Husbandry of the Blue Swimmer Crab in Aquaculture

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1998/333 Husbandry of the Blue Swimmer Crab in Aquaculture

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NON TECHNICAL SUMMARY:

OUTCOMES ACHIEVED

This project developed our understanding of the basic hatchery and early nursery rearing requirements of the Blue Swimmer Crab (*Portunus pelagicus*) in South Australia. The resultant hatchery manual also provided summary information on the hatchery and rearing operational practices required for successful rearing of the species. The work contributed to the assessment of the aquaculture potential of the species in South Australia, and indirectly assisted work in other states in their development of hatchery techniques for this and other species.

This project aimed to extend preliminary pilot scale results which suggested that juvenile blue swimmer crabs could be produced from hatcheries and that growth rates suggested commercial production may be possible.

Specifically the objectives were:

1. To define profit maximising strategies for aquaculture of the blue swimmer crab under commercial conditions.
2. To define feeding strategies for optimum growth and survival of blue swimmer crabs under aquaculture conditions.
3. To determine health impacts on blue swimmer crabs under intensive holding conditions of stocking density, water temperature and feeding strategies.

An agreement was reach with a commercial hatchery in South Australia and the project commenced operations in June 1998. Initial production of crab larvae was limited, probably due to stress on the female crabs collected from the wild and the timing being outside the normal reproductive phase of blue crabs.

Subsequent collection of berried female blue crabs from near the hatchery site produced more success and batches of blue swimmer crabs were consistently hatched between September 1998 and March 1999. All batches were fed live feed, which were also raised in the hatchery, and survival rates through the larval phases were as high as 80%. Development was impacted by temperature with best results at 27°C.

Unfortunately, there was very limited success in producing juvenile crabs with high mortality levels between megalopa and settlement in all batches. Small numbers of juvenile crabs were used for small scale grow-out work and results suggested that growth to an average 10 grams was achievable in around 100 days post hatching with high survival post settlement.

The project was halted in April 1999 due to an inability to produce commercial quantities of juvenile crabs. Subsequent discussions were held with the South Australian Research and Development Institute and the Bribie Island Aquaculture Research Centre, in an attempt to progress the project. Specifically, support was sought for addition of a further objective to the project, being to address hatchery production of juveniles. Unfortunately no progress had been made on this issue and so in February 2002 the project was cancelled.

KEYWORDS: **Blue Swimmer Crabs, Portunus, Aquaculture, Hatchery**

ACKNOWLEDGEMENTS

The author would like to thank Spencer Gulf Aquaculture for their assistance in providing access to hatchery facilities at their Port Augusta hatchery and in particular to Steven Schotten for his willingness to advise on technical issues on site.

The Yorke Regional Development Board provided access to their aquaculture incubator site at Wallaroo and assisted in some of the logistical issues during the early phases of the project.

The project was greatly assisted by members of the South Australian Blue Crab Pot Fishers Association who provided access to their vessels and fishing operations for the collection of berried female crabs during the project.

The author would also like to thank Dr Colin Paterson from Bribie Island Aquaculture Research Centre (BIARC) and Dr Steven Clarke from South Australian Research and Development Institute for their assistance in investigating alternative sites for imlementation of the later phases of the program. Special thanks go to Dr Clive Keenan of BIARC for his assistance and technical advice during the project.

BACKGROUND

During the late 1990's there was considerable interest expressed in the farming of crabs following success of the South-East Asian industry. While most interest was focussed on the tropical mud crab, due to its high market value, there had been little commercial success with the species at the time due to difficulties with hatchery production, cannibalism and relatively slow growth rates. Given this situation there appeared to be considerable potential for production of other, faster growing crab species within their natural distribution.

During 1996 and 1997, the Yorke Regional Development Board, in conjunction with Ocean Gold Investments and with financial assistance of the Department of Education, Training and Youth Affairs, undertook pilot scale trials of blue swimmer crab growout and hatchery production.

Trials were carried out at laboratory facilities at the Yorke Regional Development Board Aquaculture Centre and the old Port Broughton Prawn Farm site. Berried female blue crabs were collected from wild populations and allowed to release juveniles under controlled conditions in small (40 litre) containers.

Larval stages were maintained in the tanks with live feeding of rotifers and artemia from small culture populations. Growth rates were monitored during limited culture periods up to settlement of megalopa.

Growth and survival in these small cultures were extrapolated to suggest that time to commercial harvest (for 10cm soft shell animals) was from 4 to 6 months, that survival was greater than 80% and that viable stocking densities were achievable.

Funds were sourced through the Delicatessen to Asia program to investigate the market potential for the species and the range of product options in south-east Asia. The grant allowed further funding support for market development of the species following successful commercial scale growout.

Commercialisation of the program was dependent upon further definition of the nutritional requirements of the species and basic husbandry practices such as stocking density and water quality parameters. This project aimed to provide research support for the project to progress from the original pilot scale operations to commercialisation, focussing on investigating the husbandry practices to optimise productivity.

NEED

Pilot trials under laboratory conditions have shown that blue swimmer crabs can be successfully cultured from egg to saleable product. Investigation and refinement of husbandry techniques are required, under conditions of commercial scale production, to remove uncertainty impeding development of an industry.

Research undertaken to date lays the foundation for the development of a successful new industry. The expansion of the industry requires the development of a sound scientific basis to husbandry techniques and ongoing research support. The key impediments identified are in the areas of nutrition, health and temperature - stocking rate interaction.

OBJECTIVES

1. To define profit maximising strategies for aquaculture of the blue swimmer crab under commercial conditions.

2. To define feeding strategies for optimum growth and survival of blue swimmer crabs under aquaculture conditions.

3. To determine health impacts on blue swimmer crabs under intensive holding conditions of stocking density, water temperature and feeding strategies.

During the course of the project at fourth objective was added being:

4. To identify optimum hatchery techniques and conditions for production of commercial quantities of juvenile blue swimmer crabs for stocking into aquaculture ponds.

Addition of this objective reflected the project's difficulties in replicating the reported success of the pilot trials.

Funding for this objective was sought from sources other than FRDC.

METHODS

Literature Review

A comprehensive literature review of crab aquaculture, blue crab life cycle and crab hatchery information was undertaken to provide an information base for future work. A copy of the review is attached as Appendix 3.

Steering Committee

A Steering Committee for the project was finalised in July 1998 with Dr Gary Morgan (then Director of Fisheries, Primary Industries and Resources South Australia), and Dr Clive Keenan (Chief Scientist, crab aquaculture program, BIARC) agreeing to join Leo Haarsma (Chairman, Ocean Gold Investments) on the committee.

Hatchery Production

The immediate goal was to produce in the order of 100 000 blue crab juveniles within 10 weeks (from 29 June 1998) at the hatchery to resource the commercial scale grow-out trials.

It became clear on the commencement of the project that the Yorke Regional Development aquaculture site at Wallaroo would not be suitable for the project due to limitations with the grow-out facilities. The project required replicate trials of commercial quantities of crabs to assess various nutritional and environmental parameters. The site had a single grow-out pond available. Additionally the hatchery site was limited in its suitability due to poor water quality and availability.

A hatchery facility was established at the only commercial hatchery in South Australia at the time, the Spencer Gulf Aquaculture facility at Port Augusta. A dedicated room was fitted out with a controlled temperature water supply and controlled light/dark regimes.

