homogeneity and a least significant difference comparison of means test separated treatments.

Results

Table 60. Table of results for the two trials (DAC Batch–29 & 30).

Stocking date	Treatment	Days in culture	Number harvested	Survival (%)	Carapace width (mm)
07/03/2002	Aquamats	12	827	28.92	6.68
14/05/2002	Aquamats	15	546	19.09	5.74
07/03/2002	Bare tank	12	869	30.38	6.52
14/05/2002	Bare tank	15	902	31.54	5.99
07/03/2002	Shade cloth	12	1043	36.47	6.11
14/05/2002	Shade cloth	15	908	31.75	5.44

Table 61. Mean carapace widths for the various treatments

Treatment	Mean Carapace width (mm)
Aquamats	6.21 ± 0.47
Shade cloth	6.26 ± 0.27
Bare tank	5.78 ± 0.33

There was no significant difference in survival between treatments (P = 0.22) and the covariate had no impact on the analysis. There was also no significant difference in carapace width between treatments, however in this case the covariate was significant (P = 0.027), indicating that there was a difference between batches or runs. The second settlement trial lasted longer than the first and yielded slightly smaller crablets on average.

Discussion

These results indicated that the substrates provided did not significantly enhance the survival or growth of crabs from the megalops stage to the C3/C4 stage. This may suggest that the crabs do not require substrates at these stages when aggression between crabs is relatively low (i.e. earlier than C5) or that none of the substrates tested were suitable. Alternatively, stocking rates were low and may have been below a threshold of density dependent constraints to growth and survival.

The crablets in the first trial developed at a faster rate than the ones in the second trial possibly due to batch differences or as a result of temperature differences as the second trial was conducted during the cooler dry season months.

Assessment of the effect of settlement substrates on the survival of mud crab megalops to first stage crablets. (DAC Batches 31.1 and 31.2)

Introduction

The effect of settlement substrates on survival and growth of mud crab megalops and their subsequent development to crab stages 3⁄4 was examined in Batches 29 and 30 at relatively low density (253 m^{-2} of tank bottom area). As previously described it was found that when megalops were stocked into large tanks with either conditioned aquamats, suspended shade cloth material, or no added substrate, there was no significant difference in growth or survival. However, it was difficult to tell from the results whether the mortalities occurred during the settlement period and accompanying metamorphosis from megalopa to first stage crab or during the ensuing crablet stages C1–C3/C4.

Two experiments were carried out to gather further information on the effect of settlement substrates on successful metamorphosis from megalops to C1.

Materials and methods

Mud crab megalops used for the two experiments were produced at the DAC during batches 19 and 20. After being counted megalops were stocked into 9000l, flat bottomed tanks. Each tank received an equal number of megalops sourced from each larval rearing tank which was 937 m^{-2} or 1.17 L^{-2}. Only megalops produced from the 50ppm OTC treatments were used in the experiments.

Tanks were filled with 1μm filtered, foam fractionated, settled water which was salinity and temperature adjusted to 30ppt and 30°C respectively. Three 9000 L (base area =11.2m^2), flat bottomed fibreglass tanks were used in both trials. The tanks were kept under an outdoor shade structure. A temperature logger was used to monitor temperatures in the tanks during the trials.

Treatments

A: Floating substrate in the form of cut lengths (200mm) of swimming pool vacuum hose.

B: Provided with hides⁄refuges⁄settlement substrate in the form of submerged drain grates on the bottom of the tank and floating plastic bread crates on the surface.

C: Two cylindrical concentric rings of plastic garden mesh (25mm x25mm holes) standing with the long axis vertical provided for settlement⁄refuge.

Included in each tank was a partially submerged 100 L plastic tub with an airlift taking water from within a 500μm screen (see Figure 51). This water was directed to the floating tub which overflowed back into the main tank. Inside the 100 L tub was a 1kW immersion heater. This set up allowed the water to be heated without causing the megalopa to come into contact with the heaters

After stocking of the larvae into water of 30ppt, a very slow (~1L min^{-1}) flow through of seawater was initiated in all tanks such that the salinity gradually increased to 36ppt. The seawater was filtered to 1μm.

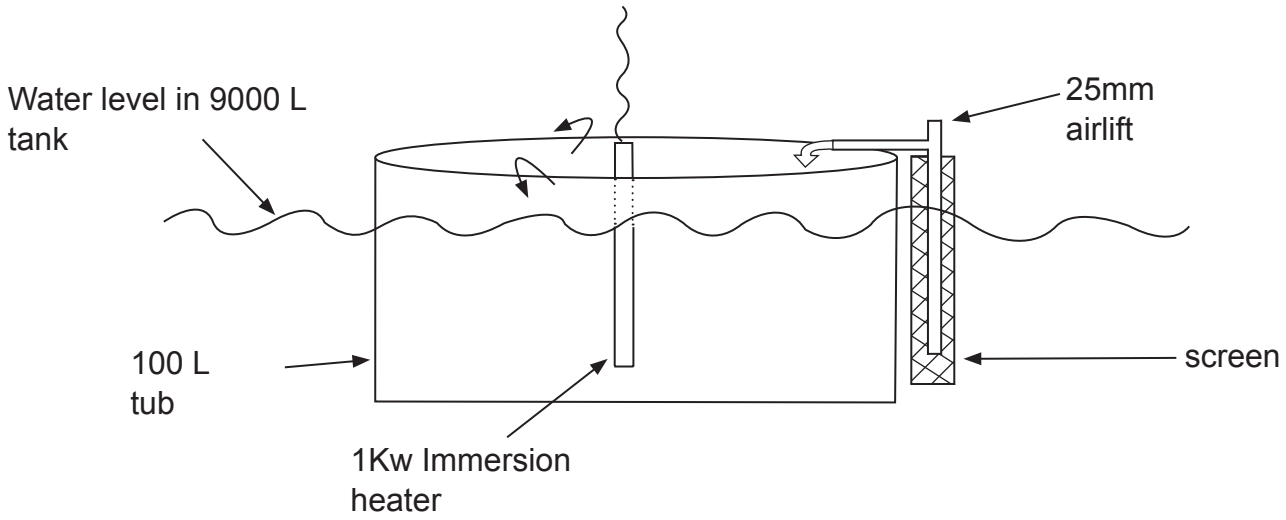

Figure 51. Diagram of set up for water heating

Megalops were fed first instar INVE AF 430 *Artemia* at approximately 0.25ml^{-1} on day 0. On days 1–4 enriched GSL *Artemia* were fed at the same rate. Also, megalops were fed 2g of an artificial prawn starter diet T1 (750*µ*m–1000*µ*m) (Grobest Pty Ltd. Jakarta Indonesia) per tank daily. On day three and on day 6 on-grown *Artemia* approximately 10 days old) were supplied. When crablets were noticed, approximately 1cm3 of frozen chironomid larvae per day were offered as food, in addition to the artificial diet.

Megalops were produced, and transferred to the 9000 Ltanks over three days. Tanks were harvested 10 days after the first stocking when the great majority of megalops had moulted to first stage crablet. Crablets were concentrated into 100 Ltubs then dispersed by mixing. Whilst the crablets were still dispersed in the tub twenty 72ml samples were taken and counted. Total survival was then extrapolated from these sub-samples.

Water quality was monitored daily.

Statistical analysis

Data from the outside settlement experiment Batch 31.2 was combined with the previous replicate Batch 31.1 and was then analysed by ANCOVA, with stocking number as the co-variate (as this was different between replicates).

Results

Batch 31.1 was carried out in September at the end of the cooler dry season and water temperatures ranged between 26.4°C to 27.8°C . Batch 31.2 was carried out at the beginning of the warmer wet season and the water temperatures were relatively higher at 29.4°C to 30.6°C.

Table 62. Stocking, yields and survival of megalops to crablet, (DAC Batch–31.1) (August 2002).

Treatment	Total Stocked	Total Harvested	Survival (%)
Garden Mesh	10491	3194	30.44
Floating tubes	10491	3588	34.20
Trays & grates	10491	2625	25.02

Table 63. Stocking, yields and survival of megalops to crablet, (DAC Batch–31.2) (December 2002).

Treatment	Total Stocked	Total Harvested	Survival (%)
Garden Mesh	8326	2100	25.22
Floating tubes	8326	2765	33.21
Trays & grates	8326	2816	33.82

When this data is combined the analysis of covariance shows that there was no significant difference (P = 0.46) in survival across treatments.

Table 64. Combined Batches 31.1 & 31.2 percentage survival of megalops to crablet.

Treatment	Rep 31.1	Rep 31.2	Overall
Garden Mesh	30.44	25.22	27.83 ± 2.61 a
Floating tubes	34.20	33.21	33.71 ± 0.50 a
Trays & grates	25.02	33.82	29.42 ± 4.40 a

Discussion

In both trials survival from megalops to crablet was approximately 30 per cent. In the absence of a “no shelter” control treatment, it is not clear whether the amount of settlement substrate or refuge provided was inadequate to make a difference to survival, or that the materials used were appropriate. Very few megalops were seen in or on the supplied refuges during the first few days when megalops remained predominantly planktonic. As the megalops took on a more benthic existence, and began to settle prior to moulting, the settlement substrate and refuges still seemed to be ignored. After the larvae moulted to the first crab stage they began to utilise the supplied refuges. This may indicate that the settlement substrate or refuges become more important during the crablet stages than they are at the megalops to crab 1 stage. When considered in conjunction with the results of Batches 29 and 30 (see Table 60) it would appear that the majority of mortalities occurred whilst the crabs were in the megalops stage or in the moult from megalops to C1 and not in the subsequent moults to higher crab stages.

In the outdoor megalop settlement experiment Batch 20 none of the treatments was any significantly better than the others. However the results of this experiment, i.e. approximately 30 per cent survival, is similar to previous experiments. It may be that not enough refuge has been supplied to make a difference to survival, or that the right material has not been used, or and most probably, refuge may not be required during the megalop stage. Very few megalops are seen in or on the supplied refuges and are predominantly planktonic during the first few days. As the megalopa become more benthic and begin to settle prior to moulting the refuges are still ignored. When the larvae moult to the first crab stage they then begin to utilise the supplied refuges and as such they may be more important during the crab stages.

Influence of various settlement substrates on survival of megalops to crablet. (DAC–Batch 32)

Introduction

Previously no difference in survival of megalops to crablet was detected between densities of 925 and 1850 m^{-2}, with and without settlement substrates (SS). Similar levels of survival were achieved with bare tanks stocked at 4000 megalops m^{-2}. In this trial Aquamats® and mussel rope were examined for their potential as SS.

Methods and materials

Megalops were stocked into 20 round plastic 100 L tubs for settlement and moulting to the first crablet stage. These tanks had a bottom surface area of 2700cm^2 and water depth of 40cm giving a working volume of approximately 100 L. These tanks were arranged in two parallel rows of 10 inside an environmentally controlled laboratory at the DAC.

Megalops were stocked to the tanks at a density of 500 individuals per tank equating to 1850 megalops m^{-2} or 5 megalops L^{-1}). Megalops were stocked to the 100 L tanks volumetrically rather than individually.

The tanks were filled with the water that had been foam fractionated and settled, 30ºC and 30ppt. After which a flow-through water exchange was initiated with 5*µm* filtered, 30ppt seawater. The flow-through water was heated to 31ºC in a 1 tonne reservoir. A magnetic drive pump drew water from the reservoir and supplied a “ring main” to ensure a consistent flow of water to each tank. Water exchange to each tank was set at 400 per cent per day and each tank had a screened over-flow near the water surface. Each tank was supplied with a single airstone to ensure aeration.. Temperature of the water in the experimental units was controlled with the environment controlled room, and photoperiod was set at 14 hours.

Megalops were fed 500–600*µ*m Vital 12™ (Higashimaru) Penaeus japonicus diet daily ad libitum. Forty-eight hour DC DHA enriched *Artemia* were also fed at 2 *nauplii* mL^{-1} on the day of stocking.

Crablets were harvested when all the megalops had moulted and individuals were counted. A total wet weight of all crablets in each tank was also determined and allowed calculation of individual weight of crablets.

There were four treatments consisting of different substrates including:

A: A bare tank with no refuge (control)

B: Two concentric cylinders of 25 x 25 mm plastic garden mesh, shelters positioned so they reached from tank base to water surface

C: A benthic Aquamat treatment (with forty 120 mm fingers = 4.8 m).

D: Lengths of weighted Mussel rope (= 4.8 m).

There were five replicates per treatment.”

Statistical analysis

Survival and mean crablet weight was analysed with a one-way ANOVA using SYSTAT 7.0 for windows (SPSS corp.). Data was assessed for homogeneity, and where significant differences were found, a least significant difference comparison of means test separated treatments.

Results

Water quality was maintained within the following ranges; temperature 28.8–29.1°C, dissolved oxygen 6.3–6.4, pH 7.81–7.88 and salinity 28–30ppt.

A high level of mortality was observed the morning after stocking, and the following day, but there was little mortality from that point onwards. Megalops remained pelagic during the first four days of the experiment before the switch to a more benthic existence.

Tanks were harvested on day 8 when all megalops had disappeared from the tanks.

Data relating to survival and mean weight of the crablets is presented in Table 65. There was no significant difference in survival to crablet (P = 0.164) or mean weight of crablets (P = 0.329) between any of the treatments with the overall mean weight of a crablet being 8.65 mg.

Table 65. Survival (%) (± se) of the megalops following settlement to crablet (DAC Batch–32).

	Survival (%)	Mean weight (mg)
Bare	19.52 ± 2.46 a	8.98 ± 0.22 a
Aquamat®	17.28 ± 0.73 a	8.27 ± 0.13 a
Mesh	14.76 ± 2.11 a	8.73 ± 0.41 a
Mussel rope	14.36 ± 0.92 a	8.64 ± 0.21 a

The data relating to survival was interesting in that there was slightly better survival in bare tanks with no added substrates at all, compared to those that had. There was no difference in mean weight between any of the treatments. There was no perceived advantage in using mesh, Aquamats® or mussel rope for settlement of megalops.

Discussion

The fact that there was no significant difference in mean crablet weight, or timing of the moult to crablet, may suggest that mud crab megalops either do not require a substrate in the water column, or that a SS has yet to be found which can improve survival. It is also possible that as a result of the initial high mortality observed, density effects and behavioural interactions with SS may not have been exhibited in this trial.

Large scale trial:

Assessment of substrates on growth and survival of juvenile mud crabs *Scylla serrata*. (DAC–Batch 33)

Introduction

Prior to development of a commercial nursery system for the production of crabs, it was considered useful to examine if there were any gross changes in the growth and survival of crablets grown in different physical environments. To assess this crablets, C1–C5, were on-grown in tanks with a variety of "habitats".

Materials and methods

The C1 crablets produced for this experiment came from a settlement experiment Batch 31.1. All 9406 crablets produced in that trial were gathered together in a 100 L tub prior to counting and distribution to treatment tanks. Crablets were divided up evenly between the three tanks used giving a stocking density of 277 ind./m^2

The trial was carried out in 11.3 m^2 round fibreglass tanks with a water depth of 600mm. Tanks were filled with seawater (36ppt) through a 1μm filter bag. After stocking a slow (~3 L min^{-1}) flow through was initiated in all tanks with undiluted unfiltered seawater.

Three habitats were examined using a single replicate of each.

Treatments

A: A tank with no added habitat was used as a control.

B: A tank with a sand substrate and floating plastic bread crates.

C: A tank to which conditioned Aquamats® were added. The floating Aquamats® used were weighted to the bottom of the tank and rolled several times, facilitating several loose rolls in thickness. The fingers of the Aquamats® floated to the surface and in some cases were floating horizontally on the water surface.

Crablets were fed ground (using a blender) and sieved Vital 12® Kuruma prawn diet (Higashimaru) and frozen chironomid larvae ad libitum. The artificial diet was initially sieved to be between 850–1400μm and was increased to 1400–1700μm after 10 days. Tanks were harvested after 17 days.

Water quality from each tank was monitored daily.

Crablets were blotted dry and then weighed on a four decimal place electronic balance.

Results

Water quality was maintained as follows; dissolved oxygen above 5.0 mgL, pH 7.59–8.09, salinity 35–36ppt and temperature 27.3–30.4°C with an average of 28.7°C throughout the trial.

Table 66. Stocking number, harvest number, survival (%) and weight of crabs at harvest after 17 days of growth. (DAC Batch–33)

Treatment	No. stocked	Stocking density (crabs/m2)	No. harvested	Survival (%)	Total mass (g)	Mean mass (g)
A: Bare tank	3135	277	1348	43.0	219	0.163
B: Sand/ floats	3135	277	1460	46.6	219	0.150
C: Aquamats®	3135	277	2167	69.1	340	0.157

The Aquamat® treatment yielded the highest survival (69.1%). Total biomass from this treatment was also higher, there being little variation in the mean mass of crablets between treatments.

