Developing techniques for enhancing prawn fisheries, with a focus on brown tiger prawns *(Penaeus esculentus)* in Exmouth Gulf

FRDC Project 1999/222

Final Report

June 2003

Neil Loneragan
Rob Kenyon
Peter Crocos
Bob Ward
Sigrid Lehnert
Mick Haywood
Stuart Arnold
Roger Barnard
Mark Bravington
Michele Burford
Nic Caputi
Mervi Kangas
Richard McCulloch
Jim Penn
Melony Sellars
Fiona Manson
Bronwyn Harch
Yimin Ye
Peter Toscas
Peter Grewe
Jennifer Meadows

CSIRO Marine Research
Mathematical & Information Sciences
Livestock Industries

Fisheries Research & Development Corporation

Fisheries Western Australia

MG Kailis Group

Developing techniques for enhancing prawn fisheries, with a focus on brown tiger prawns (*Penaeus esculentus*) in Exmouth Gulf

Bibliography.
ISBN 1 876996 37 4.

1. Shrimp fisheries - Western Australia - Exmouth Gulf. 2. Shrimps - Western Australia - Exmouth Gulf. I. Loneragan, Neil. II. CSIRO. Marine Research.

639.58099413

Published by CSIRO Marine Research

This publication should be cited as:
Loneragan NR, Kenyon RA, Crocos PJ, Ward RD, Lehnert S, Haywood MDE, Arnold S, Barnard R, Burford M, Caputi N, Kangas M, Manson F, McCulloch R, Penn JW, Sellars M, Grewe P, Ye Y, Harch B, Bravington M, Toscas P, Meadows J. (2003). Developing techniques for enhancing prawn fisheries, with a focus on brown tiger prawns (*Penaeus esculentus*) in Exmouth Gulf. Final Report on FRDC Project 1999/222. CSIRO, Cleveland. pp.276.

DEVELOPING TECHNIQUES FOR ENHANCING PRAWN FISHERIES, WITH A FOCUS ON BROWN TIGER PRAWNS (*PENAEUS ESCULENTUS*) IN EXMOUTH GULF

Loneragan NR, Kenyon RA, Crocos PJ, Haywood MDE, Arnold S, Burford M, Sellars M, Manson F, Grewe P, Ye Y, Ward RD.
CSIRO Marine Research

Harch B, Bravington M, Toscas P.
CSIRO Mathematical and Information Sciences

Lehnert S, Meadows J.
CSIRO Livestock Industries

Caputi N, Kangas M, Penn JM.
Fisheries Western Australia

McCulloch R, Barnard R.
MG Kailis Group

Blaber S, Poiner I, Rothlisberg P. (CSIRO Marine Research),
Kailis G (MG Kailis Group),
Penn JW (Fisheries WA).
Project Steering Committee

June 2003

Marine Research

Mathematical & Information Sciences

Livestock Industries

FRDC Project 1999/222

CONTENTS

Non-Technical Summary

FRDC Project 1999/222

Developing techniques for enhancing prawn fisheries, with a focus on brown tiger prawns (*Penaeus esculentus*) in Exmouth Gulf.

PRINCIPAL INVESTIGATOR: Dr Neil Loneragan
ADDRESS: CSIRO Marine Research
PO Box 120
Cleveland QLD 4163
Telephone: 07 3826 7255 Fax: 07 3826 7222

CO-INVESTIGATORS: Mr Peter Crocos (CSIRO Marine Research), Dr Nic Caputi (Department of Fisheries WA), Professor George Kailis (M. G. Kailis Group)

Report authors: Loneragan NR, Kenyon RA, Crocos PJ, Ward RD, Lehnert S, Haywood MDE, Arnold S, Barnard R, Burford M, Caputi N, Kangas M, McCulloch R, Penn JW, Sellars M, Grewe P, Ye Y, Harch B, Bravington M, Toscas P, Meadows J

Steering Committee: Blaber S, Poiner I, Rothlisberg P (CSIRO Marine Research)
Kailis G (MG Kailis Group)
Penn JW (Department of Fisheries WA)

OBJECTIVES

1. Minimise the costs of producing large numbers of juvenile prawns through research on techniques to intensively grow larvae to juvenile prawns (1 g), and developing methods of harvest, transport and release

2. Maximise the possibility of the success of releasing juvenile prawns in the environment by surveying the critical nursery habitats of brown tiger prawns in Exmouth Gulf (including the juvenile prawns and their predators)

3. Ensure that the cost and success of prawn enhancement can be rigorously evaluated by developing release protocols and monitoring strategies, and by refining the bioeconomic model developed in Stage 1

4. Minimise the risks of large changes in the genetic composition of the tiger prawn stocks and introducing disease to the wild population

NON-TECHNICAL SUMMARY:

OUTCOMES ACHIEVED

This project has been successful in developing new approaches for the production of juvenile prawns at high densities (> 2,000 prawns·m^{-3}) at both experimental and semi-commercial scales in Exmouth Gulf. This new production technology could lead to a 3 phase approach to the production of prawns: hatchery, nursery and grow-out. Surveys of benthic habitats and juvenile prawns and their predators, combined with information from fishery independent surveys, identified seagrass beds in the southern and south-eastern Exmouth Gulf as the best potential release sites for juvenile prawns. These surveys also found that the extensive loss of seagrass following Cyclone Vance is likely to have led to very low catches in the tiger prawn fishery and future work will investigate estimating the extent of the seagrass nursery habitat for inclusion as an environmental variable in evaluating stock recruitment relationships. Eight reliable microsatellite markers have now been developed for *P. esculentus* and tested to determine their effectiveness for identifying released ("domesticated") from wild prawns using new statistical methods. Results showed that these eight loci alone would not enable reliable discrimination between released and wild prawns unless the identity of the released-stock's fathers could be determined. This could be achieved in a cost-effective manner if the fathers' genotypes could be deduced by genotyping a single mixed batch of larvae from each spawning, given the maternal genotypes. The results from the bio-economic model show that enhancement is only "profitable" about 50% of the time (excluding capital costs and depreciation), but highlighted that little is known of the values for density-dependent mortality and mortality due to harvest, transport and immediately after release.

The prawn trawl fishery in Exmouth Gulf, Western Australia, is well managed and harvests a mixture of penaeid prawns. Catches of the high value, brown tiger prawn *Penaeus esculentus* have comprised about a third of the annual catch over the last 15 years. However, annual catches of tiger prawns are now about half the level they were in the 1970s and fluctuate markedly, from about 80 to 680 t. These changes in catch create uncertainty in the supply of prawns for export markets and force fishing and processing operators to have excess capacity to deal with good years. Managers, fishing industry and researchers are considering the option of enhancing the natural recruitment of brown tiger prawns by releasing domesticated juveniles in wild nursery areas to reduce natural fluctuations and increase the average annual catch.

This collaborative project (CSIRO, Department of Fisheries WA, MG Kailis Group of Companies) is Stage 2 of an overall research and development program for stock enhancement in Exmouth Gulf. Project management has been facilitated by the Steering Committee, with representation from each of the agencies and FRDC, annual face-to-face project meetings in Perth and two external reviews during the project (one of the feasibility study and Stage 2 proposal, and one of the Stage 2 results). The bio-economic model developed in this project builds on the model that was developed during discussions and workshops for the feasibility study of *P. esculentus* enhancement in Exmouth Gulf (FRDC 1998/222).

High density production techniques (Chapter 3)

Techniques for the high density production of juvenile *P. esculentus* were developed in experimental raceways in Brisbane and at a semi-commercial production scale in Exmouth Gulf. Methods for harvesting, transporting and releasing prawns were also tested in Exmouth Gulf. Prawns were grown in raceways with different structures (e.g. AquaMats™), to provide structure for the prawns and a settlement substratum for "natural" foods, and at a range of initial postlarval stocking densities (1,000 to 11,430 prawns·m^{-3} in Brisbane; 1,800 to

4,350 postlarvae·m^{-3} in Exmouth Gulf). In Brisbane, prawns took 6.5 weeks to grow from postlarvae 17 (PL17) to 1 g at a stocking density of 2,860 postlarvae·m^{-3}, compared with 8 weeks at a density of 11,430 m^{-3}. Although the mean survival rates decreased from about 50% at the lowest density to 21% at the highest density, the mean biomass at harvest was very similar across all densities, ranging from 1.5 kg m^{-3} to 1.7 kg m^{-3}. The type of substrate system in the raceway did not affect the survival and growth of prawns. The optimal initial stocking density for these raceways was estimated to be between 3,300 and 3,700 postlarvae·m^{-3}. Four large raceways were constructed in Exmouth Gulf and production runs were completed from August to December in each of two consecutive years. Prawns took between 8 and 9 weeks to reach 1 g, with a mean survival of greater than 70% in both production runs. The maximum biomass achieved at harvest was 1.38 kg prawns·m^{-3} in the first year which increased to 2.2 kg·m^{-3} in the second year (with an overall mean biomass at harvest of 1.86 kg·m^{-3}).

Development of genetic markers (Chapter 4)

Eight reliable microsatellite loci have now been developed for *P. esculentus* and used to analyse samples of wild caught tiger prawns from Exmouth Gulf. The results from this analysis suggest that *P. esculentus* in Exmouth Gulf comes from a single population. New statistical methods developed during this project showed that these eight loci would enable us to discriminate released from wild prawns, if the genotypes of both parents of the released prawns were known. However, under commercial production conditions, it is not feasible to directly genotype the fathers. The paternal genotype could be deduced by genotyping a sample of progeny (possibly a single, mixed homogenate) from mothers of known genotype. Further work is required to determine if this is feasible and how many progeny would need to be genotyped. Another approach, which would not require the paternal genotype to be deduced, would be to develop and deploy an additional 3 to 6 new microsatellite loci. The labour and operating costs for analysing the present eight loci would be about AUD $45 per individual for adults and $38 for larvae, but a two step approach (examining six loci on one gel in all individuals, and the two remaining loci only as required) could reduce the costs to about AUS$30 per individual.

Identification of potential juvenile release habitats (Chapters 5 & 6)

Surveys of the benthic habitats of Exmouth Gulf, particularly in the southern and eastern Gulf, found that the mean seagrass cover in June 1999, immediately following Cyclone Vance, was only 0.15%, which increased to about 10.3% in 2000 and 41.9% in 2001. The recolonising seagrasses *Halodule uninervis* and *Halophila ovalis* were found in all years of the study, while larger seagrasses *Halophila spinulosa*, *Cymodocea serrulata* and *Syringodium isoetifolium* were only found in 2001, indicating that the seagrasses of Exmouth Gulf have been going through a succession of recolonisation following Cyclone Vance. No juvenile tiger prawns were caught on un-vegetated habitats. The catch rates of juvenile prawns in beam trawls were very low in 1999 (<1 prawn·100 m^{-2}). Although catch rates of juvenile prawns were greater in the last 2 years of the study (2 to 3 prawns·100 m^{-2}), these are low compared to catch on seagrasses elsewhere in tropical and sub-tropical Australia. A predation index generated for each region, using a combination of predator catch rate and proportion of penaeid prawn in the diet (PPI), indicated that the threat of predation on released prawns would be lowest at Whalebone South, on the eastern side of Exmouth Gulf.

Commercial tiger prawn landings in 2000 were 82 tonnes; well below the 200 to 680 t range in the previous 10 years of the fishery, and one of the lowest on record. The low catch in 2000 matched the very low abundance of juvenile prawns late in the previous year (December 1999) and came despite the above average spawning index for the fishery in 1999. The commercial landings of tiger prawns increased to 208 t in 2001 and 330 t in 2002, indicating a recovery in the fishery following the recolonisation of the seagrass habitats.

Bioeconomic model (Chapter 8)

The model from the feasibility study of prawn stock enhancement (FRDC 1998/222) was revised to take into account new parameter values and to incorporate two new parameters: one for mortality at harvest, transport and release of the juvenile prawns, and one for density

dependent mortality from the time of release to emigration from the nursery habitats. Because of a lack of data, we assumed a mean mortality for harvest, transport and release of 7%, imposed immediately after release. The stock-recruitment relationship for tiger prawns in Exmouth Gulf was used to provide starting estimates of density dependent mortality: this was assumed to range from 8 to 23% for the 7 weeks between release and emigration from the nursery ground. From the revised model, a release of 24 million 1.0 g juvenile prawns was estimated to increase catches of brown tiger prawns by about 113 metric tonnes, or an estimated 18.8% of the released prawns are caught by the fishery. If an enhancement of 24 million prawns was carried out in one production run, 179 raceways with a final stocking density of 3,000 prawns$\cdot m^{-2}$ would need to be constructed. This scale of production requires 36 million 15-day-old postlarvae (PL15) to stock the raceways and the collection of about 1,000 fertilised females, assuming that 500 of these individuals spawn successfully. The logistics of harvest, transport and release of the 24 million juveniles is also a major consideration for an enhancement program of this scale. The median operating costs for "production" in raceways (includes operating costs for hatchery and production, harvest, transport and release) were about AUD$ 1.40 Million (M) (range = $1.16 M to $1.56 M), with about 50% of these costs due to the production of postlarvae. The median index of "profit" estimated for raceways (excluding capital costs and their depreciation) was AUD -$170,000 with a range from -$950,000 to +$1.53 M. Enhancement from production in raceways had a 48.2% chance of being profitable. The capital costs were about AUD $3.6 M to expand the existing hatchery and $4.48 M to establish the 179 raceways for producing 24 million 1 g prawns. The capital costs would be halved if two production runs of 12 million prawns were completed. However, we do not know how successful two releases would be compared with one major release. The bio-economic model was also used to evaluate enhancement using 0.5 g prawns, which was shown to be less profitable than 1 g prawns, mainly because of the greater numbers of postlarvae required (45 million PL15s) and the longer time that density-dependent mortality operates in the nursery areas. However, the total biomass of production is lower for 0.5 g than 1 g prawns, requiring about half the number of raceways and hence the capital costs of raceways are lower for 0.5 g prawns.

The model was an invaluable tool for helping to integrate research and identify parameters that have a major influence on the performance of an enhancement. However, the model only evaluates one release in a year and assumes that all prawns are released at one time. Although the model can be widely distributed because it is written in EXCEL, it is difficult to follow and modify because of the many, linked worksheets in the model. Further work on the model should make it more user-friendly and allow a wider range of enhancement scenarios to be evaluated (e.g. multiple-releases, releases spread over-time), and potentially be expanded to develop similar models for finfish and shellfish.

KEYWORDS: enhancement, tiger prawns, high density production, microsatellite DNA, genetic marker, release sites, bio-economic model, release strategies

ACKNOWLEDGEMENTS

We acknowledge the support of the following agencies:

FRDC for financial support, CSIRO Marine Research, CSIRO Mathematical and Information Services, CSIRO Livestock Industries, Fisheries WA, MG Kailis Group.

The following people kindly provided advice (mostly accepted) during the formulation and carrying out of aspects of the project:

Johann Bell (WorldFish Center)
Harry Campbell (University of Queensland)
David Die (University of Miami)
Ray Hilborn (University of Washington)
Ken Leber (Mote Marine Laboratory)

Louise Bell designed the cover.

Christa Baiano (CSIRO Marine Research) assisted with collating, formatting and layout of the final report.

Jan Vaughan (CSIRO Marine Research) for her valuable assistance in organising meetings, workshops and administration of this project.

Toni Cannard (CSIRO Marine Research) assisted with proof reading and editing.

The (Landsat ETM) image came from the Australian Centre for Remote Sensing (ACRES) – Digital Catalogue system.

SUMMARY

NR Loneragan
JW Penn
R McCulloch
PJ Crocos
RA Kenyon
MDE Haywood
PC Rothlisberg
RD Ward

1.1 Background

Enhancing fish stocks has the potential to be a useful management tool to:

- increase fishery yields;
- rebuild over-exploited stocks; and
- dampen fluctuations in catch due to variable recruitment.

High fluctuations in catches can lead to problems in processing operations and supplying fish to clients. A continuous supply of product allows the market share to be maintained and maximises the efficiency and profitability of the processing operation.

The enhancement of fish stocks also has the potential to provide more information about the biology of the species and the dynamics of the fishery, which can be used to assist managers in developing better harvest strategies. However, for stock enhancement or reseeding to be successful, the biology and ecology of the target animal must be thoroughly understood, in particular:

1. the methods for producing, transporting and releasing large numbers of juveniles
2. the environmental requirements of the animals and the carrying capacity of areas where animals are to be released
3. the factors that affect the survival of the released animals (including predation), and
4. the methods of monitoring and assessing the success of releases.

In addition, both the potential benefits of stock enhancement, and any risks to the environment and/or the wild fishery must be assessed.

A recent review of marine stock enhancement has highlighted the importance of making experimental releases before attempting commercial scale releases (Munro and Bell 1997). The size at release, timing of release and release habitat can all greatly affect the survival of released animals (Liu 1990, Leber and Arce 1996).

Overseas work on the enhancement/reseeding of prawn stocks has shown that it can be successful but that the success of the enhancement program differs greatly in different

countries, and for different sizes and species of prawn (Rothlisberg et al. 1999). Recent work suggests that 'adaptive management' of enhancement trials facilitated by an experimental evaluation of release protocols is crucial to enhancement success (Leber 1999, 2002). The outcomes of overseas studies suggests that before undertaking any commercial scale enhancement of prawns in Australia, it is essential to carefully assess the costs and benefits of stock enhancement as a management tool for Australian fisheries.

Scientists at the CSIRO Cleveland Marine Laboratories have been interested in the enhancement of prawn stocks since the early 1990s (Rothlisberg et al. 1999) and they have worked extensively on the ecology of juvenile tiger prawns (e.g. Loneragan et al. 1996). These prior studies, and discussions with Fisheries WA and the M.G. Kailis Group of Companies, lead to the FRDC-funded feasibility study 'Developing and assessing techniques for enhancing tropical Australian prawn fisheries and the feasibility of enhancing the brown tiger prawn (*Penaeus esculentus*) fishery in Exmouth Gulf' (FRDC 98/222) and its reported findings. The current study is Stage 2 of four linked stages that lead towards the commercial scale enhancement of tiger prawns in Exmouth Gulf. The progress to further stages of the study will depend on the results and outcomes of independent reviews conducted after stages one and two.

The four stages are:

1. a 12-month Feasibility study (FRDC project 98/222);
2. a multi-faceted experimental investigation that: refines the bio-economic model, including the costs of producing large numbers of juvenile prawns; surveys the critical prawn nursery habitats; develops release protocols and monitoring strategies (including biological tags); and minimises the risks of changes in the genetic composition or introducing disease to the wild population (this study).
3. an experimental scale enhancement aiming at investigating different release strategies, leading to a release of 1 to 3 million prawns;
4. a commercial scale enhancement (possibly 10 to 30 million prawns).

The long-term objectives of all stages of the project are to decrease the inter-annual variation in catches and increase the long-term average catch by 150 to 200 t (average annual catch of tiger prawns in Exmouth Gulf over the last five years = 377 t, Fisheries WA, 1998). Note that for these objectives to be achievable through stock enhancement, we are assuming that the environment is not currently at its carrying capacity for tiger prawns and that the major sources of inter-annual variation in catches act earlier in the life history than the size of released prawns e.g. mysis, protozoea or postlarvae compared with 0.5 to 1 g juveniles. In addition, enhancing prawn populations with marked individuals will provide more precise information on survival rates, productivity parameters for the early life history stages (particularly juveniles and sub-adults) and migration pathways. Because of the large scale of the releases and the use of genetic markers, population parameters will be estimated with great precision. This information can greatly increase the ability of managers to obtain the maximum benefit from wild prawn resources (by providing parameter estimates for management value-optimization models) and allow better harvesting strategies to be developed.

Exmouth Gulf is an ideal location in which to assess the feasibility of enhancing brown tiger prawn stocks (Penaeus esculentus) by releasing juvenile prawns. The reasons for this include:

1. There is the capacity to develop suitable hatcheries and aquaculture ponds in Exmouth Gulf where large numbers of juvenile prawns could be produced close to the fishing and nursery grounds, at a reasonable cost.
2. The study area is close to the facilities of the M.G. Kailis Group of Companies at Exmouth, which minimises operating costs while techniques are being developed.
3. Recent FRDC-funded studies have demonstrated that juvenile brown tiger prawns can be produced on a commercial basis (FRDC 96/302).

4. There has been extensive research on the fisheries biology of tiger prawns in Exmouth Gulf by Fisheries WA, and a comprehensive database has been developed.
5. Brown tiger prawns do not move as much as some other species of prawns e.g. grooved tiger prawns *Penaeus semisulcatus*.
6. The fishery for tiger prawns in Exmouth Gulf is a discrete fishery with a high rate of exploitation (it is regarded as fully exploited) and a very sophisticated system of monitoring catches. The probability of capturing released prawns in the fishery is therefore high.
7. The fishery has been well managed and is not in decline.
8. The fishery shows high levels of variation between years, with annual catches ranging from 80 to 682 t since 1987 (Fisheries WA 1998, unpublished data). This increases the potential benefits of stock enhancement.
9. One license holder, the M.G. Kailis Group of Companies, holds all except one of the current licenses in the fishery (12 or 13 vessels), and is one of the collaborators and contributors to the project.
10. The results from the bio-economic model of Stage 1 suggest that the stock enhancement of tiger prawns in Exmouth Gulf is a viable management option.

All stages of the project involve collaboration between CSIRO Marine Research, CSIRO Tropical Agriculture, the M.G. Kailis Group of Companies and the Fisheries Research Division, Fisheries WA. Collectively we have worked successfully on the feasibility study and we have the skills and infrastructure to undertake the subsequent stages of the project, leading to experimental and commercial releases. The outcomes of both the feasibility study and any subsequent work will have broad application to fisheries and aquaculture around Australia, particularly prawn fisheries.

1.2 References

Leber KM (1999). Rationale for an experimental approach to stock enhancement. pp63-75 *In:* Stock Enhancement and Sea Ranching (eds. BR Howell, E Moksness, T Svasand). Chapter 5. Fishing News Books, Blackwell Science Ltd., Oxford. 606 pp.

Leber KM (2002). Advances in marine stock enhancement: shifting emphasis to theory and accountability. In: Stickney R.R. & McVey J.P. (eds). *Responsible Marine Aquaculture*. CAB International, pp. 79-90.

Leber KM, Arce SM (1996). Stock enhancement in a commercial mullet, *Mugil cephalus* L., fishery in Hawaii. *Fisheries Management and Ecology* 3: 261-278.

Liu JY (1990). Resource enhancement of Chinese Shrimp, *Penaeus orientalis*. *Bulletin of Marine Science* 47: 124-133.

Loneragan NR, Haywood MDE, Heales DS, Kenyon RA, Pendrey RC, Vance DJ (1996). *The role of coastal nursery habitats in determining the long-term productivity of prawn populations in the Northern Prawn Fishery*. Final Report to FRDC (FRDC 92/45).

Munro JL and Bell JD (1997). Enhancement of Marine Fisheries Resources. *Reviews in Fisheries Science* 5: 185-222.

Rothlisberg PC, Preston NP, Loneragan NR, Die DJ, Poiner IR (1999). Approaches to reseeding penaeid prawns. In: Moksness E, Svasand, Howell B (eds). *Stock Enhancement and Sea Ranching*. pp. 365-378.

Fisheries WA (1998). 1997 Exmouth Gulf prawn fishery. Compiled by Trawl Research, Fisheries WA, in consultation with the Exmouth Gulf Prawn Fishing Industry.

1.3 Need

Prawn fisheries throughout Australia are intensively fished and some have shown signs of overfishing. In some cases, the current stocks of prawns are now lower than those which would produce maximum yields. The enhancement of Australian penaeid prawn fisheries has

the potential to be a useful management tool to increase fishery yields, rebuild over-exploited stocks, and reduce fluctuations in catch due to variable recruitment. Stock enhancement also has the ability to improve the management of fisheries by collecting more precise information about the biological characteristics of the stock (e.g. survival and growth, production in nursery grounds, migration pathways and factors affecting fluctuation in populations).

Prawn stocks can vary greatly from year to year because of environmental fluctuations and this leads to highly variable catches. Fishery managers must therefore adopt conservative harvest strategies to prevent fishers from reducing stocks to dangerous levels in years when recruitment is low. However, the harvesting and processing sector tend to be on average, over-capitalised, in order to cope with years of high recruitment. Enhancement of prawn stocks through releasing juvenile prawns has the potential to reduce fluctuations in stocks. It provides a possible way of adjusting the catching and processing capacity to more stable levels of prawn stocks, which would reduce the need for over-capitalisation.

For stock enhancement to be successful, the biology and ecology of the target animal must be thoroughly understood (including the production of the postlarvae/juveniles, environmental requirements, carrying capacity, and all factors that contribute to mortality), and methods must be available to monitor and assess the success of the releases. Much ecological information for stock enhancement is now available for many commercially important species of penaeid prawn in Australia. Novel approaches to tagging prawns (e.g. stable isotopes, rare alleles and reporter genes), release strategies, and assessment of the carrying capacity of habitats are being developed. However, the utility of stock enhancement as a management tool for Australian fisheries, particularly prawns, has not been assessed.

The feasibility study of enhancement in Exmouth Gulf has shown that it is an ideal fishery in which to evaluate the effectiveness of stock enhancement for Australian prawn fisheries. It also found that the enhancement of tiger prawns in Exmouth Gulf is potentially viable and that the risks of introducing disease and affecting the genetic composition of the wild population are likely to be low and manageable. Before proceeding to commercial scale releases, it is important to establish techniques for such releases on a smaller scale and to investigate the optimum habitats in which to make future releases. The approach adopted in this project, and the results from it, will help evaluate the feasibility of other enhancement projects in Australia, particularly those for prawns.

The farm production of prawns in arid environments, where evaporation rates are high and freshwater is scarce, has not been attempted in Australia. However, there are proposals for this to take place in the Exmouth Gulf region (Cape Sea Farm). The results from our research in Exmouth Gulf have provided new information on the production of juvenile prawns at much higher densities than previously attempted in Australia. We anticipate the techniques developed during this project would be suitable for a broad range of environments, apart from the arid conditions at Exmouth Gulf.

The beneficiaries of stock enhancement would be expected to contribute to the costs of research and monitoring, and ultimately pay for the enhancement at commercial scales. Therefore, stock enhancement must be cost-effective and a cost-benefit analysis using a bio-economic model, is an essential part of any enhancement project. The bio-economic model developed during the feasibility study (FRDC 98/222) has been revised as the results of the current proposal became available. It was used to assess the commercial viability of large scale enhancement and to optimise the design of the experimental enhancement (Stage 3). The results of the revised feasibility study suggested that it would be possible to enhance the tiger prawn fishery in Exmouth Gulf by about 100 t with releases of about 10 to 30 million juveniles. This study (Stage 2) has suggested that 24 million animals would be a more realistic estimate.

1.4 Further development

Following the second project external review by Dr Ken Leber in August 2001, the Steering Committee recommended that the next phase of research (Stage 3) should focus on investigating different release strategies, including the effects of size, season, and site at release on survival and density-dependence in the nursery ground. It was recommended that these studies should be carried out over two years, prior to a pilot release of 1 to 3 million juvenile prawns in the third year. The information from these studies should greatly reduce the uncertainty in our estimates of mortality and density-dependent effects in the nursery habitats and potentially in the fishery. These variables are likely to interact significantly with any multiple release strategy flowing from the need to reduce the capital costs of the raceway system and hence influence the bio-economics of enhancement. For example, the model showed that introducing parameters for mortality at harvest, transport and release, and density-dependent mortality in the nursery, increased the number of 1 g prawns required to achieve a 100 t enhancement from 14 to 24 million individuals. In addition to research on release strategies, research on maximising the health and fitness of released prawns prior to release would also increase the probability of successful releases.

The research outlined above on release strategies requires the use of a marker to follow the fate of enhanced prawns after release. The work on identifying microsatellite loci for use as genetic markers has highlighted the need for further work before we would be able to discriminate unambiguously between released and wild prawns, without knowledge of the paternal genotype. This research would test the feasibility of mass genotyping larvae to deduce the paternal genotype. This would be economically feasible with the existing set of loci, given that we know the maternal genotype, if genotyping a single mass homogenate of larvae enabled us to deduce the paternal genotype. If this approach was not successful, and we only knew the maternal genotype, an additional 3 to 6 microsatellite loci would need to be developed and deployed. However, it should be noted that research on oyster larvae has been successful in using microsatellite loci to genotype larvae and hence it is likely that it will also be successful for genotyping homogenates of prawn larvae. Rapid, cost-effective methods for screening large numbers of individuals and hence reducing genotyping costs would also need to be developed.

During the proposed Stage 3 research, new estimates for parameters obtained from research on optimising release strategies, would be included in the bio-economic model and the feasibility of commercial enhancement revised. The model should be further developed as a generic model so that it can be readily applied to other species. The model should be transferred to a different modelling framework to make it more user-friendly and capable of evaluating different enhancement scenarios.

The research on production has been very successful in developing systems for high-density production of juvenile prawns. However, there has been little opportunity to refine the system so that it might be even more effective for either enhancement or aquaculture production. For example, because no commercial feed, formulated for *P. esculentus* exists, expensive feeds developed for *P. japonicus* were used. Research on optimal diets for *P. esculentus* could potentially reduce the costs of production in raceways. Experimentation with the substrates used in the raceways and methods to enhance the water quality of the system may yield improvements in the production systems. These further refinements of raceway production would increase the attractiveness of the method for widespread application to the aquaculture production of prawns, potentially leading to a 3-phase system of: hatchery, high density juvenile-growout, pond growout.

1.5 Planned outcomes

The overall objective of this project has been to develop techniques that will facilitate the enhancement of prawn fisheries, particularly tiger prawns in Exmouth Gulf and hence reduce the inter-annual variation in fishery production. The focus of this stage of research has been on: production technology; genetic markers; identifying potential release sites in Exmouth Gulf and using the bio-economic model to assess the theoretical performance of enhancement.

The outcomes delivered were:

a. Production research has developed high-density culture techniques in raceways capable of producing large numbers of juvenile prawns for both enhancement and aquaculture

b. Genetic research has identified and developed 8 microsatellite loci that have been used to characterise the genetic structure of the wild population. New statistical methods developed during the project have shown that further research is needed to use these loci to identify released from wild prawns. This research would focus on cost-effectively deducing the paternal genotype from spawned larvae given of mothers with known genotypes.

c. Surveys of benthic habitats, juvenile prawns and their predators, juvenile prawns in commercial catches and information from fishery independent surveys have identified seagrass areas in the southern and south-eastern Exmouth Gulf as potential release sites for enhanced prawns. They have also provided an improved hypothesis to explain the very low commercial catch of tiger prawns in Exmouth Gulf in the year following Cyclone Vance and provided directions for improving the environmental variables used in the stock assessment for managing the tiger prawn fishery.

d. The new values for costs and new parameters fitted to the bio-economic model have revised the estimates of numbers of prawns required for a successful enhancement and the probability of making a profit (excluding capital costs and depreciation) from a commercial scale enhancement. The model has also highlighted that little is known of mortality following harvest, transport and release or density-dependent mortality in the nursery ground. It has also provided clear directions for research and development of stock enhancement in Stage 3 of the overall enhancement program.

1.6 Conclusion

Research from the first two stages of the research and development program for enhancing tiger prawns in Exmouth Gulf has provided some significant results for enhancement research. The development of high-density, raceway production systems for juvenile *P. esculentus* has been particularly successful, with benefits for both future enhancement and prawn aquaculture in regions where growing seasons are restricted. The initial nursery culture in raceways has significant potential for decreasing the field growout period required for prawn production. The raceway technology focussed on providing a 3-dimensional habitat for prawn growth and improved circulation systems in the raceways. This system will probably be applicable to other "tiger" prawn species such as *P. monodon* and *P. semisulcatus*, which occupy structured habitats (mangrove and seagrass) in the wild.

The work on the benthic habitats in Exmouth Gulf and information from fishery independent surveys has identified seagrasses in the south and south-east as potential release sites for juvenile tiger prawns. This research has secondary benefits for understanding fluctuations in

wild stocks and the reasons for reduced productivity following some severe cyclone events. The future monitoring of seagrass biomass in Exmouth Gulf is expected to generate an improved environmental variable for inclusion in the fishery stock assessment process.

The genetic results provide valuable information on the population genetics of *P. esculentus* and indicate how research might quickly progress to provide a method of identifying enhanced prawns in a production system with unknown paternal genotypes. The methodology developed, including new statistical procedures, has highlighted how the genetic methods might be developed further to discriminate between enhanced from wild prawns, and has clear applications to other enhancement studies, e.g. homarid lobsters and finfish generally.

The initial outputs from the bio-economic model indicate that the probability of enhancement being economically viable in Exmouth Gulf is low, given the current values for the product, costs of juvenile production and the estimated natural mortality rates. However, the bio-economic model that was developed during both Stage 1 and Stage 2 of the Enhancement program, now provides a comprehensive and rigorous framework for assessing the biological and economic feasibility of enhancement proposals, by incorporating growth and mortality through all stages up to capture by the fishery, as well as the production and operating costs. Of particular value is the ability of the model to assess the sensitivity of profit from enhancement against varying biological and economic parameters. This can now be used to focus areas for future research and identify where improvements could be made in the enhancement system to increase the chance of successful enhancement.

CHAPTER 2

APPROACH TO RESEARCH AND DEVELOPMENT FOR ENHANCEMENT

Neil Loneragan
Richard McCulloch
Jim Penn
Peter Rothlisberg

Parts of this Chapter will be published in the proceedings of the 2nd International Symposium on Stock Enhancement and Sea Ranching, Kobe, Japan, February 2002. The accepted manuscript is included in this Chapter as Appendix 2A:

Loneragan NR, Crocos PJ, Barnard R, McCulloch R, Penn JW, Ward RD, Rothlisberg PC (in press). An approach to evaluating the potential for stock enhancement of brown tiger prawns (*Penaeus esculentus* Haswell) in Exmouth Gulf, Western Australia. *Journal of Fisheries Management and Ecology*

2.1 Introduction

The integrated program of research and development for the enhancement of brown tiger prawns in Exmouth Gulf has involved interactions between people from different backgrounds: between researchers, managers and industry; between researchers from very different disciplines (aquaculture production at research and commercial scales, molecular genetics and statistics, prawn ecology, fisheries modelling and stock assessment); and between people in different agencies. An important part of this project has been the development of mechanisms to promote a common understanding of the objectives, progress, impediments and future directions for research and development. We have attempted to achieve this in 3 ways:

1. Stage the project with reviews of results before proceeding to the next phase of research
2. Establish a steering committee for the project with terms of reference and a meeting schedule around major project events and
3. Hold an annual project meeting of key participants from all facets of the project.

As outlined in the background to the project (Chapter 1), four stages were identified for research and development leading to the commercial enhancement of brown tiger prawns in Exmouth Gulf. These were:

1. a feasibility study to assess the potential for enhancement and develop a bioeconomic model (FRDC 1998/222 – completed)

2. the development of techniques for enhancement, including high-density production, genetic markers, and identifying juvenile nursery grounds and hence potential release sites (this project – FRDC 1999/222) and in the future
3. developing optimal release-strategies leading to an experimental enhancement (release of 1 to 3 million 1 g juvenile prawns), and
4. a commercial scale enhancement (release of 10 to 30 million juvenile prawns)

All stages of the project have involved collaboration between CSIRO (Divisions of Marine Research, Livestock Industries and Mathematics and Information Sciences), the M.G. Kailis Group of Companies and the Department of Fisheries (Western Australia).

2.2 Project Steering Committee

A Steering Committee was formed shortly after this project commenced to provide input, advice and review for the project team. The membership of the Steering Committee was chosen to represent all agencies in the collaboration, which also provides representation for managers, industry and researchers. The membership of the Steering Committee was:

Jim Penn (WA Fisheries and at that time FRDC)
George Kailis (MG Kailis)
Peter Rothlisberg (CSIRO Marine Research)
Ian Poiner (CSIRO Marine Research) (replaced by Steve Blaber during the last 12 months of the project)
Neil Loneragan (CSIRO Marine Research) ex-officio member and Project Leader.

CSIRO, through either Ian Poiner or Peter Rothlisberg, chaired the meetings. In addition to the members of the Steering Committee, observers, with technical expertise for different components of the research and development were invited to contribute to the discussions of the Steering Committee. Richard McCulloch, Mervi Kangas, and Nic Caputi were observers on the Steering Committee on a regular basis.