Hatchery operations commenced in July 1998 with collection of the first berried female blue crabs from Gulf St Vincent South Australia. The hatchery commenced continual production of live feed in volumes adequate to deal with a blue crab spawning event.

Hatchery techniques and operations were documented in a Hatchery Manual. A copy of this is provided as Appendix 4.

Several trials were carried out on the growth and survival of crabs from hatch to settlement.

Grow-out and Commercial Scale Trials

The lack of success in producing commercial quantities of juvenile blue crabs resulted in no commercial scale trials being carried out.

Small scale stocking density trials were carried out in wooden raceways (2.5m x 1m x 0.5m) and 10000 litre swimming pools.

Stocking densities used ranged from $7/m^2$ to $100/m^2$.

All juveniles in grow-out trials were fed daily on an artificial diet (Black Tiger prawn pellet 40% Protein). Sand substrate was used in all containers.

RESULTS / DISCUSSION

Hatchery Site

Several problems arose with the hatchery site which impacted on the hatchery production.

1. The water supply comes from the top of the Spencer Gulf. It is not mixed well as proven by the salinity of 44 - 48 parts per thousand all year round. The water is believed to exchange once every 11 years and is likely to be polluted with wastes associated with nearby industrial activity. There is a dodge tide once a fortnight, which is also reducing mixing. Water quality problems were associated with the poor survival of juvenile crabs.

2. During the summer months, with the increase in water temperature, the nutrient levels also rise. This is evident by frequent algae blooms in the poorly mixed inlet and outlet channels. This is also may be the cause for the dramatic increase in bacterial load, again associated with poor survival.

3. The water supply was not filtered adequately. 5um may be adequate for the growout of crabs but not for the larval rearing of this species. Ultra violet or Ozone sterilization for the entire water supply and 0.2um filtration for at least the algae appears necessary.

Hatchery Production

Females collected early in the project dropped their eggs within days of reaching the hatchery or within days of projected spawning. Early loss of eggs was attributed to stress associated with transport, handling and environmental (particularly temperature and salinity) changes. Effort has been put into addressing these issues with a variety of protocols being implemented. Minimising these stressors appears to be a priority.

Loss of eggs prior to spawning has been attributed to the time of year and the probable non-viability of eggs developing at this time. As the water temperature increased, egg production became more successful.

Collection of berried females from the local environment, with similar temperature and salinity conditions and less transport and handling, and at a time of highest spawning in the wild, provided best results.

Growth of larval stage crabs averaged 8 grams (2 - 14 grams) over the 94 day period from hatching to megalopa and Crab 1 stage (Figure 1). Temperature played a major role in this development with variations between the periods spent at each stage (Figure 2). At 27°C, development from zoeal stage 1 to megalopa was approximately 40 days while at 24°C it was 53 days.

The hatchery achieved up to 80% survival to the magalopa stage although there was considerable variability between batches (Figure 3). Settlement of Crab 1 stage juveniles was consistently poor and limited work on husbandry of juveniles.

The settlement of crab larvae in large numbers was the limiting step in the process and was where the results of this project differed markedly from those of the pilot project. It appeared that the water and feed quality were key factors in the success of this step. The hatchery site used for the project was using seawater taken from the outlet of the Port Augusta Power Station. The water was only sand filtered with no UV or other sterilisation processes.

It appears likely that a combination of poor water quality at this stage, high predation rates and possibly poor feed quality resulted in the reduced survival compared to the small scale pilot trials.

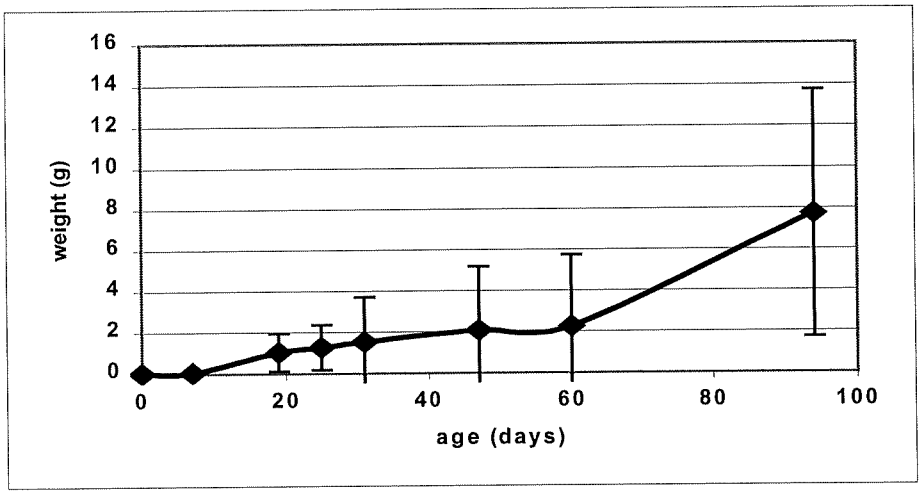

Figure 1. Growth of juvenile crabs over a 94 day period from hatch.

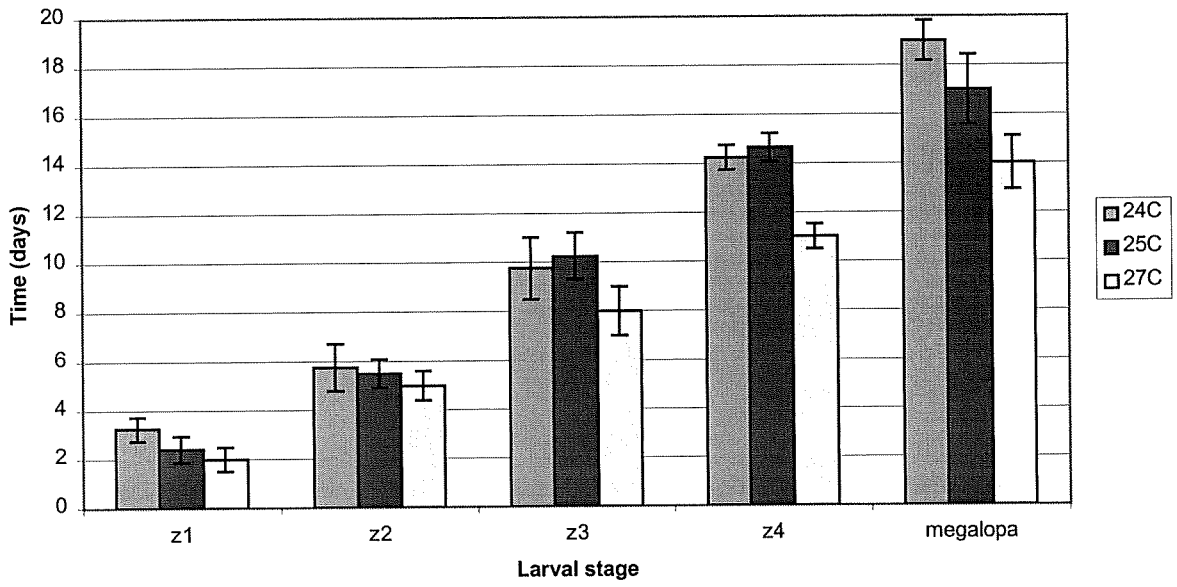

Figure 2. Growth of crab larvae at different temperatures indicating duration of the larval rearing period spent in each zoeal phase at different temperatures.