The size frequency of crablets shows that at harvest there were two distinct crablet stages present (probably C3 & C4), with the majority still at the C3 stage.

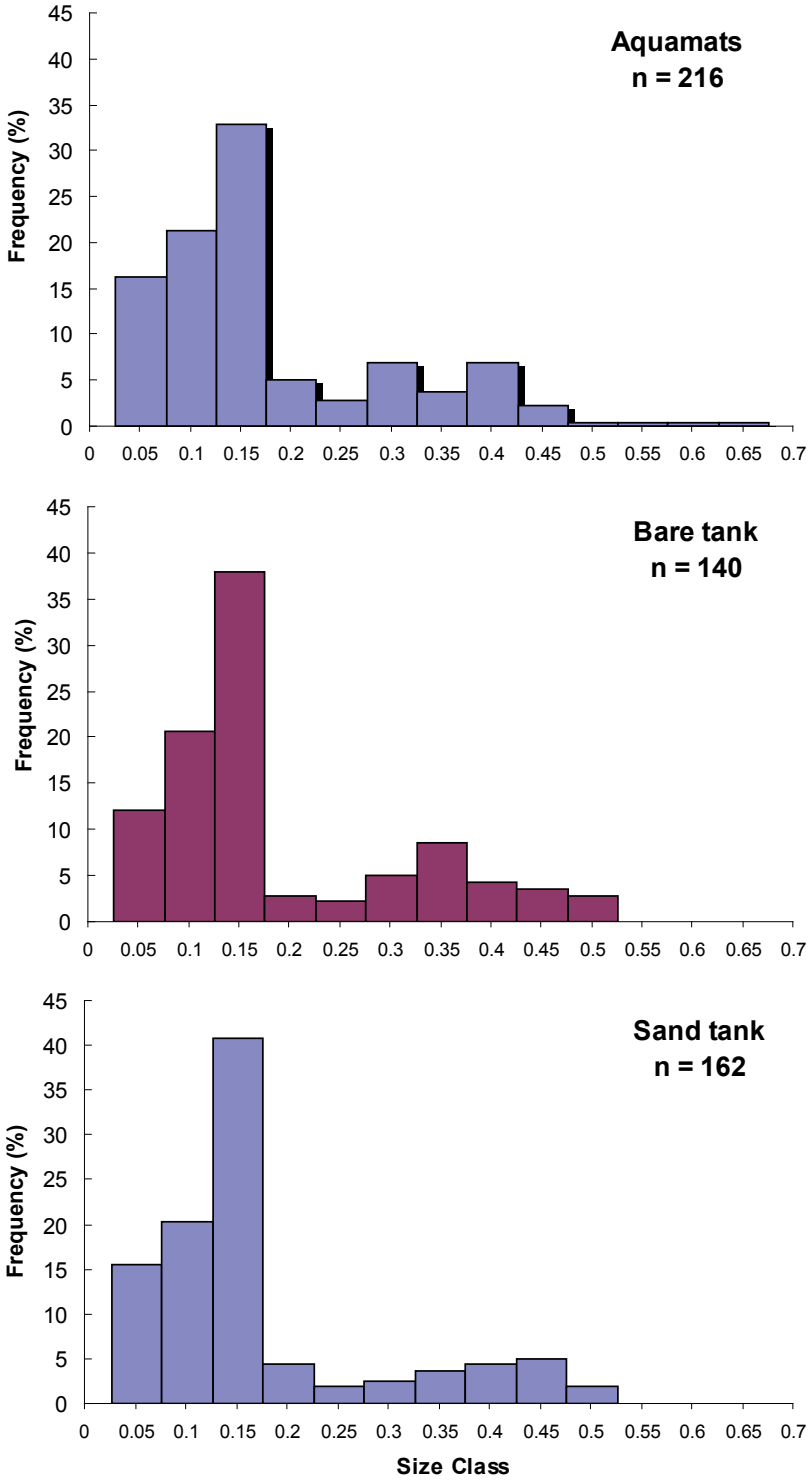

Figure 52. Size- % frequency distribution of individual crablet mass in grams. (DAC Batch–33).

Discussion

Although only one replicate of each treatment was used in this trial, it appeared that there was a difference in survival between them during the first 17 days of the crab nursery stage. The Aquamat® structure allowed crablets to move three dimensionally within the tank and presumably reduced mortality which would usually occur at the moult stage from cannibalism. The sand tank also had floating substrates or refuges, although these may have not been used as much as the Aquamats which would have provided a vertical substrate close to where the crablets are generally found i.e.: at “ground” level. Crablets may prefer a refuge

that can be climbed up rather than swam to through open water. It is also possible that the sand was too firm for the crabs to burrow into more than a few millimetres as there were not any obvious burrows formed rather just shallow depressions. This may have left the newly moulted crablets in the sand tank more vulnerable to cannibalism.

It would seem logical for crabs to move away from conspecifics during the moulting process as cannibalism is common. Moving to the surface of the water however may expose the juveniles to predation from birds or other surface feeding organisms, which may explain why the floating refuges in treatment B were not utilised by the crablets Whilst one can hypothesise that 3-D structures in the Aquamats® tank may have increased survival through minimising cannibalism, by reducing interactions with other crablets, there is another possible hypothesis to explain the difference, i.e. feed. As the Aquamats® had been conditioned, they were covered in biofilm, algae and small settled organisms which may have enhanced nutrition for the crablets in the Aquamats® tank compared to those in the other tanks, which contained no additional feed. It has been observed that crabs maintained in outdoor tanks with substrates and a matured growth of algae and detritus on the bottom of the tank often grow faster and do not suffer shell disease as much as crabs maintained for long periods in bare tanks.

Large scale trial:

The influence of live food density on megalops – crablet survival at settlement. (DAC–Batch 34)

Introduction

It is likely that food availability is a critical factor in successful settlement and survival from megalops to the stage one crab. A trial was conducted to determine the influence of water depth and food density on survival of megalops through settlement to crablet.

Methods and materials

Megalops produced from a large-scale trial were stocked into flat-bottomed fibreglass tanks with a base surface area of 11.3 m2 for settlement and moulting to the crablet stage. Three treatments were imposed, however only one replicate per treatment was possible.

In order to assess the importance of live food density, megalops were stocked to three identical tanks that were filled with varying depths of settled and foam fractionated seawater (with water quality adjusted to approximate the larval rearing tanks). This had the effect of supplying the same total amount of feed whilst at the same time increasing feed density. The water depths used were the 200 mm, 400 mm and 600 mm water depth

Tanks were stocked with 10,491 megalops over two days giving a stocking density of 937megalops m^{-2} in each tank. The remaining megalops, in excess of 51000, were stocked to a single tank and were treated in a similar manner to the experimental tanks as described below, with the exception that more artificial diet (up to 12g/day) was provided.

Three batches of different sized on-grown *Artemia* were fed to each of the experimental tanks on the day of stocking. There were 20 000 *Artemia* m^{-2} at 1.025mm in total length, plus 3 500 *Artemia* m^{-2} at 2.4mm, plus 1 800 *Artemia* m^{-2} at 6.0 mm in length.

Over the next 10 days each tank was fed an equal number of a variety of sizes of on-grown *Artemia* as well as ground and graded (500–850*µ*m) Vital 12™ Kuruma prawn diet at a rate of 6g per tank per day. In addition, 10g of dried Spirulina was fed per day in order to feed the Artemia.

On day 10 the tanks were drained with crablets harvested through the outlet and screened into a 100 L tub. These were vigorously stirred and twenty 72ml samples were taken. Total survival was then calculated through extrapolation.

Results

There was little difference in the survival rate of megalops–crablet during the trial, although the treatments were not replicated. Survival for the tanks was 57.2 per cent, 59.2 per cent and 58.6 per cent for the 200 mm, 400 mm and 600 mm water depth respectively. Survival in the high density tank was 47.0 per cent.

Discussion

The survival of the megalops to crablet was high compared to previous experiments. This was the first time significant numbers of on-grown *Artemia* have been used as a feed. The similar results between treatments would suggest that the range of live food densities used in the current experiment did not influence the feeding ability of the megalops. The tank with 200mm of water would have had three times the live feed density of the 600mm tank and yet the survivals were very similar. Given that the megalops are highly mobile and have advanced feeding appendages compared to previous zoeal stages, a lower food density may suffice as the megalops are active and efficient predators that are able to seek out prey. It is expected that the efficiency with which megalops can acquire live prey items is better than at the zoeal stages.

It is unlikely that the differences in water depth affected moulting success as the megalops settle on the bottom to moult and do not utilise the water column.

This result indicates that provided the baseline requirements for live feeds are present the megalops can seek out the prey needed to sustain themselves.

The survival in the extremely high density tank (>4500 m^{-2}) was unexpectedly high also. Indicating that megalops settlement may be carried out at higher densities than were previously expected.

Influence of stocking density and artificial refuges on megalop-crablet settlement. (DAC–Batch 35)

Introduction

Preliminary trials had examined the effect of a variety of settlement substrates on the settlement and metamorphosis of megalops to crablets. The stocking density of megalops had not been examined as a factor in the survival of megalops to crablet. This trial was set up to examine density and the presence or absence of settlement substrate on the survival and metamorphosis of megalops to stage 1 crablet.

Methods and materials

Megalops produced from the large-scale larval rearing trial that had received an OTC treatment were stocked into 16 round plastic 100 L tubs for settlement and moulting to the first crablet stage. These tanks had a bottom surface area of 2700cm2 and water height of 32cm giving a working volume of approximately 85L. The tubs were arranged in two parallel rows of eight inside an environmentally controlled laboratory at the Darwin Aquaculture Centre.

Four treatments were imposed, consisting of two megalops stocking densities with and without settlement substrate (SS). The two stocking densities were 250 and 500 megalops per tank equating to either 925 m^{-2} (LD : Low Density) or 1850 m^{-2} (HD : High Density). The settlement substrate was made from 50 per cent shade cloth material cut into 80cm x 80cm squares, weighted in the centre and placed in the tanks. Two of these were added to each tank where appropriate.

The tanks were filled with the same water as would normally be used to fill the larval rearing tanks (fractionated and settled, 30ºC and 30ppt). Megalops were screened from the tanks and counted. Megalops numbers for stocking were estimated volumetrically rather than individually. Each tank was also given a single air stone to ensure aeration. A screened, gentle flow-through of water was provided. Temperature of the water was controlled via room temperature within the environment controlled room, and day length was set at 14 hours.

Megalops were fed 500–850μm screened Vital 12 (Higashimaru) ad libitum, as well as 48 hour DCDHA enriched *Artemia* at a rate of 0.5 per mL daily. On day 0 and day 3, week old on-grown *Artemia* were fed at a rate of two *Artemia* per megalop.

Crablets were harvested after eight days when greater than 95 per cent of the megalops had moulted to C1 and individuals were counted.

Statistical analysis

Survival was analysed with a two-way ANOVA (density and Settlement Substrate). All analyses were carried out using SYSTAT 7.0 for windows (SPSS corp.). All data were assessed for homogeneity, and where significant differences were found, a least significant difference comparison of means test separated treatments.

Results

During the trial temperature was maintained between 29.1 and 30.4°C, dissolved oxygen 5.9–6.4, pH 7.96–8.07 and salinity 30ppt.

Data relating to survival of the crablets is presented in Table 67. There was no significant difference in survival between either density (P = 0.102), or in the presence or absence of SS (P = 0.674). There was also no evidence of an interaction effect of density and SS provision (P = 0.674).

Most of the mortality occurred during the first two days after stocking, and did not appear to be density-dependant as a similar proportion from both density treatments died (although this was not quantified at the time).

Table 67. %Survival (±se) of megalops to crablet (DAC Batch–35).

Treatment	Survival (%) (±se)
High Density : No SS	29.9 ± 1.91 a
High Density : SS	33.5 ± 2.00 a
Low Density : No Refuge	39.1 ± 7.21 a
Low Density : SS	39.1 ± 3.16 a

Discussion

This trial gave similar results to other trials (14c/17c) which examined settlement substrate in terms of average survival (>30%). The megalops were primarily pelagic during the first four days before switching to a more benthic existence. Very few megalops were seen in or on the provided refuges throughout the trial. The first crablets were noted on the seventh day after stocking and the tanks were harvested after eight days. These crablets were seen in, on and through the settlement substrate. The majority of mortality occurred during the first few days and seemed to be not related to density or presence of refuge, although this was only based on observations, rather than counts. There was no significant difference in survival due to density, suggesting that a greater range of densities should be tested. It may be that the presence of settlement substrate may be more important at higher densities. The larvae may also benefit from having access to more on-grown *Artemia* as these were consumed quite readily.

Influence of temperature and salinity on survival and growth of *Scylla serrata* crablets. (DAC–Batch 36)

*This trial has been published as: Ruscoe, I., Shelley, C., Williams, G., (2004) The combined effects of temperature and salinity on growth and survival of juvenile mud crabs (*Scylla serrata* Forskal). Aquaculture 238, 239–247

Introduction

The aim of the present study was to investigate the effect of temperature and salinity on growth and survival of *S. serrata* crablets, and to establish any interactive effects of the same. This data will allow us to make recommendations on these factors to culturists and to make broad recommendations on geographic ranges which can take advantage of the optimal environmental parameters.

Methods and materials

The crablets were reared from megalopa which had settled in a 3.8 m diameter flat-bottomed tank and were fed on-grown *Artemia* and an artificial crumble diet, 500–750*μ*m sieved, Vital 12™ Penaeus japonica diet, Higashimaru Pty. Ltd., Kagoshima, Japan). The crablets were harvested by draining onto a submerged screen. Only fully intact, and active, crablets were used in the experiment. Fifty-six individuals, which were not then used in the experiment, were individually weighed on the day of stocking in order to obtain a mean stocking weight.

This experiment was carried out in twenty 100 L plastic tanks inside a temperature controlled room at the DAC. These tanks were arranged in two parallel rows of ten.

Four experimental temperatures were assessed, 20ºC, 25ºC, 30ºC, and 35ºC. The room was cooled so that the water in the tanks was maintained at 20ºC, unless otherwise heated. All temperatures above 20°C were maintained with a 300W thermostat controlled immersion heater, and an airstone was used to ensure mixing of the water bath. There were five tanks maintained at each temperature in a randomised-block design. Within each tank were spaces for twelve 500ml plastic containers which were used to hold a single crab each.

Six salinities were tested. These were 0, 5, 10, 20, 30, and 40ppt. In each tank there were two crabs held individually at each salinity. Each container had a lid to prevent evaporation and subsequent changes in temperature and salinity. In total 240 individuals were used in this experiment.

The water used to create the various salinities was made from filtered sea water and a commercial scientific grade marine salt “Coralife” (Energy Savers Unlimited, Inc. Carson, CA, USA). The filtered seawater for all treatments was made up to slightly higher than 40ppt by the addition of the Coralife salt, and was then adjusted with carbon filtered freshwater to the various salinities required, except for the 0ppt treatment where straight carbon filtered freshwater was used. This was done so that each salinity received some commercial salt mixture, some filtered seawater, and some freshwater.

Three times a week, each crab was removed from the container and placed immediately into a prepared (temp and salinity adjusted) batch of new water. The container was cleaned in

freshwater, refilled with the appropriate water, and the crab restocked. In this way a 100 per cent water exchange was facilitated. Temperature of the water baths was recorded daily and ammonia tests were carried out every three days.

Crablets were fed the same artificial diet (sieved to between 1400–1700μm) each day, to excess. The following morning the containers were vacuumed to remove uneaten food and faeces, and the crabs were checked for moulting or mortalities. Each moult was recorded. Crabs were grown for 18 days. At harvest crabs from all tanks were individually weighed to 0.001g and had carapace width measurements taken (0.1mm).

Statistical Analysis

Residuals of all data sets were examined to determine a requirement for data transformation. Survival data was analysed using a two-way ANOVA with a main-effects design for the factors temperature (20, 25, 30 and 35°C) and salinity (5, 10, 20, 30 and 40ppt) (Data for Salinity = 0ppt was not included in the analysis.). This proportional data was arcsine-square root transformed prior to analysis.

Growth data, recorded as mean weight and carapace width at harvest, was also analysed with a split-plot design two-way ANOVA, with temperature (20, 25, 30 and 35°C) as the main-plot factor and salinity (5, 10, 20, 30 and 40ppt) as the sub-plot factor. The duration between moulting was also recorded and analysed in the same manner as the growth data.