The Terms of Reference developed by the Steering Committee were to:

- Develop the Intellectual Property Agreement between FRDC and the Project Collaborators
- Develop the terms of reference for the independent reviewers of the project
- Review progress of the project by: participating in annual Project workshops; providing input and advice on FRDC Milestone and Final Reports
- Provide advice on the next stage of the project in Exmouth Gulf and new opportunities for other enhancement projects
- Oversee the commercialisation (e.g. licence agreements) of Project Intellectual Property

2.3 Assessment of progress

The results from different stages in the enhancement research have been assessed at annual project meetings with representatives from all participants, regular meetings of the Steering Committee for the project, working group meetings on the model, and in two external reviews.

The comments from the first external review of the results from the feasibility study and the proposal for developing techniques (Stage 2) are included in Appendices 2B to 2D of this chapter. A written response to some of the comments by David Die is included in Appendix 2E. The written comments from the second external review in August 2001 on the progress of the second stage of research and the potential directions for Stage 3 enhancement R&D are included as Appendix 2F.

2.4 Components of Stage 3 enhancement research

Following the second project external review in August 2001, the Steering Committee recommended that the next phase of research (Stage 3) should focus on investigating different release strategies, including the effects of size, season, and site at release on survival and density-dependence in the nursery ground (Appendix 2F – Second external Project Review by Dr Ken Leber). It was recommended that these studies should be carried out over 2 years, prior to a pilot release of 1 to 3 million juvenile prawns in the third year. The information from these studies should greatly reduce the uncertainty in our estimates of mortality and density-dependent effects in the nursery habitats and potentially in the fishery. These variables are likely to interact significantly with any multiple release strategy flowing from the need to reduce the capital costs of the raceway system.

During Stage 3 research, new estimates for parameters would be included in the bio-economic model and the feasibility of commercial enhancement revised. The model should be further developed as a generic model so that it can be readily applied to other species and transferred to a different modelling framework to make it more "user-friendly" and capable of evaluating different enhancement scenarios.

Further research on genetics would test the feasibility of mass genotyping larvae to deduce the paternal genotype or develop additional microsatellite markers.

Appendices

Appendix 2A: Accepted manuscript from 2nd International Conference on stock enhancement and sea ranching, Kobe, Japan, February 2002.

An approach to evaluating the potential for stock enhancement of brown tiger prawns (*Penaeus esculentus* Haswell) in Exmouth Gulf, Western Australia

Running title: Tiger prawn enhancement

Neil R. Loneragan[1a], Peter J. Crocos[1a], Roger Barnard[2], Richard McCulloch[2], James W. Penn[3], Robert D. Ward[1b], Peter C. Rothlisberg[1a]

[1a]CSIRO Marine Research, PO Box 120, Cleveland Queensland 4163 Australia
[1b]CSIRO Marine Research, PO Box 1538, Hobart Tasmania 7001 Australia
[2]M.G. Kailis Group of Companies, PO Box 382, Fremantle WA 6160 Australia
[3]Department of Fisheries – Research Division, WA Marine Research Laboratories, PO Box 20, North Beach Western Australia 6920 Australia

e-mail: neil.loneragan@csiro.au
phone intl+ 617-3826-7255
fax intl+ 617-3826-7222

Abstract

Historically, the annual catches of tiger prawns (*Penaeus esculentus* Haswell) in Exmouth Gulf, Western Australia, have fluctuated from about 70 to 1,200 metric tonnes (t), largely because of natural environmental effects on annual recruitment. The average catch in the last 10 years (about 400 t) is about 100 t lower than in the earlier years of the fishery. This reduction in catches and the interannual variability in catches has led industry, managers, and researchers to consider enhancing the fishery by releasing cultivated juveniles on the nursery grounds. Four stages in the research program were identified: (1) a feasibility study to assess the potential for enhancement and develop a bioeconomic model; (2) the development of techniques for enhancement, including high-density production, genetic markers, and identifying juvenile nursery grounds and hence potential release sites; (3) an experimental release (1 to 3 million 1g juvenile prawns), (4) a commercial release (10 to 30 million juveniles). We are completing the second stage of the research and have successfully developed techniques for producing juvenile tiger prawns at high densities (up to 3,000 m^{-3}), identified the nursery grounds and found eight reliable microsatellite loci with potential for use as genetic markers of enhanced stock. The work has provided new estimates of parameters to be incorporated in the enhancement evaluation model and revised the plan for the next phase of research.

Key words: bio-economic model, penaeid prawn, production, nursery habitat, genetic markers

Introduction

Artificial enhancing fish stocks has been considered to be a potentially useful management tool to increase fishery yields, rebuild over-exploited stocks and dampen fluctuations in catch due to variable recruitment. Large fluctuations in commercial catches can lead to poor economic performance in fleet and processing operations and erratic supplies to markets. A continuous supply of product allows the market share to be maintained and maximises the efficiency and profitability of the fishery.

To achieve economically successful enhancement, the principles of responsible stock enhancement should be followed (Blankenship & Leber 1995) and the husbandry, biology, ecology and production costs of the target animal must be thoroughly understood, in particular:

- the methods and costs for producing, harvesting, transporting and releasing large numbers of juveniles;
- the environmental requirements of the animals and the carrying capacity of areas where animals are to be released;
- the factors that affect the survival of the released animals (including predation);
- the methods of monitoring and assessing the success of releases; and

In addition to being done on a commercial, user-pay basis, any risks to the environment and/or the wild fishery must be assessed.

Annual releases of more than 100 million juvenile prawns [=shrimp] have been made over many years in Japan (*Penaeus japonicus* Bate, Kurata 1981, Fushimi 1999) and China (*Penaeus chinensis* Osbeck, Liu 1990, Xu, Xia, Ning & Mathews 1997, Wang, Zhuang & Deng 2002). Smaller scale prawn enhancements have been carried out with *Penaeus monodon* Fabricius in Taiwan (Su & Liao 1999, Liao 2002) and Sri Lanka (Davenport, Ekaratne, Walgama, Lee & Hills 1999). Prawn enhancement has also been attempted and discontinued in the United States (*Penaeus aztecus* Ives, *P. setiferus* Linnaeus, *P. duorarum* Burkenroad, Kittaka 1981) and Kuwait (*P. semisulcatus* De Haan, Farmer 1981). Despite the number of attempts to enhance prawn fisheries, there has been relatively little rigorous evaluation of the costs and benefits of enhancement.

In Australia, scientists have been interested in the possibility of enhancing prawn stocks since the early 1990s when CSIRO researchers visited China to discuss the Chinese prawn enhancement/reseeding program. Since then, there have been workshops to assess the potential for enhancement of *Penaeus latisulcatus* Kishinouye in Venus Bay (South Australia), and *P. esculentus* in Moreton Bay (Queensland). Much of this interest in *P. esculentus* stemmed from extensive research on the ecology of juvenile tiger prawns (*P. esculentus* and *P. semisulcatus*) in northern Australia (e.g. Loneragan, Kenyon, Staples, Poiner & Conacher 1998, Loneragan, Heales, Haywood, Kenyon, Pendrey & Vance 2001a) to manage wild prawn stocks in Australia's Northern Prawn Fishery. This work, together with aquaculture development research on prawns by CSIRO, led to a paper providing an Australian perspective on approaches to prawn stock enhancement at the first International Conference on Sea Ranching and Stock Enhancement (Rothlisberg, Preston, Loneragan, Die & Poiner 1999).

Following this work, discussions were held with the M.G. Kailis Group of Companies and researchers at the Department of Fisheries (Western Australia), which led to a feasibility study into enhancing the brown tiger prawn (*Penaeus esculentus*) trawl fishery in Exmouth Gulf. The Exmouth Gulf fishery was chosen as the fishery operates in a relatively closed embayment, and one fishing company (M.G. Kailis) operates the majority of the fleet and the one processing plant. In addition, there is a complete history of the catches and effort in the

fishery, and the dynamics of the tiger prawn stock have been extensively researched and reported in the literature (Penn, Watson, Caputi & Hall 1997, Caputi, Penn, Joll & Chubb 1998). The feasibility study was undertaken as the first stage in the assessment of commercial scale enhancement of tiger prawns in Exmouth Gulf. The long-term commercial objectives of the program were defined as decreasing the inter-annual variation in catches and increasing the long-term average catch by at least 100 t (about 25% of the average annual catch, Fig. 1). During the feasibility study, the following four stages in the enhancement program for brown tiger prawns in Exmouth Gulf were identified:

Stage 1. The development of a comprehensive bio-economic model;
Stage 2. The development of production technology (including harvest, transport and release), genetic markers and obtaining information to maximise the chances of successful enhancement;
Stage 3. An experimental scale enhancement of 1 to 3 million prawns; and
Stage 4. A commercial scale enhancement (possibly 10 to 30 million prawns).

In this paper we report briefly on some of the findings from the first two stages, particularly the development and use of a bioeconomic model, studies of high-density juvenile production, the development of a genetic identification methods, and the identification of the nursery habitats of juvenile tiger prawns in Exmouth Gulf. We also outline the components of the proposed Stage 3 in the research program, which was initially going to focus on pilot releases of tiger prawns. All stages of the project have involved collaboration between CSIRO (Divisions of Marine Research and Livestock Industries), the M.G. Kailis Group of Companies and the Department of Fisheries (Western Australia).

Material and methods

Choice of enhancement location

Exmouth Gulf is an ideal location in which to assess the feasibility of enhancing a prawn fishery because:

There is the capacity to develop suitable infrastructure (i.e. hatcheries and raceways or aquaculture ponds), where large numbers of juvenile prawns could be produced at reasonable cost close to the nursery and fishing grounds.

There has been extensive research on the fisheries biology of tiger prawns in Exmouth Gulf by the Department of Fisheries (Western Australia), and data on catch, effort and fisheries-independent indices of recruitment and spawning stock have been collected over many years.

The trawl fishery for tiger prawns in Exmouth Gulf is a discrete fishery with a high rate of exploitation (it is regarded as fully exploited) and a sophisticated system for managing catches and monitoring the levels of recruitment and spawning stock. One license holder, M.G. Kailis holds 13 of the 14 licenses in the fishery, and is one of the collaborators and contributors to the project. The probability of capturing released prawns in the fishery is therefore high.

This fishery has been well managed since the early 1980s to maintain spawning stocks at adequate levels.

The fishery shows high levels of variation between years, with annual catches ranging from 70 to 682 t since 1987 due to environmental factors (Fig. 1).

Production, harvest, transport and release

Experiments on the growth and survival of juvenile tiger prawns were carried out in experimental raceway facilities in Brisbane, Queensland, and in pilot commercial raceway facilities at Exmouth Gulf in Western Australia. The effects of different structures in raceways and prawn stocking densities on growth and survival were tested (Tables 1, 2). However, details of the different raceway structures cannot be given because of confidentiality agreements. Some of the results from the high-density production in Brisbane and Exmouth are reported here.

The Brisbane experiments were carried out in two 15 m long, 1 m wide and 0.7 m deep raceways (volume = 10,500 L). Each raceway was divided into six 2.5 m long sections (1,750 L) to replicate different treatments. Each section was supplied with flow through water at an average exchange rate of 80% per day for the first 2 weeks and 250% per day for the remaining 5 to 6 weeks. Temperature was maintained at 27^{o}C with the use of a heat exchanger.

The Exmouth trials were carried out in four commercial-scale raceways (20 m long and 1.4 m deep) constructed from concrete tilt-slab panels lined with high-density polyethylene. An average water depth of 1.15 m was maintained for the trials. Two of the raceways were 2.5 m wide and two were 1.5 m wide. The raceways were supplied with flow-through seawater, with an initial exchange rate of 15% per day, gradually increasing over the trial production period. The mean water exchange per day over the trial was 130%.

During the Exmouth production trials, the mean water temperature was 25.4°C (range = 21.8 to 29.2°C) and the mean salinity was 36.3 ppt. The mean pH was 8.0 (range = 7.5 to 8.5) and was maintained by applying agricultural lime. Sucrose was added as a carbon source to establish and sustain significant heterotrophic bacterial communities, at a rate calculated on approximately 15:1 nitrogen:carbon ratio.

Development of genetic markers

Few physical tags can be used for prawns less than 1 g or 12 mm in carapace length. In this study, molecular genetic methods were assessed as a method for identifying released *P. esculentus* and describing the genetic structure of wild populations. Microsatellites were chosen as the most accurate and informative genetic marker available and because large numbers of individuals can be screened in high-throughput systems.

A large number of microsatellites have been developed for *Penaeus monodon*, the most widely cultured prawn (Xu et al. 1997). However, in contrast to mammals, microsatellite markers are not generally transferable between penaeid species (Moore, Whan, Davis, Byrne, Hetzel & Preston 1999), and it was therefore necessary to isolate microsatellites specific for *P. esculentus* (see Meadows, Ward, Grewe, Dierens, & Lehnert in press).

Microsatellites were chosen for their use as potential markers based on a number of characteristics including ease of amplification, presence of polymorphism, clear profile, accuracy of genotyping, and genotype fits to Hardy-Weinberg expectations. Allele frequency data were collected from two samples of 96 prawns in Exmouth Gulf. Eight reliable microsatellites were chosen with potential to identify released prawns. The power of these microsatellites to discriminate between individuals was then calculated. The probability of identity for a single locus i.e. the probability that two prawns taken randomly from the wild have the same genotype for that locus, was estimated from the allele frequencies in the pooled sample as:

$I = \Sigma_i p_i^4 + \Sigma_i \Sigma_{j>I} (2p_i p_j)^2$,
where p_i and p_j are the frequencies of the *i*th and *j*th alleles (Paetkau & Strobeck 1994).

However, we were more interested in the probability (*P*) that a randomly-chosen male-female pair, or randomly-chosen female, is compatible with being the parent of a recaptured individual. For example, a male-female pair with genotypes a/b and a/c at a specific locus would be compatible with being the parents of a recapture b/c, while a pair with genotypes a/b and b/b would not be compatible with these parents. An analytical solution was developed. If we only know the maternal genotype, as in the case of *P. esculentus* commercial production for enhancement, then

$$P_m = \Sigma_i p_i^2 (1-(1-p_i)^2) + \Sigma_{i<j} 2p_i p_j (1-(1-p_i-p_j)^2)$$

If we know both paternal and maternal genotypes, as in the more general case for finfish enhancement, then

$$P_{mp} = \Sigma_i p_i^2 (1-(1-p_i)^2)^2 + \Sigma_{i<j} 2p_i p_j (2(1-(1-p_i)^2)(1-(1-p_j)^2) - (2p_i p_j)^2)$$

These are *P* values for a single locus, and are multiplied across loci to calculate the overall probability of a spurious match between a random recapture and a random mother or random parental pair. If the released offspring come from more than one mother, say *x* mothers, then the probability that a random offspring will spuriously match any of the *x* mothers is: $1-(1-P_m)^x$. For a maternal-paternal pair, $P = 1-(1-P_{mp})^x$. Values for P_m and P_{mp} were estimated for each locus and over all loci.

Identification of potential release sites

Initially a broad scale survey of benthic habitat was carried out in the shallows of Exmouth Gulf since previous studies of juvenile *P. esculentus* have shown that the main areas for settlement and residence of the juveniles are the intertidal and shallow subtidal waters (e.g. Young & Carpenter 1977, Loneragan, Kenyon, Haywood & Staples 1994). This was followed by surveys of benthic habitat, juvenile prawns and fish along the eastern and south-eastern shallows of Exmouth Gulf, an area that fisheries surveys had identified as the source of recruitment to the fishery (Department of Fisheries, Western Australia, unpublished data). Benthic habitats were surveyed using both qualitative and quantitative methods. In the first survey (June 1999), the percentage cover of different benthic habitats (e.g. corals, macroalgae, seagrass, sponges) was recorded along 30 m long underwater transects at 119 sites throughout Exmouth Gulf. This survey was completed only 2 months after the very severe Cyclone Vance moved slowly through Exmouth Gulf.

From the June survey and a previous study (McCook, Klumpp & McKinnon 1995), most of the macroalgae and seagrasses were found on the southern and eastern sides of Exmouth Gulf. Seagrasses and macroalgae were sampled quantitatively at between 60 and 200 sites in the spring and summer (November/December) of each year from 1999 until 2001. At each site, a shovel of substrate (area = 0.07 m^{-2}), and a sediment sample were taken (following Poiner, Kenyon & Staples 1987). Samples of substrate processed to calculate the shoot density and biomass of aquatic plants at each site. The percentage cover of seagrasses and algae was also estimated visually at each site.

Juvenile prawns and fish were sampled in regions along the east coast of Exmouth Gulf where seagrasses were found during the surveys. Prawns were sampled using 2 small beam trawls (dimensions = 1 x 0.5 m mouth with 2 mm mesh and 1.5 x 0.5 m mouth with 12 mm mesh) and fish with gill nets (60 m panels of 76, 102, 127 and 152 mm stretch mesh) and otter trawls.

Bio-economic model

The feasibility study had two main objectives: collating the existing information relevant for stock enhancement; and developing a bio-economic simulation model of the fishery and production systems for juvenile tiger prawns. The bio-economic model was developed in EXCEL (in Office 2000) to make it readily available to industry, managers and researchers (see Loneragan, Die, Kailis, Watson & Preston 2001b for details of the model). The model contains independent modules (linked worksheets) for the hatchery, grow-out (includes harvest, transport and release), nursery and fishery. It also contains separate worksheets to follow changes in the populations of enhanced and wild prawns in Exmouth Gulf. Monte Carlo simulation with the Excel "add-in" Crystal Ball (v5.1, Werckmand, Hardy & Wainwright 1998) was used to evaluate the uncertainty associated with model predictions and thus give a more realistic prediction of the success of enhancement programs. Crystal Ball allows the user to specify the distribution of different parameters (e.g. normal, triangular, uniform).

In revising the model from the feasibility study to incorporate comments from the external reviewers and the Steering Committee, two new parameters were included: one for mortality during harvest, transport and release; and one for density-dependent mortality from release to capture in the fishery. Because of a lack of data at this stage, we assumed initial parameter estimates for a mean mortality for harvest, transport and release of 5% with a uniform distribution from 2% to 8% (imposed immediately after release). We estimated a value for density-dependent mortality of tiger prawns in Exmouth Gulf through the use of the stock recruitment relationship of the Exmouth brown tiger prawn fishery (Caputi et al. 1998, see Fig. 2). The values of α=85.3 and β=0.0468 were obtained for the Ricker equation $R = \alpha S e^{-\beta S}$. Density dependent mortality was estimated to range from 8.2 to 23% during the 7 weeks in the nursery grounds and because of the great uncertainty in this value, a uniform distribution was specified for the simulations.

The bio-economic model has many parameters, each with a different influence on the outputs of the model. A sensitivity analysis was therefore carried out to investigate how different variables affected the forecasts from the model.

Assessment of progress during the project

The results from different stages in the enhancement research have been assessed at annual project meetings with representatives from all participants (CSIRO, Department of Fisheries Western Australia, MG Kailis), regular meetings of the Steering Committee for the project (George Kailis – MG Kailis; James Penn – Department of Fisheries WA; Ian Poiner, Peter Rothlisberg – CSIRO) and in two external reviews.

Results and discussion

Production

Brisbane Experiments

At a stocking density of 2,860 m^{-3}, the growth rates of prawns over 7 weeks did not differ significantly between Structure A and Structure B (Fig. 3a) and prawns had grown to 1 g after about 6.5 weeks. Prawns cultured in raceways with Structure C at initial densities of 5,720 m^{-3} grew significantly faster than those at 11,430 m^{-3} ($P < 0.05$, Fig. 3b), reaching 1 g in 7 weeks compared with 8 weeks for the prawns at the higher densities. After 7 weeks, prawns had reached mean weights (± 1 SE) ranging from 0.68 ± 0.08 g at a stocking density of 11,430 m^{-3} (Structure C) to 1.25 at 2,860 m^{-3} (Structure A and B) (Fig. 3).

At a stocking density of 2,860 m^{-3}, 50.9 ± 18.9% and 42.5 ± 19.0% of prawns survived to a mean weight of 1 g in Structure B and Structure A, respectively. The variability in survival between replicates was high – in one replicate for each treatment survival was less than 10% compared with survivals of greater than 67% in the remaining replicates. Survival decreased at higher stocking densities and was 31.9 ± 12.0 % at 5,720 m^{-3} and 21.2 ± 2.7 % at 11,430 m^{-3}.

Exmouth Trials

Prawns were stocked in raceways at PL15 and initial stocking densities ranging from 2,610 m^{-3} to 4,350 m^{-3} (Table 2). The fastest growth and highest survival were recorded at the lowest initial stocking density of 2,610 m^{-3}, with 70% of prawns surviving and reaching a mean weight of 1.2 g after 11 weeks in one trial (Table 2, Fig. 4). Survival and growth were slightly lower at an initial stocking density of 3,480 m^{-3} (65% and 0.94 g in 10 weeks, Table 2, Fig. 4). At an initial stocking density of 4,350 m^{-3}, 75% of prawns had survived and grown to a mean weight of 0.56 g after 8 weeks (Table 2, Fig. 4). The average survival across the four raceways was 72.6% and the average biomass at harvest was 1.86 kg m^{-3}. The food conversion ratio was 1.6:1.

Development of the genetic marker

The probability that two randomly taken individuals have identical genotypes (*I*), ranged from 0.290 for CSGES120 to 0.013 for Pe1.1, which has many more alleles and genotypes (Table 3). If all eight loci were typed, the overall probability of identity value (i.e. the multilocus *I*) would be 5.07 x 10^{-10}, or 1 in 2.0 x 10^{9}, that two prawns taken at random would share the same 8-locus genotype.

For individual loci, the probabilities that the genotype of any hatchery-reared offspring might be compatible with a randomly-chosen wild-spawning female (P_m) are all quite high (Table 4). However, across all loci, P_m falls to 0.0082. In other words, using the eight microsatellites, there is about a 1 in 122 probability that there will be a spurious compatibility match between a hatchery-reared genotype and the genotype of a mother chosen at random. If the genotypes of both the hatchery mother and father are known, then the probabilities (P_{mp}) that an individual chosen at random will be compatible with a mother and father chosen at random, are very much smaller (Table 4). Across all eight loci, P_{mp} is only 1.52 x 10^{-6}.

If 10 mothers produced the progeny in the hatchery, and we only know the maternal genotypes, then P_m increases from 0.0082 for a single mother to 0.079, compared with 1.52 x 10^{-5} if we know both the maternal and paternal genotypes.

These apparently small *P* values must be assessed with respect to the number of likely parental pairs in the wild population. For example, even with the low P_{mp} = 1.52 x 10^{-6}, if there are 10^{6} wild parent pairs in the population, there is about a 78% chance that one will be compatible by chance, so finding compatibility wouldn't be a good basis for saying "this prawn was probably hatchery-raised".

The proportions of hatchery-released and wild-born prawns expected to be present in a recaptured sample can be estimated, using genetic information on the hatchery parents and wild population. The proportion of wild-born individuals in the population is given by p_w, with its estimate being $\hat{p}_w$.

Then if n_H = number of hatchery born prawns recaptured, and n = total number of recaptures,

$\hat{p}_w = 1 - (n_H/n)$

However, n_H is not known for certain because of accidental compatibilities with wild parents. This is taken into account in the formulation

$\hat{p}_w = (n-n_c)/(p_o n)$

where n_c = number of recaptures that are hatchery-compatible, and p_o (the probability of incompatibility with hatchery broodstock) = $(1-P)^h$, where P is the probability of accidental compatibility with a wild-parent pair, P_m or P_{mp} from the above depending on whether only maternal or maternal and paternal genotypes are known, and h = number of hatchery pairs.

The variance of our estimate of the proportion of wild-born prawns is:
$V\hat{p}_w = p_w(1-p_o p_w)/(np_o)$, with the square root of this term being the standard error.
For 1,000 recaptures, if both maternal and paternal hatchery genotypes are known, low estimates of Vp_w are always achieved, regardless of the number of hatchery pairs used to produce the prawns for enhancement (Table 5). However, if only the maternal genotype is known, the eight loci are unlikely to be powerful enough to estimate p_w with reasonable precision. This can only be achieved if the hatchery mothers produce huge numbers of released offspring. For example, if less than ten mothers can produce enough offspring to achieve a 3% enhancement of the wild population (p_w = 0.97), then a reasonable estimate of p_w can be derived from 1,000 genotyped recaptures. In many circumstances, such as knowing the genotypes of both parents and enhancement of 3% or greater, just 100 to 200 recaptures will provide sufficient power. Additional markers would reduce the estimates of P_m and/or P_{mp}, and hence increase our ability to detect released prawns. Note that the discrimination between released and wild prawns is more than adequate using eight microsatellites if we can determine the genotypes of both parents.

In the commercial scale production system for *P. esculentus,* it is currently not feasible to directly determine the genotype of the fathers – mated females are collected from the wild and induced to release their eggs in the hatchery. It may be feasible to deduce the genotype of the male by genotyping a sample of larvae from the spawning female. However, this sample would need to be taken quite soon after spawning because it is difficult to maintain large numbers of individual females and their progeny for long periods. If this was not feasible, maternal only genotyping would be possible if an additional 3 microsatellites with the resolution of Pe1.1 were found. At this stage, without knowledge of the identity of the male fathers of enhanced prawns, the eight microsatellites are not able to unequivocally identify enhanced from wild *P. esculentus*. However, the described methodology would be readily applicable to other enhancement projects, especially where the male genotype is known.

Identification of potential release sites

Our diving survey in June 1999, 2 months after Cyclone Vance, found evidence of a major disturbance in the shallow waters of Exmouth Gulf. In many areas, the seafloor had strong ripples at depths of up to 7 m, indicating high seas associated with the cyclone. In other areas, the substrate was covered with a few centimetres of silt and a high sediment load was still present in the water column.

The cover of both seagrasses and macroalgae in June 1999 was very low – less than 2% at all sites. Seagrasses were restricted to the southern and southwestern sections of the Gulf and in these areas, most seagrasses were found in the shallows (<3 m). In some areas, although no seagrass was found above the sediments, we found healthy rhizomes a few centimetres below the surface of the sediments. By November 1999, the seagrasses of the eastern Gulf showed some signs of recovery, with up to 20% cover at some sites in the eastern Gulf (Fig. 5). Very little seagrass was found in the western and northern parts of the Gulf. By November 2000, the cover in some areas had increased to 40% and this was 80 to 100% by December 2001. In fact by December 2001, 60 to 80% seagrass cover was recorded at most sites from about 22° 15' S, 20 km south to the south eastern end of Exmouth Gulf (Fig. 5). Our results suggest that there is now a continuous 'seagrass meadow' in this region that is about 3 to 4 km wide – an area of between 60 and 80 km^2.

Initially, the regenerating seagrasses were mostly *Halophila ovalis*, *H. dicipiens* and *Halodule uninervis*, although some sparse *Cymodocea serrulata* and *Syringodium isoetifolium* were found. In 1999, *C. serrulata* was only found at one or two sites during our survey and *S. isoetifolium* was not seen at all. In December 2001, the dominant seagrass species were high biomass, medium shoot density species such as *Halophila spinulosa*, *Syringodium* and *Cymodocea* in the 2 to 4 m depth strata. These species are taller (20 to 30 cm canopy height) than the dominant species in 1999 and 2000 (*Halodule uninervis* and *Halophila ovalis* – 2 to 3 cm canopy height). *Halophila spinulosa* now dominates the seagrass community and makes up 60-80% of the seagrass cover at most sites.

The mean catches of postlarvae in beam trawls did not differ between November 1999 and November 2000. The catches of juvenile prawns were, however, significantly higher in 2000 (3 juveniles 100 m^{-2}) than 1999 (<0.5 juveniles 100 m^{-2}) but still considerably lower than juvenile *P. esculentus* catch rates from seagrasses in Moreton Bay and the Gulf of Carpentaria, (25 to 100 juveniles 100 m^{-2}) (O'Brien 1994, Loneragan et al. 1998). The 80 metric tonne (t) commercial catch in the 2000 fishing season was the lowest recorded since 1983 (Fig. 1). Landings did however increase to about 200 tonnes in 2001, paralleling the greater extent of seagrass and higher juvenile catches in 2000, and indications are that the 2002 catch will exceed 350 tonnes, in parallel to the increase in seagrass biomass in 2001.

Estimation of the area of habitat suitable for juvenile Penaeus esculentus

During times of recruitment to seagrass beds where the total above ground biomass of seagrass ranged from 6 to 123 $g.m^{-2}$, catches of juvenile *Penaeus esculentus* ranged from 0.30 to 4.1 prawns per m^2 (O'Brien 1994, Haywood, Vance & Loneragan 1995, Loneragan et al. 1998). Caging experiments on a seagrass bed of 70 $g.m^{-2}$ found that juvenile *P. semisulcatus*, a species with similar juvenile habitat requirements to those of *P. esculentus*, survived and grew at densities of up to 32 $prawns.m^{-2}$ (Loneragan et al. 2001a). If we assume that at least 5 $g.m^{-2}$ of seagrass are needed for postlarval settlement, survival and growth, the total area of seagrass in Exmouth Gulf that meets this criterion can be estimated. This provides an indication of the corresponding number of juveniles that potentially could be stocked.

In 1999, the estimated area of seagrass that had at least 5 $g.m^{-2}$ of seagrass above-ground biomass was 2.9 km^2, which had increased to 16.5 km^2 in 2000 and about 50 km^2 in 2001. At a release density of 1 $prawn.m^{-2}$, all the seagrass in Exmouth Gulf would support a release of only 2.9 million prawns in 1999, 16.5 million prawns in 2000, and 50 million prawns in 2001. It would not have been possible to release the estimated 24 million juvenile prawns to achieve a 100 t commercial-scale enhancement (see bio-economic model section below) in 1999 or 2000. This could have been achieved in these 2 years only at release densities of 8.6 $prawns.m^{-2}$ in 1999 and 1.5 $prawns.m^{-2}$ in 2000, and assumes that density-dependent effects are not significant. The extent and biomass of the seagrass beds therefore impact on the scale of feasible enhancement, the release densities for enhancement and the potential size of density-dependent effects on growth and mortality after release.

Bio-economic model

From the assumptions and initial parameters used in the development of the bio-economic model, it was estimated that a release of 14 million juvenile 1 g prawns would be needed for the fishery to catch about 100 t of released prawns (Loneragan et al. 2001b). Introducing terms for mortality at harvest, transport and release (mean = 5%, range = 2 to 8 %) and density-dependent mortality from release to emigration from the nursery (8.2 to 23%) increased the number of prawns to be released to 24 million.

The simulations showed that the wild catch ranged from 116 to 968 metric tonnes (t) and was normally distributed with a median of 417 t and a standard deviation of 123 t (Fig. 6a). These

estimates match the range of catches recorded for the last 15 years of the Exmouth fishery. The enhanced catch was much less variable with a median of 113 t, (range = 52 t to 171 t), and was close to normally distributed with a standard deviation of 17 t (Fig. 6b). This was to be expected, as the model does not contain parameters for the impacts of cyclones, which are reflected in the wild stock estimates. If we assume an average weight at capture in the fishery of 25 g, about 4.52 million enhanced prawns are caught, with an estimated recovery rate of 18.8 %. This compares with recapture rates of *P. japonicus* [*Marsupenaeus japonicus*] in the Seto inland sea of 22 and 27.6% in two consecutive years (Tanida, Ikewaki, Aoyama, Okuyama, Nozaka & Fujiwarra 2002) and is higher than those reported for *P. chinensis* [*Fenneropenaeus chinensis*] (5 to 12.5%) and *P. japonicus* (6.8% to 10.2%) in China (Wang, Zhuang & Deng 2002).

Our simulations estimate that the production of 24 million juvenile prawns would require 179 raceways. The total cost of production in raceways ranged from AUD $1.16 million (M) to $1.56 M, with a median of AUD $1.40 M (Fig. 7a). Over one third of these costs were for the hatchery production of postlarvae (46%), with 18% for salaries, 13% for feed, 12% for electricity (pumping and aeration) and about 10% for transport. The marginal revenue for the enhanced stock ranged from AUD $410,000 to $2.93 M, with a median marginal revenue of AUD $1.35 M. The difference between costs (hatchery, production, harvest, transport and release, and monitoring) in production and the marginal revenue from enhancement (an index of "profit") varied between a loss of AUD $950,000 and a "profit" of $1.53 M, with a median loss of AUD $170,000 (Fig. 7b). There was a 48% chance of making a "profit" from enhancement. However, the "profit" used in this study should not be interpreted as net profit because no costs of capital investment (interest and depreciation) were included.

The capital costs associated with the production of 113 t of enhanced catch would be substantial and require the construction of 179 raceways, if all prawns are to be produced in one production run. We have estimated that these costs (excluding the value of land) are about AUD $4.6 M for raceways. A further AUD $3.6 M would be needed to expand the existing hatchery facilities to produce the postlarvae for stocking in the raceways. The capital costs for the production facilities would be reduced by half if juvenile prawns were produced in two runs. However, we have not been able to assess the effect of different release times, as the bio-economic model is not yet structured to handle this more complex scenario.

Using the model to complete a sensitivity analysis showed that the highest proportion of variation in forecast "profit" was for the market price of enhanced prawns (35.2%), followed by the price of wild prawns (34.7%), and the female growth parameter k from the von Bertalannfy growth equation (12.6%). No other terms contributed more than 10% to the variation in profit and density-dependent mortality after release accounted for 1% in the variation. These results suggest that the price and growth of females are influencing "profit" the most. Note that changing values in the model has a great impact on the results from the sensitivity analysis and the proportion of variation accounted for by each variable. We had to estimate values for mortality at harvest, transport and release and density-dependent mortality immediately following release. In other studies, research on developing effective release strategies for enhancement has significantly increased the likely success of enhancement. For example, experiments on the size and time of release of striped mullet (*Mugil cephalus* Linnaeus) increased the contribution of hatchery raised individuals to juvenile recruitment by over 600% in 3 years, with no apparent replacement or displacement of wild recruits (Leber & Arce 1995, Leber, Arce, Sterritt & Brennan 1996, Leber 2002).

Components of Stage 3 enhancement research

Following the second project external review in August 2001, the Steering Committee recommended that the next phase of research (Stage 3) should focus on investigating different release strategies, including the effects of size, season, and site at release on survival and

density-dependence in the nursery ground (see also Leber & Arce 1995, Munro & Bell 1997). It was recommended that these studies should be carried out over 2 years, prior to a pilot release of 1 to 3 million juvenile prawns in the third year. The information from these studies should greatly reduce the uncertainty in our estimates of mortality and density-dependent effects in the nursery habitats and potentially in the fishery. These variables are likely to interact significantly with any multiple release strategy flowing from the need to reduce the capital costs of the raceway system. During Stage 3 research, new estimates for parameters would be included in the model and the bio-economic feasibility of commercial enhancement revised. The model should be further developed as a generic model so that it can be readily applied to other species and transferred to a different modelling framework to make it more "user-friendly" and capable of evaluating different enhancement scenarios. Research on genetics would test the feasibility of genotyping larvae to deduce the paternal genotype or develop additional microsatellite markers.

Conclusions

While only the initial two stages of the planned research program have been completed, the work to date has provided some significant results for enhancement research. The development of high-density raceway production systems for juvenile *P. esculentus* has been particularly successful and has benefits for both future enhancement and prawn aquaculture in regions where growing seasons are restricted. The initial nursery culture in raceways has the significant potential for decrease the field growout period. The raceway technology developed will probably be applicable to other "tiger" prawn species such as *P. monodon* and *P. semisulcatus*, which occupy structured habitats (seagrass and mangrove) in the wild.

The work on the seagrass nursery areas in Exmouth Gulf has secondary benefits for understanding fluctuations in wild stocks and the reasons for reduced productivity following some severe cyclone events. Ongoing monitoring of seagrass biomass in Exmouth Gulf is expected to generate a new environmental variable for inclusion in the fishery stock assessment process.

The genetic results provide valuable information on the population genetics of *P. esculentus* and indicate how research might progress to provide a method of identifying enhanced prawns in a production system with unknown paternal genotypes. The methodology developed has clear applications to other enhancement studies where both parents are known, e.g. homarid lobsters and finfish generally.