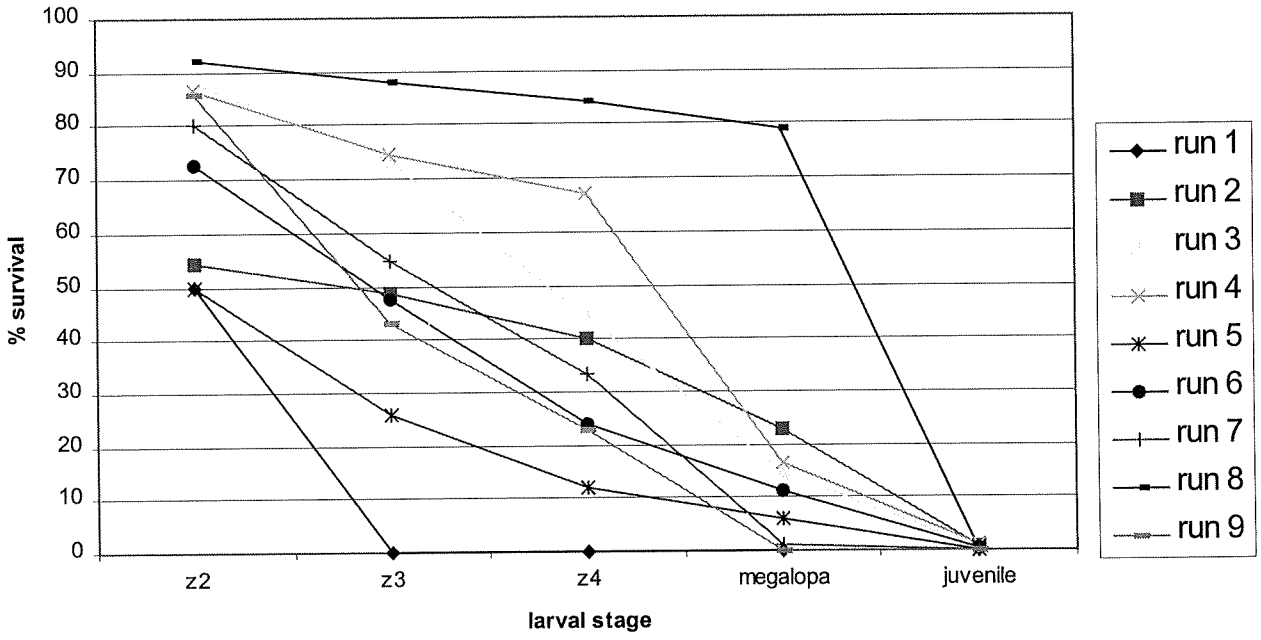

Figure 3. Larval survival at different developmental stages

GROW-OUT

Results from basic stocking density trials (Table 1) showed that the minimum stocking density for juvenile crabs up to day 90 was 25/m^2. This experiment was performed with three replicates per sample in raceways. Each raceway was split into 3 sections each of approximate area 1.2m^2.

Juvenile crabs at this stage became very cannibalistic, particularly during their first 2-3 weeks of growth. It was during this period that they moulted frequently and were therefore highly susceptible to predation.

Table 1. Survival of Crab 1 stage juveniles stocked at different densities over 90 day periods.

stocking density (#/m^2)	%survival
7	100%
10	100%
25	100%
50	56.80%
100	23.40%

Various shelter types and mechanisms were provided to the crabs at this stage, including onion bags and mussel rope, and results appear to reflect the ability of small crabs to avoid predation.

BENEFITS

Benefits flowing from the project are limited by the lack of success in meeting the objectives. The information gained from the project in terms of hatchery production would benefit future hatchery operators and may assist development of an aquaculture industry for the species.

FURTHER DEVELOPMENT

This project was severely limited by the inability to produce commercial quantities of juvenile crabs for grow-out trials. The project assumed that hatchery techniques identified in the pilot program (the precursor to this project) were replicable on a larger scale. This proved not to be the case.

Development based on the results of this project should focus on extrapolating the hatchery results from this work to fine-tune the production of juvenile crabs. The stated objectives of this project could then be addressed.

PLANNED OUTCOMES

The primary outputs from the project were a comprehensive literature review and a hatchery manual for the production of blue swimmer crabs. Secondary outputs were the preliminary results of husbandry requirements of juvenile crabs.

The planned outcomes from the project were commercial production of blue swimmer crabs in South Australia and a higher level of understanding of production and husbandry requirements of blue swimmer crabs.

The outputs provided detailed information to support commercial hatchery production of blue swimmer crabs and preliminary information on husbandry techniques. No commercial scale production has commenced although more commercially-based trials have been carried out in Queensland through BIARC.

The lack of production of commercial quantities of crabs for grow-out limited the opportunity to meet the planned outcomes.

CONCLUSION

The project was unable to meet any of its three objectives due to an inability to replicate pilot trial production of juvenile crabs on a commercial scale. The limited information available from the work undertaken suggests that high survival rates can be achieved of larval crabs under hatchery conditions. These should provide the basis for work to successfully settle larvae to the juvenile stage.

Further work is primarily required at the settlement stage to increase the survival rates. This appears to be the main limiting step in the process and was the main point of difference between this project and the pilot trials.

Growth rates from the very small numbers of juvenile crabs produced indicate that commercial production may be viable if commercial quantities can be produced.

APPENDIX 1: Intellectual Property

The only intellectual property arising from this project rests in the hatchery manual. This not considered to have any market value given subsequent work being carried out by BIARC in this area.

APPENDIX 2: Staff

The following staff were associated with this project:

Martin Smallridge	Project Manager
Paul De Ionno	Hatchery Manager
Paul Lange	Hatchery Technician

APPENDIX 3: Literature Review

Nutrition, Stocking and Health of Portunid Crabs. A Literature Review.

Paul De Ionno

Introduction

The commercial culture of portunid crabs are rapidly developing into one of the leaders in the international seafood industry. This is mainly due to the established multi-million dollar soft shell crab industry in America along with the expanding aquaculture industries in South-east Asia, the Sub-continent and Australia.

Most studies cited in this review are based around portunid crab species with an established commercial industry or those species with aquaculture potential. These species include *Callinectes sapidus*, the species farmed for the soft shell industry in America; *Scylla serrata*, (more commonly termed the mud or mangrove crab); *Portunus pelagicus*, (also termed the sand or blue swimmer crab); *Portunus trituberculatus and Carcinus maenas.*

This literature review is based around relevant nutritional, stocking and health information in relation to portunid crabs.

Nutrition

Larval Nutrition

Crab larvae are planktonic and rely on other planktonic organisms to feed on before settling and undergoing metamorphosis to the first juvenile instar, (Harms and Seeger, 1989). Current research is still revolved around optimising a larval feeding regime so as to maximise development rates and survival through the 4-6 zoeal stages, (depending on the species), the megalopa stage, and finally, to the crab 1 stage.

Freshly hatched crab larvae have been proven to feed on larger phytoplankton species (i.e. diatoms), however, it has prolonged development due to it's small cell size and larvae have not made it past the zoea 2 stage on this food source alone (Harms and Seeger, 1989).