Contour plots for weight at the end of the trial, as well as carapace width and survival were generated.

Results

Water quality remained within appropriate ranges for tropical marine crustacean species. Dissolved oxygen remained above 5.0 mg/L, pH ranged from 7.12–7.7. Ammonia peaked at approximately 3.5ppm in the 30°C treatment.

The mean weight at stocking was (±se) was 18.43 ± 0.42 mg. All crablets placed in 0ppt salinity water died within 24 hours of stocking and this data was not included in the statistical data analysis.

The remaining data showed that there was a highly significant difference in survival and growth of crablets due to treatment effects. Temperature always had a higher influence on growth and survival than salinity did, and there was never an interactive effect of the two variables.

Data for final weight and survival are presented in the following table and figures.

Table 68. Data relating to final weight, carapace width, survival and intermoult duration during C3 and C4 for the various treatments in the temperature and salinity trial (DAC Batch–36).

					Duration at C3 (days)		Duration at C4 (days)	
Temp	**Sal**	**Survival (%)**	**Wt (g)**	**CW (mm)**	**N**		**N**	
20	0	0						
	5	25	0.042 ± 0.024	6.9 ± 3.9	1	13	0	
	10	33.33	0.045 ± 0.023	7.0 ± 3.5	0		0	
	20	33.33	0.080 ± 0.040	8.13 ± 4.1	2	7.50 ± 1.5	0	
	30	33.33	0.042 ± 0.020	6.88 ± 3.4	0		0	
	40	25	0.052 ± 0.030	7.13 ± 4.1	0		0	
25	0	0						
	5	83.33	0.142 ± 0.045	9.94 ± 3.14	10	7.5 ± 0.31	2	2.0 ± 0.00
	10	83.33	0.156 ± 0.049	10.22 ± 3.2	10	7.2 ± 0.29	2	2.0 ± 0.00
	20	83.33	0.200 ± 0.063	10.89 ± 3.4	10	7.7 ± 0.45	4	4.0 ± 1.35
	30	75	0.135 ± 0.045	9.8 ± 3.3	9	7.4 ± 0.41	1	1.0 ± 0.00
	40	83.33	0.120 ± 0.038	9.5 ± 3.0	10	8.2 ± 0.63	0	
30	0	0						
	5	66.67	0.291 ± 0.103	13.39 ± 4.7	10	4.3 ± 0.21	10	10.0 ± 0.15
	10	83.33	0.337 ± 0.105	13.73 ± 4.3	10	4.3 ± 0.21	10	10.0 ± 0.25
	20	83.33	0.333 ± 0.105	13.67 ± 4.3	10	4.9 ± 0.23	10	10.0 ± 0.26
	30	83.33	0.291 ± 0.919	12.85 ± 4.1	10	5.5 ± 0.22	9	9.0 ± 0.20
	40	83.33	0.215 ± 0.068	11.69 ± 3.5	10	6.9 ± 0.28	8	8.0 ± 0.26
35	0	0						
	5	66.67	0.195 ± 0.068	11.5 ± 4.1	10	5.3 ± 0.45	8	8.0 ± 0.45
	10	83.33	0.197 ± 0.062	11.6 ± 3.7	10	4.8 ± 0.42	8	8.0 ± 0.32
	20	75	0.197 ± 0.066	11.48 ± 3.8	10	6.1 ± 0.28	8	8.0 ± .031
	30	83.33	0.172 ± 0.054	10.95 ± 3.5	10	5.3 ± 0.40	6	6.0 ± 0.42
	40	83.33	0.135 ± 0.042	9.96 ± 3.1	9	6.6 ± 0.44	6	6.0 ± 0.17

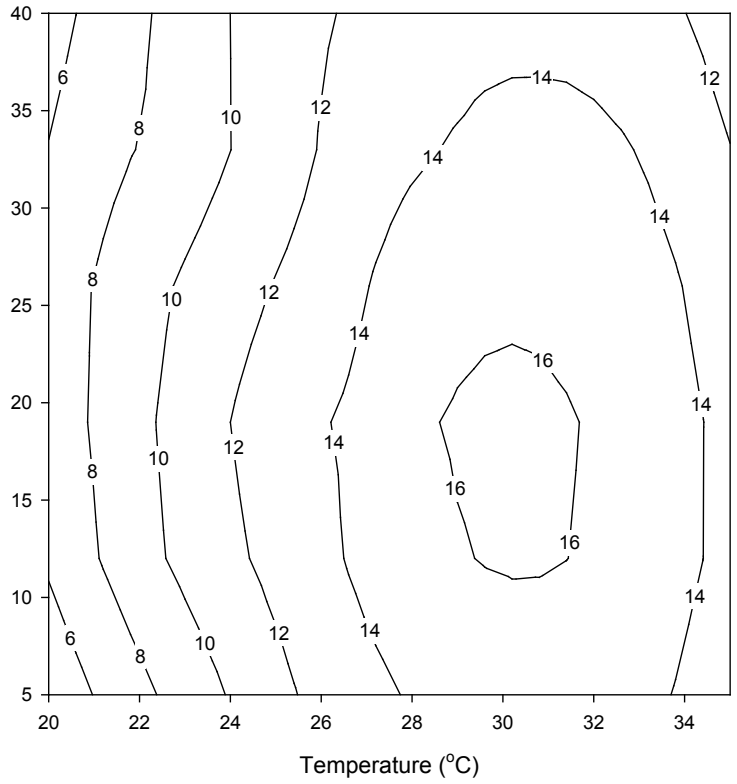

Figure 53. Contour plot of final weight (g) at harvest for the two treatments of this trial. (DAC Batch–36).

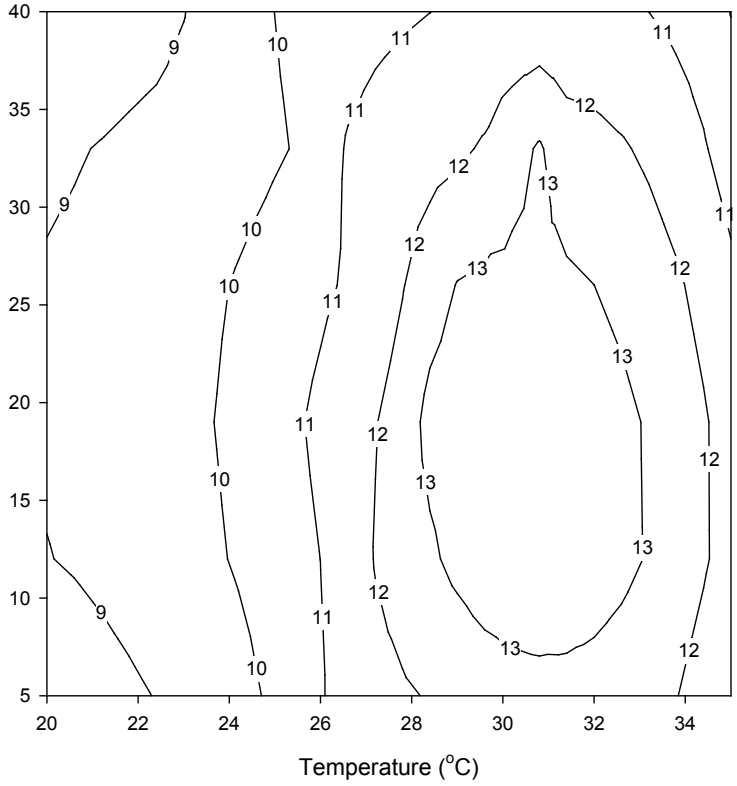

Figure 54. Contour plot of carapace width (mm) at harvest for the two treatments of this trial.

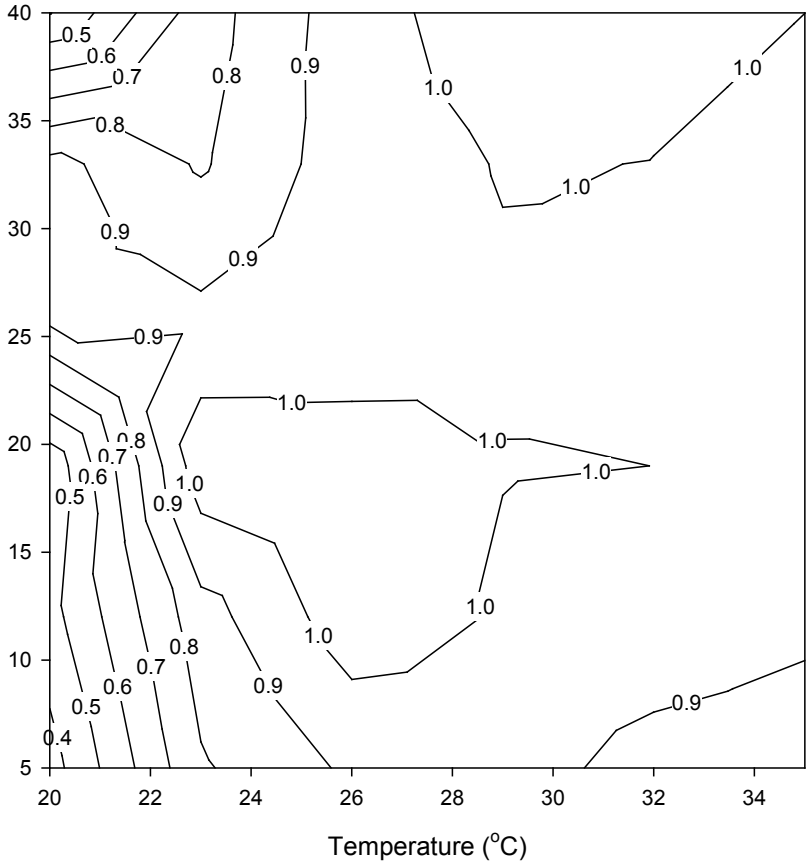

Figure 55. Contour plot of survival (proportion) for the two treatments of this trial.

Discussion

The contour plots clearly show that temperatures around 29 and 31°C and salinities between 10 and 35ppt will suit *S. serrata* crablets and will allow high levels of growth and survival. Whilst optimal production in outdoor systems will be limited to the more tropical regions of Northern Australia, farming is likely to be practical in all areas in which *S. serrata* is naturally distributed.

Larviculture systems for crabs may need to respond to the biorhythms of the larvae as it has been demonstrated that there are rhythms of digestive enzymes in mud crab larvae (Li, Tang *et al*. 2000). These rhythms were shown to vary with light, larval stage and stage within the moult cycle.

The need to maximise water quality in mud crab larviculture through such methods as filtration and treatment with UV lights (Brick, 1974), re-circulation (Heasman,1983) and more holistic system design (Mann, 1999) has been well documented. A range of parameters have been examined in attempts to improve larval production systems. Kasry (1986) found higher larval mortalities when using low salinities during the early stages of culture, whilst Baylon & Failaman (2001) suggested that salinity didn't affect the duration of larval development and that the highest survival of megalops was at a constant 32ppt for *S. serrata*. However, during the nursery stage of production Yu (2001) found a shorter moult period at lower salinities.

Survival and growth of juvenile mud crabs (*Scylla serrata*) fed commercial marine shrimp diets alone or in combination with fresh and live feeds. (BIARC Batch 17)

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Abstract:

In the early development of mud crab farming in Australia, the small market for crab feeds is unlikely to attract commercial interest in feed development. We studied the survival and growth of juvenile mud crabs *Scylla serrata* fed existing commercial shrimp diets and fresh diets (on-grown Artemia, chopped pipi, Donax deltoides) alone or in combination. The aim of this work was to identify existing feeds that could fulfil the immediate requirement for grow-out feeds for mud crabs. The work was conducted in a static bowl trial and repeated using flowing seawater, the latter conditions giving better results because of superior water quality. In contrast to the growth observed using expensive commercial Kuruma shrimp diets (AUD $6–13/kg), mud crab juveniles performed relatively poorly when fed the inexpensive commercial black tiger shrimp diets (ca. AUD $1–2/kg) alone. However, costs could be reduced by combining the high and low cost feeds without any loss of growth performance-suggesting that a nutritional compromise between the two extremes may be found with further research into crab nutritional requirements. The fresh and live feeds provided no benefit when fed solely or in combination with pelleted feeds, and present some practical issues in terms of inconvenience and water quality management. Small juvenile mud crabs grow well on existing commercial diets prepared for shrimp. This work has provided immediate information about practical options for nursery feeds as well as establishing a benchmark for growth rate in future nutrition trials.

Introduction

Mud crab aquaculture is widespread in SE Asia, where it is largely limited to collection of juvenile crabs or “seed” from the wild and on-growing them in brackish water ponds at low stocking density on a diet of so-called “trash” fish (Baliao, Santos & Franco 1999). Development of a manufactured feed for mud crabs is a priority (Williams & Primavera 2001) now that larval rearing methods are available for this species (Mann, Asakawa & Pizzutto 1999, Ruscoe, Williams & Shelley 2004b). There is a danger that the removal of the “natural” brake on industry growth by inception of hatchery production may lead to unsustainable demand for fresh feed unless a cheap alternative is found.

The larger and more readily collected instars of *Scylla* spp. are generally known in nature as predators of benthic molluscs and crustaceans (Hill 1979, Joel & Sanjeevaraj 1986), hence the ready adoption of “trash fish” as a convenient diet. However, carnivory amongst these and other portunid crabs is seen as an obstacle to their culture for two reasons. Firstly, it supposes manufactured feeds must be of high protein content (and hence expensive) and secondly, it means they will readily cannibalise other crabs if grown at high density ((Williams & Primavera 2001).

Relatively little is known about the nutritional requirements of mud crabs (*Scylla* spp), in aquaculture, particularly in the poorly understood early juvenile stages (to instar 8). Early post-settlement instars of *Scylla serrata* (to around instar 4) have a dietary lipid requirement of ca. 5 to 14 per cent and a cholesterol requirement of 0.51 per cent (Sheen & Wu 1999, Sheen 2000). Late juvenile crabs (9 to 90g, beyond instar 8) grow well on formulated diets containing 32–40 per cent protein and 6 to 12 per cent lipid (14.7–17.6 MJ/kg) (Catacutan 2002). Pond trials suggest that mud crabs fed formulated diets can grow just as well as those fed trash fish (Trino, Millamena & Keenan 2001).

The signature place that *Scylla* species occupy in mangrove ecosystems and their capacity to survive on natural pond "productivity" in their early instars suggest that their dietary preferences may be more flexible than farmer"s expect (Overton 1999, Christensen, Macintosh & Phuong 2004). Recent feeding trials show that mud crabs can readily digest defatted soybean meal and have a raft of digestive carbohydrases (Catacutan, Eusebio & Teshima 2003, Pavasovic, Richardson, Anderson, Mann & Mather 2004), suggesting that some of the marine fish meal traditionally used in aquaculture diets can be replaced by terrestrial plant sources.

The first step for pioneering industries in this position is often to trial commercial feeds from established sectors growing similar animals (Dubber, Branch & Atkinson 2004, Fiore & Tlusty 2005). Fortunately, the commercial success of the shrimp farming means that crab industry development can take advantage of the considerable scientific and commercial resources have been devoted to penaeid shrimp feed development (Dall 1992, Kanazawa 1992). Existing shrimp feeds may of course be too expensive for the use of small holder crab farmers in SE Asia, however these feeds provide the opportunity to obtain a benchmark or reference diet from which further improvement is possible (e.g. switching to plant meals) as well as providing practical feeds to sustain initial industry development in areas such as Australia where trash fish is not an option and until specific crab feeds can be manufactured.

In this study, we compared the growth and survival of early juvenile *Scylla serrata* (instar 4 to instar 8) reared individually on a range of commercial shrimp diets readily available in Australia, alone or in combination with either freshly minced pipi (Donax deltoides) or live Artemia.

Methods and materials

Experimental animals

Each cohort of sibling juvenile mud crabs (*Scylla serrata* Forskal) (Keenan, Davies & Mann 1998) used in these two trials was reared from eggs hatched from each of two wild-caught female broodstock using methods described previously (Mann *et al*. 1999).

The feeding trials

The diets tested were ones available in Australia and currently used by prawn farmers. The abbreviations for the commercial diets used in this paper are set out in Table 69. In addition to these pelleted diet treatments, treatments using live on-grown *Artemia* (LA) and fresh pipi meat (FP) were also used. Adult *Artemia* were raised on the micro algae *Tetraselmis* chuii. Pipi (Donax deltoides) were collected daily from the beach and chopped finely and rinsed before use. The first trial was conducted in (December 2000/January 2001 (summer) while the second was conducted in March/May 2001 (autumn–winter).