The initial outputs from the bio-economic model indicate that the probability of enhancement being economically viable in Exmouth Gulf is low, given the current values for the product and the costs of juvenile production and the estimated natural mortality rates. However, the bio-economic model provides a rigorous framework for assessing the biological and economic feasibility of enhancement proposals, incorporating growth and mortality through all stages up to capture by the fishery, as well as the production and operating costs. Of particular value is the ability to the sensitivity of "profit" from enhancement to biological and economic parameters. This can be used to identify areas for future research and where systems could be improved to increase the chance of successful enhancement.

Acknowledgements

We thank the Fisheries Research and Development Corporation of Australia for supporting this research. This paper summarises the efforts of many people across several disciplines: in particular we thank Stuart Arnold, Melony Sellars, Sandy Keys, Tim Quick and other staff of MG Kailis Exmouth Hatchery (Production); Jennifer Meadows, and Peter Grewe (Genetics); Mick Haywood, Rob Kenyon (Ecology); Bronwyn Harch, Yimin Ye, Nick Caputi, Mervi Kangas (Model) and Ian Poiner, George Kailis and Patrick Hone (Steering Committee). Mark

Bravington (CSIRO Mathematics and Information Sciences, Hobart, Australia) developed the analytical solutions for establishing the identity of individuals. Thanks also to the external reviewers (Harry Campbell, Ray Hilborn and Ken Leber) of the project for their constructive comments and suggestions for improving the project.

References

Blankenship H.L. & Leber K.M. (1995) A responsible approach to marine stock enhancement. *Transactions of the American Fisheries Society Symposium* **15**: 167-175.

Caputi N., Penn J.W., Joll L.M. & Chubb C.F. (1998) Stock-recruitment-environment relationships for invertebrate species of Western Australia. In: Proceedings of the North Pacific Symposium on Invertebrate Stock Assessment and Management. Jamieson G.S. & Campbell A. (eds.) *Canadian Special Publications in Fisheries and Aquatic Sciences* **125**: 247-255.

Davenport J., Ekaratne S.U.K., Walgama S.A., Lee D. & Hills J.M. (1999) Successful stock enhancement of a lagoon prawn fishery at Rekawa, Sri Lanka using cultured post-larvae of penaeid shrimp. *Aquaculture* **180**: 65-78.

Farmer A.S.D. (1981) Historical review of the Kuwait shrimp culture project. *Kuwait Bulletin of Marine Science* **2**, 3-9. (International Shrimp Releasing, Marking and Recruitment Workshop, Kuwait 1978)

Fushimi H. (1999) How to detect the effect in releasing operation of hatchery raised Kuruma prawn postlarvae? – case study of the operation in the Hamana Lake. *Bulletin of the Tohoku. National Fisheries Research Institute* **62:** 1-12.

Haywood M.D.E., Vance D.J. & Loneragan N.R. (1995) Seagrass and algal beds as nursery habitats for tiger prawns (*Penaeus semisulcatus* and *P. esculentus*) in a tropical Australian estuary. *Marine Biology* **122:** 213–223.

Kittaka J. (1981) Large scale production of shrimp for releasing in Japan and in the United States and the results of the releasing programme at Panama City, Florida. *Kuwait Bulletin of Marine Science* **2:** 149-163. (International Shrimp Releasing, Marking and Recruitment Workshop, Kuwait 1978)

Kurata H. (1981) Shrimp fry releasing techniques in Japan, with special reference to the artificial tideland. *Kuwait Bulletin of Marine Science* **2:** 117-147. (International Shrimp Releasing, Marking and Recruitment Workshop, Kuwait 1978)

Leber K.M. (2002) Advances in marine stock enhancement: shifting emphasis to theory and accountability. In: Stickney R.R. & McVey J.P. (eds). *Responsible Marine Aquaculture*. CAB International, pp. 79-90.

Leber K.M. & Arce S.M. (1996) Stock enhancement in a commercial mullet, *Mugil cephalus* L., fishery in Hawaii. *Fisheries Management and Ecology* **3**: 261-278.

Leber K.M., Arce S.M., Sterritt D.A. & Brennan N.P. (1996) Marine stock-enhancement potential in nursery habitats of striped mullet, *Mugil cephalus*, in Hawaii. *Fishery Bulletin, US* **94**: 452-471.

Liu J.Y. (1990) Resource enhancement of Chinese Shrimp, *Penaeus orientalis*. *Bulletin of Marine Science* 47: 124-133.

Liao I-C. (2002) Averting food crisis in the 21st century: the role of stock enhancement and sea ranching. Book of abstracts, *Second International Symposium on Stock Enhancement and Sea Ranching*, Kobe, Japan 28th January – 1st February 2002.

Liu J.Y. (1990) Resource enhancement of Chinese Shrimp, *Penaeus orientalis*. *Bulletin of Marine Science* **47**: 124-133.

Loneragan N.R., Kenyon R.A., Haywood M.D.E. & Staples D.J. (1994) Population dynamics of juvenile tiger prawns in seagrass habitats of the north-western Gulf of Carpentaria, Australia. *Marine Biology* **119**: 133–143.

Loneragan N.R., Kenyon R.A., Staples D.J., Poiner I.R. & Conacher C.A. (1998) The influence of seagrass type on the distribution and abundance of postlarval and juvenile tiger prawns in the western Gulf of Carpentaria, Australia. *Journal of Experimental Marine Biology and Ecology* **228**: 175-196.

Loneragan N.R., Heales D.S., Haywood M.D.E., Kenyon R.A., Pendrey R.C. & Vance D.J. (2001a) Estimating the carrying capacity of seagrass for juvenile tiger prawns (*Penaeus semisulcatus*): enclosure experiments in high and low biomass seagrass beds. *Marine Biology* **139**: 343-354.

McCook L.J., Klumpp D.W. & McKinnon A.D. (1995) Seagrass communities in Exmouth Gulf, Western Australia: a preliminary survey. *Journal of the Royal Society of Western Australia* **78**: 81-87.

Meadows J.R.S., Ward R.D., Grewe P.M., Dierens L.M., & Lehnert S.A. in press. Characterisation of 23 tri- and tetra-nucleotide microsatellite loci in the brown tiger prawn, *Penaeus esculentus*. *Molecular Ecology Notes.*

Moore S.S., Whan V., Davis G.P., Byrne K., Hetzel D.J.S. & Preston N. (1999) The development and application of genetic markers for the Kuruma prawn *Penaeus japonicus*. *Aquaculture* **173**: 19-32.

Munro J.L. & Bell J.D. (1997) Enhancement of Marine Fisheries Resources. *Reviews in Fisheries Science* **5**: 185-222.

O'Brien C.J. 1994. Population dynamics of juvenile tiger prawns *Penaeus esculentus* in south Queensland, Australia. *Marine Ecology Progress Series* **104:** 247-256.

Paetkau D., Strobeck C. (1994) Microsatellite analysis of genetic variation in brown bear populations. *Molecular Ecology* **3**: 489-495.

Penn J.W., Watson R., Caputi N. & Hall N. (1997) Protecting vulnerable stocks in multi-species prawn fisheries. In: Hancock D., Smith D., Grant A. & Beumer J. (eds.). *Developing and sustaining world fisheries resources: the state of science and management.* Proceedings of the Second World Fisheries Congress, August 1996, Brisbane, Australia, pp. 122-129.

Poiner I.R., Staples D.J. & Kenyon R. (1987) Seagrass communities of the Gulf of Carpentaria, Australia. *Australian Journal of Marine and Freshwater Research* **38**: 121-131.

Rothlisberg P.C., Preston N.P., Loneragan N.R., Die D.J. & Poiner I.R. (1999) Approaches to reseeding penaeid prawns. In: Howell BR, Moskness E, Svasand T (eds.), pp. 365-378. *Stock enhancement and sea ranching*. First International Symposium on Stock Enhancement and Sea Ranching, Bergen, Norway. Blackwell Science, Oxford.

Su M.S. & Liao I-C. (1999) Research and development of prawn stock enhancement in Taiwan. In: Howell BR, Moskness E, Svasand T (eds.). *Stock enhancement and sea ranching*. First International Symposium on Stock Enhancement and Sea Ranching, Bergen, Norway. Blackwell Science, Oxford, pp. 379-392.

Tanida K, Ikewaki Y, Aoyama E, Okuyama Y, Nozaka M, Fujiwarra M. (2002) Investigation of stocking effectiveness for kuruma prawn (*Penaeus japonicus*) in the eastern Seto inland sea. Book of abstracts, *Second International Symposium on Stock Enhancement and Sea Ranching*, Kobe, Japan 28th January – 1st February 2002.

Wang Q., Zhuang Z. & Deng J. (2002) The development and prospects of stock enhancement and sea ranching industry China. Paper for *Second International Symposium on Stock Enhancement and Sea Ranching*, Kobe, Japan 28th January – 1st February 2002.

Werckmand C, Hardy T, Wainwright E (1998). *Crystal Ball User Manual*. Decisioneering, Colorado.

Xu J., Xia M., Ning X. & Mathews C.P. (1997) Stocking, enhancement, and mariculture of *Penaeus orientalis* and other species in Shanghai and Zhejiang Provinces, China. *Marine Fisheries Review* **59:** 8-14.

Young P.C. & Carpenter S.M. (1977) Recruitment of postlarval penaeid prawns to nursery areas in Moreton Bay, Queensland. *Australian Journal of Marine and Freshwater Research* **28**: 745-773.

Table 1 **The experimental design to test for differences in growth and survival of juvenile Penaeus esculentus grown in raceways at the Cleveland laboratories in Brisbane, Australia. N = 3 replicates for each treatment.**

Comparison and stocking density (no. prawns m^{-3})	Structure
(a) Structure	
2,860	Structure A (3 structures, including Aquamat®)
2,860	Structure B (2 structures but not Aquamat®)
(b) Density	
5,720	Structure C (AquaMat® only)
11,430	Structure C (AquaMat® only)

Table 2 **Summary of raceway characteristics and results from high-density production trials in four raceways (R) in Exmouth Gulf, Western Australia.**

Volume of water in Raceway (m^3)	Initial Stocking Density (prawns·m^{-3})	Grow-out Period (days)	Number of Prawns Harvested	Mean Harvest Weight (g)	Final Harvest Density (prawns/nos m^{-3}: kg m^{-3})	Survival (%)
R1: 57.5	2,610	77	105,803	1.20	1,840: 2.21	70.5
R2: 57.5	3,480	72	129,265	0.94	2,248: 2.11	64.6
R3: 34.5	4,350	57	112,556	0.56	3,263: 1.83	75.0
R4: 34.5	2,610	57	72,396	0.62	2,098: 1.30	80.4

Table 3 **Data on the variability of eight microsatellites in two samples of 96 Penaeus esculentus Haswell from Exmouth Gulf, Western Australia.**

Locus	Number screened	Allele number	Expected heterozygosity	Probability of identity (I)
CSGES120	183	11	0.496	0.290
CSGES189	186	11	0.737	0.112
CSGES047	187	19	0.889	0.022
CSGES176	186	15	0.843	0.040
CSGES268	183	16	0.713	0.099
CSGES190	180	8	0.518	0.270
Pe1.1	186	27	0.914	0.013
Pmcd01	185	10	0.828	0.051

Table 4 **Chances of any given recapture randomly matching a randomly-chosen mother (Pm), or randomly-chosen maternal-paternal pair (Pmp), given a single hatchery mother or hatchery pair.**

Locus	P_m	P_{mp}
CSGES120	0.8653	0.5377
CSGES189	0.6691	0.3093
CSGES047	0.3634	0.0760
CSGES176	0.4663	0.1260
CSGES268	0.6516	0.2388
CSGES190	0.8542	0.5229
Pe1.1	0.2922	0.0469
Pmcd01	0.5115	0.1630
Over all loci	0.0082	1.52×10^{-6}

Table 5 **Standard errors of the estimated proportions of wild-born prawns ($\hat{p}$ w) after enhancement, with pw of 0.99, 0.97, 0.90 and 0.80, and where maternal genotypes only, or maternal and paternal genotypes, are known, and for varying numbers of hatchery mothers and recaptures, for the eight microsatellite markers. (Figures in bold are estimates of SE of pw that are reasonably acceptable, i.e. less than about half the difference between pw and 1.)**

		p_w = 0.99		p_w= 0.97		p_w = 0.90		p_w = 0.80	
Number of Hatchery mothers	Recaptures	SE of p_w Mother only	Mother Father	SE of p_w Mother only	Mother Father	SE of p_w Mother only	Mother Father	p_w Mother only	Mother Father
1	10	0.043	0.032	0.061	0.054	0.099	0.095	0.129	0.127
1	100	0.013	0.010	0.019	0.017	**0.031**	**0.030**	**0.041**	**0.040**
1	1000	**0.004**	**0.003**	**0.006**	**0.005**	**0.010**	**0.010**	**0.013**	**0.013**
5	10	0.072	0.032	0.084	0.054	0.113	0.095	0.139	0.127
5	100	0.023	0.010	0.026	0.017	**0.036**	**0.030**	**0.044**	**0.040**
5	1000	0.007	**0.003**	**0.008**	**0.005**	**0.011**	**0.010**	**0.014**	**0.013**
10	10	0.097	0.032	0.106	0.054	0.129	0.095	0.151	0.127
10	100	0.031	0.010	0.034	0.017	**0.041**	**0.030**	**0.048**	**0.040**
10	1000	0.010	**0.003**	**0.011**	**0.005**	**0.013**	**0.010**	**0.015**	**0.013**
50	10	0.227	0.032	0.229	0.054	0.234	0.095	0.238	0.127
50	100	0.072	0.010	0.072	0.017	0.074	**0.030**	**0.075**	**0.040**
50	1000	0.023	**0.003**	**0.023**	**0.005**	**0.023**	**0.010**	**0.024**	**0.013**
100	10	0.357	0.032	0.356	0.054	0.352	0.095	0.344	0.127
100	100	0.113	0.010	0.113	0.017	0.111	**0.030**	0.109	**0.040**
100	1000	0.036	**0.003**	0.036	**0.005**	**0.035**	**0.010**	**0.034**	**0.013**
1000	10	19.307	0.034	19.111	0.055	18.409	0.096	17.356	0.127
1000	100	6.105	0.011	6.043	0.018	5.821	**0.030**	5.488	**0.040**
1000	1000	1.931	**0.003**	1.911	**0.006**	1.841	**0.010**	1.736	**0.013**

Figure captions

Figure 1 **Historical landings (metric tonnes, solid histograms) and nominal effort (hours of fishing) of tiger prawns in the Exmouth Gulf prawn fishery between 1963 and 2001.**

Figure 2 **The stock recruitment relationship of the Exmouth Gulf tiger prawn stock (from Caputi et al. 1998).**

Figure 3 **Mean weights of juvenile *P. esculentus* at weekly intervals grown in a raceway system in Brisbane, Australia, from PL17, (a) stocking density of 2,860 prawns.m-3 comparing Structure A with Structure B and (b) comparing stocking densities of 5,720 m-3 and 11,430 m-3 in Structure C. Water temperature = 27 ºC during these experiments.**

Figure 4 **Mean weights of juvenile *P. esculentus* at weekly intervals grown in a raceway system in Exmouth Gulf, Australia, from PL15. Initial stocking densities were 2,610 prawns.m-3 in raceways 1 and 4 (R1, R4), 3,480 prawns.m-3 in raceway 2 (R2) and 4,350 prawns.m-3 in raceway 3 (R3).**

Figure 5 **Percent cover of seagrass estimated from 30 m underwater transects in November 1999.**

Figure 6 **Predicted catch of (a) wild and (b) enhanced prawns during a trial commercial-scale (113 metric tonnes) enhancement from 10,000 simulations of the bio-economic model. Note that the full range of values is not shown on this Figure or Figure 6a. 45 values for the wild catch and 32 for the enhanced catch are not shown.**

Figure 7 **Predicted (a) raceway production costs and (b) index of profit (difference between production costs in raceways and marginal revenue) (in millions of AUD $) obtained from the catch of the enhanced stock during 10,000 simulations of a commercial-scale enhancement (113 metric tonnes). 7 values on Fig. 7a and 82 on Fig. 7b are not shown.**

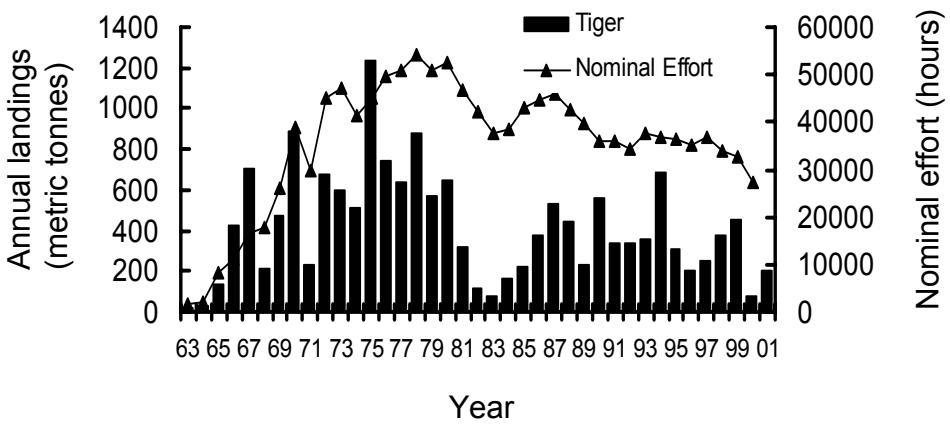


Figure 1 Loneragan et al.

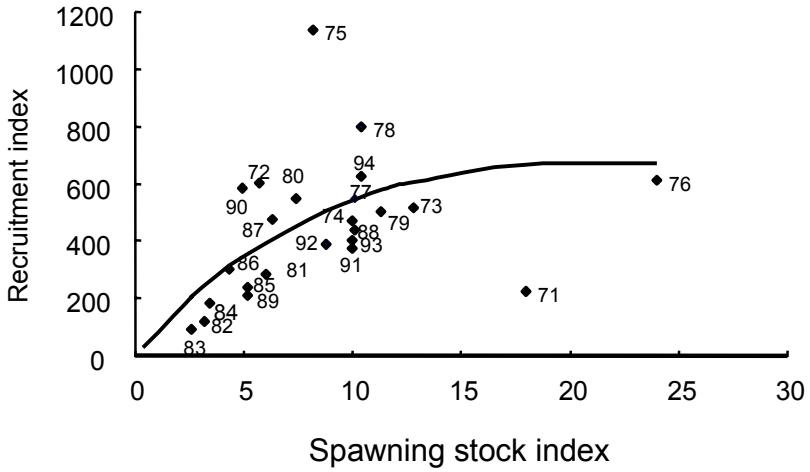


Figure 2 Loneragan et al.

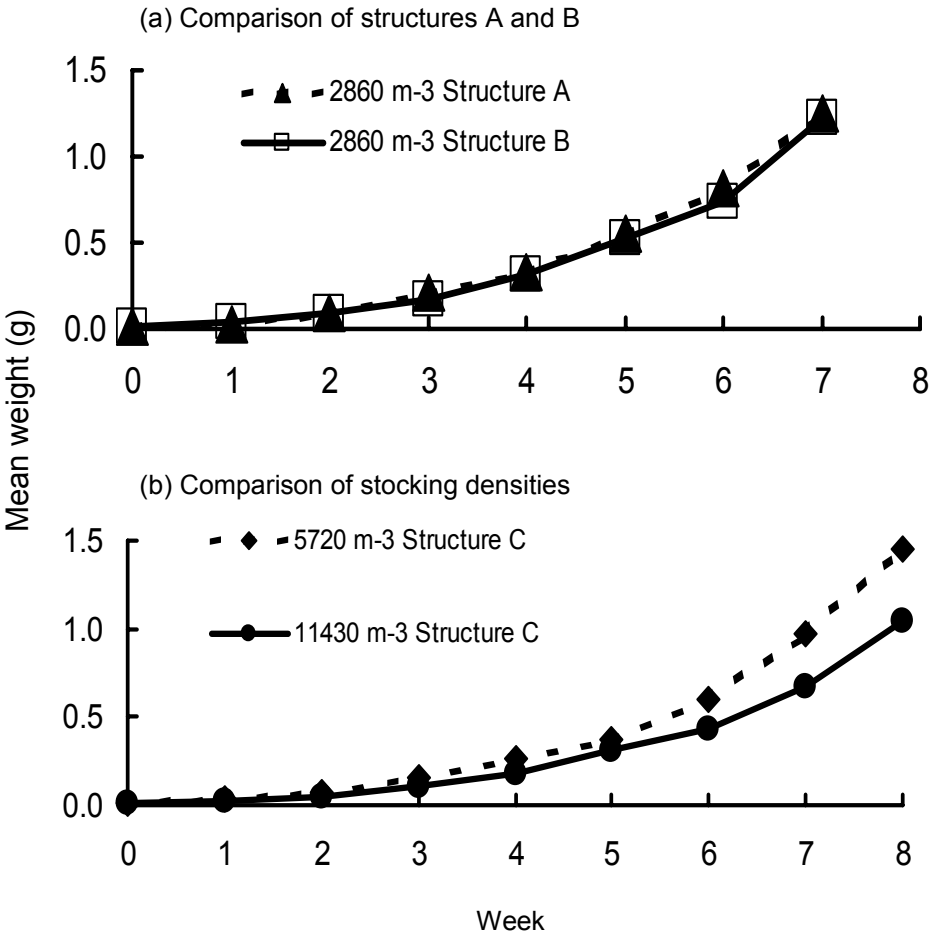


Figure 3 Loneragan et al.

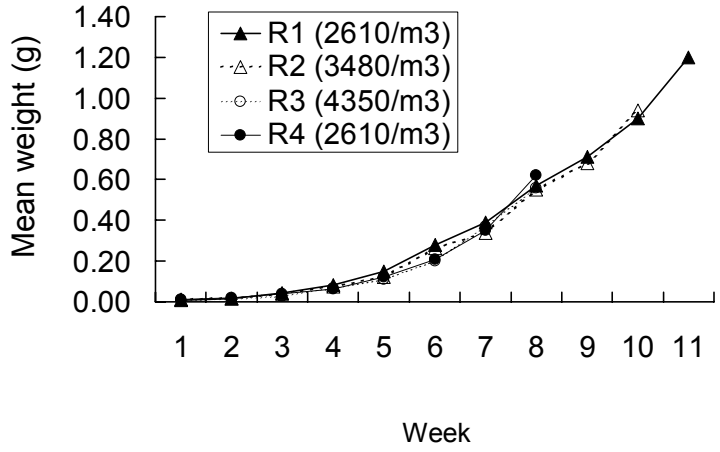


Figure 4 Loneragan et al.

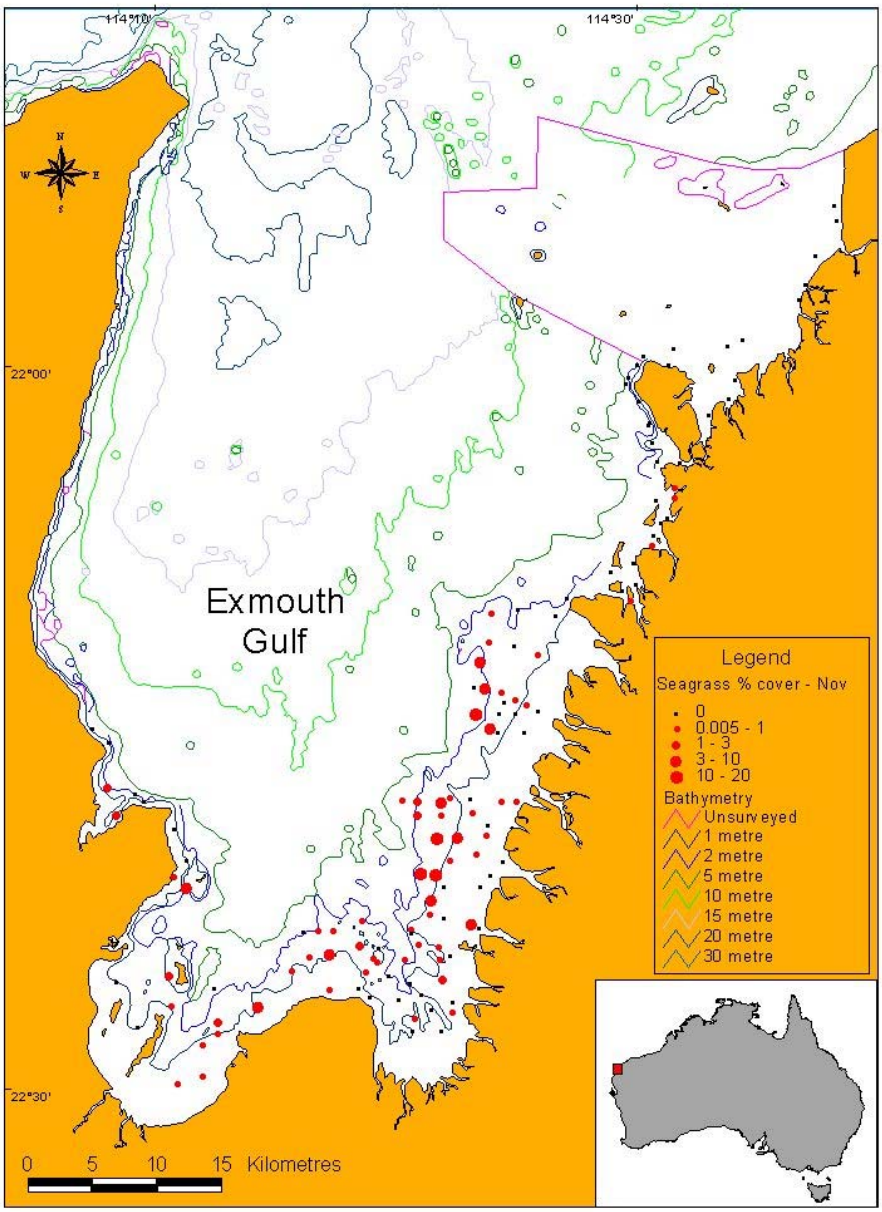


Figure 5 Loneragan et al.

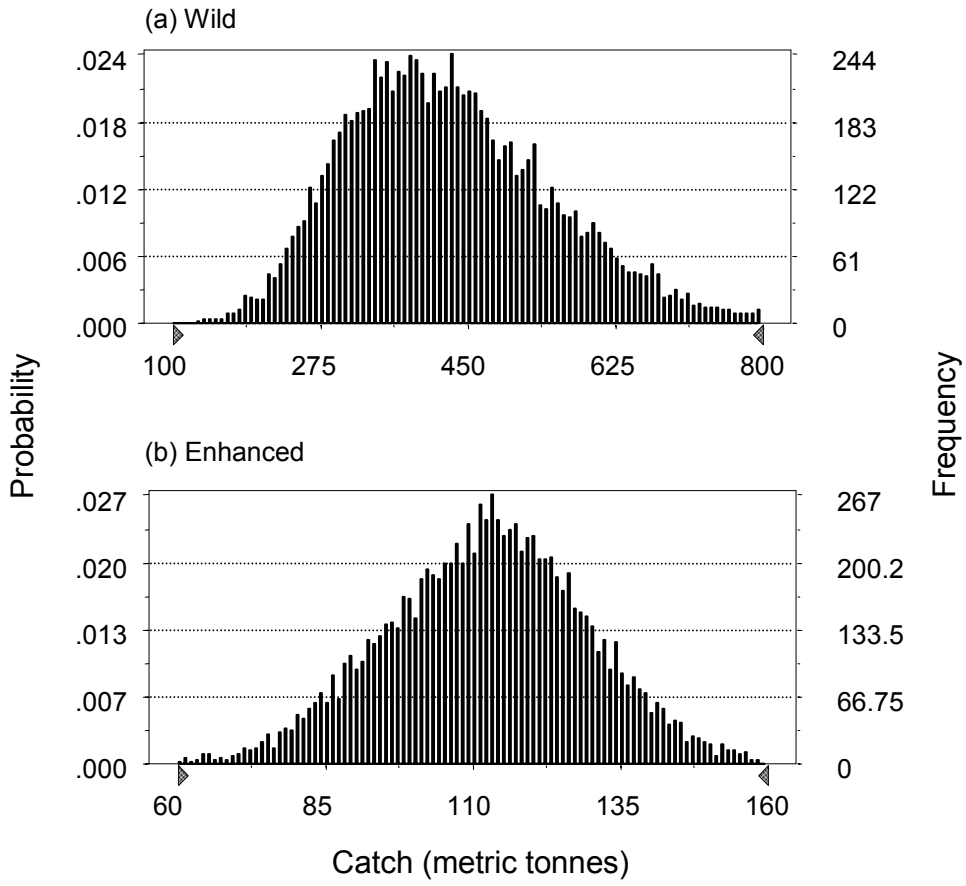


Figure 6 Loneragan et al.

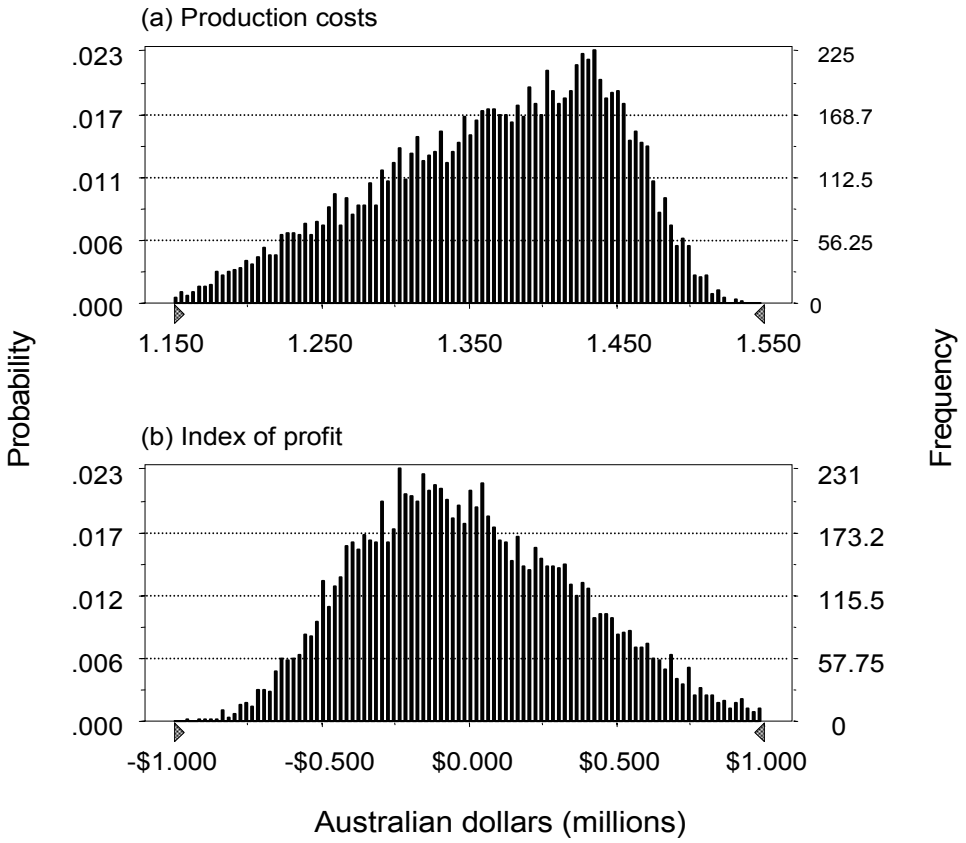


Figure 7 Loneragan et al.

Appendix 2B: Review of the Feasibility Report for FRDC 98/222 "Developing and assessing techniques for enhancing tropical Australian prawn fisheries and the feasibility of enhancing the brown tiger prawn *(Penaeus esculentus)* fishery in Exmouth Gulf", and the FRDC proposal for 1999/222 "Developing techniques for enhancing prawn fisheries, with a focus on brown tiger prawns *(Penaeus esculentus)* in Exmouth Gulf". By Professor Harry Campbell, University of Queensland.

The Feasibility Report for FRDC 98/222

(1) Assessment of the bioeconomic model

The conclusion drawn from the bioeconomic model (p. 4) is that the enhancement of tiger prawn stocks in Exmouth Gulf can be profitable. However the bioeconomic model deals mainly with the benefits of stock enhancement. The treatment of enhancement costs appears to be incomplete. For the enhancement program to be worthwhile the long-term benefits of enhancement (the new Stages 3 and 4 and subsequent enhancement) must exceed the costs. While the magnitude of the long-term benefits can be estimated by means of the bioeconomic model, we need to be confident, at the present stage of the project (Stage 2), that they are likely to be large enough to outweigh the capital costs which will be involved in Stages 3 and 4 and subsequently. I will first make some comments about the bioeconomic model and then turn to the issue of capital costs.

The bioeconomic model

The general approach of the bioeconomic model is sound. The annual returns from stock enhancement are calculated for a 10 year period, which seems a conservative estimate of the life of the assets used to produce the juvenile prawns. No additional capital costs are allowed for in the harvesting and processing sectors on the basis that excess capacity exists in those sectors. The expected net present value of the additional catch resulting from the trial commercial scale enhancement is reported as $3.4 million (p. 25). This figure is based on calculating the present value of the annual net benefits using the formula for a 10-year annuity which, at an appropriate rate of interest (10%), gives $3.4 million.

However it is not clear that this is a **net** present value as stated in the text (p. 25). While it is net of the operating costs of producing the enhanced juveniles, capital costs of $3 million are referred to (p. 25, value of land and facilities, presumably those used in producing juveniles), and it is stated earlier that the annual benefit of around $550,000 "does not include capital cost" (p. 24). The production sheet of the EXCEL spreadsheet seems to account for pumping, feed, salary and additional transport costs (SUM(L11:O11)) only, and so it seems that the $3 million capital cost should be subtracted from the $3.4 million present value of the benefits to give a net present value of $0.4 million (however, see further comments on the $3 million figure below). I assume that the capital cost of the hatchery are included in Preston et al.'s estimate. No allowance is made for capital costs of transport and release (p. 13), but they may be small.

From the above, and unless I have misinterpreted the calculations, it seems that the expected NPV of a 10 year trial commercial enhancement project is around $0.4 million, with considerable variation around that figure. However the project has a good chance of leading to full-scale commercial enhancement with substantial economic benefits. In addition, the project confers benefits on the wider community (p. 29).

I now have some more specific comments about various aspects of the economic modeling.

Prices

Prices are assumed to depend on prawn size but not on season ((1) p. 15). Unless the intra-annual distribution of the catch can be controlled (see comment below), this is a reasonable approach, provided that the price of prawns is not affected by the quantity supplied. This might be a reasonable assumption for the experimental or trial commercial projects but would require further examination in the case of a full scale enhancement.

If the additional prawn catch is to be exported then an effect on market price may be unlikely, but this could be considered (see below). If the additional catch is to be marketed domestically then there may be some effect on prices. A lower price received by producers for the existing catch would reduce the revenue to producers, but this would be offset by an equivalent gain to domestic consumers. If the additional prawns produced were valued at the lower price, this would correctly measure the benefit to producers but would understate the social benefit by the amount of the consumer surplus generated. A reasonable approach would be to value the additional prawns at an average of the prices with and without the project.

Variation in prawn prices was cited as a significant risk (p. 3) and included in the simulations. It might be possible to supplement this analysis with ABARE research results on prawn markets.

Costs

The assumption of no additional variable costs of fishing is based on the supposition that the same amount of trawling is conducted after the project as before, but that catches are simply higher because of the higher stock. No additional trawling time is allowed for. This is consistent with the harvesting model (p. 15) but may not be realistic given the schooling nature of prawns. More schools may have to be trawled to get the higher catches and this may increase fishing time and costs.

Variation in Catches

A long-term objective is to "decrease the inter-annual variation in catches and increase the long-tern annual catch" ((1) p. 7) (There is seems to be no reference to intra-annual variation and I assume there is no opportunity to influence this through the pattern of releases of farmed juveniles. If there were, then seasonal prawn prices would become relevant). According to the harvesting model (pp. 16-17) enhancement will reduce the variance of the catch, assuming the catch of enhanced prawns is constant, and it will reduce the coefficient of variation (which is a good measure of the variability processing operations have to cope with) even more, through higher mean catches. The higher mean catches, it is argued (Proposal p. 9) will allow fuller utilization of existing processing capacity, which presumably was installed in an earlier phase of the fishery when mean catches were higher. Since the capacity already exists I find this puzzling because the average catch of tiger prawns for the five most recent years cited (p. 7) is 377 tonnes, which doesn't seem to be significantly lower than the average catch in earlier periods (see Penn, Caputi and Hall (1995), Table 2). In other words, the evidence suggests that the tiger prawn fishery has been operating at its current level and variability for a long enough period that capacity would have had time to adjust to those circumstances. If that is the case, then there is likely to be no excess capacity and it could be argued that additional capacity might be required.