Zooplankton, including rotifers (*Branchionus spp.),* and *Artemia* nauplii are commonly known to be a suitable food supply for a variety of decapod larvae. Crab larvae of the portunid, *Necora puber* fed entirely on a rotifer diet have not survived metamorphosis to the megalopa stage, (Alvarez-Ossorio et al, 1990). Different species of brachyuran crab larvae fed entirely on *Artemia* nauplii, have allowed complete development to the first crab stage, but survival has generally been low (Harms and Seeger, 1989). This may be due to varying nutritional requirements and malnutrition in earlier zoeal stages. It may also be due to the vast size difference between the rotifer and the later stage zoea. In contrast, Alvarez-Ossorio et al (1990), discovered that an *Artemia* diet alone not only allowed complete development of *Necora puber* to juvenile, but also increased survival rates and decreased development times.

Recently, it has become widespread knowledge that a combination of highly nutritious rotifers and *Artemia* play a very important role in the development of most species of crab larvae (Kannupandi et al., 1993, Marichamy, 1996, O'Sullivan, 1996, Otani et al., 1996, Deering, 1997). Zainoddin (1992), discovered that survival was

increased using a rotifer/frozen *Artemia* nauplii diet, rather than using live *Artemia* nauplii alone. Nowadays, most commercial hatcheries boost the Highly Unsaturated Fatty Acid (HUFA) levels of the live feeds and this has been proven to be a success for crab larval culture (O'Sullivan, 1996).

Broodstock Nutrition

There is limited information on broodstock nutrition in portunid crabs. *Portunus trituberculatus* farmers in Japan claim that the ideal food source for broodstock is live clams, due to it's high nutritional value and ability not to foul water quality (Cowan, 1986). Mud crab farmers in the Philippines and Taiwan prefer to use live brackish water snails, but if not available, will settle for trash fish or abbatoir meats (Cowan, 1986).

Artificial Diets

Studies to establish an optimal artificial diet for the decapod crabs under culture conditions begun as early as 1977. Adelung and Ponat (1977), showed that growth rates of *Carcinus maenas* were at a maximum using artificial diets with protein levels of up to 60%. However, it was also proven that crabs fed fresh mussels of identical protein levels, exhibited faster growth rates (Adelung and Ponat, 1977).

Studies on the mud crab (*Scylla serrata*), showed that satisfactory growth rates have been achieved with compounded diets having protein levels between 35-40% (Chin et al, 1991). However, crabs fed fresh clam (*Meretrix casta),* had better food conversion efficiency than those fed artificial diets (Chin et al, 1991).

To this day, the best composition of a diet for crab growout is still to be determined. Hence, because of the lack of research in this field, some crab farmers rely on commercial prawn diet as an artificial food source (Otani et al., 1996). However, due to the current worldwide developments of crab aquaculture, a high protein micro pellet feed has recently been introduced in Japan and Malaysia (Marichamy, 1996).

Stocking rates

The maximum reported stocking density for adult crabs in the wild is 81 crabs/ha (Hill, 1975, as per Cowan, 1986). Hence to develop an economically feasible industry, the stocking density must be increased considerably (>100 times), for growout of crabs to a marketable size.

Pond growout

The pond culture of crabs to a marketable size is the simplest and cheapest way of farming these animals. Growth and survival in these ponds are linked strongly to stocking rate. Hence there has been considerable research in this field relating to the maximum at which these ponds can be stocked.

In monoculture systems it has been found that the maximum stocking density for adult mud crabs is between 4-7/m^2 (Cowan, 1986, Luo and Wei, 1986), and 10-15/m^2 for juveniles (Luo and Wei, 1986). However, recently it has been found that a stocking rate of between 0.5-1.5/m^2 gives the highest survival, growth and economic benefit (Mann and Keenan, 1998). Interestingly, data from 14 polyculture farms (farming crabs together with prawns, milkfish and *Gracilaria*), in Taiwan showed that there was only a 40% survival of crabs when stocked at 0.5-1.0/m^2 (Cowan, 1986).

Other studies have shown that experimental and commercial pond cultures of mud crabs from different countries have produced crabs successfully at a stocking range between 3,000-10,000/ha (Agbayani et al., 1990, Samonte and Agbayani, 1992, Samarasinghe et al., 1991, Marichamy, 1996)

In terms of weight, reports have shown that pond culture of *Scylla serrata* have produced between 494-2500kg/ha within 6 months, (Srinivasagam and Kathirvel, 1991, Yalin and Quingsheng, 1994, Dorairaj and Roy, 1995, Marichamy, 1996).

Larval rearing

The stocking density for crab larvae (including *Scylla serrata, Portunus trituberculatus* and *Portunus pelagicus),* has been said to range from 10-50/L (Cowan, 1986, Deering, 1997), however, survival has been proven to be considerably higher when larvae have been stocked at lower densities, i.e. 10/L (Cowan, 1986). This lower stocking density has been said to be as a direct result of cannibalism at the megalopa stage, (Cowan, 1986, Marichamy, 1996). Prior to the crabs developing to this cannibalistic stage, the stocking density can be determined by the fecundity of the female (Deering, 1997).

Health and Disease

A lot of research has been undertaken in relation to crab health. For a successful industry to be developed worldwide, the health (including diseases, prevention, clinical signs and treatments), of these species must be completely understood.

There are still many problems with disease in the hatchery production of portunids. The hatchery production of portunids has been underway for more than 30 years and it is still plagued with inconsistent stock survival (O'Sullivan, 1996). The most probable cause of this larval mortality over the years is bacterial infection (O'Sullivan, 1996).

There are many basic procedures that can be used to prevent contamination of the larval cultures. The main entry portals for contamination into a hatchery include vertical transmission, seawater, live feeds, contaminated equipment and aerosols (O'Sullivan, 1996). Hence, if prevention procedures are put in place, the potential of contamination can be greatly reduced. A link has recently been found between continuous wet floors and a decrease in larval survival (O'Sullivan, 1996). This can be avoided by a dry out period in the hatchery in between runs. All water to be used in the hatchery should be filtered and disinfected before use. Wild caught broodstock should be treated in 10ppm formalin for 10 hours upon entry to the hatchery (O'Sullivan, 1996).

Bacterial and viral infections

Vibrio spp.

Vibrio spp. are generally a pathogenic strain of bacteria that is common in crab aquaculture, especially during larviculture (Muroga et al., 1994, O'Sullivan, 1996). It is a potent bacteria, with reports of certain pathogenic strains capable of surviving short periods of boiling (Platt et al., 1995). *Vibrio* spp. is said to be most common in the live diets that must be maintained throughout the larval rearing period

(Suzuki et al., 1990). Certain strains of *Vibrio* have been proven to be the cause of mass mortalities in the zoeal larvae of portunid crabs (Muroga et al., 1994). However, studies have shown that with the addition of certain non-pathogenic bacterial strains to crab larval cultures, hence dominating the bacterial populations, *Vibrio* spp. can be made undetectable in these cultures (Nogami and Maeda, 1992). *Vibrio* spp. has also been found to be the cause of up to 80% mortality in shedding tanks in America (Sizemore, 1985).

Shell Disease

Shell disease has been found to be a very common problem of crustaceans in polluted environments, especially in regions of metal and trace element accumulation, (Engel and Noga, 1989, Weinstein et al., 1992, Noga et al., 1994). It can be caused by three different strains of bacteria, *Vibrio spp., Aeromonas spp. and Spirillum sp.* (Nowak, 1997). Crabs from riverine sites were found to be more susceptible to shell disease in comparison to crabs captured from oceanic sites (Noga et al., 1994). In commercially important *Callinectes sapidus*, shell disease is said to coincide with low serum antibacterial activity (Noga et al.,1991,1994). Hence antibacterial activity may be an important mechanism protecting crabs against shell disease and may be a useful biomarker of blue crab health (Noga et al.,1991,1994, Chisolm and Smith, 1994).