Trial 1

Trial 1 was conducted for 37 days in an enclosed room in 3L bowls each containing 600ml of static filtered seawater (30ppt salinity). These bowls were changed and washed every second day or as necessary and the crab and its label placed in a clean bowl with clean water. A fan gently circulated the air in the room to facilitate oxygen uptake by the water and maintain temperatures evenly at 27 –29°C throughout the trial room. Feed particle size was standardised by crushing and sieving to the size of the smallest diet (1–2mm). Feeding was twice a day ad libitum to provide small excess at the next feed. Low light levels were maintained to assist the crabs to catch the live Artemia.

Table 69. Summary of the declared proximate composition of commercial feeds used in this trial.

Diets	Feed	Grade	Composition (%)						Price
Abbrev.	Manufacturer		Protein	Fat	Fibre	Crude ash	0ther	Moisture	$AUD per kg
			Kuruma shrimp diets						
J1	Higashimaru, Japan	Vital, starter #9	54.9	8.7	–	16.40	11.20	8.8	11 to 16
J2	Ocean Popeye, Taiwan	Finisher #5	50	6.5		17		8.5	1.35
J3	Higashimaru, Japan	Ebistar, grower #12	>50	>8	–	19	13	<10	6.6 to 12.50
			Black tiger shrimp diets						
M1	Aquafeed, Australia	starter	40	6	2			1.9	
M2	CP, Thailand	4004	36	3	3			11	1.9
M3	Grow Best, Taiwan	crumble	40	4.5	3			12	1.7

Notes: Vital also contains liver fortification agents, carotenoids, sugars.

Experimental design

On Day^{-7} (5/12/00), 150 sibling juvenile mud crabs, (starting with instar C3–C5 crabs (0.1–0.38g)) were randomly allocated to 10 blocks of 15 containers. Each block had 1 replicate of each treatment. A single crab occupied each container.

The 15 diet treatments are presented in Table 70, each treatment had ten individually housed crabs as replicates. The design tested all the diets individually (M1, M2, M3, etc) plus combinations of live and fresh diets with selected dry diets. In Table 70, where FP2 is indicated as an additional feed this means that fresh pipi meat was substituted for the main diet for one feed time every second day. Similarly, for LA2, a small number of live *Artemia* were added to the bowls every second day in addition to the normal feeding (i.e. do not substitute for a feed). These were allowed to persist in the bowl, unless eaten, until the next water change. Treatment 15 was a combination of all ingredients.

The crabs were acclimatised to the trial conditions for 1 week before first weighing on Day 0 (12/12/00) (Crab Instar 3 –5) using an electronic balance to four decimal places. Despite frequent cleaning and water changes, there were problems with poor water quality and deaths caused by fouling by uneaten food, especially by chopped pipi and uneaten pellets. Twenty-four dead crabs were replaced with similar sized siblings at Day 0 after the 7-day acclimation period. On Day 12 (24/12/00) eight of the 10 replicates of Diet 8 (FP: chopped fresh pipi) had to be replaced. Daily cleaning was practiced thereafter. Subsequent deaths were not replaced.

The crabs were weighed on Day 10 (22/12/00) (Crab Instar 4–6), with the final weighing on Day 37 (18/1/01) (Crab Instar 6–7). Estimated instar was recorded for each crab. Carapace widths were measured on Day 37 using vernier callipers.

Table 70. Explanation of the feed treatments used in the first trial (BIARC Batch–17).

Trt #	Main diet	Additional feed
1	M2	–
2	J1	–
3	M1	–
4	M3	–
5	J2	–
6	J3	–
7	LA	–
8	FP	–
9	M2	LA2
10	J1	LA2
11	M1	LA2
12	M2	FP2
13	J1	FP2
14	M1	FP2
15	M2,J1,M1	LA2 and FP2

Trial 2

This experiment was conducted for a longer period (68 days, largely because of the lower ambient temperature) using flowing seawater distributed to the bowls (in this case 2L red plastic containers) to rectify the earlier problems using static bowls. The troughs were housed in an insulated heated room (26ºC) and temperature was checked daily and maintained 24–26ºC (lower than ideal but this was a winter trial). A 12h:12h lighting regime was provided by fluorescent ceiling lights.

Each bowl was identified with a number to simplify feeding. The 160 bowls were housed in fibreglass troughs in banks of 20 with heated 26ºC filtered seawater fed to each container via dripper taps set at 0.15 L/min flow. A 3mm outflow hole drilled 2.5cm from the top set equal water depths. Weighted pieces of black plastic oyster mesh were used to prevent the crablets

escaping from the containers. Fly screen (1 mm mesh) was glued over the outlet hole to retain the Artemia.

Greater effort was taken to have all crablets of similar starting weight and instar as possible by first choosing C3 crablets only from a sibling population (rejecting C2 and C4) and then further narrowing the variation by grading for size and moult stage following the moult to C4 (average wt 0.10g, 10mm carapace width).

The water fouling caused by uneaten pellets in Trial 1 was mainly the result of feeding a pellet size too large for the size of the crab so feed size was sieved finer for the C4–C6 crabs in trial 2. Feed of uniform sizes 500–710*μ*m (C4–C5), 710*μ*m –1mm (C5–C6), 1–2mm (C6), 1–3mm (C7–C8), 1–4mm (C7–C9) was produced by breaking and sieving the commercial pellets. Feed was fed ad libitum, daily adjusted to individual demand to maintain very slight excess. The crablets were fed morning and evening.

Experimental design

One hundred and sixty 46-day-old mud crablets were placed in the numbered bowls on day 0, randomly allocated to treatments. The bowls were cleaned twice weekly for C4 to C7, then daily for C8 and C9. Covers were removed from each block to clean, feed and record moults and immediately put back in place. When the covers were withdrawn, crabs occasionally escaped to other bowls and injuries/deaths sometimes occurred. The few escapees were restored to their correct bowls on the basis of size and colour (the feeds altered the appearance of the crabs, Figure 56).

Records were kept for each animal throughout the trial. All crabs were weighed on Days 0, 6, 26, 35, 42, 49, 58, and finally on Day 68. The crablets were dried with paper tissue and weighed on a four decimal place electronic balance with weights recorded at the start of the trial, and weekly thereafter. A two decimal place balance was used from C6 onwards. All carapace widths from spine tip to spine tip were measured on day 68 using vernier callipers. The date of each moult was recorded for each crab to calculate moult interval and to verify each instar.

The 20 treatments are outlined in Table 71. Each of the eight blocks had one of each of the 20 treatments randomised within each block. Some crablets were fed individual diets (M1, J1, J2 etc) or live *Artemia* (LA). Fresh pipi was not used in trial 2 to avoid water quality problems. Combinations of feeds were given either as mixtures or alternately. For mixtures the daily ration comprised equal proportions of the listed diets (eg. mixM1J1, mixM1J2 etc). When diets were alternated, each day a different diet from the list was the sole ration provided, with the pattern repeating depending upon the number of diets involved (altM1J1, altM1J1J2 etc). For supplementation with Artemia, each of the single feeds or mixes (above) were fed for three days and then with *Artemia* on the fourth day (M1/Art, J1/Art etc). Ebistar (J3) was fed alone and not included in the mixtures but was included to provide another benchmark against which to compare to the results of trial 1.

Table 71. Explanation of the feed treatments used in the second trial

Trial 2 Treatments		
Trt# Sole diets	**Diet Code**	**Fed as**
1	M1	alone
2	J2	alone
3	J1	alone
4	J3	alone
5	Art	alone
Mixtures		
6	M1,J2	50:50 mix
7	M1,J1	50:50 mix
8	J2,J1	50;50 mix
9	M1,J2,J1	33:33:33 mix
Alternating days		
10	M1/J2	alternate days
11	M1/J1	alternate days
12	J2/J1	alternate days
13	M1/J2/J1	alternate days
Artemia **supplemented**		
14	M1/Art	LA fourth day
15	J2/Art	LA fourth day
16	J1/Art	LA fourth day
17	M1,J2,J1/Art	Mix/ LA fourth day
18	M1,J1/Art	Mix/ LA fourth day
19	J2,J1/Art	Mix/ LA fourth day
20	M1,J2/Art	Mix/ LA fourth day

Results

Trial 1.

Wet weights

Wet weight at day 0 was closely correlated with wet weight at day 10, and overall, there was a general correspondence between weights measured on different sample days, though this tended to breakdown when correlated samples taken many days apart (Table 72).

Table 72. Correlations between weights recorded on different sampling days and SGR and carapace width (CW) recorded on day 36.

	WT0	WT10	WT24	WT37	SGR37	CW37
WT0	1					
WT10	0.758	1				
WT24	0.606	0.707	1			
WT37	0.476	0.589	0.646	1		
SGR37	-0.457	-0.158	0.023	0.484	1	
CW37	0.377	0.49	0.534	0.892	0.498	1

The treatment groups did not differ significantly when weighed at day 0 (ANOVA, F=0.53, P>0.05, Table 73). However, significant differences existed when weights were taken at day 10 (F=1.90, P=0.032) and persisted for the duration of the trial. The three lowest average weights recorded at day 10 were the diets M2, M3 and J2 (Table 73). These diets were significantly different from crabs fed the mixture of M2J1M1, crabs fed M2 augmented with live *Artemia* (M2LA2) and crabs fed live *Artemia* alone (LA), the latter showing the highest average wet weight.

Table 73. Wet weight (mean±SE, number of crablets in brackets) of juvenile mud crabs *Scylla serrata* held in individual containers and fed different diets solely or in combination. At each sampling, results with the same postscript are not significantly different at 5 per cent (BIARC Batch–17).

Treatment	Wet weight (g) Day 0	Day 10	Day 24	Day 37
M1	0.150±0.002(10)	0.299±0.003ab(10)	0.666±0.014abc(10)	0.985±0.061ab(10)
M2	0.133±0.001(10)	0.282±0.002a(10)	0.622±0.013ab(10)	0.910±0.030a(10)
M3	0.132±0.0(10)	0.274±0.003a(10)	0.612±0.015a(10)	0.844±0.052a(10)
M2J1M1	0.185±0.004(10)	0.425±0.009cd(10)	1.183±0.083efg(10)	1.931±0.105e(10)
M1LA2	0.164±0.004(10)	0.301±0.002ab(10)	0.757±0.006abcd(10)	1.407±0.074bcd(10)
M2LA2	0.180±0.003(10)	0.415±0.017bcd(10)	0.986±0.033cdef(10)	1.476±0.123cde(10)
M1FP2	0.163±0.0(10)	0.342±0.001abc(10)	0.825±0.008abcd(10)	1.196±0.066abcd(10)
M2FP2	0.152±0.001(10)	0.319±0.002abc(10)	1.074±0.047def(10)	1.615±0.046de(10)
J1	0.149±0.0(10)	0.333±0.002abc(10)	0.873±0.020abcde(10)	1.543±0.078de(10)
J2	0.144±0.0(10)	0.292±0.003a(10)	0.710±0.013abc(10)	1.052±0.064abc(10)
J3	0.162±0.004(10)	0.329±0.005abc(10)	0.817±0.021abcd(10)	1.399±0.159bcd(10)
J1LA2	0.147±0.003(10)	0.371±0.011abcd(10)	0.953±0.091bcdef(10)	1.601±0.109de(10)
J1FP2	0.176±0.002(10)	0.375±0.003abcd(10)	1.229±0.097fg(10)	1.634±0.168de(10)
LA	0.191±0.004(10)	0.480±0.020d(10)	1.432±0.206g(10)	1.911±0.156e(10)
FP	0.145±0.0(10)	0.337±0.001abc(10)	0.780±0.006abcd(10)	1.402±0.014bcd(10)

When the treatments were re-weighed at day 24, diet once more significantly influenced the results (F=4.07, P<0.001, Table 73), and the three lowest average wet weights in this table were found in the M diets. Solely feeding these M diets returned results significantly different from that of crabs fed the mixture (M1M2J2), J1 augmented with fresh pipi (J1FP2) and crabs fed solely on live Artemia. Regarding crabs fed M1LA2, these were still significantly different from crabs M2 and M3 (as at day 10) but now they were not different from crabs fed M1. M2FP2 was also significantly different from the three M diets but of intermediate response compared to crabs fed live *Artemia* alone (LA), which had the highest average wet weight.

The pattern established at earlier weighings became further consolidated at the final weighing, on day 37 (F=4.14, P<0.001, Table 73). The three M diets continued to have the lowest average wet weights, and indeed most other treatments were now significantly different from crabs fed solely on the these three P. monodon feeds, the exceptions being crabs fed M1FP2 and J2. Crabs fed M1LA2 were significantly different from crabs fed M2 and M3 but were not significantly different from crabs fed M1. The highest average wet weight was now found amongst crabs fed the pellet mixture (M1M2J2) though this wet weight was not significantly different from that of crabs fed either LA or J1 or crabs fed M2 or J1 supplemented with either fresh pipi or live *Artemia* (M2LA2, M2FP2, J1LA2, J1FP2).

SGR and carapace width

Calculating specific growth rates for the 37 day trials reinforces this pattern (Table 74), emphasising the relatively poor performance of crabs (SGR of 5 to 6) solely fed the three M diets and J2, and even crabs fed J1FP2 had an SGR not significantly different from that of crabs fed the poorest performing M diets. It is worth noting that SGR is not tightly correlated

to weight at day 37 (r=0.484, Table 74). In contrast, the crabs fed J1 or J3, or the pellet mixture (M1M2J2), or either M1 or J1 supplemented with live *Artemia* (M1LA2, J1LA2) or M2 supplemented with fresh pipi (M2FP2) showed SGR's of 6.2 to 6.9 and were not significantly different.

Table 74. Specific growth rate (SGR) and carapace width (mean ± SE), number of crablets in brackets of juvenile mud crabs *Scylla serrata* held in individual containers and fed different diets solely or in combination for 37 days. At each sampling, results with the same postscript are not significantly different at 5%.

Treatment	37 day SGR (g% day)	Carapace width (mm)
M1	5.231±0.460ab(10)	19.4±3.3abc(10)
M2	5.410±0.303abc(10)	19.0±2.2ab(10)
M3	5.012±0.569a(10)	18.5±2.4a(6)
M2J1M1	6.737±0.537e(10)	25.5±0.7f(8)
M1LA2	6.454±1.373cde(10)	20.9±2.6abcd(10)
M2LA2	5.842±0.390abcde(10)	21.6±2.1cde(10)
M1FP2	5.372±0.602abc(10)	20.7±2.6abcd(8)
M2FP2	6.680±0.559e(10)	22.5±1.4de(9)
J1	6.415±0.248cde(10)	22.0±2.4de(9)
J2	5.427±0.378abcd(10)	19.2±2.6abc(10)
J3	6.239±0.639bcde(10)	22.5±6.3de(7)
J1LA2	6.850±0.693e(10)	21.8±2.4de(10)
J1FP2	6.053±0.779abcde(10)	21.5±3.5cde(10)
LA	6.591±0.526de(10)	23.8±3.4ef(10)
FP	6.317±0.185bcde(10)	21.4±0.4bcde(9)

Carapace width at 37 days was closely correlated with wet weight at this time (Table 74). The crabs fed the pellet mixture (M1M2J2) finished the experiment with the highest average carapace width (Table 74), though this was significantly different from all other treatments except that of crabs fed solely with live Artemia, the group finishing with the next highest average carapace width. The latter group in turn was not significantly different from most other treatments, with the telling exception of the poorly performing M diets and J2, and diet M1 supplemented with live/fresh feed (M1LA2 and M1FP2).

Trial 2

Wet weights and SGR

The treatment groups didn't differ significantly when weighed at day 0 ($F=1.29$ $p>0.05$) (Table 75). Furthermore, the different treatments did not differ significantly when weighed when the experiment ended on day 68 ($F_{7,19}=1.30$ $p>0.05$). The SGR measured 68 days was also similar despite the different feeds or combinations of feeds applied ($F_{7,19}=1.20$ $P>0.05$). CW measured at the end of the trial was also not significantly effected by feed used ($F_{7,19}=1.61$ $p=0.064$).

Table 75. Wet weight, carapace width (CW) and specific growth rate (±s.e.m, (n)) of juvenile *Scylla serrata* fed commercial shrimps feeds solely, in mixtures and alternately, and supplemented with live *Artemia* during a 68 day feeding trial.