Against the latter argument is the point that capacity depends on the total prawn catch, not the tiger prawn catch alone, and that the percentage increase in total prawn catch is much less than the forecast 50% increase in tiger prawn catch.

Capital costs

I was not able to work out where the $3 million capital cost figure (p. 25) came from. Raceways are identified as the preferred production technique. They are costed at $25,000 each and 267 are required, according to the spreadsheet. This gives a capital cost of $6.675 million. However I understand from Dr Loneragan's email of April 19 that this is the cost of producing 15 million juveniles – twice the trial commercial scale (p. 23).

Raceways are identified as the preferred production technique on the basis of lower annual operating costs (p. 23). From the spreadsheet I interpret the annual operating costs of raceways as $0.47 million and those of ponds as $0.73 million. Ponds cost $50,000 each and 29 are required, according to the spreadsheet, giving a total cost of $1.45 million. Which is the lower cost alternative? It would seem to be ponds. The present values of capital and operating costs over the 10 year period at 10% are $9.56 million for raceways and $5.94 million for ponds. This comparison takes no account of possible differences in mortality and other relevant rates between the two techniques, and there may be technical reasons for preferring raceways.

One problem with the above conclusion about capital cost is that the raceways or ponds presumably have a longer life than 10 years and will be used in the full-scale project if it goes ahead. A longer economic life does not by itself change the conclusion that ponds are the lower cost alternative: an infinite life would still leave ponds about $3million cheaper in terms of NPV. However a longer life together with a lower interest rate (5%) could change the relative costs of the two techniques. This question may be worth further investigation, unless I have misinterpreted the figures.

However the main issue seems to be that of allocating capital costs between the experimental release (Stage 3), the trial commercial enhancement (Stage 4), and the full-scale enhancement program, assuming it goes ahead. No account is taken of what happens after year 10. Presumably the program goes on to its next stage if successful and the raceways or ponds continue to be used. The present value of the next stage multiplied by the probability of success could then be added as an expected benefit. The full scale enhancement is 7-10 times the scale of the experimental project, and twice the size of the trial commercial enhancement. I'm not sure what extra capital costs of producing juvenile prawns would be involved in subsequent stages (not to mention extra harvesting and processing capital costs) but if the net benefits (benefits less operating costs) of $3.4 million were scaled up and brought to a present value it would be a substantial sum.

(5) General comments.

(a) Given the conclusions of the paper by Penn et al. (1995), is there an additional benefit to the project because the enhanced stock is less likely to suffer a collapse in recruitment because of over-fishing?

(b) Exmouth Gulf is described as an ideal location for the prawn enhancement program (p. 7). One reason is that a single company holds 15/16 licences. This minimizes any possibility of wasteful competition for the additional prawns and is an important feature of this project.

The FRDC proposal for 1999/222

This proposal mainly concerns the biological research that is to be conducted and which seems to have been well thought out. However there a few points arise which are related to my comments on the Feasibility Study.

It is conceded (p. 12) that existing calculations about the production of juveniles are based on a best case scenario. More realistic figures are required to do a proper comparison of the cost of raceways relative to ponds.

If there are substantial capital costs of raceways or ponds then the economic life of these assets becomes important. It is pointed out (p. 12) that one of the risks is that of a cyclone damaging the facilities. I would have thought that the risk would be low, but this is one factor that should be taken into account in estimating economic life.

It is pointed out (p. 15) that the hatchery to be established in WA will be a shared facility to be used for pearl oyster production as well. This raises the question of cost allocation between the two activities.

Mention is made of an 11 ha site for the hatchery. The cost of that land is what it is worth in its best alternative use.

Appendix 2C: Review of the Feasibility Report for FRDC 98/222 "Developing and assessing techniques for enhancing tropical Australian prawn fisheries and the feasibility of enhancing the brown tiger prawn *(Penaeus esculentus)* fishery in Exmouth Gulf", and the FRDC proposal for 1999/222 "Developing techniques for enhancing prawn fisheries, with a focus on brown tiger prawns *(Penaeus esculentus)* in Exmouth Gulf".

By Professor Ray Hilborn, University of Washington.

Assessment of the bio-economic model

The basic structure and components of the bioeconomic model seem sound and well done. In particular I was pleased to see that the potential for competitive interaction between wild and enhanced individuals was included in the model in the equation in the middle of page 14 of the FRDC Final Report Design Standard

$$M_t = \lambda\left(N_t + N_t^*\right) + \alpha e^{\beta L_t}$$

In my analysis of other marine enhancement programs, competition between enhanced and wild stocks would appear to be the Achilles heel of marine enhancement programs. Hilborn and Eggers (2000) found this in pink salmon in Prince William Sound, Alaska. This is the largest salmon hatchery program in North America and the evidence suggests there has been a very small net increase in production. In a review (Hilborn 1998) I found evidence for replacement rather than enhancement in a number of other marine programs, perhaps best demonstrated in the experimental work the Norwegians have conducted on cod enhancement (Smedstad et al. 1994 cited in FRDC report). There is a review currently in press by a group of Norwegians on all the cod enhancement programs around the world showing there is no evidence for increasing wild production by enhancement.

So inclusion of the possibility of competition between wild and enhanced prawns is an essential part of a realistic bioeconomic model. Having said that, I would suggest several improvements.

Wild and enhanced prawns may be differential competitors. If the enhanced prawns are larger than the wild prawns, I would expect them to be better competitors. This could be implemented by modifying the equations as follows

$$M_t = \lambda\left(\tau N_t + N_t^*\right) + \alpha e^{\beta L_t}$$

where τ is the relative competitive ability of enhanced prawns when set >1. Alternatively one could weight the competitive ability by the length or weight of the wild and enhanced prawns. The model assumes that once prawns recruit, any density dependent mortality ceases and the mortality is density independent. This is a common assumption in fisheries models, but work by Ram Myers shows that for finfish density dependence continues through the first several years of life.

The value of λ isn't given in the analysis. On page 31 of the FRDC Final Report example parameter values are given, and under Mortality Larvae-Juvenile there are values for α and β but no mention of λ. Perhaps the variable "culture" on that page is λ but given that the text back on page 14 says "Survival **can** be made dependent on the total abundance of prawns" perhaps it has been assumed in the runs made that λ is zero. If this is the case, then all model runs are highly optimistic and do not, in fact, allow for replacement of wild production by enhanced prawns.

The traditional assumption in marine enhancement is that there is no such competition, that every additional individual that survives from enhancement is an increase in production. With the exception of the Norwegian work cited above, I know of know of no marine enhancement programs that have an experimental design to test for replacement rather than enhancement.

The basic biology of invertebrates, where most show little increase in average recruitment with increases in spawner abundance, suggest strong density dependence. I believe it would be possible to estimate the intensity of this density dependence for tiger prawns in Exmouth Gulf from the work of Penn and Caputi. I cannot tell from the documentation provided if this has been done. I recommend it should be done.

The hope in marine enhancement is to try to rear the enhanced individuals to sizes beyond which the most intense mortality has taken place, with the expectation being that most density dependent mortality is also over by that point. I don't know enough about the biology of prawns to know if this might be the case with tiger prawns, but again drawing on my experience with fishes, we have continued to be surprised by how much density dependent growth and survival there appears to be throughout the life history.

In Pacific salmon enhancement there are documented negative impacts on wild production due several other mechanism in addition to pure competition. Development of genetically distinct hatchery stocks with lower fitness in wild spawning, and interbreeding with wild fish, thus lower wild fish fitness.

Introduction of disease
Increase in predator populations due to enhancement
Assess the risks of enhancement of tiger prawns in Exmouth Gulf

Having highlighted the modeling concerns about replacement rather than enhancement and the consequent overestimation of benefits, I believe this needs to be addressed in the design of the program evaluation. The problem of detecting enhanced production is very similar to that of detecting oil spill impacts (Hilborn 1996). I can think of three ways that the monitoring program might assess the risk that the enhancement replaces rather than enhances production.

Spatial controls. To have some sites enhanced and some un-enhanced. I suspect this is the only experimental design that will provide the power to determine the true net production from enhancement. However I don't see that it is possible within the Exmouth Gulf. I would very much like to see the authors address the question of feasibility of spatial controls.

Temporal controls. One could use before and after comparison. This is a very weak experimental design as it provides no controls on environmental factors between years. However for before and after comparison to work the scale of the enhancement needs to be very large, and the planning documents suggest that initial trials will be small.

Model based comparison. You can just assume model parameters are true! I do not recommend this at all.

Assess the approach to developing techniques for production
Not my area of expertise

Assess the approach to selecting release sites
My only comment would be to explore the potential for having enhanced and un-enhanced sites and following wild and enhanced prawn growth and survival. I recommend that this be examined closely.

Provide general comments on the approach to enhancement
I will admit to great reservations about the basic design of this project. In my experience enhancement projects are most likely to provide net benefits where there is little if any interaction with wild stocks, and the enhancement is a replacement for wild production rather than enhancement of wild production. The only marine enhancement projects with significant wild production where a convincing case can be made that enhancement has augmented wild production are for sedentary invertebrates. This isn't to say that they don't exist, it is just that in the places where the data are available to evaluate replacement or enhancement, the data suggest replacement. The Exmouth Gulf wild stocks are described as healthy and well managed, and the motivation is to smooth natural variability. The presence of healthy, and economically valuable, wild stocks makes this intrinsically a very high risk project.

Given that there is a significant, and indeed I would suggest probably overwhelming, expectation of replacement rather than enhancement, there does not appear to be any possibility of experimental evaluation of whether replacement or enhancement has occurred. The lack of potential for spatial controls on enhanced and un-enhanced means that small scale projects cannot be evaluated. Before and after comparisons are the only realistic possibility and these require that the enhancement be large in relative to wild production, and such a large scale enhancement would pose significant genetic and health risks to the wild population, as well as great financial risk.

Clearly much of the motivation for this project is to determine the economic feasibility of prawn enhancement in Australia. I suggest that Exmouth Gulf is not a particularly good place for such trials. I suggest that an alternative location be found where spatial controls on enhancement could be conducted and where a large well managed wild stock is not at risk.

References

Hilborn, R. 1996. Detecting population impacts from oil spills: a comparison of methodologies. American Fisheries Society Symposium 18: 639-644.

Hilborn, R. 1998. The economic performance of marine stock enhancement projects. Bulletin of Marine Science 62: 661-674.

Hilborn, R. and D. Eggers. 2000. A review of the hatchery programs for pink salmon in Prince William Sound and Kodiak Island, Alaska. Transactions of the American Fisheries Society 129: 333-350.

Appendix 2D: Review of the Feasibility Report for FRDC 98/222 "Developing and assessing techniques for enhancing tropical Australian prawn fisheries and the feasibility of enhancing the brown tiger prawn *(Penaeus escultentus)* fishery in Exmouth Gulf", and the FRDC proposal for 1999/222 "Developing techniques for enhancing prawn fisheries, with a focus on brown tiger prawns *(Penaeus escultentus)* in Exmouth Gulf". By Dr Ken Leber, Director, Center for Fisheries enhancement, Mote Marine Laboratory.

Review Summary

I have carefully reviewed the Feasibility Report for FRDC 98/222, and the FRDC proposal for 1999/222 relating to developing techniques for enhancing prawn fisheries, with a focus on brown tiger prawns (*Penaeus esculentus*) in Exmouth Gulf. I have also reviewed all of the background materials included in the review packet. After careful consideration of these documents, I find the approach described therein to be quite well thought out, but seriously lacking in some areas that are critical to achieving the project objectives. These are not fatal flaws, as they can be resolved.

I strongly support the scientific rigor and background that has been infused into the design of this project. Stock enhancement, and marine stock enhancement in particular, has been poorly developed as a fishery management tool, in part because of lack of involvement of ecologists in the field. As important as the development of viable aquaculture production technology is in this field, the principal bottlenecks to successful development of marine stock enhancement today are unanswered ecological questions about the fate and effects of stocking — critical uncertainties, which must be addressed and understood to gain any semblance of control of enhancement effect. By including variables for some of the more important of these ecological uncertainties in the bioeconomic model (even though some of the key ones are not activated at this point), the Principal Investigators have set the stage for a rigorous test of stock enhancement potential to effect production of brown tiger prawns. The fact that there is a bioeconomic model at all attests to the focus on defining indicators of success from the outset of this project. This is rare in the field. By recognizing which uncertainties will have the most impact on the costs and benefits of stocking, using Monte Carlo simulations of parameter values in the bioeconomic model, the Principal Investigators have a good vehicle for directing research towards resolving the most critical uncertainties.

Given the poor history of marine stock enhancement (Hilborn, 1999; Leber, 1999), and the fact that enhancement programs are receiving a huge influx of funding into this poorly developed branch of fisheries science, and given that marine stock enhancement research is expensive, and thus rarely even attempted, we need programs with a scientific basis like this one to step forward and help evaluate the potential of this management tool. With incorporation and resolution of the issues listed below and in the following review, this project should be fully funded.

Attention is needed to incorporate approaches to the six issues highlighted below that are key to success of the project:

(1) Specific attention to conserving genetic diversity of wild stock

— Good consideration has been given to avoidance of hatchery-mediated translocation of exogenous genes into the Exmouth Gulf brown tiger prawn stock targeted for enhancement. However, attention is also needed to minimizing the potential for genetic swamping of tiger prawns and to the minimum number of *effective* hatchery breeders needed to conserve genetic

diversity of the wild stock. Attention is also needed to minimizing and hatchery selection, domestication, stochastic allele frequency changes, and reduced levels of variation can occur in the F1 generation.

(2) Testing assumptions about release strategies

— Although the critical importance of pilot experiments to determine optimal release strategies is discussed, there is no discussion of testing assumptions about release-strategy related (post-release) mortality as part of Stage 3 experimental releases. Evaluating enhancement impact on yields in the fishery across a range of release parameter values is a crucial step in planning the large scale test-of-concept planned for Stage 4 (e.g. field testing a range of size-at-release values; field testing a range of microhabitats; field testing different release methods to liberate prawns from tanks on deck to the nursery habitats below; by examining the differential effects of such experimental treatment conditions on yields in the fishery). Without such tests of these particular critical uncertainties, adaptive management potential is seriously curtailed, and the project objectives may be compromised by invalid assumptions about optimal release strategy. This area certainly needs to be reconsidered in both Stage II development work and Stage III experimental release work. I see difficulty in approaching multiple treatment conditions in pilot releases using genetic tags. Although the genetic tags can afford an adequate mark for examining the contribution of hatchery prawns to the enhanced stock, marking a portion of the prawns in each release with a high-information tag is needed to investigate the effect of release strategies on relative survival. Adapting use of a high-information content tag (e.g. coded-wire microtags) to tiger prawns is a research activity that should be pursued as part of Stage II work.

(3) Enhanced production versus displacement of wild individuals

— There is no discussion of the experimental control condition(s) that will be needed to evaluate the effect of stocking on the actual production of both wild and hatchery tiger prawns in Exmouth Gulf. Assumptions made about carrying capacity need to be tested by comparing wild recruitment in the enhanced stock(s) in Exmouth Bay with recruitment into wild stocks that are not enhanced.

(4) Enhancement is but one of several fishery management tools

— Clearly the Principal Investigators recognize that enhancement is but one on several tools that could be used in concert to affect sound management of the Exmouth Gulf tiger prawn fishery. But attention is needed to some key management criteria that need to be developed: a) Will the fleet take all enhanced production? Or will spawning stock biomass be allowed to increase in Exmouth Gulf to increase natural production in subsequent years? (b) How will it be decided how much to adjust Total Allowable Catch to take advantage of the added production that may occur as a result of the enhancement activities? An overestimate of stock enhancement effect, with subsequent increase in catch allowances, could lead to over fishing the wild stock. (c) If the project is successful and full-scale stock enhancement of Exmouth Gulf tiger prawns is initiated, under what conditions would stock-enhancement activities be temporarily sidelined or stopped?

(5) Intensive prawn culture technology transfer into the project

— There is no discussion of how the project would integrate advances already made elsewhere in intensive penaeid production technology. At the very least, the other systems should be considered in addition to (or even instead of) the raceway techniques described here. There has been much successful work in Japan, the USA, and in Taiwan, for example, in development of intensive prawn production technology.

(6) Disease control in the hatchery

— Besides preventing release of any batch of prawns that is identified as diseased, what protocols are needed to prevent disease transfer *into the stock-enhancement hatchery* from other aquaculture facilities? from wild broodstock? Are specific-pathogen-free protocols considered to prevent spread of, for example, IHHN? Taura syndrome? Etc. Is IHHN already prevalent in the wild stock? Are other prawn diseases?

Although these six issues have not been specifically addressed in the feasibility study or proposal, there are clear steps that the Principal Investigators can take to well integrate them into the project with some added expense. Because of a clear intention in the feasibility study and in the proposal to incorporate a responsible approach to tiger prawn enhancement, this project will likely make substantial contributions to the development of marine stock enhancement. The rigorous attention to resolving critical uncertainties that will be needed to evaluate tiger prawn enhancement potential should be ensured here by beefing up the stage II, III, and IV plans by considering the issues stated above and detailed in the body of the project review.

Project Review

1) Assessment of the bio-economic model — Is the approach to assessing the effects of enhancement on the production and economics of the fishery appropriate?

While I have little expertise in modeling, I can provide some general comments on improving the approach taken with the bioeconomic model that should be useful.

— What proportion of hatchery yield is actual increase in production?
A key question about using the model to assess the effects of enhancement on the fishery is how to factor in the ecological effects that carrying capacity and competitive displacement could have on the increase in yields afforded by stocking hatchery prawns. Whereas the contribution of hatchery prawns to fishery yields is a good gauge of the magnitude of hatchery prawns that survive and enter the fishery, hatchery contribution alone may not reflect the realized increase in catch afforded by stocking. What if the assumption that all hatchery prawns landed represent additional yield is wrong? If, say, 50 tonnes of hatchery prawns are landed, at the expense of 30 tonnes of wild prawns lost because of displacement by the hatchery stock, then the net increase is 20 tonnes. Although there is a variable in the model for carrying capacity, which is currently turned off for lack of data, I see no provision in the model for evaluating displacement. There is also no discussion of the experimental control condition(s) that will be needed to evaluate the effect of stocking on the actual production of both wild and hatchery tiger prawns in Exmouth Gulf. Eventually, assumptions made about stocking effect on yields need to be tested by comparing wild recruitment in the enhanced stock(s) in Exmouth Bay with recruitment success in nearby wild stocks that are not enhanced. The difficulty here is in factoring recruitment variability out of this comparison. One way to deal with this is through replication of both the “enhanced” and “control” stocks.

From an economic standpoint, this is the “question of the day” in the field of stock enhancement. Thus any progress gained in understanding *realized* increase in yields afforded by stocking prawns in Exmouth Bay will make a major contribution to the developing field of stock enhancement. This issue is undoubtedly of prime importance to MG Kailis Group. For if the fleet considers they have landed 50 tonnes of “additional” (hatchery) prawns, as in my “what if” example above, when they might have landed 30 tonnes of this yield anyway, in the absence of stock enhancement, this has important implications for the bioeconomic analysis. Alternatively, the tiger prawn population in Exmouth Bay may be far from any resource limitations on prawn production capacity, and all of the hatchery contribution may represent

increase in yield. But this assumption should be field tested through comparison of hatchery enhanced and control fisheries.

— A term for release-related mortality is needed in the enhanced stock sub-component.

Much emphasis on the benefits of enhancement in the model is placed on uncertainty in prawn prices and production yields in the hatchery. Variability in enhancement effect on yields in the fishery is acknowledged, but ignored in the model because of high uncertainty. However, yield from enhancement is clearly as important an uncertainty as hatchery production and market price are in evaluating enhancement potential.

There is a "general approach" issue about maximizing enhancement yield, which I will discuss here (and will bring up again under "General comments on enhancement approach"), that is directly related to the appropriateness of the bioeconomic model for assessing the effects of enhancement on the production and economics of the fishery.

There is a valuable approach that can be used to maximize stocking yield in the fishery by learning to control the effects of release strategy on prawn survival after release — using pilot release experiments to evaluate effects (on survival and yield) of a range of values for various release variables. Yet the approach outlined in the proposal will rely on the bioeconomic model and life history information to establish release protocols.

Emphasis is given in the bioeconomic model to survival during hatchery production, transport and release. There is also a submodel for post release survival. That submodel is based on the assumption that survival of hatchery prawns is no different from survival of wild prawns the same size, which are already acclimated to the release habitat. Yet post-release survival can vary greatly depending upon release strategy (e.g. see Leber et al., 1996, 1997; Munro and Bell, 1997; Leber, 1999; and see discussion about this in the FRDC feasibility study).

The nursery-ground model for the enhanced stock sub-component lacks a parameter for *release-related* mortality, which often can be mediated by release strategies. Such mortality could be attributed to several interactive factors, such as attraction of predators to the release containers; density-dependent mortality during the period immediately after release (when densities of hatchery prawns at the release microhabitat are naturally orders of magnitude greater than densities of wild prawns); learning deficits that may increase risk to predation in naive hatchery prawns (exploratory learning is highly developed in some invertebrates, including crustaceans); stress-induced differential predation rates (of hatchery prawns compared to wild prawns) during the stress-recovery period following hatchery releases; differential mortality as hatchery prawns learn to forage in the wild; differential choice of refugia within the microhabitat, etc. Natural mortality of wild shrimp is not a good predictor of the combined interactive effects these and other sources of mortality that are directly affected by release protocols.

I suggest that the bioeconomic model be revisited with attention to developing a *release-related* mortality variable in the nursery-ground model for the enhanced stock sub-component, and make this variable a function of release strategies. This will focus attention on this important source of variability in yields from enhancement and afford a way to incorporate data on relative survival, obtained from pilot experiments.

The feasibility study recognizes the importance of defining optimal size-at-release, release habitat and microhabitat, timing (release season, day/night), and release magnitude. But the proposal does not show any focus on optimizing these release strategies in pilot release experiments as part of the Stage III releases. Rather, release strategies will be selected based

solely upon life-history information about wild prawns and according to bioeconomic model predictions.

However, pilot release experiments need to be used in tandem with the model to ground truth model assumptions and provide information about *release-related* mortality that occurs during some period soon after releases (hours or days) (Leber et al., 1996). Release-related mortality may differ significantly from natural mortality of wild prawns. Thus, pilot releases, in addition to life-history and model predictions, are all needed to test whether assumptions about optimal release strategies are correct. To err, for example, on choice of optimal size-at-release could compromise the entire project.

2) Assess the risks of the enhancement of tiger prawns in Exmouth gulf — including those of introducing disease, affecting the genetic diversity of the stocks and to the environment

— Disease

Diseases of penaeid prawns spread around the globe at a much accelerated rate during the past decade owing to rapid expansion of penaeid aquaculture technology. These diseases have had disastrous effects on aquaculture production on a global scale. Careful attention is needed to prevent transfer into the wild of pathogens from diseased prawns. Besides preventing release of any batch of prawns that is identified as diseased, what protocols are needed to prevent disease transfer *into the stock-enhancement hatchery* from other aquaculture facilities? from wild broodstock? Are specific-pathogen-free protocols considered to prevent spread of, for example, IHHN? Taura syndrome? Etc. Is IHHN already prevalent in the wild stock? Are other prawn diseases?

By working with Fisheries W.A., and specifying a certification process for released tiger prawns, the project is taking important steps to proceed in a responsible manner. Access to the primers to test for specific viruses is a particularly important step. Serious attention should be given to preventing translocation of diseased shrimp into the stock enhancement hatchery.

— Genetics

There are numerous ways in which cultured organisms can have a direct genetic impact on recipient stocks (reviewed by Utter, 1998). The majority of genetic hazards may be grouped into three categories (Tringali and Leber, 1999). “Type 1" genetic hazards are those that occur by way of hatchery-mediated translocation of exogenous genes into native populations (e.g. by releasing hatchery progeny derived from breeders belonging to a genetically divergent stock from the recipient stock).

“Type 2" hazards may be broadly defined as those stemming from genetic changes in a hatchery population, irrespective of the source of broodstock, that directly result from the processes of broodstock sampling, breeding, and rearing. Typically, the number of breeders selected to found the hatchery stock represents a small percentage of the available breeders in the source population. When insufficient numbers of breeders are used, sampling error can cause large stochastic differences in allelic and genotypic frequencies (Taniguchi and Sugama, 1990) or reduced levels of genetic variation in hatchery broods compared to the wild stock (Bartley et al., 1995). Hatchery populations can also be genetically compromised if the initial broodstock sampling fails to capture a sufficient range of phenotypic variability available in the source population (Leary et al., 1986). Other types of genetic changes to hatchery populations include artificial selection and domestication and inbreeding depression (Tave, 1993). Artificial selection, domestication, stochastic allele frequency changes, and reduced levels of variation can occur in the F1 generation. However, hatchery populations must usually be propagated over multiple generations without sufficient input of additional

wild genotypes before experiencing the deleterious effects of inbreeding (Tringali and Leber, 1999).

"Type III" genetic hazard is represented by a singular mechanism — the possible genetic swamping of natural populations through successful enhancement efforts. This mechanism can lead to post-stocking alterations in the native gene pool even when hatchery populations lack Type I and Type II genetic risk factors. Because of the disproportionate contribution of hatchery-derived progeny to the gene pool of a supplemented stock, an inevitable reduction occurs in the genetically "effective" population size (Ne) of the enhanced stock in the following generation (Ryman and Laikre, 1991). Reductions in Ne, if severe, can result in substantial allelic and genotypic frequency changes over time and, depending upon future population abundance, excessive loss of genetic diversity (Waples and Do, 1994). Tringali and Bert (1998) evaluated the sensitivity of the model parameters in the Ryman/Laikre model for Ne over a range of values that may be typical for marine stock enhancement programs. To minimize Type III genetic hazard, some minimum number of *effective* hatchery breeders should be used per generation interval, and the maximum relative contribution of hatchery raised prawns to the wild stock should be limited to appropriate levels per generation interval. Tringali and Leber (1999) derived these for snook stock enhancement using the Ryman/Laikre model, anticipated survival of released juveniles to reproductive age, and spawning stock abundance of the enhanced subpopulation.

In the FRDC feasibility study and proposal, good consideration has been given to avoidance of hatchery-mediated translocation of exogenous genes into the Exmouth Gulf brown tiger prawn stock targeted for enhancement. However, serious attention is also needed to minimize Type II and Type III genetic hazards. The feasibility study gave scant attention to these hazards, and made reference to questions at the genetic workshop in Perth about whether genetic differences even mattered. The proposal incorporates recommendations by the panel for minimizing genetic risks. But no mention is made of protocols to minimize Type II or Type III hazards. Given the objective of increasing catches of tiger prawns by adding 100 tonnes of hatchery prawns to the fishery, there is clear need to develop such protocols to conserve wild stock genetic diversity.

3) Assess the approach to developing techniques for the production, harvest and release of brown tiger prawns in Exmouth Gulf

— The need for a high-density production capability is recognized in the feasibility study and proposal. Mass production technology is clearly needed to be able to begin Stage III, and large-scale production techniques must be in place to proceed with Stage IV. Given the short time frame for development of intensive prawn production technology, the Principal Investigators are urged to look closely, if they haven't already, at intensive prawn farming technology that has already been developed for other penaeids. There is no discussion of how the project would integrate advances already made elsewhere in intensive penaeid production technology. At the very least, the other systems should be considered in addition to (or even instead of) the raceway techniques described here. There has been much successful work in Japan, the USA, and in Taiwan, for example, in development of intensive prawn production technology.

For example, intensive prawn production technology was developed as early as 1991 by the U.S. Marine Shrimp Farming Program, a research consortium involving researchers in several U.S. states (Wyban and Sweeney, 1991). Wyban and Sweeney (1991) provide detailed methods for all stages of intensive production of *Penaeus vannamei*. In this example, postlarvae are stocked at 800 to 1200 per square meter in 20,000-liter cylindrical tanks and grown to 1 gm in 40 days. Algal monocultures were used to accelerate growth rates. It is highly recommend that the Principal Investigators consider consultation with an expert in

intensive prawn culture. Dr. James Wyban (now a consultant on the big island of Hawaii), James Sweeney (now with CEATECH on Oahu), or Dr. Shaun Moss (who is running the penaeid aquaculture program at Oceanic Institute on Oahu) would all be well worth considering.

4) Assess the approach to selecting release sites for juvenile brown tiger prawns in Exmouth Gulf

— Although the critical importance of pilot experiments to determine optimal release strategies is discussed, there is no discussion of testing assumptions about release strategies as part of Stage 3 experimental releases. Evaluating enhancement impact on yields in the fishery across a range of release parameter values is a crucial step in planning the large scale test-of-concept planned for Stage 4 (e.g. field testing a range of microhabitats; field testing different release methods to liberate prawns from tanks on deck to the nursery habitats below; and examining differential effects of such experimental treatment conditions on yields in the fishery). Without such tests of critical uncertainties, adaptive management potential is seriously curtailed, and the project objectives may be compromised by invalid assumptions about optimal release strategy. This area certainly needs to be reconsidered in both Stage II and Stage III work. See discussion about pilot releases and need for a high-information content tag under the general comments in the next section.

5) Provide general comments on the approach to enhancement

— Pilot experiments and adaptive management

To make rapid progress in the rather poorly developed field of marine stock enhancement, a rigorous assessment of stock enhancement impact is needed that can provide answers about lingering critical uncertainties (Leber, 1999) from every release (e.g. do we stock now or later; is stocking 1 gm prawns really going to give us the biggest bang for the buck, or do we get optimal yields in the fishery per dollar spent rearing them by stocking 5 gm prawns? In spite of our concerns about predation, would half-gram prawns show even greater yields per dollar? Is this cage method giving us high survival after releases, or would we be better off bagging them and wet packing to the bottom? Is our release method resulting in prawns occupying optimal refuge from predators? Can we improve survival 10 fold by making x, y, or z changes? How important, if at all, is acclimation in benthic cages for a few hours prior to release?).

This issue relates to item no. 8 in the list of points (in the FRDC Feasibility Study) quoted from the "Responsible Approach" paper (Blankenship and Leber, 1995) — Use an empirical process for defining optimum release strategies. Using the example about optimizing size at release, the bioeconomic model predicts that 0.5-gm juveniles is the optimal size to release, and the feasibility study recommends 1-gm juveniles as a conservative effective release size. These predictions are based on assumptions that size-dependent hatchery-prawn mortality immediately following release is similar to size-dependent mortality of wild prawns. This crucial assumption must be tested to determine actual optimal size at release because the release of naive, stressed hatchery prawns into the environment, at localized release densities that are clearly greater than natural densities, will likely induce greater predation rates than normal. The information from China about size at release is good additional background for guiding choice of size at release. But if the Principal Investigators don't test this crucial issue with tiger prawns in Exmouth Gulf, and if the assumption of equal size-dependent mortality is invalid, then both Stage 3 and Stage 4 releases are a weak test of stock-enhancement potential.

Omitting tests of assumptions about release variables in the Stage 3 experimental design would be a fundamental weakness in the project, which must be shored up — there needs to

be an attempt in the Stage 3 release experiment to embed experimental tests of critical assumptions that require multiple treatment conditions. Without this ability, use of adaptive management will be seriously curtailed over the life of the project. Resolve this issue and you have an outstanding opportunity for successfully determining enhancement potential, given the scientific rigor that the rest of the project is receiving.

Clearly, adopting a strategy of constant refinements based on the results of pilot experiments embedded within the design for every release can provide crucial information needed early in the project to improve the potential for success. To use this form of adaptive management requires integration of high-information tag technology into the project design now, so that it can be used in Stage III experimental releases to perform factorial design evaluations of multiple questions simultaneously (e.g. Leber et al., 1998).

— The need for a high-information content tag for pilot release experiments

I see difficulty in approaching multiple treatment conditions in pilot releases using genetic tags. For example, a typical pilot release experiment to evaluate release strategies can easily require 60 to 80 distinct tag codes if you embed replication into the design by blocking replicates of each treatment condition over time, as in Leber et al. (1998). This is an extremely important issue, because the only way of determining, for example, optimal size at release is to do the pilot experiment to evaluate the effects of multiple *release-related* sources of size-dependent differential predation. Lacking this information could result in premature rejection of stock enhancement as an economically viable management tool (if, for example, the 1 gm prawns suffer disproportionately high [or even total] mortality compared to, say, 2 or 5 gm prawns [e.g., Leber et al., 1997]). One simply has to look at yields in the fishery across a range of size-at-release groups and compare those yields with rearing costs to determine optimal release strategy. The same could be said for the timing of releases (day/night; season) and release habitat. So, that is why it is highly recommend that as part of Stage II, the investigators also explore adapting a high-information content tag (such as coded-wire tags -- they make a half length tag, too, which may be just what is needed with small penaeids). Not all of the released prawns would need to be coded this way; just enough to yield a large enough sample size to evaluate experimental treatment effects. The genetic tags would suffice for identifying the rest of the hatchery-released prawns (i.e., the ones not tagged with CWTs) to evaluate enhancement contribution to the fishery.

— Stock Enhancement is but one of several tools for managing yields in the fishery

Clearly the Principal Investigators recognize that enhancement is but one on several tools that could be used in concert to affect sound management of the Exmouth Gulf tiger prawn fishery. But attention is needed to some key management criteria that need to be developed: a) Will the fleet take all enhanced production? Or will spawning stock biomass be allowed to increase in Exmouth Gulf to increase natural production in subsequent years? (b) How will it be decided how much to adjust Total Allowable Catch to take advantage of the added production that may occur as a result of the enhancement activities? An overestimate of stock enhancement effect, with subsequent increase in catch allowances, could lead to over fishing the wild stock. (c) If the project is successful and full-scale stock enhancement of Exmouth Gulf tiger prawns is initiated, under what conditions would stock-enhancement activities be temporarily sidelined or stopped?

Otherwise, this is a well thought out project, and we need more like it to advance this branch of fisheries science.

References Cited

Bartley, D.M., D.B. Kent and M.A. Drawbridge. 1995. Conservation of genetic diversity in a white seabass hatchery enhancement program in southern California. Pp. 249-260 *In* H.L. Schramm and R.G. Piper (eds). Uses and Effects of Cultured Fishes in Aquatic Ecosystems. American Fisheries Society Symposium 15.

Blankenship, H.L. and K.M. Leber. 1995. A responsible approach to marine stock enhancement. AFS Symposium 15: 167-175.

Hilborn, R. 1999. Confessions of a reformed hatchery basher. Fisheries

Leary R.F., F.W. Allendorf, and K.L. Knudsen. 1986. Genetic differences among rainbow trout spawned on different days within a single season. Progressive Fish Culturist 51: 10-19.

Leber, K.M. 1999. Rationale for an experimental approach to stock enhancement. Pp. 63-75 *In* B.R. Howell, E. Moksness, and Terje Svasand (eds) Stock Enhancement and Sea Ranching. Fishing News Books. Blackwell Science, Ltd. Oxford. 606 pp.

Leber, K.M., N.P. Brennan, and S.M. Arce. 1998. Recruitment patterns of cultured juvenile Pacific threadfin, *Polydactylus sexfilis* (Polynemidae), released along sandy marine shores in Hawaii. Bulletin of Marine Science 62(2): 389-408.

Appendix 2E: Response to comments in Hilborn review by David Die (now CIMAS-RSMAS, University of Miami, Florida, United States)

Ray is correct in saying that we did not include density dependence in the model that we used to conduct the initial evaluations of the feasibility of enhancement. The reason for that is two fold:

1. All the trials we have run corresponded to scenarios were the enhanced catch was less than 1/4 of the average yearly catch. According to the S/R relationships estimated for tiger prawns in WA and the NPF, there is no way of detecting density-dependence over such small range of recruitment. Thus, although in theory there might be some decrease in the survival of recruits, we would not be able to detect it given the noise in the data.