Viral infections

There are no major reports of serious viral infections in portunids. However, Sindermann (1970), claimed that gross disease signs of viral infections in crabs are evident by the slow development of paralysis and a slight darkening of the exoskeleton.

Fungal infections

Fungal infections caused by *Lagendiales* spp. are very common in the eggs and larvae of most crab species (Millikin, 1978, Hamaskai and Hatai, 1993, Nakamura and Hatai, 1995). Eggs infected by fungus can be recognised by their smaller size and greater opacity (Millikin, 1978). Infected eggs will fail to hatch or give rise to abnormal zoea larvae (Sindermann, 1970). However, the fungal diseases caused by *Lagendiales* spp. on newly hatched larvae can be can be treated in the hatching tank with 25ppm formalin bath treatments (Hamasaki and Hatai, 1993).

Protozoan infections

Hematodinium spp.

Infection from this parasitic dinoflagellate has been widely researched. It is a blood protozoan that infects the hemolymph of many crustacean species, including commercially valuable portunid crabs (i.e. *Scylla serrata, Portunus pelagicus and Callinectes sapidus),* (Hudson and Lester, 1994, Messick, 1997, Whittington et al., 1997, Shields, 1997, Whittington et al., 1997(b)). Infected crabs frequently show signs of weakness and lethargy, and often die due to stress-related handling or fishing, (Whittington et al., 1997). Light infections of this blood protozoan can become fatal within 2-3 weeks (Shields and Messick, 1997). Hematodinium infections are said to follow a seasonal cycle, with prevalence generally low during the prebreeding and ovigerous season, (Messick, 1994, Messick, 1997, Shields, 1997). Infections are said to be heaviest and most prevalent in juveniles (Messick, 1994, Messick, 1997). The

disease can be minimised by low salinities, cooler water temperatures and a static water flow (Messick, 1997, Shields, 1997).

Recently, a new member of the genus *Hematodinium*, has been discovered in Moreton Bay, Australia. It is said to be specific to the sand crab, *Portunus pelagicus*, (Hudson and Shields, 1994).

Ciliates

Another protozoan that can be parasitic to portunid crabs are ciliates. The hemolymph infecting ciliates *Mesanophrys* sp., and the gill parasites, *Epistylis* sp., *Operculariella* sp., and *Acineta* sp. are ciliates that have been found to occur in portunids and could cause potential problems in aquaculture, (Messick, 1992, Shields, 1992, Hudson and Lester, 1994, Messick and Small, 1996)

Microsporidians

Microsporidian infections have been known to cause mortalities of the commercially important portunid crabs, *Callinectes sapidus* and *Portunus pelagicus* (Messick, 1992, Sumpton, 1994). This infection can be easily identified by severe muscle necrosis and the coarse white appearance of the muscles (Sumpton, 1994).

Other Parasites and Symbionts

Parasitic crustaceans

The rhizocephalans, *Sacculina granifera* and *Loxothylacus ihlei*, are parasitic crustaceans common to both adult and juvenile sand crabs, *Portunus pelagicus* and mud crabs, *Scylla serrata*, respectively (Shields and Wood, 1993, Sumpton et al., 1994, Knuckey et al., 1995). These parasites are said to follow a seasonal cycle and appear to be more prevalent in the summer months (Sumpton et al., 1994). Signs of this parasitic infection are slower growth rates, castration, underdeveloped gonads, small egg masses on ovigerous females and larger abdominal flaps, (Shields and Wood, 1993, Sumpton et al., 1994, Knuckey et al., 1995).

Other parasitic crustaceans known to affect portunids include isopods and copepods.

Egg predators

Egg predators have the potential of becoming a problem in the aquaculture of portunid crabs. Egg predators responsible for being major sources of egg mortality in many species of portunids include the nemerteans, *Carcinonemertes spp.*, and copepods, *Choniosphaera spp.* (Millikin, 1978, Shields and Wood, 1993, Torchin et al., 1996).

Gill parasites and symbionts

There are many gill parasites and symbionts commonly found in portunids that have been found to increase stress through respiratory distress (Hudson and Lester, 1994). Parasites include the isopod, *Portunion maenadis*, and the trematode, Levinseniella sp., (Shields, 1992, Searle and Crompton, 1995). Symbionts include the barnacles, *Octolasmis sp.* and *Chelonibia patula*, (Gannon, 1990, Gannon and Wheatly, 1992, Shields, 1992, Hudson and Lester, 1994).

Metal toxicity

Metal toxicity has been found to have sublethal effects on both larval and juvenile crabs (Mortimer and Miller, 1994). Chromium, nickel and copper levels less than an order of magnitude higher than the Australian guidelines for marine aquatic ecosystems, have proven to have severe effects on larval and juvenile *Portunus pelagicus* larvae (Mortimer and Miller, 1994). Signs included the inhibition of moulting, an increase in the duration of development period and reduced size achieved by successive juvenile crab instars (Moritimer and Miller, 1994).

Summary

In conclusion, this literature review has summarised relevant information relating to nutritional information, stocking densities and the health of larval, juvenile and adult portunids. Hopefully, this review, will assist in the future research of diet formulation, stocking and health studies.

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APPENDIX 4: Hatchery Manual

The Husbandry of Blue Swimmer Crabs, *Portunus pelagicus*, in Aquaculture

Hatchery Manual and Project Summary

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March, 1999

Introduction

The portunid, **Portunus pelagicus,** *or more commonly known as the blue swimmer or sand crab, is the most common crab species found in the upper gulf regions of South Australia. The aquaculture of this species has the potential to be a very profitable industry for South Australia, mainly due to its fast growth rates and international marketing potential.*

The life cycle of blue swimmer crabs is quite simple. In the spring months, female blue swimmer crabs come out of the deeper water where they were fertilized, to spawn in the shallow, warmer water. Once spawned, the egg loads (berry) are attached to the abdomen of the females. The berry is said to carry up to 3 million eggs, depending on the size and age of the female. Within 7-14 days (depending on temperature), these eggs will progress from an orange coloration with no definition, to a distinctive dark grey coloration with two purple/red spots (believed to be the eyes of the larval crab) and extensive definition. The definition of the egg is clearly evident with the use of a dissecting microscope. Within 2-3 days of the eggs changing to a dark grey coloration, the crabs will hatch, generally overnight and simultaneously, bringing to life thousands of hungry zoea 1 stage larvae.

Once hatched, the larvae progress through 4 zoea stages (i.e. zoea 1-4), and a megalopa stage prior to moulting to a crab 1 stage juvenile. The process from zoea 1 to juvenile takes approximately 18 days at 25C, however it is highly temperature and light dependant (i.e. under 24 hour light at 27C we managed to reduce this larval period to 13 days).

This manual summarizes the hatchery site, facilities required, problems encountered and techniques used to rear blue swimmer crabs throughout the course of the project.

Site and Facilities

LOCATION

The project was carried out in conjunction with Spencer Gulf Aquaculture Pty. Ltd., at their site in Port Augusta, South Australia. The facility pumps water from the inlet and outlet channels of the Northern Power Station, hence providing a year round supply of warm water. Water temperatures were never lower than 20C in the winter months.