Feed		Wet weight (g) day 0	day 68	CW(mm) day 68	SGR(%/day) day 68
	M1	0.11±0.00(8)	1.84±0.46(5)	24.0±2.1(5)	4.05±0.29(5)
	J2	0.12±0.01(8)	2.54±0.33(8)	26.4±1.1(8)	4.42±0.21(8)
	J1	0.13±0.01(8)	3.77±0.73(8)	30.6±2.1(8)	4.81±0.27(8)
	J3	0.12±0.01(8)	3.66±0.62(8)	29.8±1.7(8)	4.94±0.27(8)
	Art	0.12±0.00(8)	2.66±0.33(8)	27.4±1.1(8)	4.42±0.22(8)
Mix	M1,J2	0.12±0.01(8)	3.37±0.8(8)	28.5±2.3(8)	4.61±0.32(8)
	M1,J1	0.13±0.01(8)	3.37±0.27(8)	30.9±1.5(8)	4.8±0.18(8)
	J2,J1	0.12±0.01(8)	3.78±0.67(8)	30.2±1.6(8)	5.02±0.32(8)
	M1,J2,J1	0.12±0.01(8)	3.54±0.48(8)	29.2±1.4(8)	4.86±0.26(8)
Alternate	M1/J2	0.12±0.00(8)	2.91±0.32(7)	27.7±0.9(8)	4.63±0.14(7)
	M1/J1	0.13±0.01(8)	3.71±0.51(8)	30.1±1.7(8)	4.87±0.26(8)
	J2/J1	0.12±0.00(8)	3.25±0.51(8)	28.1±1.7(8)	4.71±0.27(8)
	M1/J2/J1	0.13±0.00(8)	3.43±0.53(7)	29.3±1.8(7)	4.71±0.27(7)
75%/25%Art	M1/Art	0.11±0.01(8)	2.79±0.32(7)	27.4±1.1(7)	4.63±0.17(7)
	J2/Art	0.12±0.01(8)	3.21±0.48(7)	28.8±1.4(7)	4.73±0.19(7)
	J1/Art	0.12±0.01(8)	3.97±0.33(8)	30.9±0.8(8)	5.14±0.06(8)
	M1,J2,J1/Art	0.11±0.01(8)	3.17±0.47(8)	27.7±1.8(8)	4.75±0.25(8)
	M1,J1/Art	0.13±0.01(8)	4.16±0.61(8)	31.4±1.4(8)	4.94±0.28(8)
	J2,J1/Art	0.12±0.01(8)	3.88±0.16(8)	31.4±0.4(8)	5.12±0.07(8)
	M1,J2/Art	0.14±0.01(8)	3.54±0.51(7)	29.5±1.6(7)	4.66±0.24(7)

However, feed treatment had a telling effect on weight measured earlier in the study (on days 6 to 58) (Table 76). At the day 6 weighing (Wt6, $F=2.36$ $p<0.01$), the weight of crabs fed M1 was significantly different from that of crabs fed J1. When different feeds were given alternately, alternating M1/J2 was no improvement on feeding M1 or J2 alone. However, the weight of crabs fed J1/M1, while different from those solely fed M1 was not significantly different from those fed J1 alone. Again, alternating M1 with both J1 and J2 was an improvement on solely feeding crabs with M1, but was not significantly different from alternating M1 and J2. Supplementation of live *Artemia* into single or mixed treatments generally brought no significant improvement upon the weight measured feeding pellets alone, save for crablets fed M1/J2.

Table 76. Wet weight (±s.e.m) of juvenile *Scylla serrata* fed commercial shrimps feeds solely, in mixtures and alternately, and supplemented with live Artemia.

At each weighing, means with the same letters are not significantly different at 5%. The number in brackets is the sample size.

		Wet weight (g) day 6	day 26 day 35	day 42	day 49	day 58	
Feed							
	M1	0.22±0.03ab(8)	0.55±0.03a(8)	0.57±0.04a(7)	0.85±0.16a(6)	1.18±0.09a(6)	1.17±0.13a(4)
	J2	0.28±0.02bcde(8)	0.66±0.04abcd(8)	1.11±0.13bcde(8)	1.22±0.14ab(8)	1.60±0.09abcd(8)	2.22±0.25abcd(8)
	J1	0.30±0.03de(8)	0.74±0.06bcdef(8)	1.21±0.16bcdef(8)	1.70±0.15bc(8)	2.23±0.35de(8)	2.33±0.40bcde(8)
	J3	0.23±0.03abc(8)	0.71±0.06abcdef(8)	1.20±0.20bcdef(8)	1.42±0.29bc(8)	1.90±0.26bcde(8)	2.43±0.42bcde(8)
	Art	0.28±0.02bcde(8)	0.64±0.04abc(8)	0.90±0.11abc(8)	1.29±0.17abc(8)	1.75±0.28abcde(8)	2.06±0.25abc(8)
Mix	M1,J2	0.27±0.04bcd(8)	0.74±0.04bcdef(8)	0.90±0.11ab(8)	1.58±0.32bc(8)	1.86±0.29bcde(8)	2.30±0.35bcde(8)
	M1,J1	0.28±0.02bcde(8)	0.74±0.02bcdef(8)	1.14±0.19bcdef(7)	1.39±0.21bc(6)	2.22±0.39de(7)	2.57±0.37bcde(8)
	J2,J1	0.27±0.02bcd(8)	0.77±0.10bcdef(8)	1.37±0.25bcdef(8)	1.67±0.27bc(8)	1.82±0.24bcde(8)	2.43±0.33bcde(7)
	M1,J2,J1	0.19±0.02a(8)	0.68±0.03abcde(8)	1.12±0.16bcde(8)	1.32±0.15bc(8)	1.64±0.19abcd(8)	2.62±0.38bcde(8)
Alternate	M1,J2	0.25±0.02abcd(8)	0.60±0.05ab(8)	1.16±0.15bcdef(8)	1.20±0.16ab(8)	1.37±0.11ab(8)	2.45±0.41bcde(7)
	M1,J1	0.29±0.02cde(8)	0.85±0.13f(8)	1.39±0.21cdef(8)	1.51±0.20bc(8)	1.93±0.37bcde(8)	3.11±0.49cde(8)
	J2,J1	0.27±0.02bcd(8)	0.75±0.03bcdef(8)	1.39±0.21def(8)	1.44±0.23bc(8)	1.76±0.09abcde(8)	3.11±0.47cde(8)
	M1,J2,J1	0.31±0.01de(8)	0.78±0.04cdef(8)	1.17±0.21bcdef(8)	1.51±0.22bc(7)	1.52±0.23abc(7)	2.47±0.55bcde(7)
75%/25%Art	M1	0.26±0.03abcd(8)	0.68±0.05abcde(8)	0.89±0.13abcd(7)	1.25±0.16abc(7)	1.38±0.13ab(7)	1.97±0.31ab(6)
	J2	0.27±0.03bcd(8)	0.84±0.11ef(7)	1.21±0.23bcdef(7)	1.53±0.15bc(7)	1.84±0.31bcde(7)	2.70±0.56bcde(7)
	J1	0.30±0.02de(8)	0.74±0.06bcdef(8)	1.62±0.19f(8)	1.78±0.14c(8)	1.81±0.14bcde(8)	3.36±0.49e(8)
	M1,J2,J1	0.29±0.01cde(8)	0.78±0.09cdef(8)	1.47±0.14ef(8)	1.62±0.09bc(8)	1.59±0.11abcd(8)	2.77±0.43bcde(8)
	M1,J1	0.34±0.03e(8)	0.86±0.06f(8)	1.31±0.17bcdef(8)	1.79±0.19c(8)	2.38±0.22e(8)	3.28±0.42de(8)
	J2,J1	0.27±0.02bcd(8)	0.72±0.03bcdef(8)	1.35±0.19bcdef(8)	1.70±0.07bc(8)	2.06±0.32cde(8)	3.0±0.40bcde(8)
	M1,J2	0.34±0.02e(8)	0.83±0.05def(8)	1.22±0.20bcdef(8)	1.69±0.17bc(7)	1.92±0.14bcde(7)	2.54±0.43bcde(7)

Abbreviations: M1= Aquafeeds, J1=Vital; J2= ocean pop-eye; J3=Ebistar

At the day 26 weighing, (Wt26 F=1.82 p=0.026), the weight of crabs fed M1 was significantly different from that of crabs fed J1 and crabs fed mixtures of M1/J1, M1/J2 and J1/J2 but not the mixture of all three pellets. Alternating M1/J2 was again no improvement on feeding M1 or J2 alone. However, the weight of crabs fed J1/M1 alternately, was again different from those solely fed M1 but not significantly different from those fed J1 alone. Again, alternating M1 with both J1 and J2 had some merit over solely feeding crabs with M1, but it was an improvement over alternating M1 and J2. Supplementation of live *Artemia* brought no significant improvement upon the weight measured feeding pellets alone, except for crablets fed with J2.

By day 35 (Wt35 F=1.92 p=0.017) the weight of crabs fed M1 was significantly different from that of crabs fed J1, J2 and J3 and crabs fed all mixtures except M1/J2. Alternating M1 with japonicus diets (J1 and J2) had significant benefits over that of feeding M1 alone, but alternating J1 and J2 was no improvement over feeding J1 alone or M1/J2. Addition of live *Artemia* brought no significant improvement upon the weight measured feeding pellets alone.

At day 42, (Wt42 F=1.79 p=0.031)feeding M1 was now significantly different from all other treatments except for J2 and M1/Art. Mixing M1 with J1 and/or J2 was not significantly different from solely feeding J1 or J2. Live *Artemia* generally brought no significant improvement upon the weight measured feeding pellets alone, thought the means for J1/Art and M1/J1/Art were the highest observed at this sampling.

Day 49,(Wt49 F=1.76 p=0.034) consolidating many of the earlier patterns with crabs fed solely M1, J2 or live *Artemia* performing poorly besides those of crabs fed J1 and J3. Crabs fed a mixture of M1 and either J1 or J2, (or crabs fed J1 mixed with J2) gave a significantly different mean crab weight from those fed M1 alone, but none were significantly different from

crabs fed solely on J1 or J3. The mixture of M1,J2 and J1 was not significantly different from that of M1 alone. Alternating M1/J2, J2/J1 and M1/J1/J2 was no improvement on feeding M1 alone, but alternating M1 and J1 was significantly different from that of feeding M1 alone but not significantly different from feeding the two pellets as a mixture. Live *Artemia* again generally brought no significant improvement upon the weight measured feeding pellets alone.

At the day 58 weighing ($F=1.68$, $p=0.049$), the pattern seen at day 42 persisted. Feeding *Artemia* alone was no better than feeding pellets, and pellet mixtures or alternating M1 with japonicus feeds (J1 and J2) performed better than crabs fed M1 alone, and similar to performance of crabs fed solely with the P. japonicus diets. This is another occasion when crabs fed live *Artemia* with either J1 or M1,J1 mix showed relatively high average sizes.

Weight of instars.

Treatment had no significant effect on the weight of crab 4, (AOV $F_{19,159}=1.29$ $P>0.05$) (Table 77). Some evidence of a response is already occurring as the crablets moulted to crab 5 (AOV $F_{19,158}=1.64$ $P=0.056$) but the one-way AOV showed that the effect of feed treatment did not become significant until applied to crab 6 ($F_{19,158}=2.24$, $P<0.01$). Further examination using LSD tests shows that feeding J2, or *Artemia* or alternating M1/J2 were not significantly different from feeding M1 alone. On the other hand, combining M1 with J1 either as a mixture or alternating them produced crablets not significantly different from those of solely feeding the more expensive J1. Supplementing these combinations with *Artemia* gave no advantage. The feed treatment applied continued to contribute to the size of crab 7 and crab 8 (AOV, $F_{19,152}=2.31$ $p<0.01$ and $F_{19,112}=2.38$ $p<0.01$ respectively). The LSD comparisons confirm that combinations of M1 with either japonicus feed are not significantly different from giving J1 alone. And as seen with crab 6, alternating the feeds appeared to have some benefit over mixing them while treatments supplemented with *Artemia* continued to be matched by the pellet only combinations.

These changes arise via the moult increment and not the periodicity of moulting (below). The weight change at moulting to crab 5 (Table) 78 was not significantly altered by feed treatment (AOV, $F_{19,158}=1.59$, $P>0.05$) however the weight change on moulting to crab 6, 7 and 8 all showed a significant influence of feed treatment, (crab6: $F_{19,157}=2.86$, $p<0.001$; crab 7 $F_{19,152}=2.05$, $p<0.05$; crab 8: $F_{19,110}=4.17$, $p<0.001$). Examining the LSD comparisons of the means suggests that the patterns follow those of the weight data itself, particularly for crabs fed the poor performing M1, the expensive J1 and combinations of these.

Table 77. Wet weight (±s.e.m) upon moulting to each instar of juvenile *Scylla serrata* fed commercial shrimps feeds solely, in mixtures and alternately, and supplemented with live Artemia.

At each instar, means with the same letters are not significantly different at 5%. The number in brackets is the sample size.

	Crab 4	Crab 5	Crab 6	Crab 7	Crab 8
M1	0.111±0.005(8)	0.255±0.015(8)	0.547±0.029a(8)	1.178±0.088a(6)	2.680±0.930a(2)
J2	0.120±0.006(8)	0.284±0.017(8)	0.664±0.037abc(8)	1.599±0.091bcd(8)	3.310±0.307abcde(4)
J1	0.127±0.005(8)	0.303±0.029(8)	0.744±0.065bcde(8)	1.746±0.159bcde(8)	3.773±0.402bcdef(6)
Art	0.125±0.005(8)	0.306±0.014(8)	0.638±0.035ab(8)	1.519±0.119bc(8)	3.063±0.297ab(6)
M1,J2	0.125±0.007(8)	0.311±0.017(8)	0.740±0.037bcde(8)	1.749±0.083bcde(8)	3.970±0.090cdef(4)
M1,J1	0.126±0.005(8)	0.305±0.017(8)	0.736±0.021bcde(8)	1.693±0.065bcde(6)	3.680±0.152bcdef(6)
J2,J1	0.116±0.009(8)	0.268±0.023(8)	0.678±0.074bc(8)	1.591±0.148bcd(8)	3.183±0.202abcde(6)
M1,J2,J1	0.121±0.006(8)	0.279±0.017(8)	0.677±0.030bc(8)	1.555±0.090bcd(8)	3.454±0.201abcdef(7)
M1,J2	0.120±0.003(8)	0.269±0.009(8)	0.652±0.031ab(8)	1.495±0.080bc(8)	3.103±0.297abcd(6)
M1,J1	0.128±0.008(8)	0.293±0.017(8)	0.736±0.054bcde(8)	1.773±0.136bcde(8)	3.892±0.437def(6)
J2,J1	0.120±0.004(8)	0.292±0.015(8)	0.748±0.030bcde(8)	1.755±0.094bcde(8)	4.198±0.341f(5)
M1,J2,J1	0.130±0.005(8)	0.312±0.013(8)	0.779±0.044cde(8)	1.754±0.087bcde(8)	4.208±0.295f(5)
M1/Art	0.114±0.007(8)	0.272±0.021(8)	0.681±0.051bc(8)	1.482±0.115b(6)	3.106±0.308abc(5)
J2/Art	0.121±0.006(8)	0.285±0.021(8)	0.740±0.061bcde(7)	1.570±0.153bcd(7)	3.503±0.466bcdef(6)
J1/Art	0.119±0.007(8)	0.302±0.022(8)	0.745±0.057bcde(8)	1.809±0.143cde(8)	3.974±0.332ef(8)
M1,J2,J1/Art	0.114±0.006(8)	0.292±0.014(8)	0.696±0.030bc(8)	1.633±0.092bcde(8)	3.760±0.355bcdef(6)
M1,J1/Art	0.134±0.006(8)	0.343±0.025(8)	0.855±0.055e(8)	1.942±0.140e(8)	4.198±0.341f(6)
J2,J1/Art	0.119±0.005(8)	0.292±0.014(8)	0.722±0.029bcd(8)	1.761±0.068bcde(8)	3.884±0.165def(8)
M1,J2/Art	0.140±0.007(8)	0.344±0.019(8)	0.826±0.047de(8)	1.872±0.128de(8)	4.198±0.373f(5)
J3	0.117±0.008(8)	0.263±0.019(7)	0.713±0.056bcd(8)	1.694±0.153bcde(8)	3.623±0.359bcdef(6)

Table 78. Moult increment (g, ±SE) upon moulting to each instar for juvenile *Scylla serrata* fed commercial shrimps feeds solely, in mixtures and alternately, and supplemented with live Artemia.

At each instar, means with the same letters are not significantly different at 5%. The number in brackets is the sample size.