2. Furthermore, although we know there is density dependence on recruitment we do not know the life history stages when it operates. Using the estimated stock recruitment parameters to define density dependence of the enhanced stock would assume that density dependence processes only operate between the size at which we release enhanced prawns and the size at which they recruit. We would be assuming no density dependence during the larval, postlarval or early juvenile phases. We found it difficult to make such assumptions, and even harder to parameterize different S/R scenarios. That is why we choose to calculate the extra mortality (from enhancement to recruitment) that would erode the benefits from reseeding. If density-dependent mechanisms were to be capable of producing such mortality then it would be obvious that the benefits from reseeding would be eroded.

I have tried to follow Ray's suggestion and made some quick calculations by using the average S/R curves from NPF (Wang and Die 1996) and Exmouth (Penn and Caputi 1985) and assuming (as I said above) that all density-dependence operates between the size at seeding and recruitment.

For Exmouth, if we assume than an average spawning stock of 10 corresponds to an average catch of 400 tons then the SR relationship predicts a recruitment of 22 (all units are here the same as in Penn and Caputi 1985), increasing the spawning stock to 12.5 (by a 1/4) will yield a recruitment prediction of 24. This implies quite a drop in the numbers of recruits per spawner from 2.2 to 1.9 and implies a decrease in survival of 13%. These numbers are, however, very uncertain because if I use as average spawning of 8 rather than 10, then the drop in survival is only 10% but if I use an average spawning stock of 12 then survival drops by 15%. These calculations suggest that depensation may erode some of the benefits of enhancing the stock and we knew that. So far they suggest that even if all depensation occurs between seeding and recruitment it is unlikely that all the benefits will be eroded (although we still have to add extra sources of mortality due to seeding).

If I do similar calculation in the NPF the results suggest a lot less depensation for the stock of *P. esculentus*. I estimate only an extra 7% mortality due to depensation if the spawning stock was to be increased by 25% through enhancement.

In summary I think Ray's suggestions are well founded and I will consider them in the next version of the model. In fact the EXCEL model had a S/R relationship already built in, but the parameters were set so that it did not operate. I now will be able to parameterise it to run scenarios of depensation.

Appendix 2F: August 21-22, 2001, Review of FRDC project "Developing techniques for enhancement of prawn fisheries, with a focus on brown tiger prawns (Penaeus esculentus) in Exmouth Gulf.

Ken Leber
Center for Fisheries Enhancement
Mote Marine Laboratory, Sarasota, Florida 34236 USA

Assessment of production, harvest, transport, release

The gains made here are very impressive. In particular, I was impressed with the results of the production trials by CSIRO and MG Kailis. There is a clear capability established to meet the production and harvest goals of the project. The work to develop higher stocking densities and the successful application of the novel new method to increase surface area is a major accomplishment of the project to date. The choice of raceways over ponds for producing juveniles for release gives added control over production variables. The ease of harvest afforded by the raceways, and the ease of removing the 3-D substrate, without loss of prawns is a strong achievement.

The release method could use some improvement. There are several aspects that need attention during stage 2.5.

The Xactic transport tanks are totally appropriate for pilot-scale work, but there needs to be some improvement over the lack of ability to drain all of the prawns out of the transport tanks at the time of release. Netting up to one-quarter of the prawns and releasing them on the surface invites high post-release mortality. Rather than use significant resources to evaluate experimentally the relative survival of prawns released at the surface compared to survival of those delivered through the release hose close to a benthic or seagrass substratum, the project team needs to simply remove the necessity to net-prawns-and-release-them-at-the-surface. One idea is to use ~ 3hp water pump, with a J-valve on the delivery end, to reduce pressure (and stress on the prawns), to pump additional water into the transport tank as needed during draining.

The "stranding" (my term) of prawns within the water column after release is a red flag. These individuals may be suffering from stress. Attention is needed to reduce or eliminate this behavior, as there is high probability that such behavior is a source of high post-release mortality. It is unusual for nocturnally active penaeids not to seek cover during the daytime. The project investigators should look at the vertical orientation of released prawns within the water column as an opportunity to improve post-release survival, by eliminating or reducing the cause of this behavior. Is this a response to thermal shock? How does water temperature within the transport tank at the time of release compare to the temperature of seawater at the end of the delivery hose. Crustaceans are very susceptible to rapid change in water temperature, which can be lethal. I've learned this the hard way in previous experiments with penaeids (e.g., preparations for work described in Leber, 1985). Is this a response to pressure-induced stress during the flushing motion of a gravity-fed drain? Are the "stranded" individuals those that were released by net on the surface? Are there air bubbles under the carapace? Seek to improve (their behavior) this constraint to placing the released prawns directly near their benthic and seagrass habitat.

Selecting the best release sites for juvenile tiger prawns in Exmouth Gulf

Selection of release sites is a concern. Work is needed (in Stage 3) to compare relative survival at the site chosen (South of Whalebone) with survival at alternative sites. Although seagrass biomass was greatest at Whalebone South, juvenile prawn abundance (a good initial

proxy for habitat suitability) was greatest at Gales and Simpson. This raises a red flag about the choice of Whalebone site as the best release location. This is a very important assumption, which needs to be evaluated in the experimental (pilot) release stage, as choice of release site will have huge influence on the outcome of the project. Post-release survival should be compared at several release sites during stage-3 pilot release experiments.

The work to identify major predators has not provided particularly useful information so far, but this is certainly not from lack of effort. The data are simply too sketchy to use to plan release strategies. The work done so far to identify seagrass distribution and biomass is crucial. The team might gain more at this phase of the project (Stage 2.5) by seeking abundant seagrass/prey/refugia-from-predators now, and look later in more detail at predators. As the team already realizes, one would expect much greater densities of the crustacean prey, consumed by penaeids, to be located within and on seagrasses, e.g. amphipods, caridean shrimps, xanthid crabs (Leber, 1985). There may be much to gain from looking at prey abundance and correlating this with prawn abundance to help determine release microhabitats. Masuru Tanaka has shown a correlation between survival of released juvenile Japanese flounder and mysid abundance.

Genetic studies

It is presumed that genetic stock identification of tiger prawns in Exmouth Gulf will be accomplished prior to large-scale releases. This is needed to verify the hypothesis that the Exmouth tiger-prawn fishery is indeed fishing a single stock, which has important ramifications for managing broodstock genetics in a stock enhancement program.

Although genetic studies of stock structure at Exmouth Bay have not been completed yet, significant progress has been made in developing a genetic capability to identify the offspring of parents with known genetic identities using microsattelite technology. This capability is crucial to carrying out Stage-3 pilot release experiments. This technology provides a high-information content, benign mark to identify treatment groups of released hatchery-reared prawns. Because MG Kailis is reluctant to use physical tags, owing to high likelihood of a negative marketing effect, the development of a genetic mark is quite valuable to the project. If MG Kailis is successful at rearing prawns for release without cross contamination of genetic material (without mixing prawns) among raceways, then the genetic marking capability developed has high potential for identifying treatment groups and replicates in Stage-3 pilot releases. A high-information content mark is crucial to work needed to identify and resolve sources of post-release mortality and to optimize release strategies. The ability to distinguish at least 9 separate groups of prawns per release season is an important requirement of work needed to identify release tactics that will help achieve high post-release survival.

Given the importance of a reliable mark, the efficiency and reliability of using genetic technology to identify different groups of released prawns must be documented. A “blind” test of genetic identification of prawns from various parental combinations (where the genetic identity of all male and female pairs is known, but the parental source of offspring is not provided to the genetic team until after analysis) is an activity that should be considered for work during stage 2.5. This will enable any factors affecting prawn identification that might arise during transport, storage, and laboratory analysis of individuals collected in stage-3 work to be identified ahead of time. It will also provide documentation of the effectiveness of using microsattelites to identify groups of released prawns. This is not unlike holding back (from a release experiment) a sample of coded-wire tagged animals to verify tag retention, or doing a blind test to check coded-wire tag decoding accuracy (both are routine procedures in our lab). This issue will undoubtedly come up during peer review of any scientific results of stock enhancement research/evaluation that might be published using genetic marks to identify release groups.

Revisions to the model to assess the feasibility of stock enhancement in Exmouth Gulf

Revisions were needed in the model to incorporate new information and two factors identified in the first review – a term for density dependence and a term for post-release mortality; these are being incorporated. A strength of the modeling strategy used here is the incorporation of a chance-based stochastic approach using Monte Carlo simulations to estimate a whole range of risk conditions, and to test the sensitivity of the model to those conditions.

At the August 21-22 workshop in Perth the need to incorporate all the elements of risk that are important was highlighted and well summarized. The model could be further strengthened by including more details about post-release mortality. For example, post-release mortality is strongly influenced by release strategy. The model would be strengthened by adding an equation for optimal size at release (I have given MG Kailis a manuscript that is currently in review, which includes a simple model for this. I will send this manuscript to Neil Loneragan as well), and consideration of the effects of release season on (1) production costs, (2) growth rates, (3) survival, and (4) optimal size-at-release, all of which can vary significantly with release season.

Assessment of the risks of the enhancement of tiger prawns in Exmouth Gulf

It appears that significant precautions are being taken to reduce risks of increasing disease, affecting the genetic diversity of Exmouth stocks, and to the environment. The incorporation of a procedure to certify prawns for release by a government aquatic-health expert, indicated in the feasibility report, is a smart decision. Besides histological examination, it may also be wise to include the use of recently developed viral primers to assay for specific pathogens of prawns.

Genetic risk will be greatly reduced by the decision that has been made to use 40 to 60 different pairs of wild parents for each release. This will minimize the probability of reducing genetic diversity by including rare alleles from wild prawns in the hatchery offspring. Collection of wild parents from Exmouth Bay will prevent translocation of exogenous genes into the population, assuming subsequent research on genetic stock identity verifies only a single prawn population in Exmouth. Otherwise, source material (parents) from separate parental stocks will be needed should more than one stock of tiger prawns be identified in Exmouth Gulf. This latter topic was not discussed in the workshop.

What elements of research and development should be considered in the next stage of the project?

Based on the planning and results to date, I strongly encourage the project team to move forward with the next stage of the project (Stage 3), given that the model indicates that profit potential is encouraging. Stage 3, in its original form a trial release of 1 to 3 million brown tiger prawns in Exmouth Gulf, should be modified to include pilot release experiments designed to explore minimizing post-release mortality. If the team conducts pilot release experiments to evaluate release strategies (e.g., size at release, release habitat, release season, and release magnitude effects on growth and survival) there is every indication of maximizing the potential of prawn enhancement by identifying optimal release strategies. A test release built upon the results of this phase would be a strong test of stock enhancement potential, owing to development of an understanding of release strategy effects on post-release survival.

To make Stage-3 an effective step in designing the test of prawn stock enhancement potential, envisioned in the Stage-4 commercial trial, some development work is needed.

(1) A suitable sampling gear must be adopted to collect prawns on the nursery ground before and after Stage-3 pilot experiments are initiated. I suggest deployment of a prawn try net (~ 4 m ottertrawl) towed behind a small boat (~ 7 m, equipped with at least a 60 hp outboard motor), which is a common method for sampling prawn populations in Gulf of Mexico seagrass meadows (Leber and Greening, 1986). Sampling effectiveness should be explored as part of Stage 2.5.

(2) MG Kailis is strongly encouraged to produce prawns for Stage-3 pilot releases in a minimum of 9 raceways. This is the minimum number of rearing units needed to provide enough genetic coded groups to make enough progress in Stage 3 to plan the commercial trial in Stage 4. 9 raceways would afford 3 replicates of 3 treatment conditions. Any less will compromise the ability to generate reliable information about how to reduce post-release mortality (see Leber et al., 1996 and 1997, and Leber and Arce, 1996 for examples). Great care will be needed to prevent mixing of genetic identities of prawns among raceways.

(3) I encourage use of a randomized-block analysis of variance experimental design for Stage-3 pilot release experiments. This simply entails blocking all treatment conditions together in time or space with separation (in time or space) among replicate blocks of treatments (as shown in my Powerpoint presentation of 22 August, 2001, in Perth). I suggest blocking treatments along the north-south axis of the nursery habitat in Exmouth Gulf. If releases are stretched over multiple days, care should be taken to attempt to release at least one replicate of all of the treatment conditions on the same day (e.g., Leber et al., 1996, 1997, 1998).

(4) Attempt to identify the level of stress in released prawns by subsampling and caging overnight at the release site some of the prawns at the time of release (some of the first and last individuals being released). This will afford an immediate evaluation of stress-induced mortality.

(5) Close attention should be given to acclimation prior to release (e.g. water temperature, especially).

General Comments

This is a well organized, strong scientific and businesslike approach to increasing yields in the Exmouth Gulf brown tiger prawn fishery. The project team realizes that stock enhancement is only at an intermediate stage of development and that, for them to be successful, they must develop and test some new technology. This project has high probability for successful evaluation of *Penaeus esculentus* enhancement potential in Exmouth Gulf. This is among the best stock enhancement efforts I have seen, and employs a state-of-the-art approach. The strength of the project is exemplified in the bioeconomic model, the ability to evaluate model sensitivity of various key variables, the willingness to test model assumptions, the public-private partnership and the clear trust that I see among CSIRO, Fisheries WA, and MG Kailis (a major asset, which helps elevate potential for success in a multidisciplinary project), the already well-managed fishery, the involvement of the fishery in obtaining project data, the clear attention to applying a responsible approach to enhancement efforts, the attempt to adapt genetic marking to the forefront of enhancement research and development without compromising genetic diversity of the wild stock, the use of pilot release experiments to identify optimal release strategies, the attempt to get unbiased reviews, and the subsequent attention that was given to key review issues. Certainly this project, and the field of stock enhancement in general, will benefit by addressing the questions posed in the proposal, and in the recent workshop, for Stage 3.

Literature Cited

Leber, K. M. 1985. Influence of decapod foraging and microhabitat complexity on seagrass communities: a field test of the refuge hypothesis. Ecology 66(6): 1951-1964.

Leber, K. M. and H. S. Greening. 1986. Community studies in seagrass meadows: a comparison of two methods for sampling microinvertebrates and fishes. Fishery Bulletin 84(2): 443-450.

Leber, K. M., S. M. Arce, D. A. Sterritt, and N. P. Brennan. 1996. Marine stock-enhancement potential in nursery habitats of striped mullet, *Mugil cephalus*, in Hawaii. Fishery Bulletin 94(3): 452-471.

Leber, K. M. and S. M. Arce. 1996. Stock enhancement effect in a commercial mullet *Mugil cephalus* fishery in Hawaii. Fisheries Management and Ecology 3: 261-278.

Leber, K. M., H. L. Blankenship, S. M. Arce, and N. P. Brennan. 1997. Influence of release season on size-dependent survival of cultured striped mullet, *Mugil cephalus*, in a Hawaiian estuary. Fishery Bulletin, 95(2): 268-280. (April, 1997).

Leber, K. M., N. P. Brennan and S. M. Arce. 1998. Recruitment patterns of cultured juvenile Pacific threadfin, *Polydactylus sexfilis* (Polynemidae), released along sandy marine shores in Hawaii. Bulletin of Marine Science 62(2): 389-408.

CHAPTER 3

TECHNOLOGY DEVELOPMENT FOR THE HIGH-DENSITY PRODUCTION OF JUVENILE *PENAEUS ESCULENTUS*

Peter Crocos
Stuart Arnold
Melony Sellars
Michele Burford
Roger Barnard
Richard McCulloch

3.1 Summary

The development of techniques for the production of juvenile *P. esculentus* at high densities has been carried out as an essential component of the overall aim to develop techniques for releasing farmed prawns into natural nursery grounds to enhance prawn fisheries. The strategy was to develop and test the high-density production concepts at the CSIRO Cleveland site, and then to further develop and test these protocols at a semi-commercial scale at the MG Kailis Group facility at Exmouth, WA.

In addition to the development of techniques for the high-density production of juveniles, the scope of the project research was extended to incorporate an evaluation of the fitness of these juveniles for release into the natural environment. This included an evaluation of potential physical damage sustained during the high-density production process, and an evaluation of the behaviour (e.g. burying response, seeking shelter in seagrass etc) of these juveniles when released into the natural environment. As well, a study to evaluate the relative nutritional contributions from natural and artificial feeds in the high-density production system was completed. Evaluations of water quality dynamics and the economics of production in the high-density production system were also carried out.

High-density production of juveniles
The approach taken by CSIRO Marine Research's laboratory in Cleveland, Queensland, was to develop a pilot ultra-high density production system in land-based raceways with the use of an artificial substitute for the sea-grass beds which juvenile prawns naturally inhabit. The use of artificial substrates in a system increases the surface area that the prawns occupy, thereby reducing the effective density. Artificial substrates also create added surface area for colonisation by biota, which provides a natural feed supplement to the prawns throughout the production run.

Between March and November 2000, CSIRO developed technology that successfully produced *P. esculentus* juveniles in a raceway system at harvest densities of around 1000 prawns m^{-3} with the use of AquaMats™ as artificial substrates.

The pilot system developed at the Cleveland facility was then further developed and modified by MG Kailis at their facility in Exmouth, WA and scaled-up to a semi-commercial production system between August and December 2000. Prawns were grown to between 0.5 - 1 g at harvest densities of between 851 and 2487 prawns m^{-3} with a mean survival rate of 77.7%. The maximum biomass achieved at harvest was 1.38 kg m^{-3} .

Utilising the developments made at Exmouth, further trials at Cleveland from December 2000 to March 2002 successfully grew prawns initially stocked at densities of up to 12,000 m^{-3} to ascertain the optimal stocking density for culture of juveniles to 1g. These trials also evaluated several different artificial substrate systems: commercial products versus the structures developed in Exmouth, and combinations of the two.

Juvenile *P. esculentus* were cultured from PL17 (seventeen-day-old postlarvae) to a mean weight of 1g in 6.5, 7 and 8 weeks at a stocking density of 2,860 m^{-3}, 5,720 m^{-3} and 11,430 m^{-3}, respectively. Mean survival rates ranged from 21.23% at the highest density to 50.85% at the lowest density. The final mean biomass at harvest was very similar across all densities, ranging between 1496g m^{-3} and 1742g m^{-3}. Three different artificial substrate systems were evaluated, with each substrate made up of a different combination of AquaMats™ and MG Kailis 3-D structure The type of substrate system used had no effect on prawn performance. An optimal density to stock the raceways was estimated to range between 3,300 and 3,700 prawns m^{-3}. This optimal density was based on the results of prawn performance (growth, survival, biomass and size variation), labour input requirements and risk associated with the different stocking densities.

A second intensive juvenile prawn production trial was carried out in Exmouth between August and December 2001. The raceways were stocked at between 2,610 and 4350 prawns m^{-3} with a final harvest density ranging between 1,840 and 3,263 m^{-3}. Mean survival was 72.6% and mean biomass achieved at harvest was 1.86 kg m^{-3}, with a maximum of 2.2 kg m^{-3} .

This chapter details the development and testing of techniques at CSIRO, Cleveland, and the results from initial pilot-scale production trials at Exmouth.

Additional components of the study
The scope of the research was expanded to include several additional components considered to add value to the overall objective of the production of large numbers of juveniles suitable for release into the natural environment to enhance natural prawn stocks. Therefore, each of the following additional components are reported separately:

Section 3.5	Water quality dynamics of the high-density production system.
Section 3.6	Evaluation of juvenile prawn health, physical damage and recovery following harvest from the high-density production system.
Section 3.7	Determination of the primary sources of nutrition for prawns produced in the high-density system, using a stable isotope analysis approach.
Section 3.8	Post-production behaviour of juvenile tiger prawns in relation to fitness for release
Section 3.9	Economics of high-density production – an analysis of the CSIRO Cleveland trial.
Section 3.10	Suggested further development of technology for the high-density production, transport and release of *Penaeus esculentus*, for stock enhancement

3.2 High density production of juvenile P. esculentus in a raceway system

3.2.1 Objectives

- To ascertain the optimal density for culturing juvenile *P. esculentus* to a mean weight of 1g in an experimental raceway system
- To evaluate different artificial substrate systems

3.2.2 Methods

3.2.2.1 System design and operation

Raceway
The experiment was carried out in two 15 m long, 1 m wide and 0.7 m deep raceways (10,500 litres). Each raceway was divided into six 2.5 m long, 1 m wide and 0.7 m deep sections (1,750 litres) for treatment replication (Fig. 3.1). Each section was supplied with flow through water at an average exchange rate of 80% per day for the first two weeks and 250% per day for the remaining five to six weeks. Temperature was maintained at 27°C with the use of a heat exchanger.

Figure 3.1 Raceway system setup showing the six sections used for treatment replication.

Artificial substrates

The two main concepts behind the use of artificial substrates for the high-density production of juvenile *P. esculentus* are:

1) To increase the surface area available for the prawns to inhabit. This distributes the prawns more evenly throughout the culture system, thereby reducing the effective density within the system.
2) To provide a suitable substrate for colonisation of biota, which in turn provides the prawns with a continuous natural food supply throughout production.

Through experimentation, an artificial substrate has been designed with different components that serve specific functions throughout the production (Table 3.1).

Table 3.1 Specific functions of the artificial substrate components.

Component	Function	Materials
Upper layer.	Provides a substrate for heavy colonisation of biota, which then provides a natural food supply to the prawns (Fig 3.2).	AquaMat™ and/or proprietary structure
Weighted frame.	Holds the substrate components together and keeps it submerged. The legs of the frame suspend the substrate 100mm off the bottom, allowing the laminar flow to prevent any waste buildup underneath.	Sand-filled PVC pipe.
Mid-water horizontal base.	Creates a shaded layer, which a majority of the prawns occupy during the day. This is important especially as the prawns get older and tend to hide more frequently.	Proprietary structure
Lower layer.	Increases the surface area available to the prawns under the horizontal base.	Sinking AquaMat™ or proprietary structure

Figure 3.2 A close-up of postlarvae grazing amongst algae which colonises the artificial substrates and also the walls of the raceway. The algae and associated biota provide a natural food supply throughout the production.

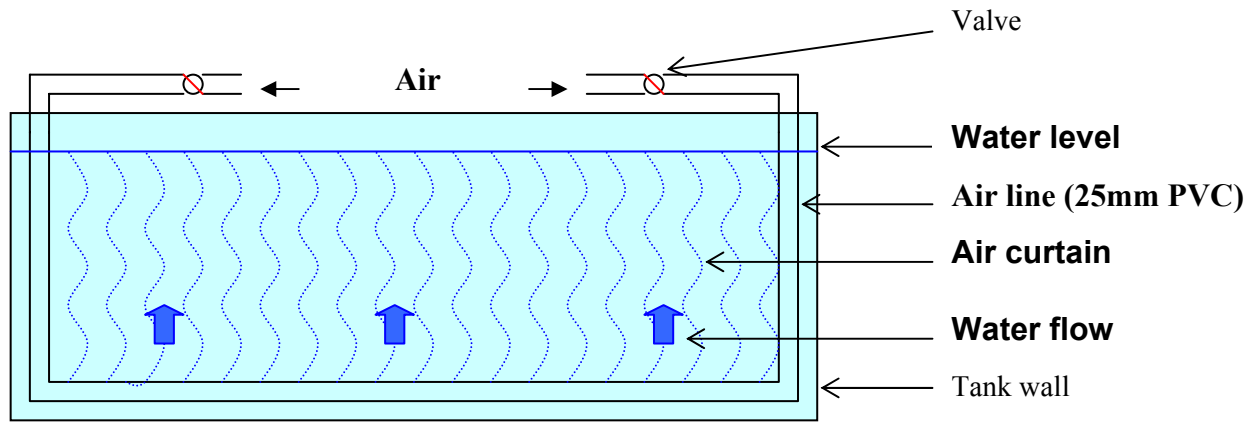
Three different artificial substrate systems were used in this experiment. Each system consisted of different combinations of AquaMat™, a preconditioned structure and a non-preconditioned structure (Table 3.2).

Table 3.2 Description of the different artificial substrate systems.

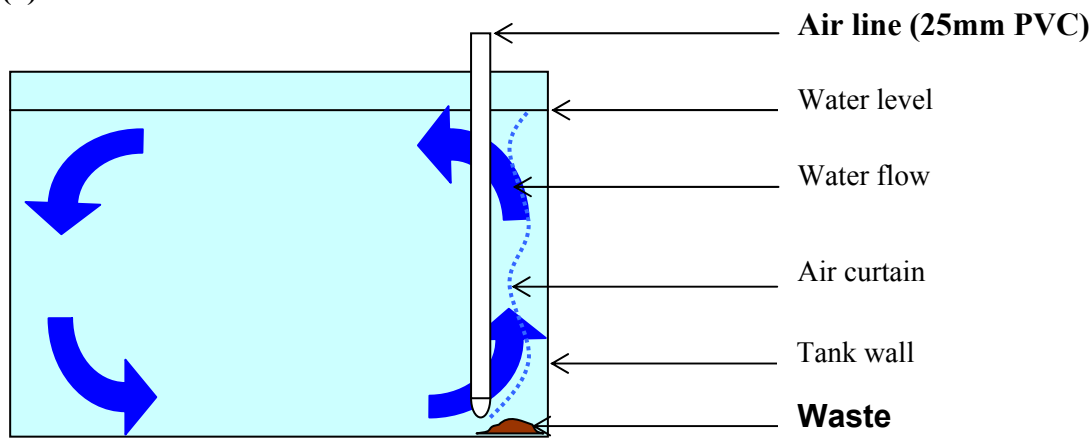
Substrate	Description	Surface area (m^2)	Resulting increase in tank surface area (%)
A	AquaMat™, preconditioned and non-preconditioned structure	24	340
B	Preconditioned and non-preconditioned structure	25.6	360
C	AquaMat™	8.6	120

Aeration system

The aeration system consisted of 25mm PVC pipe running along one wall and about 100 mm off the bottom of the tank (Fig. 3.3a.). The side of the PVC facing the wall had holes (1mm in diameter) every 100mm to produce an even curtain of air bubbles up one side of the tank. The air curtain forced water up the side of the tank creating a laminar flow of water across the surface of the raceway (Fig. 3.3b).



(a)



(b)

Figure 3.3 (a) Longitudinal section of the aeration system showing the PVC air line running along the bottom of the tank creating a curtain of air bubbles, and (b) a cross sectional view of the aeration system showing how the air curtain creates a laminar flow of water across the surface of the raceway, and the buildup of waste under the airline.

Advantages this system creates include:

- Suspends particles in the water longer, which increases the chance of being removed through the outlet
- Concentrates the waste build-up along one wall, enabling easier removal through siphoning and also monitoring of feed rates
- Maintains a cleaner tank bottom under the substrates
- Prevents thermal stratification
- Encourages good water mixing, preventing pockets of oxygen depleted water from forming

3.2.2.2 Production of postlarvae

Wild caught broodstock of *P. esculentus* for the Cleveland raceway experiments were obtained from Cairns through a commercial supplier (Bill Izzard) and conditioned to maturation at CSIRO. The ripe females were then transferred to Rocky Point Prawn Farm hatchery for spawning and larval rearing to PL17 (seventeen-day-old postlarvae).

3.2.2.3 Experimental design and stocking

The experiment was designed to make two comparisons: One between different artificial substrates and the other between different stocking densities (Table 3.3). Each comparison was conducted in a separate raceway.

Table 3.3 Experimental design for high-density rearing and substrate comparisons.

Comparison	Stocking density (no. prawns m^{-3})	No. replicates	Substrate
Substrate	2,860	3	AquaMat™ and structure(Substrate A)
	2,860	3	No aquamat (Substrate B)
Density	5,720	3	AquaMat™ (Substrate C)
	11,430	3	AquaMat™ (Substrate C)

The raceway sections were stocked at the appropriate density with PL17's and grown until a mean weight of 1g was achieved for each treatment.

3.2.2.4 Feeding regimes

Artificial diet was fed three times daily to satiation at 7:30 am, 12:00 pm and 7:30 pm. The feed composed of a mixture of different sized crumbles of commercially formulated diets. The feed rates and composition were adjusted on a day today basis, depending on the amount of uneaten food observed in each replicate and how the prawns handled the different size crumbles. In general the feed was administered at 20-25% of the estimated biomass at PL17 and gradually reduced to 4-6% of the estimated biomass at a mean weight of 1g.

3.2.2.5 Growth, survival, biomass and size variation estimations

Weekly samples of 100 prawns from each replicate were weighed individually to estimate the mean weight of prawns from each treatment over time. Once the mean weight was greater than 1g the treatment was harvested. From the harvested prawns a sample of 200 prawns were weighed individually to obtain a final harvest weight and the remaining prawns were counted to calculate survival and biomass.

The growth results were expressed as the mean weight of prawns within each tank at weekly intervals. Differences in growth among treatments over time were compared using the heterogeneity of slopes test (PROC GLM, SAS Institute Software). Differences in the mean weights among treatments at weekly intervals were compared by one-way analysis of variance using the Generalised Linear Models procedure (SAS Institute software).

Survival was calculated as the percentage of remaining prawns in each tank from the number originally stocked. Biomass was calculated from the mean weight of prawns for each tank at harvest multiplied by the number of prawns remaining per m^{-3} at harvest. Differences in survival and biomass among treatments were compared by one-way analysis of variance (PROC GLM, SAS Institute Software).

Size variation was expressed as the coefficient of variation of the individual prawn weights in each tank. The size variation between treatments at two standardised mean weights, 0.5 g and 1.0 g, and after 7 weeks growout was compared by one-way analysis of variance (PROC GLM, SAS Institute Software).

3.2.3 Results and discussion

3.2.3.1 Growth

Comparison between artificial substrates

At a density of 2,860 m^{-3} there was no significant difference ($P>0.05$) in the growth rate of prawns over 7 weeks growout between Substrate A and Substrate B (Fig. 3.4a). This indicates that, at a density of 2,860 m^{-3}, substrate type had no effect on growth rate.

Comparison between densities with the same substrates

The growth rate of prawns was significantly faster ($P<0.05$) over 8 weeks at a density of at 5,720 m^{-3} (Substrate C) than at 11,430 m^{-3} (Substrate C) (Fig. 3.4b). In this case an increase in stocking density above 5,720 m^{-3} caused a decrease in the growth rates of the prawns.

Comparison across all treatments

The time taken to culture prawns from PL17 to a mean weight of 1g was 6.5, 7 and 8 weeks at densities of 2,860 m^{-3}, 5,720 m^{-3}, and 11,430 m^{-3} respectively (Fig. 3.4c). Prawns grew faster at lower densities throughout the experiment and after 7 weeks the mean weights (± SE) were 1.26 ±0.07g, 1.23 ±0.04g, 0.97 ±0.13g and 0.68 ±0.08g for the 2,860 m^{-3} (Substrate A), 2,860 m^{-3} (Substrate B), 5,720 m^{-3} (Substrate C) and 11,430 m^{-3} (Substrate C) treatments respectively.

After only one week of culture, prawns stocked at 11,430 m^{-3} were significantly lighter ($P<0.05$) than prawns stocked at 2,860 m^{-3}. This trend continued throughout the grow-out. After 2 weeks, prawns stocked at 5,720 m^{-3} were significantly lighter ($P<0.05$ than those stocked at 2,860 m^{-3}. This trend continued for the remaining 5 weeks although it was not always significant due to variation within the replicates.

It is important to remember that Substrates A and B, used in the 2,860 m^{-3} density treatments, were made with different materials and had a greater surface area than Substrate C, used in the 5,720 m^{-3} and 11,430 m^{-3} density treatments. Therefore, the lower growth rates at a density of 5,720 m^{-3} compared with the density of 2,860 m^{-3} could be a result of either stocking density or substrate, or a combination of both. Because of this difference in substrates, a direct comparison of the effect of density on prawn growth cannot be made between these treatments. However, there are a number of reasons that strongly suggest that higher stocking densities were the major factor that caused reduced growth rates and not the different substrates.

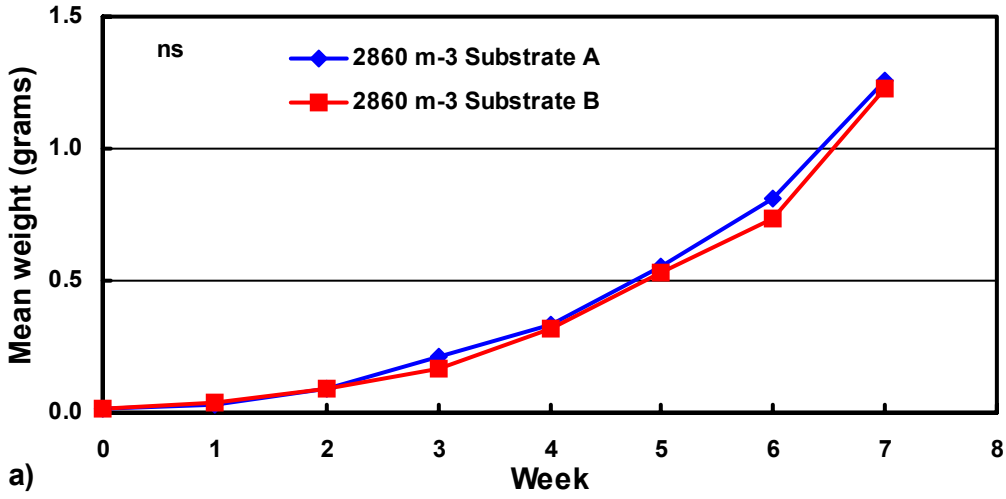
The ANOVA results (Appendix 1) indicate that growth was significantly affected by density but not substrate type, with the Mean Squares for density accounting for 1.7 % of the variation in the

ANOVA, compared with only 0.1 % for substrate. These values are low because the biggest effect on growth was time (week), which indicates that the prawns grew significantly larger from week to week. Although not significant, the % Mean Squares values for density for the ANOVA of survival and size data were also greater than those for substrate.

It has been well documented for different penaeid species that an increase in stocking density causes a reduction in the mean weight of prawns in the population. Williams *et al*. (1996) cultured *P. vannamei* and *P. setiferus* in an indoor re-circulating system at seven different densities that ranged between 28 and 284 prawns m^{-2}. They found an inverse linear relationship between density and growth. Apud *et al*. (1981) also found an inverse linear relationship between density and final mean weight of *P. monodon* reared for 3.5 months from PL53-PL54 in earthen ponds at densities of 2.5, 5, 10 and 20 prawns m^{-2}.

The difference between Substrate C (used to culture prawns at 5,720 m^{-3} and 11,430 m^{-3}) and Substrates A and B (used to culture prawns at 2860 m^{-3}) was the use of sinking AquaMats™, which provided the lower substrate layer. The function of the lower layer is to provide extra surface area for the prawns during the day as they start to seek lower light levels at an older age. However, different materials used in the upper layer are more likely to effect prawn performance because this layer is the primary substrate for natural colonisation by biota. If the material in this layer is not suitable for optimal colonisation, then the amount of natural feed supplements will be reduced resulting in slower growth rates in the early stages of prawn development. As there was no difference in prawn growth rates between the two different upper layer substrates at a density of 2,860 m^{-3}, the growth rates are unlikely to have been affected by using different lower layer substrates. Therefore, a comparison across all the treatments has been made based on the assumption that Substrates A, B and C had a minimal effect on prawn growth, but stocking density is likely to have had a major effect on prawn growth.

Overall, the results suggest that there was a direct relationship between stocking density and growth rate, with prawns stocked at 2,860 m^{-3} growing to a mean weight of 1g half a week and one week earlier than prawns stocked at 5,720 m^{-3} and 11,430 m^{-3} respectively. The best growth performance, a mean weight of 1.26g in 7 weeks, was achieved at a density of 2,860 m^{-3} using Substrate A. However, a similar result was achieved using Substrate B at the same density. Substrate type did not affect growth rates at a stocking density of 2,860 m^{-3} and is unlikely to affect growth at stocking densities up to 11,430 m^{-3}.