Facilities

Ocean Gold Investments Pty. Ltd. were provided with the following facilities at the site in Pt. Augusta.

1) hatchery space comprising of facilities for larval rearing, live feed production, and open water algae cultures
2) nursery space including facilities for stocking density trials and pond space for growout.
3) Access to all the essential components of a commercial aquaculture facility i.e. cold rooms, workshop, work area, office facilities and a staff room.

Staff

A scientist (0.75 FTE) and a full time labourer staffed the project.

Water supply

Below is a flow diagram of the filtered water supply provided to Ocean Gold Investments Pty. Ltd. throughout the duration of the project.

Inlet/Outlet channel (Pt.Augusta Power Station)

Sand Filter (30um)

Storage tanks (gravity flow => nursery; pump => hatchery)

D.E. filter (Diatomaceous earth; 5um)

Boiler

Header Tanks (salinity adjusted with charcoal filtered freshwater - 30ppt and 44ppt water)

Gravity flow to hatchery

Hatchery

Broodstock conditioning

Wild caught broodstock were used throughout this project, hence berried females were only available during their natural spawning season, (September-February). Berried females were captured by staff and professional fishermen from Adelaide and Whyalla during the project, however successful hatches were not obtained until crabs were captured from the same location (i.e. water supply) as the hatchery.

Once captured, the eggs were examined under the microscope to determine development stage, viability, damage and also to observe whether they were contaminated with a pathogenic source. Commonly found in the berry were parasitic nematodes, ciliates and occasionally slight fungal infections. The berried females were then transferred to a 1500 litre hatching tank. The crabs were generally kept in a low light environment and hides (150mm PVC pipe offcuts), were added so as to reduce stress. Filtered seawater was added through a flow through system exchanging at approximately 500%/day. Water temperature was kept constant at 25C. Prior to hatching, the crabs were fed daily with cockles (*Donax deltoides)*. The hatching tank was vacuumed daily so as to remove the build up of detritus, hence minimising the possibilities of contamination.

Generally, the berried females were very active during their period in the hatchery. The females were fed 1-2 cockles per day and often seen swimming around the tank. The berry from the blue swimmer crabs used in this project were generally quite small in comparison to literature with hatches generally around the 300,000-400,000 mark. The berry progressed from a distinct orange colour post spawning to a dark grey colour within 2 days of hatching. At 25C, this egg development period took approximately 2 weeks. Regular egg examinations were performed and the egg development was monitored closely.

Once hatched, the zoea 1 stage larvae were harvested through a 250um screen and transferred to 4 x 20L buckets containing aerated seawater. They were then counted by taking 5 x 10mL samples per bucket, examined and transferred to 3000 litre conical based tanks. The larval tanks were pre-filled with algae and rotifers (refer to Appendix 1).

ALGAE

2 species of algae were used throughout this project, *Isochrysis tahitian* (brown), and *Nannochloropsis occulata,* (green). The inoculations were provided by the SGA hatchery in sterile 20 litre carboys and then scaled up to 2500 batch cultures. Chlorinated 30ppt seawater, nutrients, vitamins and trace elements were then added in accordance with the protocol below.

Both cultures were grown successfully at 25C within 4-5 days of inoculation as open, continuous cultures under a constant 400W metal halide light source. The main role of the algae was to enrich live feeds and provide a greenwater culture for the larval tanks.

Algae protocol

1) Fill tank and aerate water
2) Chlorinate tank (using 10% active chlorine)
   - 100 ppm for 1 hour treatment or 10ppm for overnight treatment
   - formula to calculate chlorine requirements:

     Chlorine Required = Tank Volume x conc.required (ppm) x 100%/P

     (Where P = % active ingredient, i.e. 10%, and 1ppm=1mg/L)
3) Dechlorinate tank (using sodium thiosulphate)
   - Formula to calculate thiosulphate requirements:

     Sodium thiosulphate required = Chlorine used (mL) x 0.125 (assuming 12.5% actual chlorine)
4) Leave to stand for 10 minutes
5) Add aquasol @ 80g/1000L
6) Add Iron @ 80 mL/1000L
7) Add Vitamins (to be stored in fridge) @ 0.1mL/L
8) Add trace metals @ 1mL/1000L
9) Add 1-2 carboys of algae per tank (assuming tank is around 2000 litres)
10) Heat tank to between 22-27 degreesC.

N.B. Whenever topping up an algae culture, fill up with seawater once you reach the bottom 10% of the culture and replenish the nutrient supply as per the whole tank.

LIVE FEEDS

1) Rotifers

Rotifers are an essential food source for early stage crab larvae (i.e. zoea stages 1&2), hence the requirement for a rotifer population in the hatchery. An initial starter culture of 300 million rotifers, *B.plicatilis* was obtained from the SGA hatchery at the commencement of the project. For the remainder of the project, we have maintained a population in accordance with demand ranging from 500 million to 2 billion. Rotifer populations were maintained in 3 x 3000 litre flat-bottomed tanks.

The two main food sources used in this project were algae and bakers yeast. However, prior to feeding out the rotifers to the larvae, they were enriched in pure algae for a minimum of 8 hours. They were then rinsed with saltwater through a 60um screen for 10 minutes to remove dead cells and other detritus.

Yeast was fed at approximately 1.3 g/million/day, with the morning feed being 1/3 of the evening feed. Vitamin B12 was added (see protocol), to each yeast feed so as to assist in maintaining good egg ratios (>20%). Algae was added at approximately 20% of tank volume/day.

Dacron was added to the tanks to control ciliates and to remove wastes. Daily maintenance involved morning counts, dacron cleaning, 3 feeds (2 x yeast and 1 x algae), screening (if required), harvesting and enriching (if required).

Counts were performed by taking 3 x 1mL samples from the tank and then counted in drops under a dissecting microscope. Egg percentages were also counted and recorded. High egg percentages was a good sign of a healthy rotifer culture.

The dacron was removed from the tank daily and rinsed with freshwater.

Screening involved transferring the entire rotifer population from the tanks into 62um nylon screens and then giving them 2 rinses before replacing them in a freshly scrubbed tank with new seawater and algae. The initial rinse was performed with freshwater for 1-2 minutes so as to kill ciliates and other potential pathogens that could cause the cultures to crash. Seawater was used for the second rinse for periods of up to 2 hours, depending on the cleanliness of the culture. This rinse would remove most of the wastes from the population and assist in maintaining good, clean, healthy cultures.

It should be noted that rotifers are very temperamental and subject to crashing for no particular reason. However maintenance of good water quality at all times and the use of algae daily has proven to prevent most population crashes. To be on the safe side, we always maintained higher population numbers than required.

Rotifer protocol for Blue Swimmer Crab Project at Pt.Augusta

To start a rotifer culture:

Day 0

1) Add 1200 litres of 32 ppt seawater at 24 degrees C and aerate
2) Chlorinate and dechlorinate (as per protocols)
3) Add 300 litres of algae (i.e. 20% of tank volume)
4) Add rotifers (preferably around 200/mL for a 1500L culture)
5) Feed yeast - if less than 200/mL => 0.92 g/million/day
   - if greater than 200/mL => 1.3 g/million/day
   - split the daily yeast requirement into thirds and feed 1/3 in am, and 2/3 at p.m.
   - when feeding yeast, ensure yeast is well blended
   use 40 mL of vitamin B12 (conc=1gm/L) for every 500g of yeast used
6) Add dacron to tanks to minimize detritus and ciliate build up

Days 1-3

1) daily count in am
2) add 200 litres of algae
3) feed yeast 2 times/day

Day 4

1) screen tank

N.B.