	Crab 5	Crab 6	Crab 7	Crab 8
M1	0.144±0.011(8)	0.291±0.016a(8)	0.640±0.055a(6)	0.860±0.0a(1)
J2	0.164±0.013(8)	0.381±0.031bc(8)	0.934±0.086bc(8)	1.825±0.177bcde(4)
J1	0.176±0.027(8)	0.441±0.038cdef(8)	1.003±0.096bc(8)	1.987±0.227bcdefg(6)
Art	0.182±0.010(8)	0.332±0.031ab(8)	0.881±0.088bc(8)	1.568±0.170b(6)
M1,J2	0.187±0.012(8)	0.429±0.022cde(8)	1.009±0.048bc(8)	2.145±0.039cdefg(4)
M1,J1	0.179±0.012(8)	0.431±0.010cdef(8)	0.977±0.047bc(6)	2.010±0.107bcdefg(5)
J2,J1	0.152±0.016(8)	0.410±0.052bcde(8)	0.913±0.078bc(8)	1.762±0.116bcd(6)
M1,J2,J1	0.157±0.012(8)	0.398±0.023bcd(8)	0.878±0.063bc(8)	1.846±0.126bcdef(7)
M1,J2	0.148±0.008(8)	0.384±0.027bc(8)	0.843±0.052b(8)	1.637±0.214bc(6)
M1,J1	0.165±0.009(8)	0.443±0.038cdef(8)	1.036±0.085bc(8)	2.153±0.258defg(6)
J2,J1	0.172±0.014(8)	0.456±0.019cdef(8)	1.007±0.066bc(8)	2.348±0.217fg(5)
M1,J2,J1	0.182±0.010(8)	0.467±0.033def(8)	0.975±0.060bc(8)	2.352±0.214g(5)
M1/Art	0.157±0.016(8)	0.409±0.031bcde(8)	0.836±0.073b(6)	1.602±0.181b(5)
J2/Art	0.164±0.016(8)	0.454±0.042cdef(7)	0.830±0.104b(7)	1.915±0.287bcdefg(6)
J1/Art	0.183±0.015(8)	0.443±0.035cdef(8)	1.064±0.088c(8)	2.165±0.190defg(8)
M1,J2,J1/Art	0.178±0.012(8)	0.404±0.023bcde(8)	0.937±0.066bc(8)	2.102±0.236cdefg(6)
M1,J1/Art	0.209±0.020(8)	0.512±0.031f(8)	1.087±0.096c(8)	2.287±0.198efg(6)
J2,J1/Art	0.172±0.009(8)	0.431±0.021cdef(8)	1.039±0.047bc(8)	2.123±0.107cdefg(8)
M1,J2/Art	0.204±0.014(8)	0.482±0.031ef(8)	1.047±0.088bc(8)	2.232±0.230defg(5)
J3	0.151±0.011(7)	0.412±0.030cde(7)	0.981±0.098bc(8)	1.982±0.223bcdefg(8)

The full inter-moult period leading to the first moult in the experiment (crab 5) is unknown. The inter-moult period before moulting to each instar (Table 79) was not significantly influenced by the feed treatments applied (moult to crab 6: $F_{19,158}=0.75$ $p>0.05$; moult to crab 7: $F_{19,157}=1.01$ $P>0.05$) with the exception of the moult to crab 8 (AOV $F_{19,114}=2.79$, $p<0.001$) which saw a pronounced delay in the moult of the M1 treatment and surprisingly J1. The quicker moulting treatments appear to consist of mixtures of the various pellet feeds and some cases where mixtures were supplemented with live Artemia. Examining the timing of moulting as days elapsed in the experiment showed no significant effect of feed treatment on the schedule of moulting to crab 5, 6 and 7 (crab 5: $F_{19,159}=1.36$ $p>0.05$; crab 6 $F_{19,158}=0.65$ $p>0.05$; crab 7 $F_{19,157} = 0.97$ $p>0.05$ and the AOV for the average day of the moult to crab 8 lay on the threshold of significance (AOV, $F_{19,115}=1.70$ $P=0.051$).

Table 79. The inter-moult period preceding the moult to each instar In the experiment

	Crab 6	Crab 7	Crab 8
M1	13.0±1.1(8)	23.1±1.9(8)	30.0±6.0g(2)
J2	12.0±0.5(8)	20.0±2.4(8)	24.0±0.7cdef(4)
J1	12.8±0.7(8)	17.5±1.4(8)	27.3±3.3fg(6)
Art	11.5±0.7(8)	20.9±1.4(8)	22.8±2.0abcde(6)
M1,J2	13.4±0.5(8)	22.3±2.5(8)	19.3±2.5a(4)
M1,J1	13.0±0.6(8)	17.1±1.9(8)	20.0±1.7ab(7)
J2,J1	12.6±1.5(8)	19.1±3.3(8)	20.5±2.8abc(6)
M1,J2,J1	11.8±0.5(8)	19.4±2.6(8)	20.7±1.1abcd(7)
M1,J2	12.9±1.1(8)	18.5±2.1(8)	24.0±0.8bcdef(7)
M1,J1	12.3±0.7(8)	19.0±2.9(8)	22.7±1.5abcde(6)
J2,J1	12.1±0.4(8)	19.0±2.4(8)	24.8±0.5def(5)
M1,J2,J1	13.5±0.4(8)	21.1±3.0(8)	23.6±1.0bcdef(5)
M1/Art	12.1±0.5(8)	24.0±2.5(8)	23.6±1.0bcdef(5)
J2/Art	12.1±1.0(7)	17.6±1.5(7)	23.0±1.1bcdef(6)
J1/Art	12.4±0.7(8)	15.9±1.1(7)	23.5±1.8bcdef(8)
M1,J2,J1/Art	11.4±0.8(8)	17.0±1.8(8)	25.3±1.2ef(6)
M1,J1/Art	12.3±0.6(8)	18.5±1.8(8)	20.8±2.4abcde(6)
J2,J1/Art	11.4±0.9(8)	17.8±1.6(8)	22.6±1.2abcde(8)
M1,J2/Art	13.6±0.7(8)	20.0±1.6(8)	23.6±0.9bcdef(5)
J3	12.3±0.8(8)	21.3±2.8(8)	21.5±2.4abcde(6)

The changes in weight that set in as early as the moult to crab 6 may have established the differences in weight between the treatments. It is clear that there are strong positive correlations between weights of individual crabs as they grow from instar to instar (Table 80). Even the weight of individuals at crab 4 (at the start of the experiment) correlates very well with their subsequent weight up to four instars later! In part this is possibly explained by the modest correlations between the pre-moult weight of the crablet (eg. weight5 in the table) and the increment when the crab moults (incr6 in the table). The compounding of this covariate effect across instars must have the effect of amplifying rather than confounding earlier differences in size.

Table 80. Correlations between moult increments to reach that instar (g), the preceding moult period and the weight of the instar

Rows	incr 5	incr 6	incr 7	incr 8	incr 9	period 6	period 7	period 8	period 9	weight 4	weight 5	weight 6	weight 7	weight 8
incr5														
incr6	0.17													
incr7	0.80	0.57												
incr8	0.40	0.64	0.76											
incr9	0.46	0.56	0.69	0.95										
period6	0.35	0.30	0.37	-0.11	-0.25									
period7	0.28	-0.37	-0.16	-0.33	-0.17	-0.39								
period8	-0.08	0.53	0.18	-0.13	-0.31	0.58	-0.14							
period9	-0.30	-0.43	-0.35	0.12	0.15	-0.42	-0.32	-0.79						
weight4	0.31	0.78	0.69	0.78	0.65	0.10	-0.19	0.42	-0.30					
weight5	0.82	0.58	0.92	0.72	0.68	0.28	0.06	0.20	-0.37	0.81				
weight6	0.40	0.96	0.75	0.73	0.66	0.32	-0.26	0.47	-0.46	0.87	0.78			
weight7	0.69	0.77	0.96	0.80	0.72	0.37	-0.21	0.31	-0.42	0.81	0.92	0.90		
weight8	0.54	0.73	0.88	0.97	0.90	0.09	-0.30	0.05	-0.11	0.84	0.85	0.84	0.92	
weight9	0.50	0.64	0.78	0.98	0.99	-0.13	-0.22	-0.18	0.06	0.73	0.76	0.74	0.82	0.96

Reanalysing the results using pre-moult weight as a covariate confirms this. Once the significant treatment effect was established on the occasion of the moult to crab 6 (ANCOVA, covariate weight5) $P<0.001$; treatment effect on either weight6 or incr6 both $p<0.001$) it turns out that the sustained effect observed when the feed treatments continued for later instars is easily accounted for the outcome of this earlier moult, perpetuated by the strong pre-moult weight covariate ($p<0.001$). The ANCOVA for weight of crab 6 is summarised in Table 81.

Table 81. ANCOVA of effect of treatment on weight of crab 6 using weight of crab 5 as a covariate.

Source of variation		s.s.	m.s.	F	cov.ef.	p
	d.f.(m.v.)					
BLOCK stratum						
Covariate	1	0.041471	0.041471	2.38		0.174
Residual	6	0.10463	0.017438	5.31	1.2	
BLOCK.*Units* stratum						
treat	19	0.213636	0.011244	3.42	0.99	<.001
Covariate	1	1.669132	1.669132	508.3		<.001
Residual	130(2)	0.426892	0.003284		4.87	
Total	157(2)	2.937925				

Once the covariate effect is removed, the LSD comparisons for the treatment effects (Table 82) nevertheless confirm that M1 and Artemia-fed crablets performed poorly at this moult and that crabs benefited when fed combinations of M1 and japonicus feeds, performing as well as they did when fed J1 alone.

Table 82. LSD Comparisons of weight of crab 6 in different treatments following ANCOVA using weight of crab 5 as a covariate. ++ LSD=0.05703 (BIARC Batch–17).

Treatment	tr	n	Mean	
M1	1	8	0.6295	ab
J2	2	8	0.6852	bc
J1	3	8	0.7233	cde
Art	4	8	0.6106	a
M1,J2	5	8	0.7019	cde
M1,J1	6	8	0.7118	cde
J2,J1	7	8	0.7324	cde
M1,J2,J1	8	8	0.7093	cde
M1,J2	9	8	0.7065	cde
M1,J1	10	8	0.7373	cde
J2,J1	11	8	0.7523	de
M1,J2,J1	12	8	0.7399	cde
M1/Art	13	8	0.7279	cde
J2/Art	14	8	0.7567	e
J1/Art	15	8	0.7269	cde
M1,J2,J1/Art	16	8	0.6984	cd
M1,J1/Art	17	8	0.7482	de
J2,J1/Art	18	8	0.7264	cde
M1,J2/Art	19	8	0.7172	cde
J3	20	8	0.7468	de

Discussion

The immediate practical outcome of this work is that it is clear that juvenile mud crablets (*Scylla serrata*) in these experiments grew well when fed nothing but commercial pelleted diets formulated for penaeid shrimp. This indicates that mud crabs, at least at the sizes used here, may have no specific dietary requirements other than those already present in shrimp feed and so development of specific diets tailored to mud crabs seems unlikely to present any major technical obstacles. Some work has already been undertaken on development of broodstock diets for maturation of pond-reared broodstock, showing that the females performed better as broodstock when the natural diet is supplemented with formulated ingredients (Millamena & Quinitio 2000, Millamena & Bangcaya 2001).

Nursery feeds may not be required under all circumstances. Mud crab farmers overseas stock ponds extensively (0.5 crabs/ m²) and currently rely upon fresh feed (fishery by catch) to supplement natural pond productivity, though at such low stocking densities the latter only seems to be crucial later in growth (Christensen *et al*. 2004). The extensive farming model may not apply in Australia, where labour costs are higher, and higher per hectare returns

are expected from pond aquaculture. Furthermore, it is anticipated that mud crab hatcheries in Australia will grow the post-larvae to around crab 4 at which time they can be readily packaged and transported without seawater to farms. To restrict the demand on nursery ponds, it will be more efficient for the hatcheries to grow crablets at higher density, with suitable habitat either in large tanks or confined to netted enclosures in ponds (Mann *et al* in prep).

In this study, sole or supplemental feeding of crablets with fresh or live feeds provided no benefit above that which could be obtained from pelleted feeds alone. As the difficulties experienced here with water quality when using pipi flesh show, manufactured feeds stored in nothing more complicated than a feed shed have considerable practical advantages over supplying fresh seafood wastes or live adult *Artemia* to a nursery pond- particularly at the high stocking densities anticipated.

Shrimp diets differ considerably in quality and expense and it is unfortunate that crablets fed the three examples of inexpensive black tiger shrimp feeds used in this experiment (M1, M2 and M3) were outperformed by crablets fed the more expensive Kuruma shrimp feeds (J1 and J3). The better quality Kuruma shrimp feeds are not immediately ruled out for nursery use because of expense-after all, relatively little feed is required because the biomass of crablets in a nursery remains very low for a long time. The expense can anyway be contained simply by mixing or “diluting” the expensive feed with a cheaper alternative without any significant loss of growth rate, as these results show. This perhaps indicates that the black tiger shrimp diets tested may be limiting in something that can be ready satisfied by the addition of some Kuruma shrimp feed to the diet, or that the presence of the higher quality pellets in the mix makes the other pellets more attractive to the crablets. However, instances where the effect occurs when the feeds are alternated rather than mixed suggests that the former explanation may be more likely.

Feeds for Kuruma shrimp tend to have higher protein levels than black tiger shrimp feeds (Table 69) but because of the greater value of the former species, there is more incentive to include high quality ingredients in the formulation. What little information there is about dietary requirements of mud crabs (Sheen & Wu 1999) has not indicated that such high protein levels (>50%) are a particular requirement of mud crabs–but more information may be needed. The performance of crabs fed mixtures of black tiger shrimp and Kuruma shrimp feeds indicate that the protein requirements may fall somewhere in the 40–50 per cent range. It should be noted that Kuruma feeds also tend to contain boosted levels of astaxanthin and a richer variety of additives–and the colour of crablets noticeably improved here when fed these diets above that of crablets fed solely on cheaper feeds (Figure 56).

Figure 56. Effect of feed on appearance of crablets. The top row shows crablets from treatments including expensive Kuruma diets (J1 and J3). The bottom row shows crablets from treatments including cheaper feeds, (M1 and/or J2).

The problems experienced in matching granule sizes to crablet instars in the first trial also highlight the important of particle size. While all commercial shrimp feeds are generally available as nursery-sized “crumbles” some further grinding and tailoring of particle size may be needed to apply these to crablet rearing, particularly for the first few crablet instars. Seemingly, the crablets prefer to eat particles that they can fit into their mouth, so feeding large pellets too soon will lead to waste. Growout of mud crabs using shrimp pellets will potentially also encounter another size problem–mud crabs grow considerably larger than shrimp and cannot be expected to prosper on the largest commercial shrimp pellets available.

There is little data for which to compare these results to previous studies. Some mud crab growth and diet studies are pond based (Trino, Millamena & Keenan 1999, Trino *et al*. 2001) so it is difficult to compare when details about moult increment and period are unknown while others examine growth at earlier or later instars than those studied here (Catacutan 2002, Ruscoe, Shelley & Williams 2004a).

Previous studies report that mud crabs survived and grow well on experimental formulated feeds (Sheen & Wu 1999, Catacutan 2002) but calculations show that these findings are based on relatively low WSGR’s; around 2 to 3. Mud crabs appear to grow even better on commercial shrimp feeds, with SGR’s in this study of between 4.0 and 6.8, depending upon seasonal temperature differences. These SGR’s are comparable to, though are slightly lower on average than, the growth rate reported by Heasman (1980) which were for mud crab

juveniles fed a fresh flesh diet (SGR ranging from 4.6 to 11). Temperature needs to be kept in mind when discussing growth of mud crablets (Ruscoe *et al*. 2004a) and this is probably one reason for the lower WSGR seen in the only other published mud crab growth study to use similar-sized crablets to those reported here (particularly trial 1), which finished with 1g individuals after 63 days at 23–27 °C (Sheen & Wu 1999).

Previous studies have not found any significant effect of diet on moulting period (Sheen & Wu 1999, Catacutan 2002), and this trial generally supports this. Significant differences in crab weight between feed treatments arise because of differences in weight change at moulting, and not because the crabs moult at different times. However, we found in trial 2 that the period leading to the moult to instar 8 was significantly delayed for two treatments. Paradoxically, these were one of the worst and one of the best performing feeds, M1 and J1 respectively so it is unlikely to be simple feed treatment differences that caused this.

Escape and cannibalism can be a serious problem in portunid crab diet trials, (Fitz & Wiegert 1991). In the second trial, mortality in the M1 diet at the final harvest ruined the power of the analysis but the results of earlier weighings nevertheless reinforced the picture. The asymmetrical nature of encounters between crablets may explain why crablets fed M1 alone suffered so much late mortality–by this time they were much smaller than their siblings. Prevention of these deaths in future trials is important–though intermediate weighing can also be used as a safeguard.