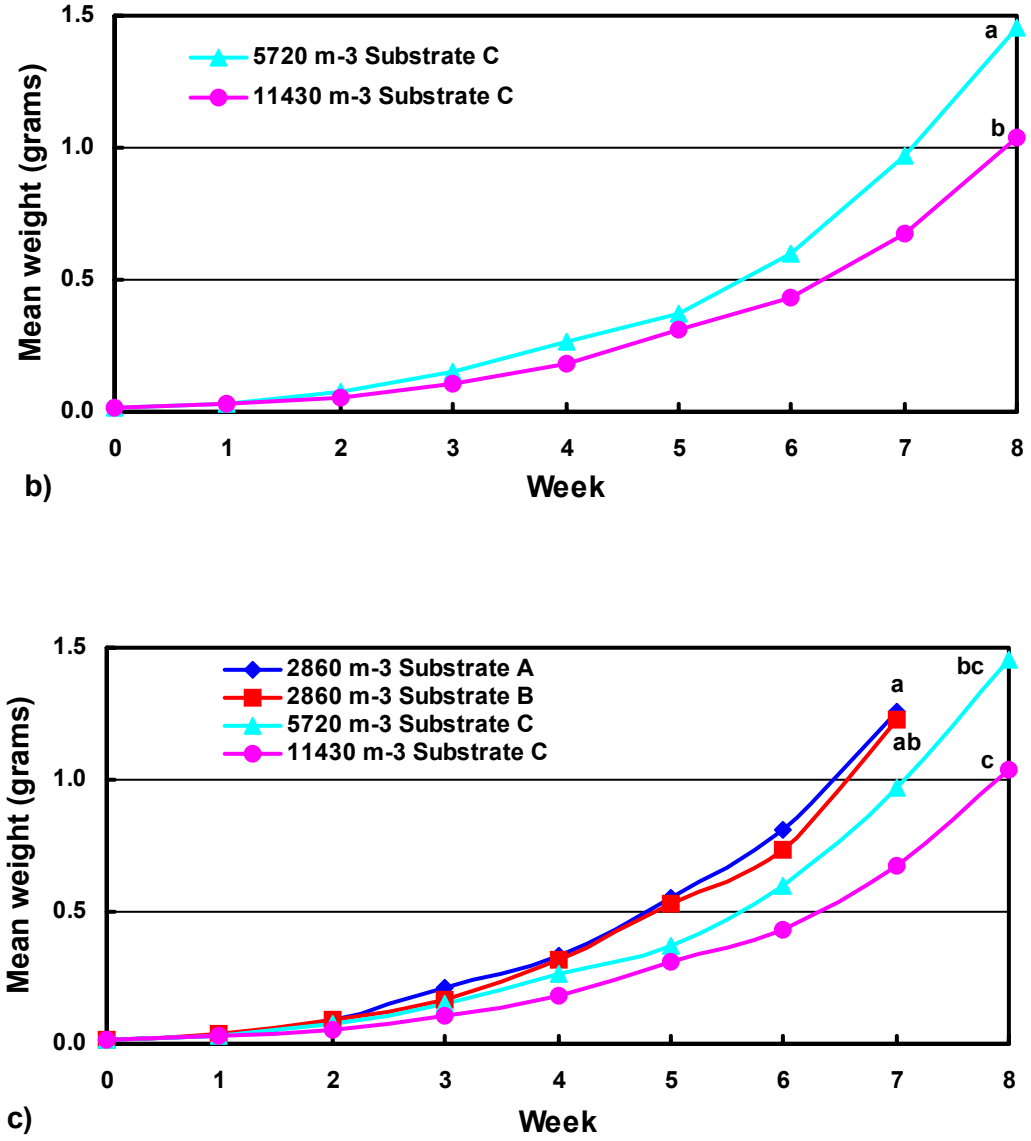


Figure 3.4 **Mean weights of *P. esculentus* juveniles grown for 7-8 weeks in a raceway system from PL17, a) stocking density of 2860 prawns m^{-3} comparing Substrate A with Substrate B, b) Substrate C comparing stocking densities of 5720 m^{-3} and 11430 m^{-3}, c) comparison across all treatments (growth curves with the same superscript are not significantly different (*P*>0.05)).**

3.2.3.2 Survival

Comparison between artificial substrates

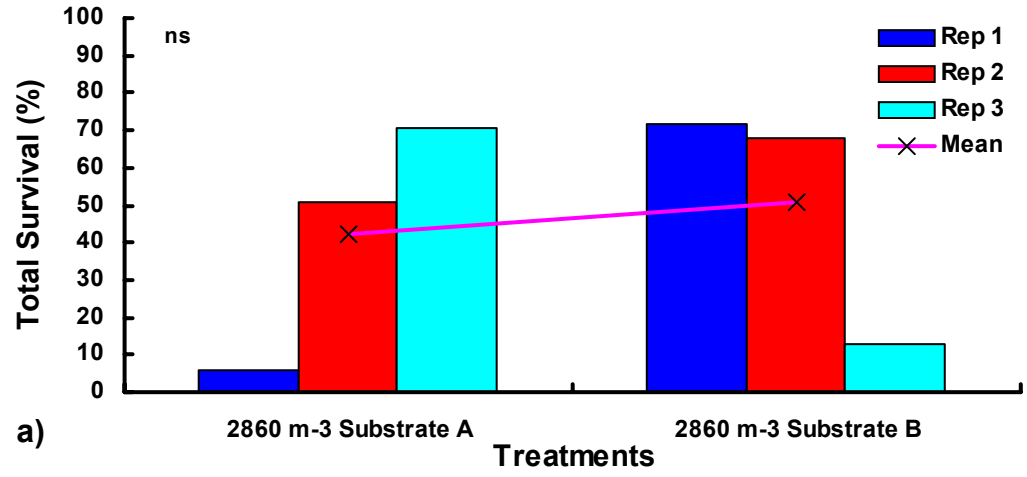
At a mean weight of 1g, 50.85 ± 18.91% and 42.51 ±19.02% of the prawns survived in the 2,860 m^{-3} (Substrate B), and 2,860 m^{-3} (Substrate A), treatments respectively (Fig. 3.5a.). However, this difference was not significant (*P*>0.05) because of the high variability within treatments. One replicate within each treatment had very low survival (10%), while the each had greater than 67% of prawns surviving.

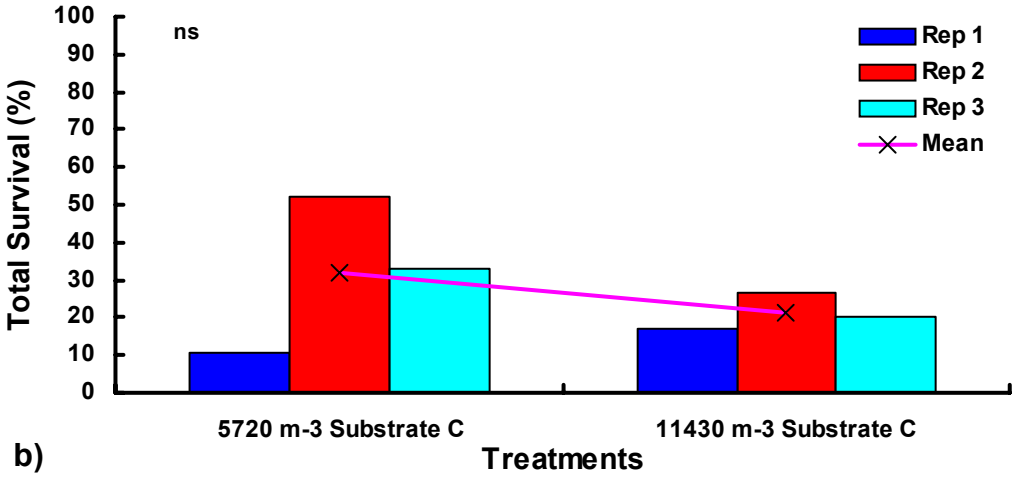
Comparison between densities with the same substrate

As stocking density increased, survival decreased with 31.9 ± 12.0% and 21.2 ±2.7% remaining in the 5,720 m^{-3} and 11,430 m^{-3} treatment respectively (Fig. 3.5b). Again this difference was not significant (P>0.05) because of the high variability within treatments.

Comparison across all treatments

Survival did not differ significantly among all treatments (P>0.05) because of the high variation between replicates within each treatment (Fig. 3.5c). However, the mean survival decreased as stocking density increased. The highest recorded survivals for each treatment were 71.8%, 70.3%, 52.3% and 26.5% for the 2,860 m^{-3} (Substrate B), 2,860 m^{-3} (Substrate A), 5,720 m^{-3} and 11,430 m^{-3} treatments respectively. These results also suggest that survival decreases as stocking density increases.





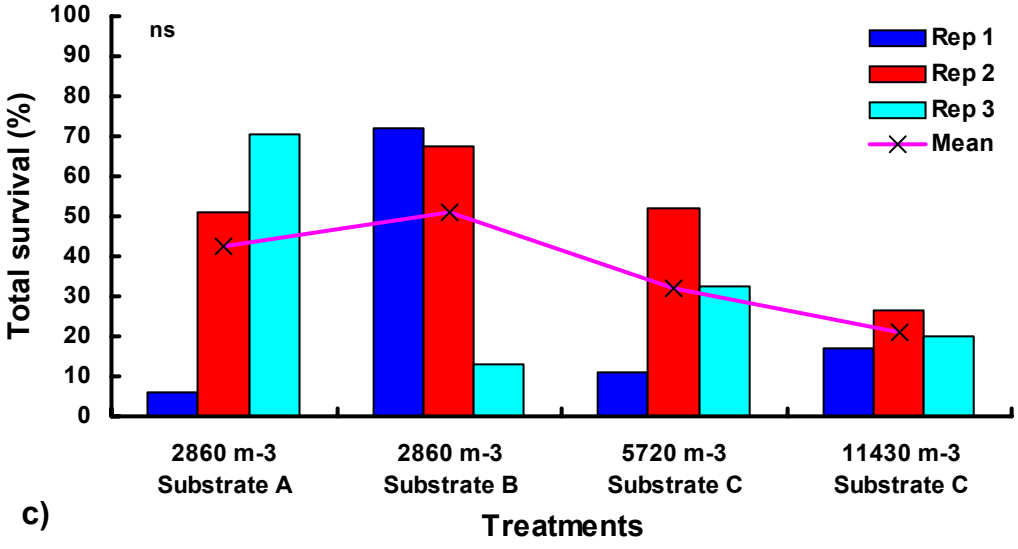


Figure 3.5 **Survival of *P. esculentus* juveniles grown from PL17 to a mean weight of 1g in a raceway system, a) stocking density of 2860 prawns m^{-3} comparing Substrate A with Substrate B, b) Substrate C, comparing stocking densities of 5720 m^{-3} and 11430 m^{-3}, c) comparison across all treatments.**

3.2.3.3 Biomass

Comparison between artificial substrates

At a density of 2,860 m^{-3} there was no significant difference (P>0.05) in total mean biomass between prawns cultured with Substrate A and prawns cultured with Substrate B (Fig. 3.6a).

Comparison between densities with the same substrate

Total mean biomass was not significantly different (P>0.05) between prawns cultured at 5720 m^{-3} and 11430 m^{-3} using Substrate C (Fig. 3.6b).

Comparison across treatments

Across all treatments there was no significant difference (P>0.05) in total mean biomass after 7 weeks of culture (Fig. 3.6c). The 2860 m^{-3} (Substrate B) treatment produced the highest mean biomass of 1742 grams m^{-3}, while the 2860 m^{-3} (Substrate A) treatment produced the lowest mean biomass of 1496 grams m^{-3}.

Biomass can be related to survival, number of prawns harvested, and stocking density. As stocking density increased survival decreased, however, the biomass remained constant because the number of prawns harvested was still greater at the higher densities (Fig. 3.7). The same relationship can be seen with mean weight. As mean weight decreased with an increase in stocking density, the number of prawns harvested increased, resulting in an even biomass across densities (Fig. 3.8). This relationship suggests that there is an optimal biomass that can be maintained within a system. As the optimal biomass is approached, growth slows and mortality rates increase. If the system is pushed beyond its optimal biomass, poor water quality and high stress levels can result in almost complete mortality within the system. Therefore, it is important to gain an understanding of the limits of a particular system so events of high mortality due to over stocking can be avoided.

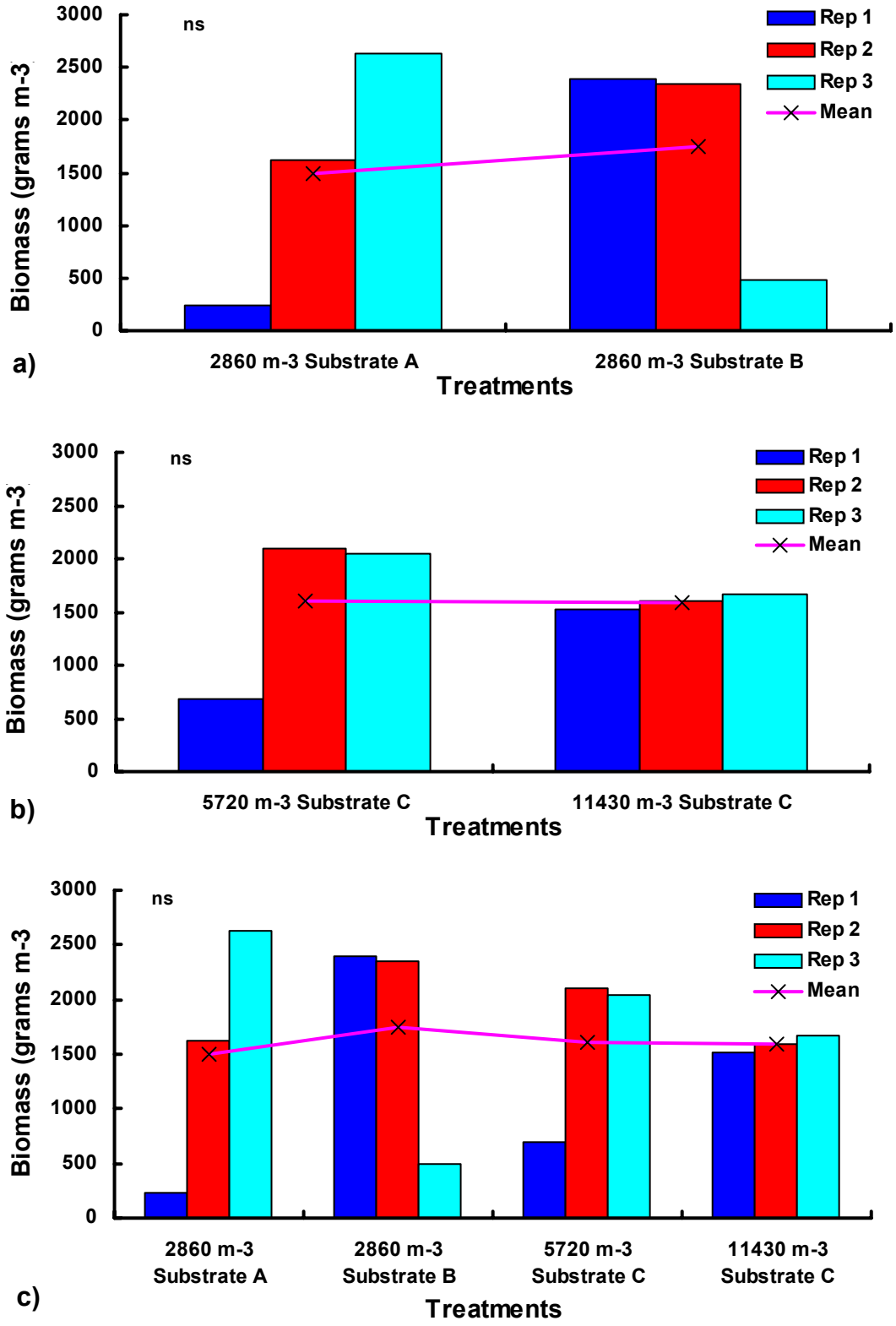


Figure 3.6 **Total biomass of *P. esculentus* juveniles grown for 7 weeks from PL17 in a raceway system, a) stocking density of 2860 prawns m^{-3} comparing Substrate A with Substrate B, b) Substrate C comparing stocking densities of 5720 m^{-3} and 11430 m^{-3}, c) comparison across all treatments.**

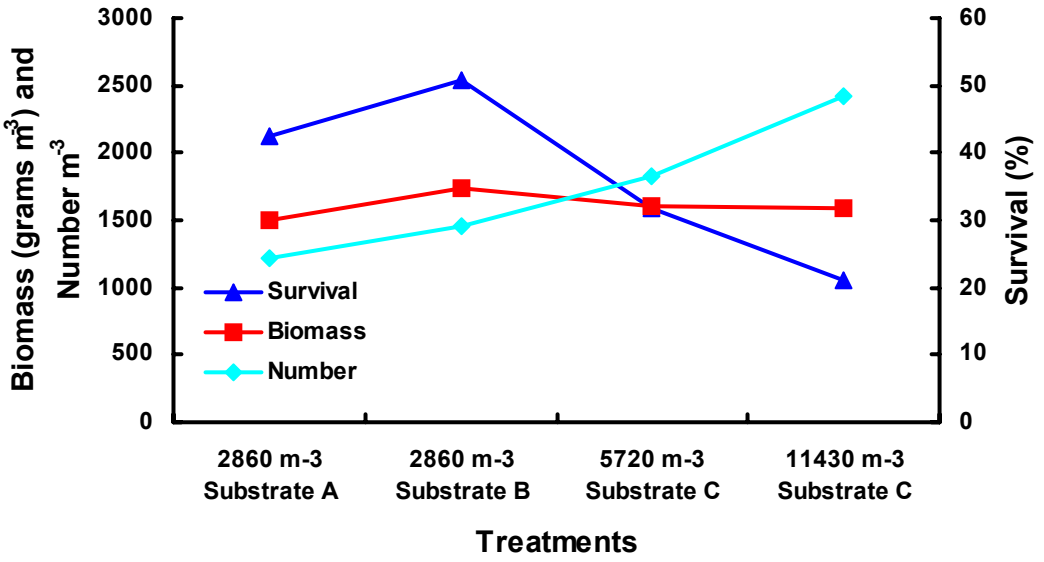


Figure 3.7 **The relationship between survival, number of prawns harvested and biomass of *P. esculentus* juveniles grown from PL17 to a mean weight of 1g, at different densities with different substrates in a raceway system.**

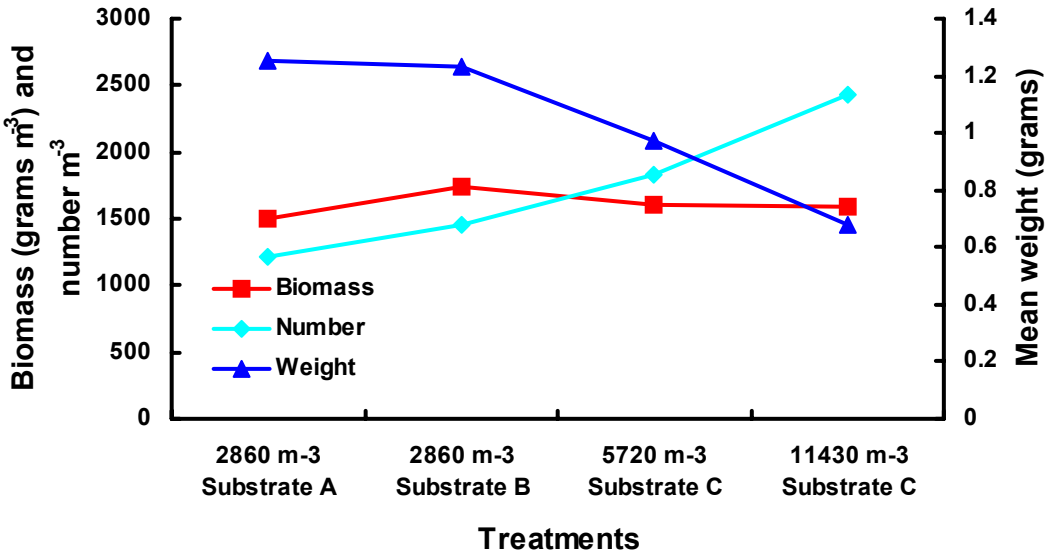


Figure 3.8 **The relationship between mean weight, number of prawns harvested and biomass of *P. esculentus* juveniles, grown for 7 weeks from PL17 at different densities with different substrates in a raceway system.**

3.2.3.4 Size variation

The distribution of individual weights did not differ significantly (P>0.05) between the four treatments when prawns were compared at a mean weight of 0.5g (Fig. 3.9). However, after 7 weeks there was significantly greater variation (P<0.05), in prawn size grown at 11,430 m^{-3} than

at the two 2,860 m^{-3} densities (Fig.3.10). The same result was expressed when compared at a mean weight of 1g (Fig. 3.11).

After 7 weeks and also at a mean weight of 1g, the size variation of the 5,720 m^{-3} treatment was between the 11,430 m^{-3} and 2,860 m^{-3} treatments. This suggests that an increase in stocking density above 2,860 m^{-3} will result in an increase in size variation after the prawns grow beyond a mean weight of 0.5g.

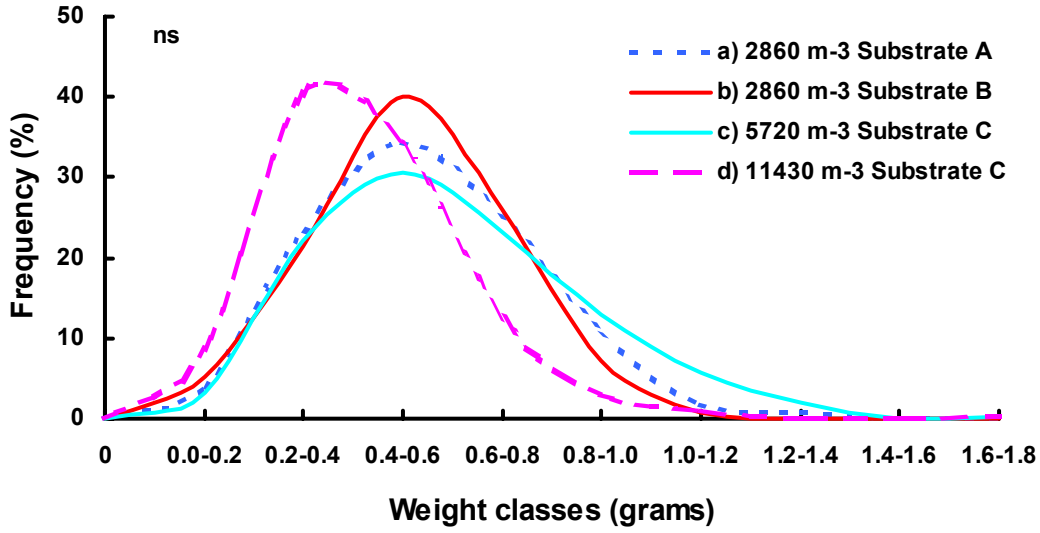


Figure 3.9 **Size distribution of *P. esculentus* juveniles grown to a mean weight of 0.5g from PL17 at different densities with different substrates in a raceway system,** a) **mean weight of 0.527g after 5 weeks,** b) **mean weight of 0.550g after 5 weeks,** c) **mean weight of 0.597g after 6 weeks,** d) **mean weight of 0.429g after 6 weeks.**

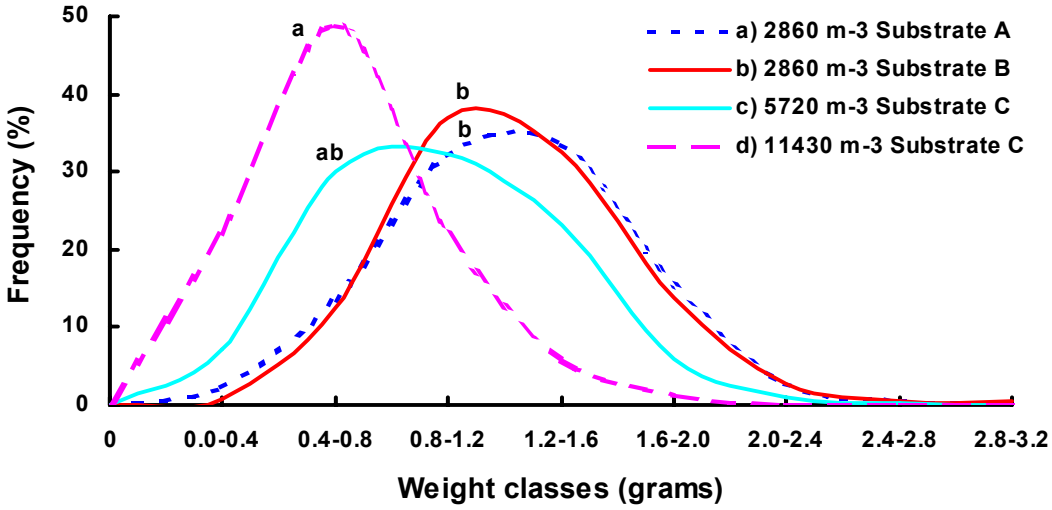


Figure 3.10 **Size distribution of *P. esculentus* juveniles grown for 7 weeks from PL17 at different densities with different substrates in a raceway system. Distributions with the same superscript are not significantly different**

(P>0.05). a) **mean weight of 1.229g,** b) **mean weight of 1.255g,** c) **mean weight of 0.971g,** d) **mean weight of 0.676g.**

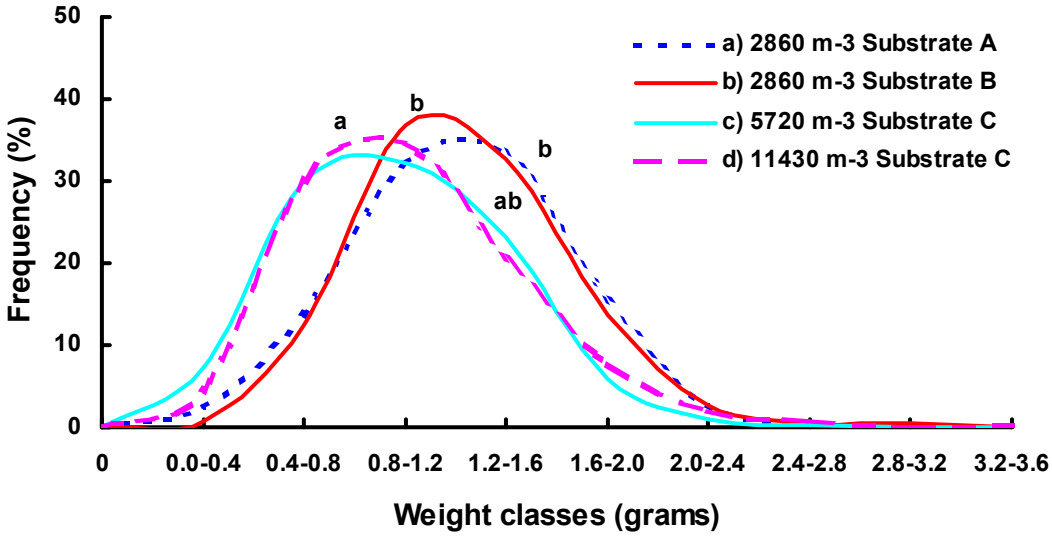


Figure 3.11 **Size distribution of *P. esculentus* juveniles grown to a mean weight of 1.0g from PL17 at different densities with different substrates in a raceway system. Distributions with the same superscript are not significantly different (P>0.05), a) mean weight of 1.229g after 7 weeks, b) mean weight of 1.255g after 7 weeks, c) mean weight of 0.971g after 7 weeks, d) mean weight of 1.039g after 8 weeks.**

3.2.3.5 Feed rates, regimes and composition

Feed rate data was recorded and summarised at the end of the experiment to produce a recommended feed regime (Table 3.4). Feed rates are expressed as a percentage of the estimated biomass of prawns. A minimum and maximum value is given because the rates varied between tanks and depended on the accuracy of estimating the biomass. In general the feed was administered at 20-25% of the biomass at PL17 and gradually reduced to 4-6% of the biomass by the time they averaged 1g. The composition of the feed was also recorded and summarized at the end of the experiment (Table 3.5). The feed was composed of a mixture of different sized crumbles of commercially formulated diets. It was important to steadily increase the range of sizes as the variation in prawn size increased with time.

Table 3.4 Summary of recommended feed rates and mixes for feeding *P. esculentus* juveniles from PL17 to 1g, at high densities in a raceway system.

Week	PL Age	Mean Weight (g)	Feed rate (%)*	Feed Mix
1	17-23	0.017-0.034	20-25	M1-M2
2	24-30	0.034-0.09	20-25	M2-M3
3	31-37	0.09-0.19	18-23	M3-M4
4	38-44	0.19-0.33	10-15	M4
5	45-51	0.33-0.54	7-11	M5-M6
6	52-58	0.54-0.78	5-8	M7
7	59-65	0.78-1.24	4-6	M8

* Feed rates are expressed as a percentage of the estimated prawn biomass.

Table 3.5 Composition of feed mixes fed to *P. esculentus* juveniles from PL17 to 1g at high densities in a raceway system.

Feed Mix	Percent of each Feed						
	PL DIET*	HIG5	HIG6	HIG7	HIG8	OP	HIG9
M1	60%	40%					
M2	30%	50%	20%				
M3		40%	50%	10%			
M4		20%	50%	30%			
M5			20%	50%	20%	10%	
M6				40%	40%	20%	
M7				20%	40%	40%	
M8				10%	50%	30%	10%

HIG = Higashi; OP = Ocean Popeye.
* PL diet comprises one part each of Artemia Flake, Higashi#2, Higashi#3, Frippak 300+ and CP Star 300.

3.2.4 Optimal stocking density and artificial substrate

Optimal stocking density
Based on the results of the high-density production, an optimal stocking density to culture *P. esculentus* juveniles to 1g would range between 3,300 m^{-3} and 3,700 m^{-3}. This optimal density was calculated from the following observations of the production data:

1) The higher densities, 5,720 m^{-3} and 11,430 m^{-3}, were excluded on the basis of:
   - Slower growth rates
   - Higher size variation at 1 g
   - Lower survival
   - Higher labour input required because prawns died in large numbers throughout the grow-out and waste build up was higher due to excess feed and faeces. This all had to be continually removed through siphoning
   - Risk was higher because the biomass was pushed beyond its maximum limit early. This caused poorer water quality that resulted in frequent events of mass mortality for the remainder of the grow-out.

2) The recommended optimal density is based on the results of the 2,860 m^{-3} density treatments due to:
   - Faster growth rates
   - Lower size variation at 1g
   - Higher survival
   - Less labour is needed to clean the raceways because wastes only appeared to build up towards the end of the grow-out
   - The risk of a crash was lower because the biomass was under its maximum limit until the end of the grow-out.

3) Calculation of optimal density.

The mean biomass at the three densities was very similar after 7 weeks. However, the mean biomass at the 2,860 m^{-3} density treatments could have been higher except for a two replicates that crashed just before the harvest at week 7. This result suggests that the biomass in the system at this density was close to its maximum limit and hence provides an indication of the optimal harvest biomass. Three of the 2860 m^{-3} replicates produced between 2,300g m^{-3} and 2,600g m^{-3}, and the survival ranged from 68% to 72%. Assuming 70% survival to a mean weight of 1 g, these results suggest that a stocking density of between 3,300 prawns m^{-3} and 3,700 prawns m^{-3} would result in a harvest biomass between 2,300 g m^{-3} and 2,600 g m^{-3}.

Optimal artificial substrate

There was no difference in prawn performance between the different artificial substrates tested. Therefore, the optimal artificial substrate is Substrate B, because this substrate is the easiest to maintain. Substrate B consisted mostly of preconditioned structure while Substrates A and C consisted mostly of AquaMats™. Substrate B has the following advantages over the use of AquaMats™ to commercial operators, especially the MG Kailis Exmouth Hatchery, WA

- Significantly cheaper;
- Easier to handle, clean and disinfect;
- Provides more even distribution of artificial feed;
- Are not prone to produce areas of anaerobic waste buildup;
- Do not produce moult fibres which block screens and filters;
- Preconditioned with biota which provides added surface area for benthic organisms to colonise;
- Less bouyant thereby reducing the number of weights needed to submerge the substrates. This reduces capital costs and also the amount of labour required to construct the weights, and;
- Less degradable therefore reducing the frequency of replacement.

3.3 High-density production of juveniles at Exmouth, WA.

Trial 1: August – December 2000

Trial 2: August – December 2001

3.3.1 Objectives

- Transfer the technology developed to date for the high-density production of P. esculentus juveniles to industry

- To produce between 50,000-100,000 P.esculentus juvenile prawns with a mean weight of between 0.5-1.0 g by developing high-density production techniques using commercial-scale raceways.
- Trial harvest, transport and release techniques for stocking juvenile prawns into Exmouth Gulf

3.3.2 The M.G. Kailis raceway system in Exmouth, WA

Four commercial-size raceway systems were designed and constructed at Exmouth. The design differed from the system used at CSIRO's laboratories, Cleveland, in that they needed to be significantly longer and wider. The aeration and water movement system developed in Cleveland, which created a water circulation effect that prevented waste build-up under the structures and concentrated waste for easy removal, was further developed at Exmouth. A simpler and more efficient aeration system was used to circulate water and concentrate the waste in the larger, deeper and significantly wider raceways.

Instead of using fiberglass, the raceways were constructed from concrete tilt-slab panels and steel universal beams, half above ground level and half below ground level, and were lined with 1mm High Density Polyethylene (HDPE). The raceways were 20m in length with an average depth of 1.35m (with 200mm free-board the average water depth was 1.15m. Two raceways were 1.50m wide and two were 2.50m wide. The volumes of the raceways were 34.5 m^3 and 57.5 m^3 for the 1.50m and 2.50m wide tanks respectively (Table 3.6). The raceways were housed in a shade-cloth tunnel. Artificial substrates were put into each of the raceways to increase the surface area. The substrates were made up of AquaMat™ and 3-D structures developed by MG Kailis, preconditioned and non-preconditioned structures.

Table 3.6 Raceway dimensions, volume and Trial 1 stocking densities.

Raceway	Dimensions (LxWxD)	Volume (m^3)	Stocking density (prawns m^{-3})
1	20mx2.5mx1.15m	57.5	909
2	20mx2.5mx1.15m	57.5	1,818
3	20mx1.5mx1.15m	34.5	3,636
4	20mx1.5mx1.15m	34.5	1,818

3.3.3 Methods

3.3.3.1 Production

Two weeks prior to the prawns being stocked, the raceways were spiked with bay water to introduce natural biota into the tanks. Around 20,000 L was pumped into Raceways 1 and 2 and around 10, 000 L was pumped into Raceways 3 and 4. Cultures of microalgae and microalgae nutrients were also added to promote phytoplankton blooms in the water column and the colonisation of natural biota on the artificial substrates. Each raceway was stocked with PL15 (fifteen-day-old postlarvae) *P. esculentus* at different densities (Table 3.6). The prawns were fed a mixture of different sized crumbles of commercially formulated diets over an 8-9 week period. Weekly samples of 100 prawns from each raceway were weighed individually to estimate the mean weight of prawns from each raceway.

3.3.3.2 Harvest, transport and release

The raceways were harvested on five separate days between 11th Dec. (7 weeks and 3 days after stocking) and 20th Dec. (8 weeks and 5 days after stocking). Harvested prawns were bulk weighed before being put into transport containers so that survival and biomass could be estimated and

also to trial different transport densities. To estimate the number of prawns harvested from a raceway on a given day, 500 g of harvested prawns were counted to provide a mean harvest wet weight. The total wet weight harvested was then divided by the mean harvest wet weight to estimate the total number of prawns harvested.

Once the prawns were harvested from the raceways they were stocked into four plastic transport tanks. Each tank had a capacity of 1 m^3 and was fixed with a lid and aerated with medical grade oxygen. The tanks were transported 30 min by truck to Exmouth, then loaded onto the "Blue Horizon" (the mother ship for the Kailis Pearl Oyster dive crew). The prawns were released into a designated release site about 8 nm to the south of Whalebone Island in Exmouth Gulf. This release site is about a 2-2.5 hour boat ride from the Exmouth harbour, including the loading of the tanks onto the vessel.

3.3.4 Results

3.3.4.1 Growth

The highest growth rate was recorded in Raceway 1 with a stocking density of 909 PL's m^{-3}, in which prawns grew to a mean weight of 0.99 g after 8.3 weeks (Fig. 3.12). The lowest growth rate of 0.553 g after 7.7 weeks was recorded in Raceway 3, with a stocking density of 3,636 PL's m^{-3}. Due to the commercial size of the trial each of the raceways were harvested over a number of days.

The results from Exmouth (prior to the start of harvest) are consistent with the results obtained from raceways at Cleveland whereby growth was inversely related to stocking density. It should be noted that the mean initial stocking weight of PL 15s was very low at 2.87 mg. The culture temperatures experienced in this trial were far from optimal with mean temperature in the first 4 weeks of 23.75'C, with a minimum of 21.4'C. Nevertheless good growth and high survival was achieved.