1) When blending yeast, maintain less than 550g of yeast per litre of water
2) When rinsing or adding water to rotifer cultures, ensure the water going in is the same temperature as the previous supply (rotifers are very sensitive to sudden changes in water temperature)
3) Rotifers are highly sensitive to chlorine. Even when mixing up yeast for feed do not use tap water or a water source which contains chlorine.
4) When harvesting/screening, ensure that the rotifers are screened through dacron.

2) Artemia

Artemia is also a major dietary component for blue swimmer crab larvae. We have learnt that the larvae do not feed on Artemia nauplii until they reach zoea 3. But without feeding crab larvae Artemia, they do not survive the moult to the megalopa stage. 2 stages of Artemia were fed to the larvae; freshly hatched nauplii and on-grown Artemia nauplii enriched with Super Selco.

Artemia were hatched and on-grown in 90L conical hatching tanks. Each tank was kept under a constant light source, maintained at 28C and fitted with 3-4 airstones. Water was sterilized prior to use, and no more than 120g were hatched at one time in a hatching tank (higher stocking rates tended to decrease hatching rate). 100% water exchange was performed on the cultures daily. The nauplii were rinsed daily and prior to feeding using a 150um screen, for at least 10 minutes.

Cysts were hydrated and decapsulated prior to hatching so as to increase hatching rate (refer to protocol). Due to the high bacterial load of these zooplankton, a lot of care was taken to minimize contamination in the larval tanks. All water used in the culture of Artemia was sterilized using chlorination/dechlorination techniques and light was used to separate hatched/unhatched cysts prior to addition to the larval tanks.

Artemia protocol for Blue Swimmer Crab project at Pt.Augusta

Decapsulation (begin at ~3:30pm for next morning feed)

For every 100g:

1) Hydrate in 2 litres of water for 1 hour
2) Decapsulate with 15g NaOH (add 1st as pH must stay >7) and 400mL Chlorine
3) Add ice (when hatching >200g) to control temperature increase as reaction is exothermic
4) Stir until all cysts are orange in colour, then rinse until chlorine smell is removed
5) Hatch overnight in Artemia hatching tank (90litres) in 28C seawater.
6) Ensure water is chlorinated/dechlorinated prior to the addition of cysts.

Enrichment

1) Enrich with Super Selco/Protein Selco
2) Super Selco req. = (No.hours to be enriched x tank vol. x 0.6)/24
3) When harvesting, rinse Artemia thoroughly before feeding out to larvae

	AF Cysts	EG Cysts
Day 1	4pm decap	6pm decap
Day 2	8am hatched & harvest	starve
Day 3		8am enrich/4pm enrich
Day 4		8am harvest

General

1) There are approximately 250,000 naups/gram of cysts.
2) EG grade cysts are bigger and better for enriching, whilst AF grade cysts are smaller and better quality for initial larval feeds.
3) Always check Artemia quality (survival) before enriching, feeding, etc.
4) Selco must be blended prior to enriching.
5) Always ensure air is turned on in art tanks no matter what you're doing.
6) When weaning from nauplii to enriched Artemia, ensure a 12-hour enrichment only for the first 2 days.

LARVAL REARING

The crab larvae were reared in 3000 litre conical based tanks. The cultures were maintained under a photoperiod of 18-20 hours light. Water temperature was kept constant at 25C, with good aeration and all tanks were fitted with a protein skimmer so as to remove the film from the surface, hence keeping cultures clean and dissolved oxygen levels at a maximum. The salinity generally remained constant at 44ppt, Trials performed at lower salinities showed that there was no difference in growth/survival. Tanks were held in both static and flow through systems throughout larval growth, with 20% water exchange performed daily in static systems, whilst flow through systems were exchanging water at ~200%/day. The revised larval feeding regime and system design for the larval rearing period are summarised in the larval rearing protocol attached (see Appendix 1).

Static systems were used whilst the larvae were feeding solely on rotifers (i.e. until zoea 3). Once the larvae began to feed on Artemia, it was essential to change the system to flow through (through a 500um screen), to flush older feed out of the system and maintain water quality.

Larvae were stocked in initial larval stages at up to 100/L, but once they reached the megalopa stage where they proved to be very cannibalistic, stocking densities were dropped to 5-10/L after severe cases of cannabalism were observed.

When the larvae moulted to the megalopa stage, they underwent a complete metamorphosis and began to look and behave very similar to a juvenile crab. They showed signs of settlement, become very cannibalistic and consumed more food. Vertical substrates in the form of mussel rope, onion bags and shade cloth were added to the water column so as to provide a refuge for these aggressive, cannibalistic larvae. Daily maintenance of the larval tanks involved larval observation and live feed counts, feeding, tank vacuuming (this was essential so as to decrease the bacterial load in each tank), 20% water exchange (when the cultures were run as static systems), and general observations and cleaning.

BACTERIAL CONTAMINATION AND OTHER PATHOGENS

Bacterial contamination was a major area of concern throughout the course of this project.

The first signs of contamination in the hatchery appeared in the eggs of the wild caught berried females. Close observation revealed parasitic nematodes and ciliates (these pathogens are commonly known to feed on the eggs), and a fungal covering over the egg load. However, we were able to reduce this without treatment solely by maintaining water quality, (i.e. 500% water exchange daily and keeping the tank free of detritus build up).

As the summer progressed, so did the level of pathogenic bacteria in the water supply. Widespread research in the larval rearing of portunid crab larvae has shown that unlike many species of fish, crab larvae are highly susceptible to the slightest bacterial load, and is said to be the major cause of poor larval survival in many crab hatcheries worldwide, even after 30 years of research. I think our results can justify the above statements.

Prior to November, we successfully produced approximately 13,000 juvenile crabs. However, following this period we did not produce one. Considering our improvements in feeding regimes and nutrition, (80% survival from zoea 1 to megalopa in our final run in late January/early February), stocking densities and general knowledge on the larval rearing of blue swimmer crabs, I think that the contaminated water supply may have been a major cause of larval mortality.

The most evidence of bacterial contamination in our hatchery was the prevalence of a bright red substance, that accumulated on areas of detritus build up (i.e. on the bottoms of the rotifer, Artemia, algae and larval tanks). Some days during its peak, the Dacron detritus collectors in the rotifer tanks were bright red. It was possible to minimize this bacteria from contaminating our larval stocks until they reached megalopa. At this stage the larvae required a substrate to be added to the water column, hence restricting flow and also providing a haven for bacterial growth.

If this project is to continue in future months, I think it is essential that the water supply be filtered to a greater level (i.e. U.V./Ozone sterilization and/or 0.2 um filtration), or the project be moved to a site which does not have as high a nutrient/bacterial load in the water supply in the summer months.