As the covariate analysis shows, how big a portunid crab is after it moults depends a lot on how big it is before it moults. This is why covariance analysis is strongly recommended in crab growth trials and why care has to be taken to reduce the initial size variation of the crabs, (Cadman & Weinstein 1988). The crabs here more than double their weight in the process of moulting. It was in a sense surprising how early the outcome became established in trial 2, since once it did so, at the moult to crab 6, the powerful pre-moult weight covariate then almost renders the rest of the experiment superfluous. Once a difference in size sets in between treatments then if nothing else changes then the “laws” of crab growth cannot fail to amplify that difference into subsequent instars. This also apparently means that the feed difference seems to have most of its statistical effect during one instar. If the crablets could later be switched back to “cheaper” diets the question arises whether the earlier boost would be sustained or would their growth rate falter. Cost saving by longer term switching between high and low cost feeds should probably be considered.

While costs can be reduced by mixing feeds, this is not to say that further cost reductions will not occur. The ability of mud crabs to digest starches and cellulose (Pavasovic *et al*. 2004), a spin-off perhaps of them spending considerable part of their lifecycle amongst mangroves, suggests that not only might some of the protein in their diet be supplied from terrestrial plant meals but also that there may be a nutritional role for cheap carbohydrate sources in formulated mud crab diets.

Effect of stocking density and habitat on growth and survival in intensive nursery systems for mud crabs *Scylla serrata*. (BIARC–Batch 18)

David L. Mann*, Tom Asakawa, Beverley Kelly, Trent Lindsay and Brian Paterson

Abstract

Intensive nursery systems are designed to culture mud crab post-larvae through a critical phase in preparation for stocking into growout systems. This study investigated the influence of stocking density and provision of artificial habitat on the yield of a cage culture system. For each of three batches of post-larvae, survival, growth and claw loss were assessed after each of three nursery phases ending at crab instars C1/C2, C4/C5 and C7/C8. Survival through the first phase was highly variable among batches with a maximum survival of 80 per cent from megalops to a mean crab instar of 1.5. Stocking density between 625 and 2300 m^{-2} did not influence survival or growth in this first phase. Stocking densities tested in phases 2 and 3 were 62.5, 125 and 250 m^{-2}. At the end of phases 2 and 3, there were five instar stages present, representing a more than 20-fold size disparity within the populations. Survival became increasingly density-sensitive following the first phase with higher densities resulting in significantly lower survival (phase 2: 63% vs 79%; phase 3: 57% vs 64%). The addition of artificial habitat in the form of pleated netting significantly improved survival at all densities. Mean instar attained by the end of phase 2 was significantly larger at lower stocking density and without artificial habitat. No significant effect of density or habitat on harvest size was detected in phase 3. The highest incidence of claw loss was 36 per cent but was reduced by lowering stocking densities and addition of habitat. For intensive commercial production, yield can be significantly increased by addition of a simple net structure but decreases rapidly the longer crablets remain in the nursery.

Introduction

Growout of mud crab *Scylla serrata* (Forskål) has, until recently, been restricted to use of wild-caught juvenile crabs as the technology for mass production in hatcheries was still in developmental stages (Keenan and Blackshaw, 1999). However mud crab aquaculture is now entering a new phase of development with the expansion of commercial hatchery production. It is now being reported from several countries that commercial scale hatcheries are supplying the industry with seed in increasing quantities (Allan and Fielder, 2003). Hatchery produced seed supplied to farmers is typically in the form of small post larval crabs between 6 and 20 mm carapace width (C2–C5).

The nursery period is a necessary intermediate step in mud crab production between hatchery and growout, to grow post-larvae to a size suitable for transport and release into large extensive to semi-intensive production systems. Additionally, those with experience in crab hatchery operation generally recognise that the early post larval phase, which includes the megalops stage, is prone to highly variable survival rates. A controlled nursery phase therefore facilitates management and production predictability of the growout phase.

The nursery period, from megalops stage to crabs of around 2.5 to 5g body weight (25–35 mm carapace width), encompasses several phases that are characterised by developmental changes. The first phase includes the metamorphosis from megalops to crab instar 1

(C1) and is a critical step that can be prone to high losses and is a time when the animal is seeking substrate for settlement (Mann *et al*., 1999). During the next phase, the crabs increase dramatically in size, moulting progressively through to the C4 instar. The third phase begins at C5 and marks the attainment of shell proportions similar to that of large juvenile crabs (Heasman, 1980). For the purposes of this investigation, instar C7 to C8 crabs with 2 to 6g body weight, were considered to be at the upper end of crab size produced within a specialised intensive nursery system.

Loss of mud crabs during communal rearing in semi-intensive to intensive conditions is generally attributed to aggression associated mortality and cannibalism which are considered to be the main constraints on yields from culture systems (Mann and Paterson, 2003, Allan and Fielder, 2003, Quinitio *et al*., 2001, Williams and Primavera, 2001). Other crab species exhibit similar behavioural density limitation during early development, including the snow crab Chionoecetes opilio (O. Fabricius) (Sainte-Marie and Lafrance, 2002), the spider crab Mithrax spinosissimus (Lamarck) (Wilber and Wilber, 1991), the blue crab Callinectes sapidus (Rathbun) (Moksnes *et al*., 1997) and the blue swimmer crab Portunus pelagicus (Linnaeus) (Marshall *et al*., 2005). In these studies, damage to the animals such as missing limbs and puncture wounds, also resulted from agonistic interactions. Attacking behaviour and predation among small mud crabs are consistently observed in clear-water mud crab nursery systems.

Environments that provide greater habitat complexity have a higher carrying capacity for benthic, cannibalistic crustaceans such as the Australian freshwater crayfish Cherax quadricarinatus (von Martens) (Parnes and Sagi, 2002, Jones, 1995a), homarid lobsters (Aiken and Waddy, 1988) and the blue crab Callinectes sapidus (Moksnes *et al*., 1997). Even relatively simple habitat systems such as that provided by crevices between bricks significantly enhance the carrying capacity of the rearing environment for blue swimmer crabs P. pelagicus by reducing the incidence of agonistic interactions leading to cannibalism (Marshall *et al*., 2005). Similarly, inclusion of additional habitat structures into mud crab culture systems is considered a key way of achieving higher productivity levels. It has already been shown that inclusion of discrete shelters in the rearing environment of mud crab juveniles reduces the incidence of agonistic behaviours (Hutchinson, 1999) and improves survival rates in highly stocked tanks (Fielder *et al*, 1988)..

An experiment was conducted to assess the influence of stocking density and addition of artificial habitat structure on the productivity of a simple nursery system, using hatchery-produced post-larvae. It was designed to gain further insight into the dynamics within crab populations during the nursery phase and determine the yield of a pilot-scale intensive culture system. The results also provide baseline productivity estimates for application in commercial farms.

Materials and Methods

Three successive hatchery batches from different females, producing respectively 42 500, 37 000 and 22 300 megalops of normal morphology, were used to stock replicate batches of the nursery experiment in February, April and September 2003.

Nursery culture units were 1mm mesh cages (called hapa nets) suspended within an outdoor 400m3 plastic-lined pond or 25m3 concrete tanks. The hapa nets were 2m long x 1m wide x 1m deep suspended from a floating PVC frame and held to the correct form by an external, weighted PVC frame attached to the bottom corners. The artificial habitat was

constructed from woven synthetic netting (20mmx20mm) threaded onto a rigid wire frame in a pleated configuration. A single layer of this mesh structure was placed on the bottom of the hapa net to provide a complex 3D habitat zone, covering 95 per cent of the bottom surface area (Figure 57).

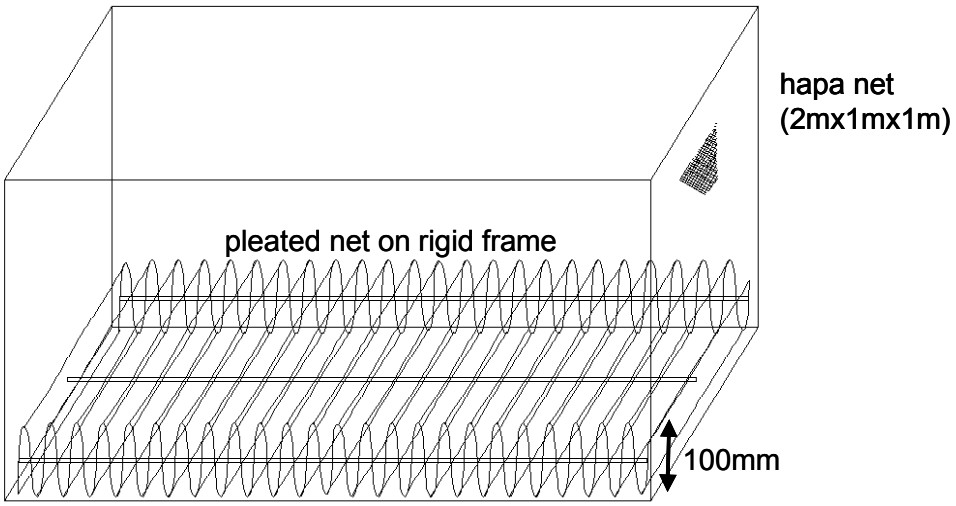

Figure 57. Diagram of hapa net with artificial habitat structure installed on the bottom.

The nursery period was separated into three phases corresponding with apparent transitional steps in development. Phases 1 to 3 terminated when mean crab instar was within the range of C1 to C2 (0.01–0.04g), C4 to C5 (0.09–0.4g) and C7 to C8 (1.0–6.0g) respectively. The duration of each phase ranged from 5 to 11 days, 19 to 21 and 16 to 22 days for phases 1, 2 and 3 respectively largely due to differences in prevailing temperature. At the end of each phase, each hapa net was harvested and all crabs counted and assigned to a size class based on body size. During early growth of crabs carapace width size frequency distributions follow discrete modes that correspond to instar groups. For the purposes of this experiment, this allowed accurate size class allocation, and therefore instar determination, by the trained eye. The carapace width and weight of a random sample of at least 20 intact crabs from each instar group were measured. These measurements confirmed the accuracy of the size classification. For phases 2 and 3, the occurrence of missing claws was also recorded. For phases 1 and 2, the harvested crabs were then reassigned to treatments in the following nursery phase. Phase 2 was stocked with C1 and C2 instar crabs and phase 3 with only C5 instar crabs.

Concrete tanks were used for the first nursery phase of batches 1 and 2 and were maintained with flow through filtered seawater at ambient conditions. All other experimental work were conducted in the lined pond where a “natural” plankton bloom was maintained, with a minimum secchi depth reading of 0.5m. Water colorant (Aquatic Blue WSP–Nuturf Pty Ltd, Australia) was added when phytoplankton bloom failure occurred. For all batches, salinity ranged from 34 to 36gL^{-1}, pH 7.8 to 8.6 and DO was greater than 80 per cent saturation. Temperature ranged between 24 to 29ºC for batches 1 and 3 and 22 and 26ºC for batch 2. Water circulation in the pond and tanks was driven by air-lifts and pumps. Approximately three times per week, the nets were lifted for observation and cleaning as necessary.

In the first nursery phase, the stocking densities across all batches ranged between 600 and 5000 m^{-2} and in batch 3, only densities approximating 700, 1400 and 2300 megalops m^{-2} were used. No additional habitat was added to the hapa nets for this phase. In the subsequent nursery phases experimental treatments consisted of duplicated combinations of

two densities with and without artificial habitat. The treatment densities were 125 and 250 m^{-2} for the second phase and 62.5 and 125 m^{-2} for the third phase. However in the third nursery phase, batch 1 and 2 treatments were limited to varying density only.

Megalops and crabs were fed slightly in excess with Kuruma prawn post-larval diet (Ebistar #4, 6 and 8; Higashimaru, Japan) three times per day. In the first phase of batches 1 and 2, sub-adult *Artemia* were given at <10 L^{-1}.

Survival rate was calculated as the proportion of crabs remaining at harvest of the number initially stocked and rate of claw loss as the proportion of harvested crabs missing one or both claws. Growth was assessed as the mean instar of the population within each hapa net as per the following formula:

mean instar = [(No. instar 1 crabs x 1) + (No. instar 2 crabs x 2) + ...]/total No. crabs

Analysis of phase 1 data was restricted to batch 3 as the very high mortality rates across all densities in batches 1 and 2 confounded any treatment effect. The analyses investigated the influence of stocking density and phase duration on survival and final mean instar. Data analysis of the second and third nursery phases investigated the influence of batch, stocking density and habitat on survival rate, mean instar at harvest and proportion of crabs missing a claw. Preliminary analyses of variance for each batch identified that their residual variances were approximately equal, so pooled (cross-trials) meta-analyses were conducted using generalized linear models (McCullagh and Nelder 1989). “Mean instar” and per cent survival were analysed with a normal distribution and identity link, and the residuals from these analyses proved to be approximately normally distributed. All count data were analysed using the Poisson distribution with a logarithm link function. The figures expressed in the results are the means and standard errors adjusted to account for unbalanced data.

Results

Nursery phase 1

The average survival rates of megalops to C1/C2 crab instars in batches 1 and 2 were uniformly low at 8.3 per cent and 7.0 per cent respectively when harvested at mean instar 1.1 to 1.2. The survival of batch 3 strongly contrasted with the two prior results, showing an average survival across all hapa nets of 80 per cent to mean instar 1.5. In batch 3, stocking density in the range of 625 to 2300 m^{-2} did not significantly influence survival ($p=0.8$) or growth rate ($p=0.18$). However, for this same batch, the survival rate was significantly lower with extended duration of the first phase from 5 to 11 days ($p<0.01$) corresponding with mean instars at harvest of 1.1 to 1.9, respectively (Table 83).

Table 83. Adjusted means and standard errors for survival and instar of post-larval *S. serrata* for batch 3 in nursery phase 1 (megalops to C1/C2) (BIARC Batch–18).

Duration	Survival* (%)		Mean instar	
days	mean	se	mean	se
5	90	0.02	1.1	0.10
7	84	0.02	1.5	0.06
11	60	0.05	1.9	0.10

* = differences at $p<0.05$ within column

Nursery phases 2 and 3

While some treatment interactions of batch and either density or habitat were marginally significant ($p<0.05$), it was the main effects of batch, habitat and density that were the dominant sources of variation, and are therefore the main focus of analyses interpretation.

Addition of net habitat significantly improved survival rate at all crab densities tested in nursery phases 2 and 3 (Table 84 and Table 85). In nursery phase 2, survival rate was significantly lower at a stocking density of 250m^{-2} than at 125 m^{-2} (Table 84). A similar trend is evident in nursery phase 3 for densities of 125 m^{-2} and 62.5 m^{-2}, however no significance was detected ($0.05<p<0.1$) (Table 85).

In phase 2 the average crab size, measured as mean instar, was significantly greater at the lower density and when no additional habitat was provided (Table 84). However in phase 3, there was no significant difference in mean instar between the two stocking densities ($p>0.1$) and between with or without habitat ($0.05<p<0.1$) (Table 85).

Table 84. Adjusted means and standard errors for survival, instar and claw loss for all three batches and stocking density and habitat treatments for post-larval *S. serrata* in nursery phase 2 (C1/C2 to C4/C5).

		Survival (%)		Mean instar		Claw loss (%)	
		mean	se	mean	se	mean	se
Batch	#1	81.0	4.7	4.3*	0.07	6.1*	0.6
	#2	63.7	4.6	5.7	0.12	6.0	0.9
	#3	65.8	4.2	3.5	0.15	26.5	1.8
Stocking Density	125/m^2	78.5*	3.9	4.6*	0.03	11.0*	1.0
	250/m^2	62.9	3.5	4.4	0.02	13.5	0.8
Artificial Habitat	With	82.8*	3.5	4.4*	0.02	11.1*	0.7
	Without	54.6	3.8	4.6	0.03	15.9	1.1

*= differences at $p<0.05$ within each variable group

Table 85. Adjusted means and standard errors for survival, instar and claw loss for all three batches and stocking density and habitat treatments for post-larval *S. serrata* in nursery phase 3 (C5 to C7/C8) (BIARC Batch–18).