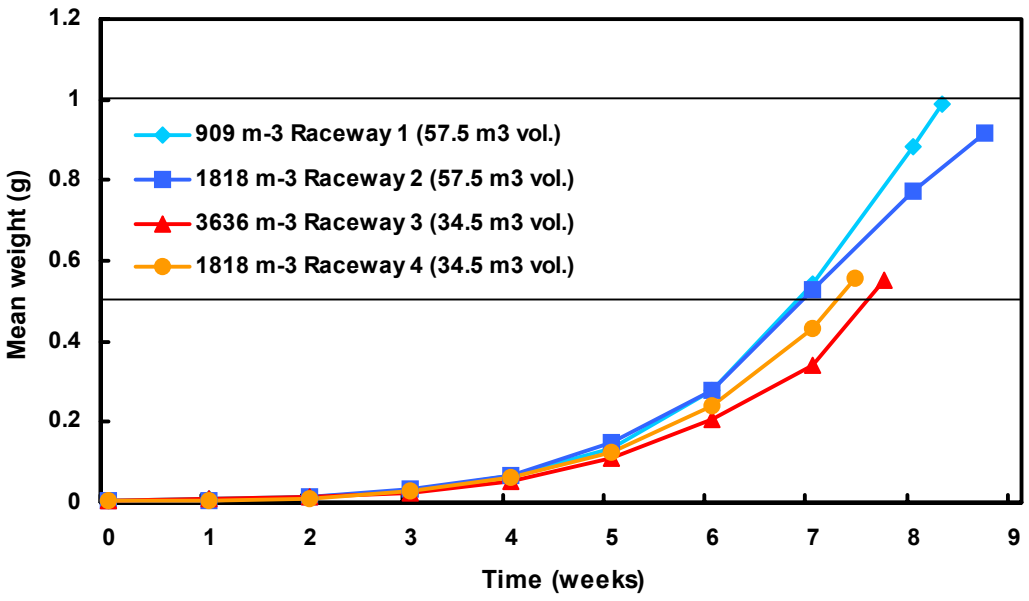


Figure 3.12 **Trial 1 Mean weights of *P. esculentus* juveniles grown for 7.7-8.7 weeks from PL15 in two different size raceway systems and different stocking densities. Raceway 1 (57.5m^3 volume), stocking density of 909 prawns m^{-3}. Raceway 2 (57.5m^3 volume), stocking density of 1818 prawns m^{-3}. Raceway 3 (34.5m^3 volume), stocking density of 3636 prawns m^{-3}. Raceway 4 (34.5m^3 volume), stocking density of 1818 prawns m^{-3}.**

3.3.4.2 Survival

Prawn survival throughout the production was very high ranging from 65% in Raceway 3 to 89% in Raceway 1 (Fig. 3.13). It is important to note that the survival values don't all correspond to the same day because of the different harvest times for each raceway. Also, when Raceway 1 was harvested, 15% of the harvested prawns were moribund, due to having no aeration for 10 hours overnight, a result of human error. However, Raceway 1 was ready for harvest prior to this mortality event. Therefore the survival estimate of 89% is indicative of the survival rate the day prior to harvest.

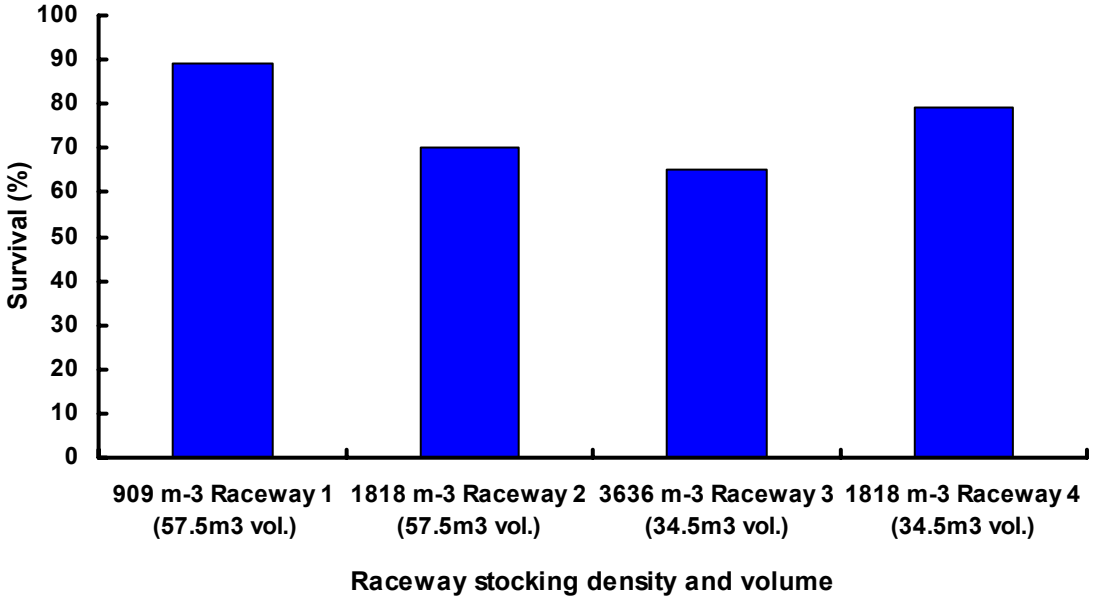
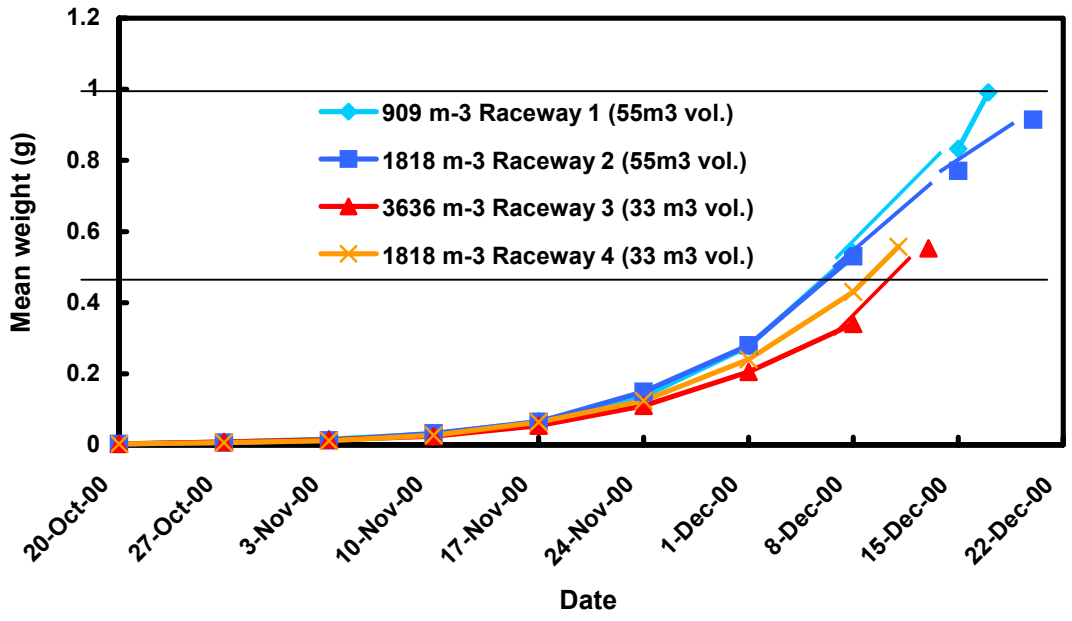


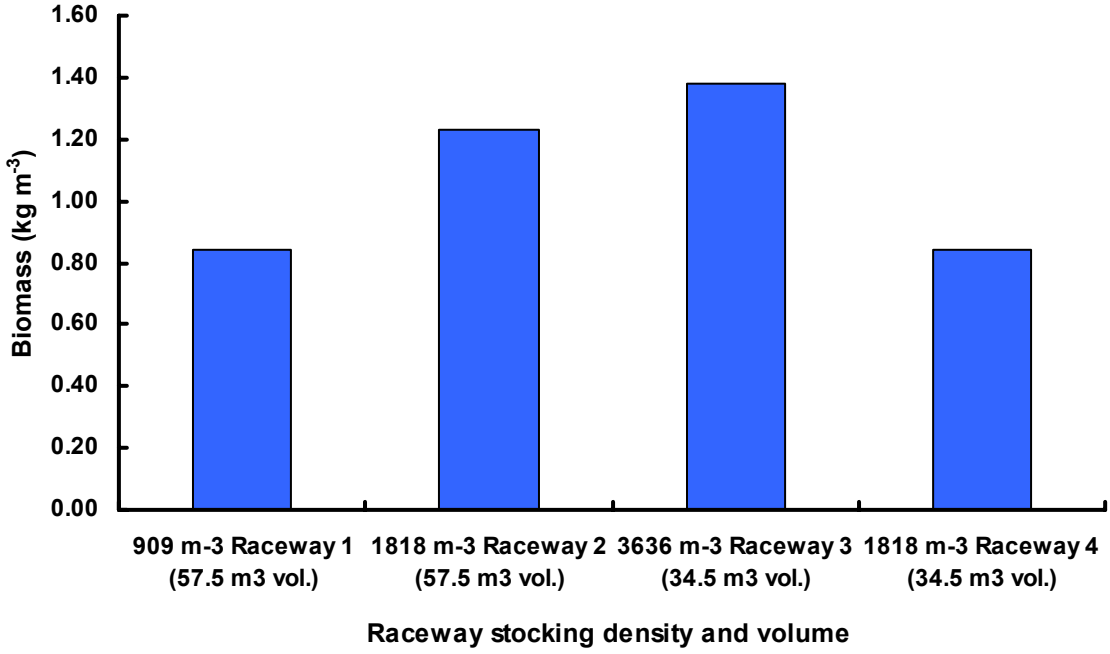
Figure 3.13 **Survival of *P. esculentus* juveniles grown from PL15 for 7.7-8.7 weeks in two different size raceway systems and different stocking densities. Raceway 1 was completely harvested after 8.3 weeks. Raceway 2 was completely harvested after 8.7 weeks. Raceway 3 was completely harvested after 8 weeks. Raceway 4 was completely harvested after 7.7 weeks.**

3.3.4.3 Biomass

The total biomass produced by each of the raceways ranged between 29.04 kg in Raceway 4 and up to 70.46 kg in Raceway 2 (Figure 3.14a).
In terms of biomass produced per m^3, the highest biomass of 1.38 kg m^{-3} was produced in Raceway 3, whereas the lowest biomass of 0.84 kg m^{-3} was produced in Raceways 1 and 4 (Fig. 3.14b).



a)



b)

Figure 3.14 **(a) Total biomass (kg) and (b) biomass per m^3 of *P. esculentus* juveniles grown for 7.7 to 8.7 weeks from PL15 in two different size raceway systems and different stocking densities. Raceway 1 was completely harvested after 8.3 weeks. Raceway 2 was completely harvested after 8.7 weeks. Raceway 3 was completely harvested after 8 weeks. Raceway 4 was completely harvested after 7.7 weeks.**

3.3.4.4 Harvest, transport and release

Harvesting started on the 11th December, seven weeks and three days from when the raceways were stocked, and ended nine days later on the 20th Dec. (Table 3.7). During this time prawns from Raceways 2, 3 and 4 were harvested and transported to the release site in Exmouth Gulf on five separate days. Prawns from Raceway 1 were harvested but not released because of concern due to the stress from an aeration failure just prior to harvest.

The transport tanks were initially stocked at a density of around 4.5 kg m^{-3}. 100% survival throughout the transport phase was observed during the first two days of release and by the third day the transport tanks were being stocked at densities up to 14 kg m^{-3}. An estimated total of 198,623 prawns were released into Exmouth Gulf over the five days of harvesting.

It is important to note that the mean individual wet weights taken at harvest are not the same values as that used for the growth rate data. The mean individual weights at harvest include a percentage of water on the prawns, which is impossible to remove without subjecting the prawns to an unnecessary amount of stress prior to release. For growth data the prawns were dried with paper towel prior to weighing.

3.3.4.5 Water Quality

The lowest recorded pH was 7.25 in week 7, whilst the highest pH was 8.75 during week 2. The maximum daily pH swing (difference in pH from 07.00 to 16.00) was 0.51. The maximum ammonia concentration recorded was 1.8 mg $litre^{-1}$ during week 7. The lowest recorded DO was 1.3 mg $litre^{-1}$ after the aeration had been inadvertently left off overnight. During normal operation the lowest recorded DO was 4.6 mg $litre^{-1}$.

The temperature varied from a minimum of 21.4°C in week 3 to a maximum of 31.0°C in the final week of the trial. During week 1 the mean water temperature was 23'C and by week 9 the mean water temperature was 29.3°C.

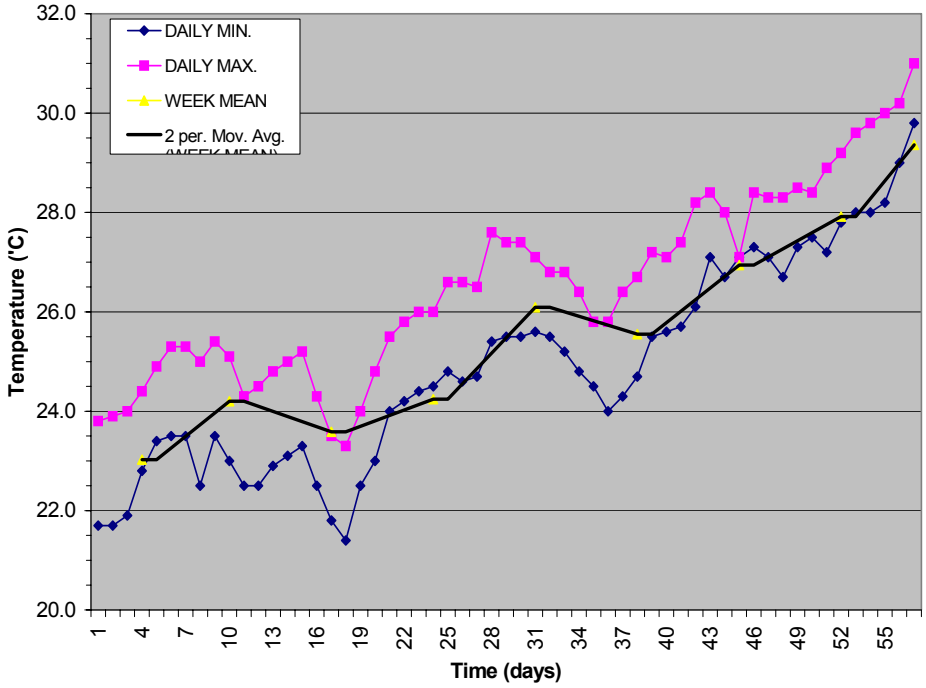


Figure 3.14A Raceway water temperatures in Exmouth Gulf during production trials between September and December 2000.

Table 3.7 Total wet weight, mean individual weight and estimated number of prawns harvested from the raceways and stocked into the transport tanks.

Date	Transport Tank No.	Raceway No.	Total Wet Weight (g)	Mean Individual Wet Weight at Harvest (g)	Estimated No. Prawns
11/12/00	1	4	4200	0.589	7130
	2	4	4550	0.589	7725
13/12/00	1	2	6450	1.160	5560
	2	2	8050	1.160	6940
	3	4	8750	0.714	12250
	4	4	9400	0.714	13100
15/12/00	1	3	9950	0.587	16950
	2	3	12100	0.587	20613
	3	3	11950	0.587	20358
	4	3	13950	0.587	23765
18/12/00	1	2	11600	1.179	9839
	2	2	11250	1.179	9542
	3	2	6550	1.179	5555
	4	2	6500	1.179	5513
20/12/00	1	2	11250	1.110	10135
	2	2	13850	1.110	12477
	3	2	7000	1.110	6306
	4	2	5500	1.110	4955
		Total	162,850		198,713

3.3.5 Trial 2 Production Results (2001)

3.3.5.1 Super-Intensive Juvenile P. esculentus Production Summary

MG Kailis

Super-Intensive Juvenile *P.Esculentus* Production Summary

2001, Exmouth

		RACEWAY				
		1	**2**	**3**	**4**	Total / Mean
Production						
Volume (m3)		57.5	57.5	34.5	34.5	
Initial Stocking Density / m3		2,610	3,480	4,350	2,610	3,263
Number Stocked		150,075	200,100	150,075	90,045	590,295
Production Duration (days)		77	72	57	57	
Survival		70.5%	64.6%	75.0%	80.4%	**72.6%**
Number Harvested		105,803	129,265	112,556	72,396	**420,020**
Number Harvested / m3		1,840	2,248	3,263	2,098	2,362
Mean Weight (g)		1.20	0.94	0.56	0.62	**0.83**
Biomass		126.96	121.51	63.03	44.89	
Biomass / m3		2.21	2.11	1.83	1.30	**1.86**
FCR		1.52	1.73	1.54	1.49	**1.57**
Water Quality						
Temperature ('C)	mean	25.50	25.21	25.74	25.19	**25.41**
	max	29.15	27.80	29.03	29.15	28.78
	min	22.31	22.32	21.95	21.80	22.10
	sd	1.28	1.35	1.62	1.80	1.51
pH	mean	7.89	7.86	8.04	8.13	**7.98**
	max	8.34	8.24	8.38	8.53	8.37
	min	7.48	7.50	7.60	7.65	7.56
	sd	0.18	0.18	0.17	0.22	0.19
Salinity (ppt)	mean	36.31	36.40	36.39	36.14	**36.31**
	max	37.58	37.37	37.24	37.40	37.40
	min	35.86	35.90	35.94	35.89	35.90
	sd	0.46	0.43	0.41	0.46	0.44
DO (ppm)	mean	4.97	5.03	5.52	4.97	**5.12**
	max	7.64	6.73	8.57	7.64	7.65
	min	3.21	3.40	3.79	3.21	3.40
	sd	0.76	0.73	0.71	0.76	0.74
Ammonia (ppm)	mean	0.11	0.19	0.21	0.15	**0.17**
	max	0.25	0.46	0.56	0.48	0.44
	min	0.04	0.01	0.04	0.01	0.03
	sd	0.06	0.14	0.17	0.16	0.13
Water Exchange (% / day)	mean	119	157	141	106	**131**
	max	210	250	230	200	223
	min	30	30	25	15	25

3.3.5.2 Trial 2 Transport Densities (2001)

Transport Tank (effective volume in m^3)	Number of Prawns	Mean Weight (g)	Total Biomass (kg)	Stocking/Transport Density (prawns/m^3) (kg/m^3)	Survival (%) (5days post-transport)
1 (1)	35,432	0.6	21.26	35,432 21.26	93
2 (1)	52,300	0.6	31.38	52,300 31.38	92

3.3.6 Conclusion

The semi-commercial raceway system developed at the MG Kailis hatchery in Exmouth WA, successfully produced 270,000and 420,020 0.5-1 g juvenile brown tiger prawns, in trials 1 and 2 respectively, at stocking densities of up to 3,636 prawns m^{-3} with a maximum biomass at harvest of 2.21 kg m^{-3} and a mean biomass of 1.86 m^{-3} in the second trial at Exmouth. The success of the production was a result of technology developed at CSIRO's laboratories in Cleveland being incorporated into the system, and the further development of this innovative technology at Exmouth.

The high growth rates (PL15 to around 1 g in 7 to 8 weeks), and survival levels (65% to 89%), achieved produced enough prawns that enabled harvest, transport and release methods to be tested. This in turn provided valuable information for the further development of production techniques in Cleveland. With minor modifications and up-scaling, the system developed has the potential to provide sufficient numbers of juvenile prawns to satisfy stock enhancement protocols.

3.4 Summary of findings from the CSIRO and Exmouth production trials

The combined research efforts of CSIRO and a pilot production run at the MG Kailis facility in Exmouth has led to the successful production of juvenile *P. esculentus* at high densities in land-based raceway systems. The following summarises the technology developed and the major results from the different production runs. This demonstrates the success of the high-density production approach.

Technology developed:

- A raceway tank design that's suitable for ultra-high density production, manageable and space efficient
- A 3-dimensional artificial substrate design that provides ecological benefits to the prawns throughout the different stages of production. This 3-dimensional substrate approach is essential for the ultra-high density production of juveniles.
- An innovative aeration system that prevents waste from building up underneath the substrates and enhances the management of feed regimes, water quality and ultimately prawn health
- Harvest, transport and release strategies have been developed and tested. The outcomes from this research has is provided a firm basis for the development of improved technology for future release operations.

Production outcomes:

- Growth from PL15 to 1 g in $6^1/_2$ to $8^1/_2$ weeks at stocking densities between 909m^{-3} and 1818 m^{-3}
- Survival rates up to 89%
- Biomass production up to 2.21 kg m^{-3}
- Calculation of an optimal stocking density between 3,300 PL's m^{-3} and 3,700 PL's m^{-3}
- Confirmation that preconditioned structures developed by MG Kailis are a suitable artificial substrate alternative to AquaMat™ fronds.
- Development of feed protocols throughout production
- Harvest, transport and release of around 200,000 juveniles into specific sites in Exmouth Gulf with no observed deaths
- Juvenile densities of up to 14 kg m^{-3} transported with no observed deaths.

3.5 Water Quality Management

3.5.1 Environmental characteristics of the raceway system

Good water quality is essential for the successful grow-out of prawns at high densities as it directly impacts on prawn health, which in turn influences growth and survival. Culture water is polluted through decomposition of uneaten food and prawn faecal products. The stringent monitoring program used in this experiment ensured water quality parameters remained within tolerable limits.

3.5.1.1 Temperature

Temperature is one of the most important physical parameters to maintain at its optimum as it:

- directly influences a prawns rate of respiration, feeding, digestion, assimilation, behaviour and growth;
- effects the solubility of oxygen in water;
- effects the rate of oxygen consumption by microbes decaying organic matter and excess feed;
- effects the rate at which fertilisers, fungicides, herbicides and toxicants (of anthropogenic or natural sources) dissolve and act in water, and;
- sudden changes in temperature of more than 5^0C can result in high mortality rates as prawns become stressed and more susceptible to disease (a sudden change from cold to warm can be more detrimental to prawn health than a change from warm to cold).

The optimal growing temperature for juvenile *P. esculentus* in the laboratory has been recorded at 30^0C, with an optimal range of 25^0C to 30^0C (O'Brien 1994a).

3.5.1.2 Ammonia

Ammonia is produced as a by-product from prawn protein catabolism and the decomposition of organic matter by microbes. As ammonia concentrations in the water increase, prawns minimise their rate of ammonia excretion resulting in increased ammonia concentrations in their blood and body tissues (Chien 1992). This ammonia retention causes increased blood pH and impacts on the prawns biological processes by;

- reducing its ability to carry out osmoregulation,
- reducing the ability of blood to transport oxygen,
- increasing oxygen consumption by body tissues, and
- causing gill filament haemorrhaging.

Furthermore, ammonia is present in water in two forms, a relatively non-toxic ionised form (NH_4^+), and a highly toxic unionised form (NH_3), which readily diffuses across cell membranes. The ratio of NH_3 to NH_4^+ increases with increased temperature and pH, and to a lesser extent is affected by salinity. Within a super intensive system such as the raceway, ammonia concentrations become dangerously high at a fast rate making monitoring an essential part of routine management.

Optimum ammonia concentrations vary with prawn species and size. For *P. japonicus* PL12's, ammonia concentrations should optimally be kept below 2.83 mg ammonia-N/l (Chen and Lei 1990), where concentrations for PL 30-50 *P. monodon* should be kept below 1.8 mg ammonia-N/l (Chin and Chen 1987). However, the safe concentrations of ammonia increase as the prawns get bigger. Chien (1992) reported that safe concentrations of ammonia for 13.4mm length juvenile *P. japonicus* and 35.5mm length juvenile *P. monodon* were 4.03 mg and 3.7 mg ammonia-N/L respectively.

3.5.1.3 Dissolved oxygen

The solubility of oxygen in water decreases as temperature increases. The primary loss of oxygen from the water occurs through respiration of the culture species and other micro-organisms in the tank. Oxygenation of water in this experiment was supplied through mechanical aeration, flow through water and photosynthesis. As respiration occurs 24 hours a day and photosynthesis only occurs in the light hours, oxygen depletion most commonly occurs in the early hours of the morning up until sunrise. It is important that mechanical aeration is used to compensate at these times. In a super intensive system such as the one used it is important to have 24 hour mechanical aeration as fluctuations in dissolved oxygen concentrations are most common in such a system where autotrophic populations are high, tanks are small and prawn density is high.

For the closely related species *P. monodon*, it was suggested by Law (1988) that dissolved oxygen concentrations should be kept above 2.0 mg/l for postlarvae. However, for *P. japonicus,* which can tolerate lower levels of dissolved oxygen, it is thought that concentrations of less than 1.0 mg/l are lethal (Egusa 1961).

3.5.1.4 pH

Water pH fluctuates, increasing during the day due to the removal of carbon dioxide by photosynthesis and decreasing during the night. High pH increases ammonia toxicity due to increased NH_3 levels, and increases the fraction of toxic hydrogen sulfide (H_2S). Extremely low pH can severely stress prawns by causing their calcium carbonate exoskeleton to dissolve, resulting in high moralities. When moulting, low pH has an exacerbated detrimental effect on prawn health, as prawns have no hard outer exoskeleton to protect their soft body tissues.

It is thought that the extremely low pH of the black anaerobic sediment that accumulated in the raceway system from prawn waste and uneaten food was the cause of many prawn deaths in previous attempts to rear juvenile *P. esculentus* at high densities. A pH range of 7.5 to 8.5 has been recommended by Wickens (1976) for the culture of *P. monodon*.

3.5.2 Objectives

- To observe the effects of stocking density and total tank biomass on water quality parameters during the raceway rearing process
- To maintain water quality parameters within optimal limits for the duration of the experiment

3.5.3 Methods

In all treatments temperature was recorded every half hour using a Hastings Data Logger. Dissolved oxygen and pH were measured twice weekly using a TPS machine. Ammonia was

measured one to two times per week using either a standard technique as outlined in Appendix 2 or the HACH® technique.

3.5.4 Results and discussion

3.5.4.1 Temperature

For the duration of this experiment temperature remained around the optimal level of 28^0C, except on one occasion when it approached a critical limit of 34^0C due to exceptionally warm weather (Fig 3.15). At this extreme temperature, prawn health was compromised, and did result in some deaths. Flow rates were not increased to alleviate this peak in temperature as they had already been increased previously. A further increase in flow rate can not be applied in the commercial situation at Exmouth, Western Australia and was therefore not applied. Apart from this peak, temperature was at its optimum throughout the rearing process, allowing optimal prawn growth and health.

3.5.4.2 Ammonia

For the duration of the raceway experiment ammonia concentrations remained within acceptable limits with the maximum level detected being 2.75 mg ammonia-N/l (Fig. 3.15). When such high levels were detected they were alleviated by increasing flow rates and siphoning tanks to reduce organic matter and microbial loads. An overall trend shows that as total biomass increases over time, ammonia concentrations become greater and need to be monitored more tightly.

3.5.4.3 Dissolved oxygen

Throughout this experiment dissolved oxygen concentrations remained within acceptable limits ranging from 2.0 mg/l to 9.0 mg/l (Fig. 3.15). When concentrations around 2.0 mg/l were detected mechanical aeration was increased along with flow rates, and tanks siphoned to reduce microbial oxygen consumption.

As the total biomass of each tank increased, it was observed that dissolved oxygen concentrations were lower than average for the duration of the day. This may have a severe impact when a series of cloudy days or poor weather sets in, as photosynthesis rates are compromised in these conditions, leaving mechanical aeration and flow through water to oxygenate the system. Dissolved oxygen concentrations recorded throughout this experiment were within acceptable ranges, showing that prawn growth and health were not compromised by this parameter.

3.5.4.4 pH

For the duration of this experiment pH ranged from 7.5 to 9.0 (Fig. 3.15), and appeared to have no detrimental impact on prawn health and growth. It is likely that pH remained within this optimal range as a result of other water quality parameters being well managed.

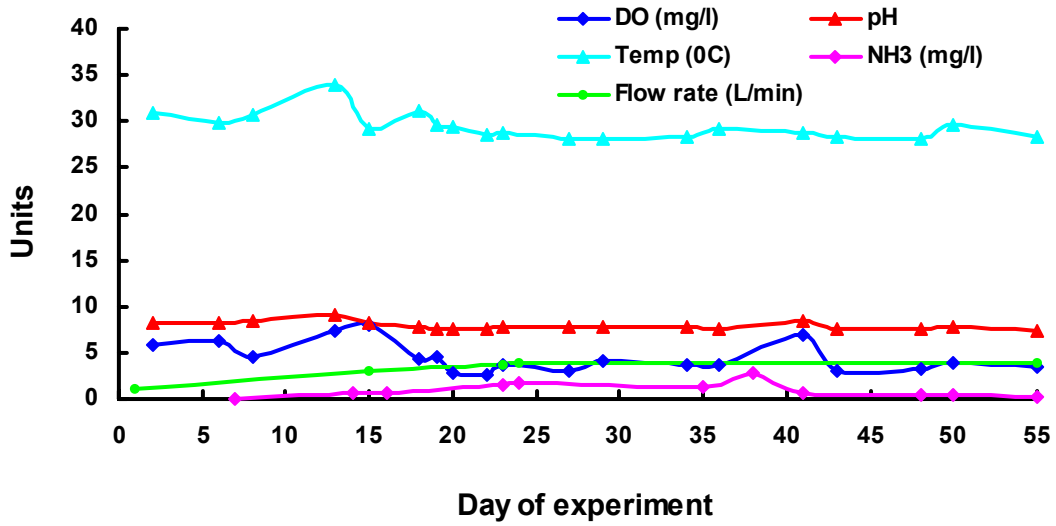


Figure 3.15 **Water quality data for five different parameters over the duration of the raceway experiment. This graph represents the average reading from all twelve tanks.**

3.5.5 Conclusion

When parameters approached or fell outside acceptable limits various management practices were undertaken to alleviate the problem, for example:

- siphoning to remove built-up particulate waste
- shading the raceway
- altering flow rates periodically
- increasing mechanical aeration.

The four water quality parameters monitored varied little between treatments, remaining within acceptable limits for the majority of the experimental period (Fig. 3.15).

General analogies can be drawn from these results. For example, peaks in temperature caused by warm weather, increased prawn activity and consequently increased the production of ammonia. These elevated ammonia concentrations were detected through routine water quality monitoring and managed by increasing flow rates. With a corresponding lag in time, the increased flow rates caused elevated dissolved oxygen concentrations and pH.

3.6 Analysis of juvenile prawn health and post production recovery

3.6.1 Background

Optimal survival and growth of prawns is affected by their health status. Many factors can affect prawn health such as water quality parameters and diet quality and quantity. Prawns reared at high densities may have their health compromised by this rearing environment. Also, poor quality postlarvae (PL) can affect the initial performance of prawns within a high-density rearing system, slowing down their overall growth rate. Following the growout period, the harvest, transport and release procedures can cause additional stress and damage to the juvenile prawns.

When reared at high densities the interactions between prawns increases, making them more prone to attack by cannibalism. As prawns grow and the total biomass in each tank increases,

prawns have even less room to escape attack. At the same time, the susceptibility of prawns to cannibalism increases when they moult. It is estimated that PL's when initially stocked in the raceway would moult 4 to 5 times per fortnight, but decreasing to once per week for 1 gram prawns. Heales et al. (1996) suggest that in the wild , they are moulting every 3 days at this stage – i.e. 4 or 5 moults in a fortnight). The potential for damage to prawns therefore would be expected to increase with increased rearing density.

After being released into the natural environment during enhancement, the prawns must adapt to a new environment, evade predators, search for food, find a habitat and recover from any external damage sustained during high-density rearing. From a preliminary examination of prawns recaptured immediately after a trial harvest and release in Exmouth Gulf, Western Australia, it was observed that virtually all prawns were without antennae, and most had missing legs (Haywood, CSIRO Marine Research, pers. com. 2001). The type of damage to the juveniles suggested that this it had occurred during the production phase, and not during the harvest, transport and release. These observations raise the concern that damage during high density production could lead to poor initial survival rates after the prawns are released. This study was designed to identify the damage sustained during high-density rearing, and to evaluate recovery from this damage.

3.6.2 Objectives

- To ascertain the health of prawns at stocking in the high-density rearing system and the impacts of the stocking procedure on the health of postlarvae.
- To monitor the health of prawns throughout the high-density rearing process and ascertain whether prawns reared at high densities become more damaged than prawns reared at lower densities.
- To investigate how long it takes prawns to recover from damage sustained during the high-density raceway rearing process and whether prawns reared at different densities recover at different rates.

3.6.3 Methods

3.6.3.1 Assessing initial postlarval health

Four measures of initial health were taken:

- initial PL dry weight, and the response of PLs to three stress tests
- a salinity stress test
- a formalin stress test
- a stocking stress test.

Initial PL dry weight

Prawn weight at PL17 was calculated by averaging the dry weight of 100 PL's from the same batch of PL's used to stock the raceway. Dry weights were converted to wet weights based on the assumption that 77.2% of a PL's wet weight is water (This was calculated from the wet and dry weights of 200 individual PL's in earlier experiments).

Salinity stress test

One hundred postlarvae were put in aerated water at a salinity of 10% for two hours and survival rates were recorded one and two hours after this. Salinity was then increased to 35% over 15 minutes and survival was recorded after at one and two hours. Postlarvae were then fed a diet of equal proportion of higashi #2, higashi #3, CP star300, artemia flake and Frippak 300+. The

level of digestive tract fullness was examined 24 hours after feeding to assess any effects of salinity stress on feeding behaviour.

Formalin stress test

One hundred postlarvae were put in water with a formalin concentration of 200 ppm for two hours with aeration and then transferred back to fresh seawater. Survival rates were recorded after one and two hours of exposure.

Stocking stress test

Two 100 L tanks with aeration and water exchange were stocked with 250 postlarvae which were put through the same stocking stresses from counting and stocking as prawns stocked in the experiment. They were fed a diet of equal proportion of higashi #2, higashi #3, CP star300, artemia flake and Frippak 300+. Their survival was recorded over a 7-day period to assess any level of stress associated with counting and stocking.

3.6.3.2 Assessing prawn condition during grow-out

Characterising damage

Prawn condition was recorded weekly for the duration of the experiment by microscopically examining the degree of damage sustained by the different body parts of each prawn. Twenty prawns from each treatment were examined, and the main characteristics examined included categories for the gut cavity, weight and appendages (Fig. 3.16). A unit of damage was assigned based on the degree of damage observed in each category. A total score was given for each prawn based on all units of damage. Appendix 3 outlines the different categories for the scoring system used.

The relationship between damage, density and substrate, and the time from stocking, were initially compared using the heterogeneity of slopes test (PROC GLM, SAS Institute Software). Where slopes of the regressions were found consistent among treatments, differences in intercepts were then analysed by analysis of covariance.

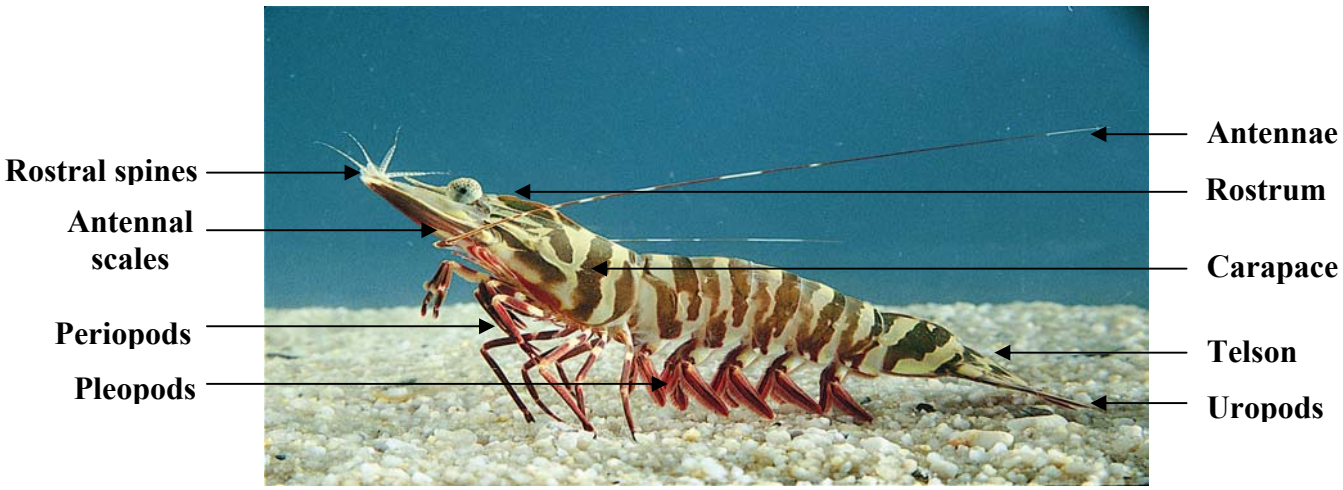


Figure 3.16 Lateral view of adult *Penaeus esculentus* showing appendages examined during routine health checks.