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Appendix
Summary of scientific results obtained throughout project

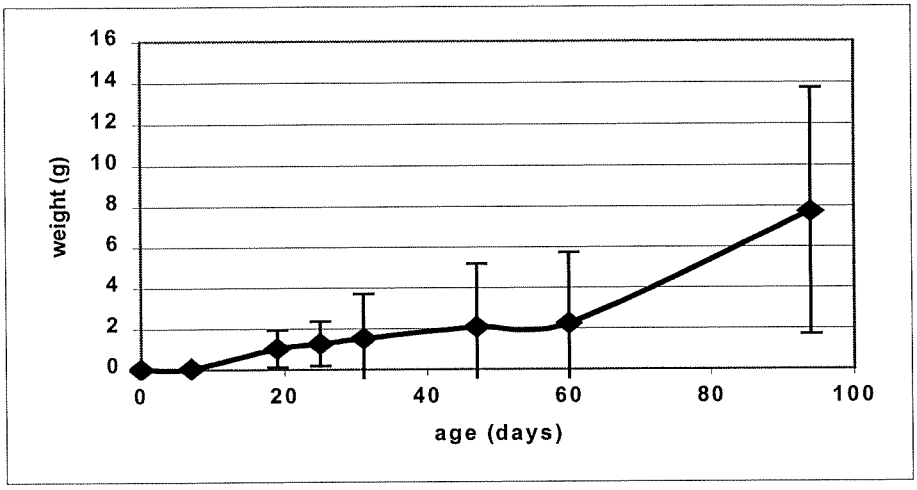

Figure 1. This graph highlights the growth of juvenile crabs over a 94 day period from hatch.

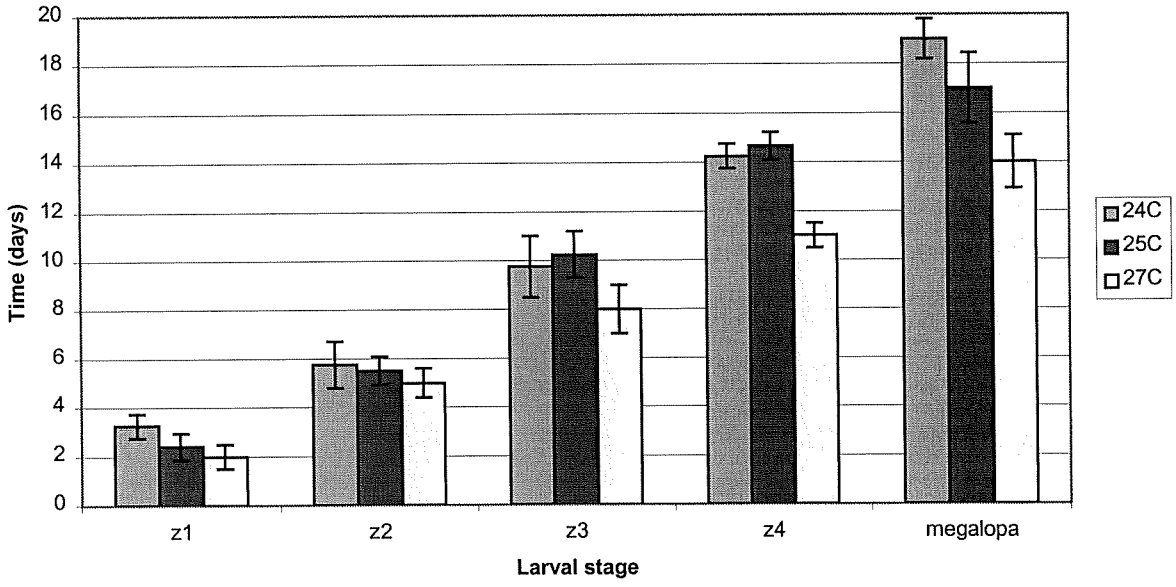

Figure 2. Figure 2 highlights the growth of crab larvae at different temperatures. The graph indicates the duration of the larval rearing period spent in each zoeal phase. i.e. At 24C, the larvae were at zoea 1 stage for approximately the first 3.5 days of their cycle, whilst they were zoea 2 until approximately day 5.

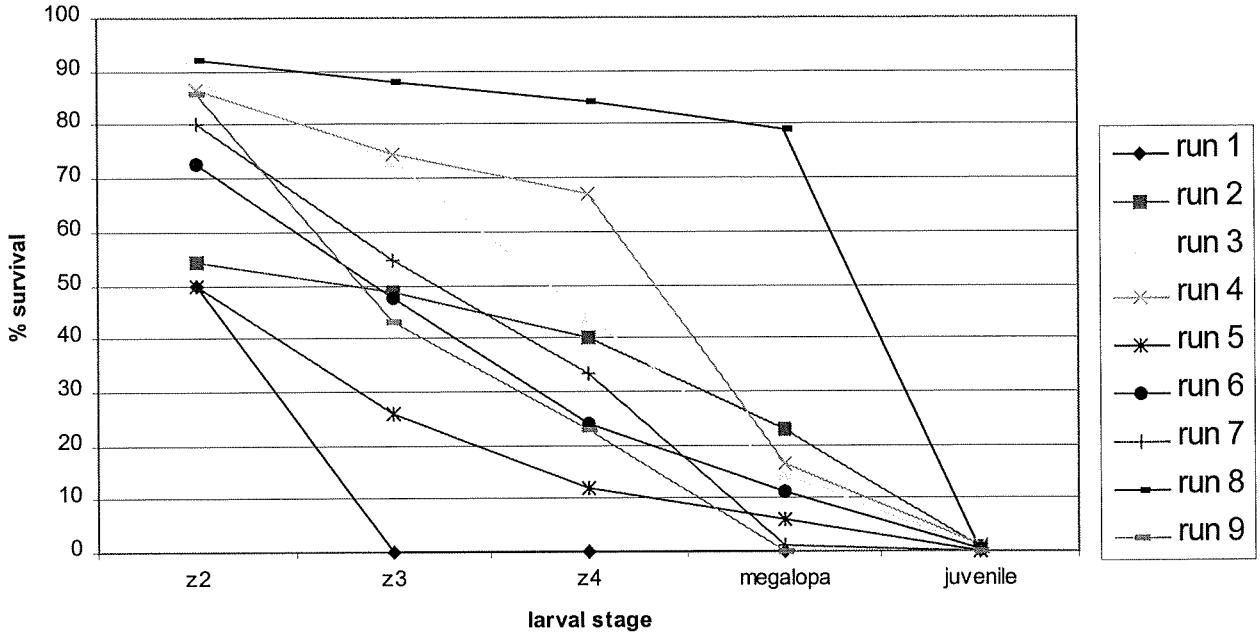

Figure 3. Figure 3 highlights larval survival at different stages throughout the course

e 3. This graph highlights larval survival at different stages throughout the course of the project.

Table 1. Table 1 highlights the summarizes the approximate number of crab larvae to survive to megalopa and then on to a crab 1 stage juvenile throughout the course of the project.

	No. crabs		% survival	
Run #	Megalopa	Juvenile	Megalopa	Juvenile
1	0	0	0	0
2	80,000	2800	22.9	0.8
3	100,000	1800	14.7	0.26
4	130,000	10,000	16.5	1.27
5	20,000	200	6.1	0.06
6	100,000	1800	11.4	0.2
7	10,000	0	1.3	0
8	300,000	0	78.9	0
9	0	0	0	0

Table 2. Results from basic stocking density trials showed that the minimum stocking density for juvenile crabs up to day 90 was **25/m^2**. This experiment was performed with three replicates per sample in raceways. Each raceway was split into 3 sections of an approximate area of 1.2m^2.

stocking density (#/m^2)	**%survival**
7	100%
10	100%
25	100%
50	56.80%
100	23.40%