		Survival (%)		Mean instar		Claw loss (%)	
		mean	se	mean	se	mean	se
Batch No.	#1	75.9*	4.4	5.9*	0.08	19.4*	2.3
	#2	63.4	3.9	6.9	0.06	35.1	3.0
	#3	55.1	3.0	7.7	0.05	36.2	2.9
Stocking Density	62.5/m2	63.9	3.4	7.5	0.05	28.8*	2.8
	125/m2	56.8	2.9	7.2	0.05	2.3	1.8
Artificial Habitat	With	64.1*	2.6	7.3	0.04	31.4*	1.7
	Without	36.6	3.9	7.5	0.06	35.5	3.9

* = differences at $p<0.05$ within each variable group

Among the three batches tested, there were no differences in the average weight of crabs within each instar in phases 2 or 3, indicating that moult increment (that is the proportional increase at each moult) remained similar across batches.

In both phases 2 and 3, the rate of claw loss was highest at the higher stocking densities of 250m^{-2} and 125 m^{-2} respectively for phases 2 and 3 (Table 84 and Table 85). In both phases, there was also a significantly higher proportion of crabs missing claws when no artificial habitat was provided (Table 84 and Table 85).

In phases 2 and 3, stocking density and habitat treatments did not significantly interact for survival, growth or claw loss ($p>0.1$), indicating the treatments were acting independently of each other.

There was a difference in the frequency distribution of crab instar among the three batches in both nursery phases 2 and 3 ($p<0.01$). In phase 2, the distribution of crab instars was not significantly influenced by habitat or density. In phase 3 only the habitat treatment significantly influenced the final distribution of crabs among instars ($p<0.01$)

The rate of claw loss was strongly influenced by both density and habitat treatments and batch for both phase 2 ($p<0.01$) and 3 ($p<0.05$). Additionally, the smaller instars had a significantly higher rate of claw loss (Table 84).

From stocking at megalops, harvests of C1/C2, C4/C5 and C7/C8 crabs (mean weight ~0.01g, 0.2g and 3.5g respectively) were produced in 7, 26 and 42 days, respectively. Productivity, in terms of final crab density, dramatically dropped with increasing harvest size. The best performing treatments in phases 1, 2 and 3 attained harvest densities (crabs/m^{-2}) and per cent survival rates of 1024 (81.2%), 203 (81.0%) and 70 (56.2%), respectively.

Table 86. Percentage of harvested post-larval mud crabs missing either one or both claws for each instar across all three batches and treatments for nursery phases 2 and 3. Figures are adjusted means and standard errors (in italics).

Phase 2: Instar* (Mean ± SE)				
2	3	4	5	6
23.3±8.4	19.1 ± 2.2	15.9 ± 1.3	8.6 ± 0.6	5.0 ± 1.6
Phase 3: Mean Instar*				
5	6	7	8	9
40.1±4.8	39.6 ± 3.9	31.0 ± 2.8	26.3 ± 2.5	25.6 ± 6.3

* = differences at $p<0.05$ across instars

Discussion

Productivity during the first phase of the nursery cycle is influenced to a far greater extent by quality of megalops than density dependent factors, such as cannibalism, up to a density of at least 2000 crablets m^{-2}. The poor result through metamorphosis in batches 1 and 2 and the far superior result of batch 3 correlates positively with the observed vigour and feeding behaviour of the megalops although no numeric rating or assessment criteria was applied. The absence of a similar pattern of mortality among the three batches in nursery phases 2 and 3 indicate that survival rate of the megalops to C1 is not related to the subsequent performance of the batch. Seemingly the crabs that are unfit for continued development are eliminated at this critical point.

In the transition through nursery phases 1,2 and 3 there is a dramatic reduction in the stocking density that yields acceptable survival levels of greater than 80 per cent. This strong trend indicates that post-larval crab populations become increasingly density-sensitive through the short period of early development and this is apparently exhibited as lethal interactions among the population. Similar responses to density have been found in other crab species. Examination of mortality within a captive population of M. spinosissimus sibling juveniles revealed that aggression related mortality accounted for at least 60 per cent of all deaths (Wilber and Wilber, 1991). Additionally a large proportion of these attacks were on recently-moulted soft crabs. In small, closely monitored populations of juvenile P. pelagicus mortality was almost entirely due to cannibalism and the main risk factors for the victim were being post-moult and small size (Marshall *et al*., 2005). Observations made during the course of the present experiment are consistent with cannibalism of both hard shell and soft shell crabs being a dominant cause of mortality. However, it was not possible to quantify the data. It was apparent that damaged or dead crabs were rapidly consumed, as intact crab carcasses were not found in the nets during observations or harvests.

Inclusion of a three-dimensional habitat structure in the rearing environment of cannibalistic crustaceans to reduce losses caused by aggressive encounters is well established. It is used for freshwater crayfish pond production (Naranjo-Pa´ramo *et al*., 2004, Jones and Ruscoe, 2000) and crab growout (Trino *et al*., 1999) and has been applied to development of homarid lobster aquaculture (Carlberg *et al*., 1979). Short lengths of pipe, bundles of mesh, blocks, corrugated plastic sheets and benthic algae are examples of structures used to increase the complexity of the benthic habitat zone and provide what various authors describe as either

refuge, shelter or habitat. This experiment demonstrated that the use of a simple pleated net structure results in significant reduction of cannibalism from the very first crab instar. The net structure, as applied in these experiments, was designed to be simple, durable and easy to manage, with the expectation that similar structures may be readily applied at a commercial level. The significant benefit to productivity gained by using the structure should mean that intensive crab nurseries employ the practise even if only growing crabs for a short period through the early instars. It should be noted that these experiments used an artificial habitat structure that was confined only to the bottom portion of the culture container. Under typical rearing conditions in earthen ponds, tanks or mesh cages, it is apparent that the majority of the crab population remain on or close to the bottom, even when there are suitable surfaces for climbing vertically (Mann *et al*, unpublished data). For this reason, the habitat tested in this experiment was installed on the net bottom and did not extend far into the water column. However, it may be possible to design rearing and habitat systems that make effective use of the water column by attracting the normally benthic oriented crabs vertically into raised habitat Vertical structure may, however, impede water movement, cleaning and management that need to be considered when designing the system.

Claw loss is considered a measure of the degree of agonistic interaction among conspecifics and has been used as an indicator in population studies of other crustacean species (Linnane *et al*., 2000). Claws are the limbs most frequently lost in aggressive interactions (Juanes and Smith, 1995). In this experiment, incidence of claw loss had an inverse relationship with survival, and was higher at high density and lower with addition of habitat. This is indicative of the strong link between incidence of agonistic encounters and mortality rate. Moreover, claw loss has a functional and physiological cost and also renders the individual more vulnerable to attack (Juanes and Smith, 1995). For juvenile P. pelagicus, loss of one or both claws reduces the body weight of subsequent instars (Paterson *et al*, this issue).

Crab growth, as measured by the average instar attained during each phase, was significantly influenced by density and habitat in phase 2 and a similar trend was evident in phase 3,for habitat. Growth of other decapod crustaceans is considered a density dependent characteristic, including the crab C. opilio (Sainte-Marie and Lafrance, 2002), the freshwater crayfish C. quadricarinatus (Jones, 1995b, Naranjo-Pa´ramo *et al*., 2004), the lobster H. americanus (Aiken and Waddy, 1988) and the crab P. pelagicus (de Lestang *et al*, 2003). In this experiment the modal crab size attained within both density treatments was similar. However at higher densities, a larger proportion of crabs were in the smaller size class, lowering the average size. In the habitat comparisons there is a lower proportion of the smallest instar crabs in the “without habitat” treatment, raising the overall average size. As survival is also lower without habitat it may be interpreted that the smallest size group, in particular, gained some level of protection from predation from the artificial habitat. In small experimentally structured populations of P. pelagicus sibling juveniles with equal proportions of small and large size class crabs, the smaller size class consistently suffered significantly higher mortality (Marshall *et al*., 2005). It was determined that large predatory crabs preferentially attacked smaller individuals. Similarly, in a study of cannibalism within post-larval C. sapidus populations of mixed cohorts, the smallest cohort was heavily preyed upon by larger crabs even within complex habitats (Moksnes *et al*., 1997). Size disparity within a population is therefore a significant factor influencing survival rates.

The five instars present at harvest represent a many fold size range of approximately 0.02 to 1.0g for phase 2 and 0.4 to 7.5g for phase 3. There appears to be a large proportion of the population (at least 20%) that are growing significantly slower than the modal group.

A large size range was also reported for sibling mud crab post larvae grown under similar conditions in the Philippines (Quinitio *et al*., 2001). This characteristic is likely a significant factor contributing to high rates of cannibalism once the crabs grow beyond the C3 and C4 instars and there is some evidence that size grading may improve survival rates for C10 instar mud crabs (Quinitio *et al*., 2001). However in this experiment, a greater than four-fold weight disparity was established within days in phases 2 and 3, despite only two instars being stocked.

Rapid development of a wide size range within a population of mud crab post-larvae is exacerbated by the large growth increments characteristic of the species. A single moult increment is typically greater than 100 per cent in terms of weight, which means that with only two instars present there can be a 2-fold size difference within the population. Social interaction within the population has also been shown to contribute to development of a wide size range in communally reared juvenile H. americanus (Aiken and Waddy, 1988). It has not yet been demonstrated whether the smallest size group of crabs continues to exhibit low growth rates throughout the subsequent growout phase. However, for H. americanus lobsters, the lower size class exhibits more normal growth following separation from larger conspecifics (Aiken and Waddy, 1988).

A critical consideration for commercial scale crab nursery culture is that, under relatively intensive culture conditions such as those used in this experiment, harvest size is one of the main predictors of survival rate and harvest density. As growout systems are usually stocked at low densities, there is an advantage in transferring crabs from the nursery to the growout system as soon as possible. Crabs as small as C3 and C4 (0.025 to 0.1g) appear sufficiently robust to tolerate transport without immersion in water and stocking into typical brackish water earthen growout ponds. It is also feasible to harvest at the C1 or C2 instar, soon after the critical metamorphosis to the crab stage. However, harvest and handling of the very small, fragile crabs were more difficult than older and larger stocks.

The information generated by this experiment can be used to formulate a production and economic model that optimises an integrated commercial production regimen for mud crabs, considering the three main production steps, hatchery, nursery and growout.

Investigation of mud crab nursery culture systems at BIARC.

In addition to the primary investigations on nursery diets and density vs habitat interactions further work collected information on other nursery system design aspects. Post larval crabs were grown in a variety of different systems:

1. outdoor concrete tanks and indoor fibreglass tanks
2. with and without light transmitting and shading covers
3. with and without assorted solid and flexible habitat structures
4. with and without green water

The aim was to assess the operation of different culture units, including hapa nets, tanks and small ponds and investigate various parameters affecting productivity, particularly stocking density and provision of habitat structures.

It proved difficult to quantify the performance of the systems and individual variables for valid comparisons. Problems included high “within treatment” variability in crab survival, inability to match environmental conditions among treatments, difficulties in stabilising blooms in green water tanks and irregular high post-larval mortality (likely due to variable larval quality in the hatchery).

Future nursery system development

The following outlines a summary of approaches to nursery culture of mud crabs that can be used as a guide to future development and commercial operation. The outline was developed in reference to recent project results and liaison with operators. It presents three different production models which a crab industry may adopt for the post hatchery component of the production cycle. A further discussion of the aspects presented can also be found in Mann and Paterson 2004.

Model 1. Low intensity pond nursery continuous with growout

Megalops are harvested from the hatchery and stocked directly into the growout pond and cultured through the growout cycle so that there is no discrete nursery phase. The stocking rate is relatively low at around 10 to 30 per m^2 as the greatest limiting factor to the density at harvest is cannibalism, particularly in the later stages. This method has been used successfully at BIARC for blue-swimmer crab production but is usually not the desired option for mud crab pond farmers. The megalops stage is the equivalent of a first post-larval stage and still needs to undergo a second metamorphosis to the first crab instar. A high variability has been observed in the rate of megalopa transition to crablet for mud crabs. For the pond farmer it can be up to a month before the outcome of a stocking with megalopa can be assessed as the early stages are extremely difficult to sample from the pond and meaningful estimates of total population determined. Another draw-back is that production pond area is occupied for a longer period than if crablets are stocked at a later stage. In an analogous situation, prawn farmers prefer to stock at a minimum stage of PL15, typically necessitating a short nursery phase.

Model 2. Semi-intensive pond nursery

Megalops are harvested from the hatchery and stocked into small (<0.5ha) nursery ponds and grown to a large crablet size (~5g) or into early juvenile stages up to 20g. The nursery duration is six to 10 weeks and to ensure continuity of production a number of ponds would be used. The ponds need to be modified to include a high level of habitat. The hapa net experiment at BIARC indicates that in excess of 40 crabs m^{-2} is achievable at a crab size of approximately 5g.

The advantage of this model is that it is that the early juvenile sizes may be appropriate for stocking directly into the intensive, compartmentalised growout systems that are currently under development.

Model 3. Short intensive nursery

Megalopa are harvested from the hatchery and stocked into intensively managed culture units and cultured through to the earliest appropriate stage for transport and stocking into culture systems such as earthen ponds. Crab instar 3 (C3, 8mm) or instar 4 (C4, 10mm, 0.1g) are considered to be the most appropriate for this purpose. The advantage of this approach is that the hatchery operator would be able to produce large numbers of crablets utilising relatively small volume tanks, raceways or mesh cages (hapa nets) at a crab stage that would be acceptable to pond farmers to stock into ponds. In these culture systems the nursery is more controlled (potentially heated to allow early season production). The cycle is short, 3 to 4 weeks, and it is anticipated that relatively high densities of crabs can be economically produced as cannibalism is less intensive up to C4 than in later stages (see density vs habitat report). A C4 crab has a tough carapace and can remain viable in air for long periods, allowing ease of harvest and packaging and long distance transport. Another advantage is that nursery culture units can be tailored specifically to the early crab stages up to C4 which have a different habit to later stages.

This nursery model is analogous to that commonly practised by prawn hatcheries which have a short nursery phase in large outdoor tanks following harvest at early PL stage from hatchery tanks.

It is considered that model 3, short intensive nursery systems, is initially the most appropriate for adoption by pond farmers for the reasons outlined above. Farmers will prefer the largest possible crabs for stocking ponds due to a shorter growout period however it is anticipated that there will be a big jump in the cost of crabs for sizes over C4. Culture of larger sized crabs requires a far lower density of production and therefore more facilities and production area are utilised. It has already been shown that this model can be effectively used by prawn hatchery operators as demonstrated by the Tropical Mariculture prawn hatchery in mud crab production trials.

Cellular crab growout systems where the crabs are maintained individually through to market size, such as that being developed by the Bowen Aquaculture Centre, are expected to require a more advanced crab for stocking. In this instance model 2, with an extended nursery phase, will be required. The nursery system used is essentially similar to that used for short nursery culture however the larger crabs will require a different habitat structure to maximise productivity. Greater pond area will also be needed to supply the crab numbers due to a lower harvest density.

Benefits and Adoption

The commercialisation of larviculture and nursery systems for the mud crab will be valuable for existing and future, land based farmers with access to marine water, providing a new species for diversification and also to coastal aboriginal communities interested in developing aquaculture compatible with traditional skills and knowledge.

At the beginning of this project survival rates for the production of megalops and crablets was low and unreliable at a large-scale. The new systems designed in this project have increased average survival rates for large-scale production and are significantly more reliable.

The systems and technology developed in this project have been adapted successfully to two other crab species, the blue swimmer crab (Portunus pelagicus) and the three-spot crab (P. sanguinolentus), demonstrating the impact of this investment by the Fisheries Research and Development Corporation together with that of the governments of the Northern Territory and Queensland.

The main beneficiaries of this work will be the aquaculture industries of the Northern Territory and Queensland, together with aboriginal communities across northern Australia. In northern New South Wales and across northern Western Australia, where mud crabs are found, the technology from this project may also be of value in supporting industry development.

In the Northern Territory (NT), following the success of this project the government has scaled up its hatchery at the DAC to be able to produce batches of 100 000 crablets at a time to support initial industry development. In addition the NT Government is supporting a trial (hopefully the first of many) farming crabs in mangrove enclosures with the indigenous community at Maningrida. The NT Government, in partnership with the Gwalwa Daraniki aboriginal community, utilising funds from the Commonwealth and NT, is also undertaking pilot development of crab farming in ponds at an ex-prawn farm in Darwin.

In Queensland, partnership arrangements between the crab team at BIARC and the first industrial scale crab farm being constructed in Bowen are in progress, as are similar arrangements with the first soft-shell crab farm in Brisbane.

At a meeting in October 2004 a workshop was held in Brisbane between industry pioneers and government agencies to consider how best to develop crab aquaculture in Australia, to build on the success of this project. The proceedings of that workshop were recently published (Shelley, 2005).

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