3.6.3.3 Assessing post-production recovery

The recovery rate of juvenile prawns was assessed and compared between prawns grown for 7-8 weeks in different tank systems and stocking densities (Table 3.8).

Table 3.8 Treatment descriptions for assessing post-production recovery

Treatment	Tank System	Stocking Density	Internally Tagged
1	Raceway	2860 m^{-3}	Yes
2	Raceway	5720 m^{-3}	Yes
3	Raceway	11430 m^{-3}	Yes
4	Sandy-bottom	75 m^{-3}	Yes
Control	Sandy-bottom	75 m^{-3}	No

Each treatment and the control had three replicates and ten prawns per replicate. All prawns were internally tagged with fluorescent elastomer implants for individual identification, except for those in the control treatment (5).

The condition of prawns was recorded at 0, 7, 15 and 22 days after release into sandy-bottom tanks by recording the level of damage (Fig. 3.17). The same examination procedure as used for the 'prawn condition during grow-out' study was applied, allowing each prawn to be given a total score of damage for each assessment day (Appendix 3). Differences in the rate of recovery within and between treatments were compared by analysis of variance using SAS (SAS Institute Software).

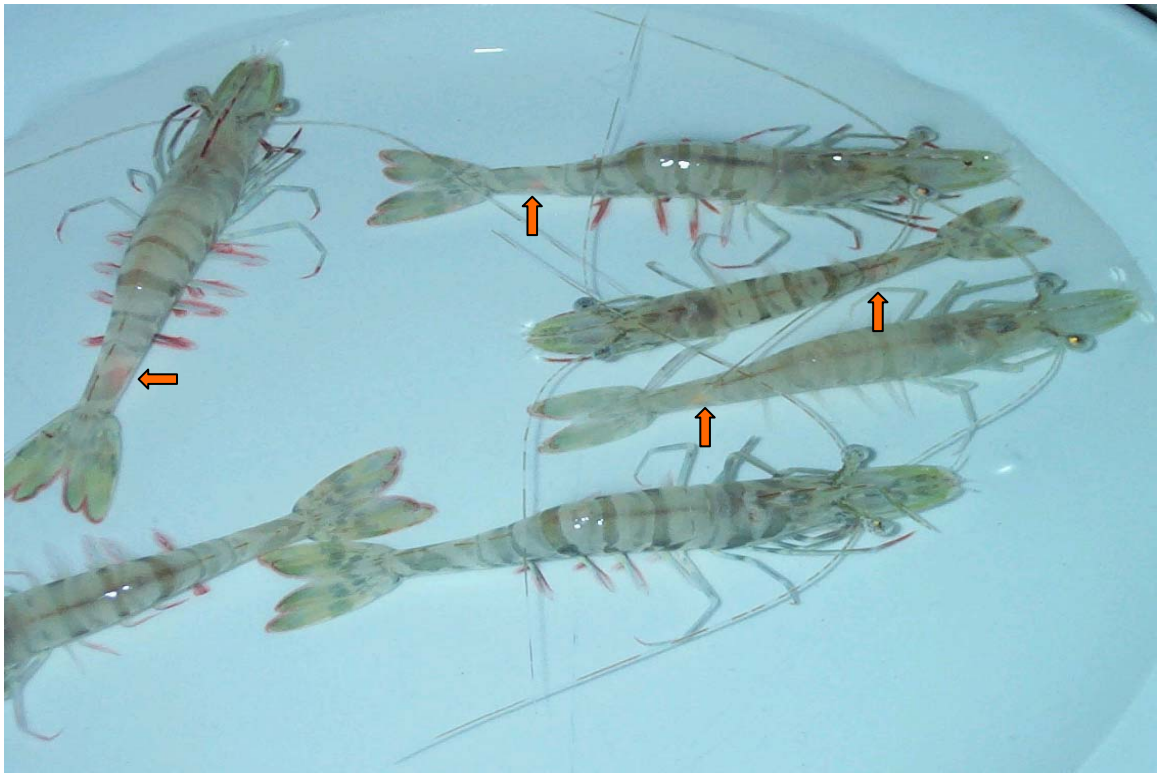


Figure 3.17 Prawns with internal tags (indicated by arrows) after damaged appendages have recovered.

3.6.4 Results and discussion

3.6.4.1 Initial postlarval health

Prawns stocked in the high-density rearing experiment at PL17 had an average wet weight of 0.017 g. For the three previous high-density rearing experiments the initial weights of the PL's ranged between 0.004 g and 0.008 g (Fig. 3.18). The high initial PL weights for this fourth experiment gave the prawns a head start when stocked into the raceway system, and may be partly responsible for the successful growth rates achieved.

The PL's exposed to 10 ppt salinity had 100% survival after 1 and 2 hours, and the PL's exposed to 200 ppm formalin also had 100% survival after 1 and 2 hours. These animals were fed and kept over night, and survival was 100% the next day. Also, 65% of the PL's had full digestive systems suggesting that their feeding behaviour wasn't severely affected by the stress tests. Two 100L tanks were stocked with 250 postlarvae which were put through the same stocking stresses as postlarvae stocked into the experiment. After 1 week the survival was 100%. From these results we concluded that the PL's used to stock the raceways were in good health and condition and that our stocking technique had no impact on prawn health.

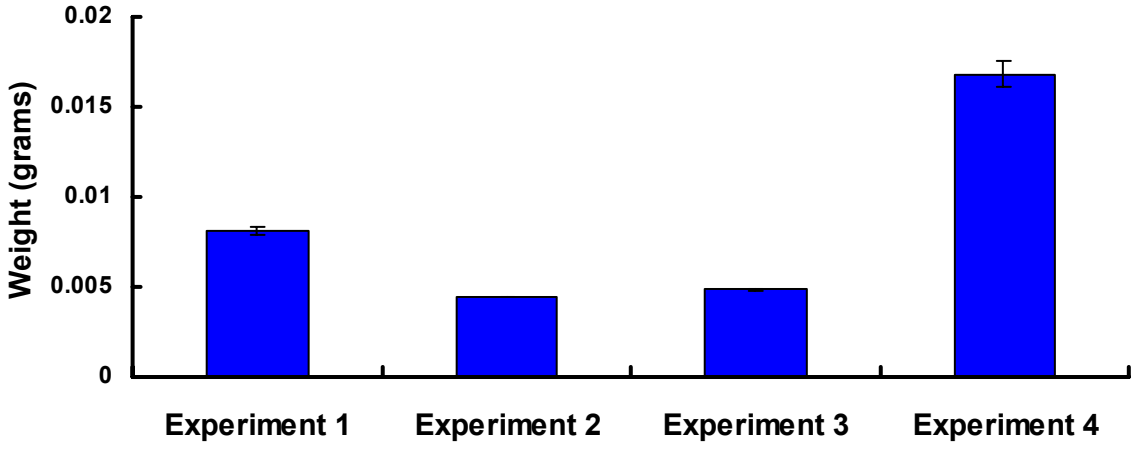


Figure 3.18 Estimated mean postlarval wet weight (± 1 SE) at stocking for the three previous raceway experiments and experiment 4, which is the focus of this report.

3.6.4.2 Prawn condition during grow-out

High-density raceway reared prawns were significantly more ($P<0.05$) damaged over time, whereas prawns reared at a low density in sandy-bottom tanks were significantly less ($P<0.05$) damaged over time (Fig. 3.19). Although the mean level of damage sustained by prawns increased with density, it did not differ significantly ($P>0.05$). There were also no significant differences ($P>0.05$) between the level of damage sustained by prawns reared on the different substrate types.

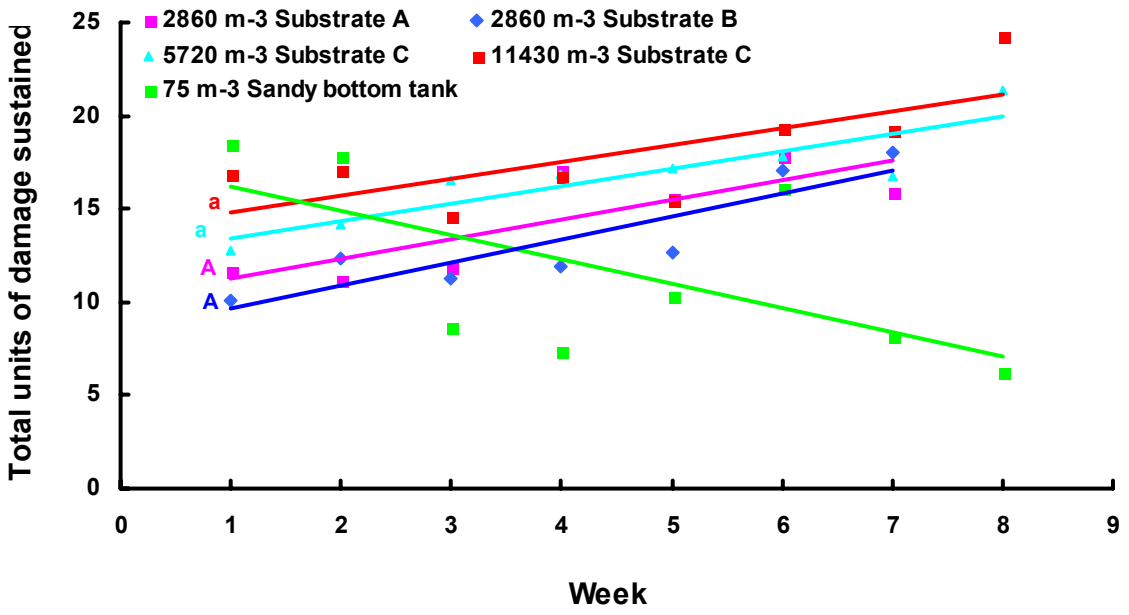


Figure 3.19 **Condition of juvenile *P. esculentus* when reared at different densities in a raceway system and at low densities in a sandy-bottom tank. Trendlines with the same superscript are not significantly different (P>0.05).**

3.6.4.3 Post-production recovery

The rate of appendage recovery did not differ significantly (P>0.05) between densities over time (Fig. 3.20). Prawns recovered more than 65% of the units of damage sustained during the high-density raceway rearing process within seven days of rehabilitation (Fig. 3.21). Rearing density had no apparent effect on this recovery rate.

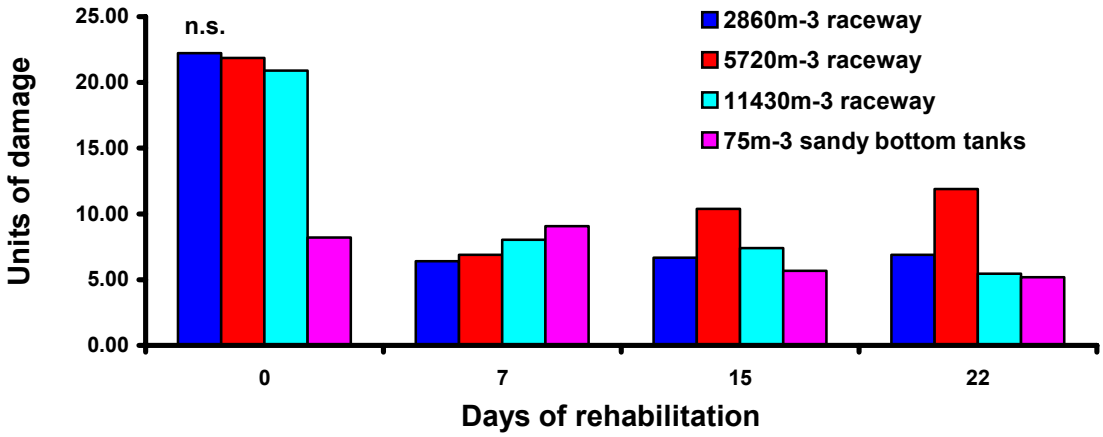


Figure 3.20 **Units of damage sustained by juvenile *P. esculentus* reared at high densities in a raceway and low densities in a sandy-bottom tank, after being rehabilitated in sandy-bottom tanks (including necrosis).**

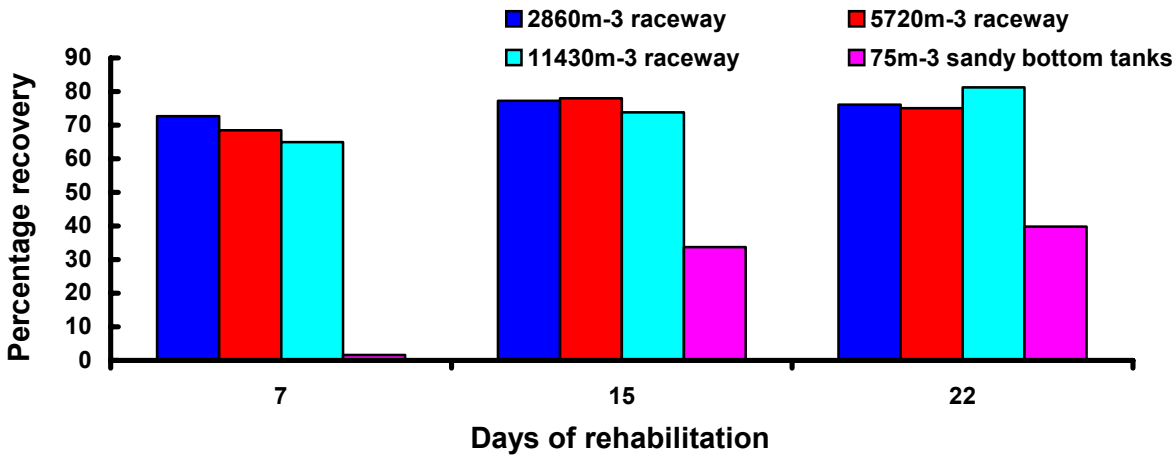


Figure 3.21 Percent recovery of juvenile *P. esculentus* reared in different tank systems at different densities.

3.6.5 Conclusion

The high-density raceway rearing process resulted in an increase in prawn appendage damage over time. Prawns recovered 65% of this damage within seven days of rehabilitation in a sandy-bottom environment at low densities.

Understanding the moult cycles could potentially reduce the recovery time post-release by using it as an indicator of the optimal harvest time. Moult stage C is likely to be the ideal stage to harvest the prawns because this is when the exoskeleton achieves maximum rigidity (Smith & Dall 1985). However, future research needs to focus on:

- prawn appendage recovery during the first seven days after release and how this recovery relates to the frequency of moult cycles and moult stage;
- how synchronous moult cycles are throughout the high-density production, especially when they reach harvest size, and;
- the acceptable percentage of prawns at the "desirable" moult stage so that survival is optimal during post harvest and release.

3.7 Determination of the primary sources of nutrition for juvenile prawns using isotope analysis

3.7.1 Background

In their natural habitat, juvenile *P. esculentus* are omnivorous, with gut content analysis showing that protozoa, diatoms, seagrass and zooplankton are the preferred food sources (O'Brien 1992). Much of the microalgal and protozoan species consumed are likely to be seagrass epiphytes (Loneragan, Bunn & Kellaway 1997). Other juvenile and postlarval penaeid species also consume considerable amounts of plant material (Newell et al., 1995; Dittel et al., 1997). However, as *P. esculentus* grow, their feeding behavior shifts from omnivorous to carnivorous (Smith et al. 1992, O'Brien 1994).

In intensive pond and tank systems, the nutritional requirements of shrimp are generally met by the addition of formulated feed. However a number of studies have shown that the natural biota can contribute substantially to shrimp growth (Leber & Pruder 1988; Moss 1995; Otoshi et al., 2001). Based on these findings, trials have been conducted to assess the usefulness of substrates which promote the growth of epiphytes and microbial biofilms as a food source for the shrimp, *Litopenaeus vannamei* and *Farfantepenaeus paulensis* (Bratvold & Browdy 2001; Thompson et al., 2002). The presence of substrates has also been shown to improve the growth of the freshwater prawn, *Macrobrachium rosenbergii* (Tidwell et al., 2002).

Stable isotope ratios of food sources and consumers can be used as a measure of the importance of different food sources to prawn nutrition (Gearing 1991; Shearer & Kohl 1993). The $\delta^{15}N$ and $\delta^{13}C$ ratios are most commonly used. Using mixing models, the proportion of prawn nutrition that was contributed by each food source can be calculated. This method has been used to determine the contribution of natural foods to prawn nutrition in aquaculture ponds (Parker et al., 1989, 1991). However, this method has some disadvantages. If the isotopic signature of food sources are the same or very similar, it is not possible to differentiate between them. Additionally, due to the natural variability in ratios, values for the contribution of food sources have significant errors associated with them.

Another approach is to label the natural food with an enriched form of either $\delta^{15}N$ or $\delta^{13}C$. The ^{15}N-nitrogen can be traced through the food web and into the prawns by measuring $\delta^{15}N$ or $\delta^{13}C$ ratios in the prawns and food sources. This approach has been used to determine the contribution of various food sources and ingredients to prawn nutrition (Preston 1996; Burford 2000; Burford et al., 2002).

This study used two methods to determine the contribution of natural food to the nutrition of juvenile *P. esculentus* in high-density tank experiments: $\delta^{15}N$ and $\delta^{13}C$ natural abundance ratios in the natural and artificial feeds, and prawns; and spiking tanks with ^{15}N-ammonium, then tracing ^{15}N-nitrogen into the prawns.

3.7.2 Objectives

- To ascertain the source of nutrition for juvenile P. esculentus in the early stages of the high density rearing process.
- To compare the use of natural abundance δ15N and δ13C ratios to that of an enriched δ15N tracer for determining the contribution of the epiphytes to the nutrition of juvenile P. esculentus.

3.7.3 Methods

3.7.3.1 Experimental and tank design

The experiment was conducted in tanks during a high-density *P. esculentus* experiment (Section 3.2). Treatments included, prawn stocking density and substrate comparisons (Table 3.9). In total, there were three substrate types, all varying in material components and hence surface area for growth of epiphytes. Treatments A, B, C and D are referred to in the Section as: Substrate A 3000, Substrate B 3000, Substrate C 6000 and Substrate D 11000 respectively (see section 3.2.2.1 for detail on substrates).

Table 3.9 Experimental design for high-density rearing and substrate comparisons.

Comparison	Stocking density (prawns m^{-3})	Reps	Substrate	Description	Surface area (m^2)
Substrate	2860	3	A	AquaMat™ + other structure	24
	2860	3	B	Other structure	25.6
Density	5720	3	C	AquaMat™	8.6
	11430	3	C	AquaMat™	8.6

3.7.3.2 Artificial and natural feeds

Natural food items such as epiphytes and zooplankton were encouraged to grow on the substrates and tank walls by spiking tanks with 55 L unfiltered seawater from adjacent Moreton Bay and fertilising with: 35 g of Yates Gro-Plus Complete Plant Food; and soluble nutrients (1.6 g potassium nitrate, 0.13 g sodium dihydrogen orthophosphate and 0.2 g sodium metasilicate). Tanks were then maintained for 5 d prior to stocking to allow time for the natural biota to become established on the substrates.

Two formulated feeds were used: a dried diet which was a combination of commercial feeds for postlarval shrimp; and a high protein shrimp feed ground in a hammer mill to a size of 1 – 1.5 mm. After stocking, prawns were fed the artificial diets three times daily to satiation. The feed rates and composition were adjusted on a day-to-day basis. In general, the feed was administered at 20-25% of the estimated biomass. See Section 3.2.2.4 and 3.2.3.5 for more detail.

3.7.3.3 Sampling

Natural abundance isotope experiment

Samples were taken between days 10 and 13 after stocking when the prawns were between PL 27 and PL 30 (approx. 0.032 ± 0.0011 g wet weight). Material sampled included:

1. epiphytes
    (a) 1 g ww sample from the substrates (AquaMat™ (Fig. 3.22) and other structures)
    (b) 1 g ww sample from the wall with the air supply (see Figs. 3.23 and 3.1 Section 3.2.2.1)
    (c) 1 g ww sample from the wall adjacent to the air supply (see Figs. 3.1 Section 3.2.2.1)
2. 1 g ww sample of prawns (approximately 20 in total)
3. 1 g of each of the different artificial diets.

Epiphytes were collected with a scalpel by scraping the surface of the substrates. Prawns were collected by random netting throughout each tank. Samples were stored frozen and later dried at 60^0C for 24 h on acid washed glass petri dishes. They were then ground with mortar and pestle. Prawns sampled from each tank were combined before grinding.

^{15}N-enrichment experiment

Fourteen days after stocking, when prawns were PL 32 (approx. 0.077 ± 0.005 g wet weight), the ^{15}N isotope ratios were elevated in all tanks by adding ^{15}N-ammonium chloride (NH_4Cl, 99 atom%) at a rate of 10% of the background ammonia concentration (Table 3.10). Ammonia

concentrations were determined using the phenol-hypochlorite method (American Public Health Association, 1995). Flow-through water was turned off for 4 h to allow the epiphytes time to assimilate and cycle the ^{15}N-ammonium. One hour after the addition of ^{15}N-ammonium chloride, biota samples were taken and 24 h later, prawn samples were taken. Collection and processing protocols for this study were the same as for the natural abundance isotope experiment.

The biomass of epiphytes and shrimp was estimated. In the case of epiphytes this involved scraping off the biomass on a known area of substrate then weighing and extrapolating weights for the entire tank. The wet weight: dry weight ratio was calculated by weighing epiphytes before and after drying at 60°C for 24 h. Shrimp biomass was calculated from the stocking density, estimated mortality and average shrimp wet weight (100 animals were weighed per tank).

Table 3.10 Amount of ^{15}N-ammonium chloride added to each tank.

Tank	Treatment	Background ammonia concentration on 16/1/01 (mg L^{-1} NH_3)	Amount of $^{15}NH_4Cl$ added (mg N $tank^{-1}$)
1	Substrate B 3000	0.04	10.90
2	Substrate B 3000	0.02	10.90
3	Substrate A 3000	0.02	3.63
4	Substrate B 3000	0.00	10.90
5	Substrate A 3000	0.00	3.63
6	Substrate A 3000	0.00	3.63
7	Substrate C 6000	0.04	10.90
8	Substrate C 11000	0.23	92.65
9	Substrate C 6000	0.01	10.90
10	Substrate C 11000	0.14	92.65
11	Substrate C 11000	0.14	92.65
12	Substrate C 6000	0.01	10.90

3.7.3.4 Analyses and calculations

Natural abundance samples of epiphytes and prawns were analyzed for $^{13}C/^{12}C$-carbon ratios and $^{15}N/^{14}N$-nitrogen ratios, and carbon and nitrogen content using a mass spectrometer (Europa). ^{15}N-enriched samples were analysed for $^{15}N/^{14}N$-nitrogen ratios and nitrogen content using a mass spectrometer.

The following formula was used to calculate the $\delta^{15}N$ and $\delta^{13}C$ ratios:

$$\delta^{15}N \text{ or } \delta^{13}C = [(R_{sa}/ R_{std}) - 1] \times 100$$

Simple mixing models, assuming only two sources of carbon and nitrogen, were used to determine the contribution of carbon and nitrogen from epiphytes to the nutritional requirements of the prawns (Gearing 1991, Gu et al. 1996).

The formula for the carbon natural abundance data is:

$$F_x = \frac{\delta 13C_m - \delta 13C_y}{\delta 13C_x - \delta 13C_y}$$

F_X is the fraction of carbon from source X
x = amount of carbon from source X
y = amount of carbon from source Y
$\delta^{13}C_m$ = isotope ratio of the mixture

$\delta^{13}C_X$ = isotope ratio of source X
$\delta^{13}C_m$ = isotope ratio of source Y

The formula for nitrogen natural abundance data is:

$$F_{Nx} = \frac{x - y}{e}$$

F_{NX} is the fraction of nitrogen from source X
$x = \delta^{15}N$ of prawns
$y = \delta^{15}N$ of food source
$e = \delta^{15}N$ enrichment factor resulting from trophic transfer during feeding (assumed to be 3.5 %)

To calculate the contribution of epiphytes to the nitrogen requirements of prawns using the enriched ^{15}N data, it was assumed that prawns consumed 25% of their body weight in food each day, and thereby total nitrogen intake was determined. The formula used to determine the total amount of nitrogen contributed from the epiphytes was:

$$Y = \frac{P_{15N} * 100}{E_f}$$

Y is the total amount of nitrogen consumed by the prawns (mg)
P_{15N} = total amount of ^{15}N-nitrogen in the prawns (mg)
E_f= ^{15}N enrichment of the epiphytes (atom%)

3.7.4 Results and discussion

3.7.4.1 Substrate and tank wall communities

Visual examination of the epiphytes showed that there were considerable differences between treatments. Initially the density comparison treatments had a high level of epiphyte coverage on the walls, especially the wall where the air bubbles were generated. The epiphytes were dominated by low profile algal turf with short filamentous threads. The epiphytes also varied considerably between substrate types. The additional structure on Substrates A & B, had very low profile algal turf with few short filamentous strands, whereas the AquaMat™ in Substrates A & C had a dense mat of short filamentous algae.

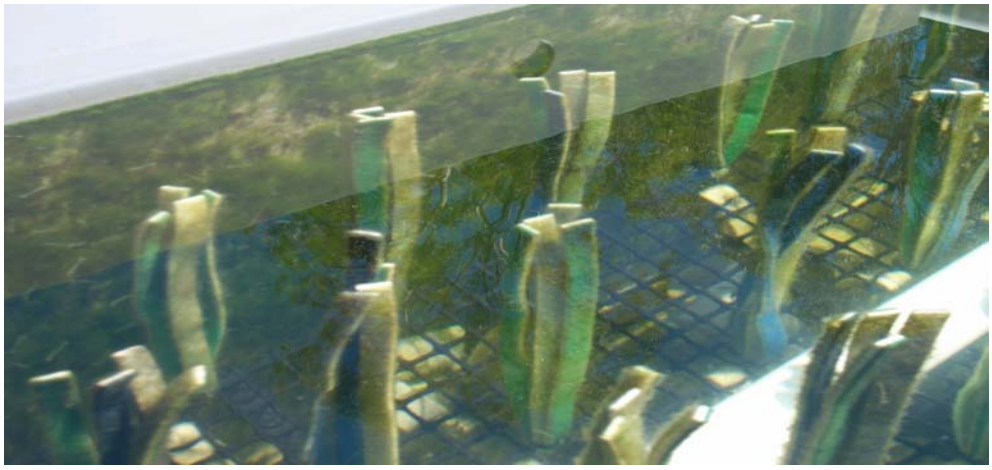


Figure 3.22 Epiphyte growth on the Substrate C.

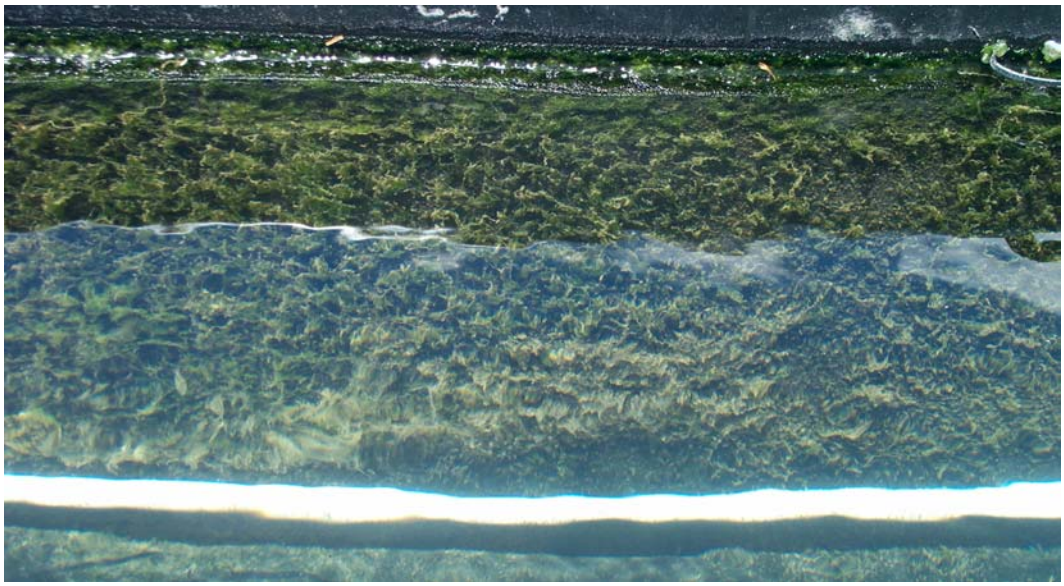


Figure 3.23 Epiphytic growth on a tank wall where the aeration was highest prior to stocking.

3.7.4.2 $\delta^{15}N$ and $\delta^{13}C$ natural abundance experiment

The natural food, principally algal epiphytes, which were present on the walls and other substrates on the tanks, was sampled separately but had similar isotopic signatures irrespective of location or treatment. For this reason a mean isotopic signature for all the epiphytes in each tank was used.

There were distinct differences in the natural abundance ratios of the epiphytes between treatments: the $\delta^{13}C$ ratios in the Substrate B 3000 treatment were considerably higher than in the other three treatments (Fig. 3.24); and the $\delta^{15}N$ ratios of Substrate C 6000 were higher than in the other three treatments. The Substrate B 3000 treatment also had the highest C:N ratio (Table 3.11). The differences may be due to the types of epiphytes that colonised the different substrates. This, in turn, may be affected by the substrate surface characteristics, e.g. the added structure has a smoother surface than the AquaMats™.

There were distinct differences between the $\delta^{15}N$ and $\delta^{13}C$ natural abundance ratios of the artificial feeds (RPPF mix and Lucky Star), and the natural food, principally algal epiphytes, in the tanks (Fig. 3.24). However, the $\delta^{15}N$ and $\delta^{13}C$ natural abundance ratios of the prawns in all the treatments were similar (Fig. 3.24). The contribution of the epiphytes to their nutrition was calculated using mixing models (Gearing 1991, Shearer and Kohl 1993). Carbon from epiphyte sources contributed to prawn nutrition in all the treatments, the highest being in the Substrate C 11000 treatment (60%) and the lowest being in the Substrate B 3000 treatment (25%) (Table 3.11). Nitrogen from the epiphyte sources also contributed between 52 and 84% of the nitrogen requirements of the prawns.

The C:N ratio of epiphytes in the Substrate B 3000 treatment was higher than the other treatments relative to the C:N ratios of the prawns (Table 3.11). This combined with the lower calculated proportion of carbon assimilated by prawns in the Substrate B 3000 treatment suggests that the biota in this treatment were a less nutritionally beneficial food source for the prawns. Despite the differences between treatments, there was no statistical difference in prawn growth with substrate type (see Section 3.2.3.1) over the entire nursery growout period (6-8 weeks).

Figure 3.24 **Mean (± SE) $\delta^{15}N$ and $\delta^{13}C$ ratios in formulated feed, epiphytes and prawns in the four treatments.**

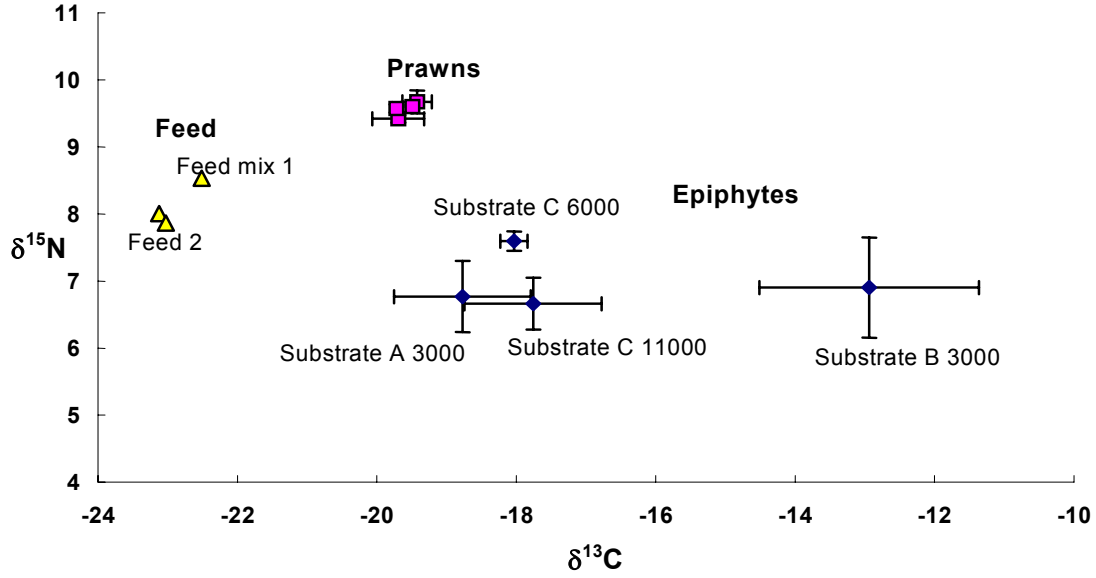


Table 3.11 **Contribution of epiphytes to carbon and nitrogen nutrition of *P. esculentus* in tanks as determined by natural abundance stable isotope ratios, and molar C:N ratios of the epiphytes and the prawns.**

Treatment	% carbon from the epiphytes	% nitrogen from the epiphytes	C:N ratio of epiphytes	C:N ratio of prawns
Substrate A 3,000	48	84	7.7 (0.5)	4.8 (0.1)
Substrate B 3,000	25	79	8.7 (0.5)	4.9 (0.1)
Substrate C 6,000	51	52	6.7 (0.4)	4.8 (0.1)
Substrate C 11,000	60	80	6.2 (0.1)	4.9 (0.2)

3.7.4.3 ^{15}N-enrichment experiment

The addition of ^{15}N-enriched ammonium chloride resulted in isotopic enrichment of the epiphytes within 1 h, and prawns within 24 h in all the treatments (Table 3.12). Previous studies have shown that the natural foods in tank systems become enriched within 1 h of the addition of ^{15}N-ammonium (Burford 2001). The contribution of nitrogen from the epiphytes to the nitrogen requirements of the prawns was calculated. Over 24 h, prawns derived between 33 and 71% of their nitrogen from the epiphytes. These results support the natural abundance data that showed that prawns derive nitrogen from the epiphytes, however, the percentages in each treatment are generally lower than for the natural abundance data. One of the main differences between the two methods is that the natural abundance data integrated feed consumption over the first 10 to 13 d after stocking. In contrast, the ^{15}N-enrichment method only reflected feed consumption in the 24 h since the tanks were spiked. Additionally, the spiking experiment was commenced two weeks after stocking, by which time grazing by prawns may have reduced the biomass of suitable biota.

Table 3.12 Mean (± SD) ^{15}N-nitrogen enrichment (atom% excess) in the epiphytes and prawns in four treatments in the tanks, and the contribution of nitrogen in the epiphytes to the nitrogen requirements of the prawns.

Treatment	Epiphytes Atom% excess	Prawns Atom% excess	% nitrogen from the epiphytes
Substrate A 3,000	0.011 (0.002)	0.004 (0)	59
Substrate B 3,000	0.024 (0.003)	0.005 (0)	33
Substrate C 6,000	0.008 (0.001)	0.004 (0)	71
Substrate C 11,000	0.020 (0.003)	0.007 (0.001)	42

There have been few previous studies tracing ^{15}N-enriched dietary sources in aquaculture. ^{15}N-enriched formulated feeds have been fed to *P. monodon* to determine short- and long-term assimilation of nitrogen (Preston et al. 1996, Burford and Williams 2001, Burford et al. in press). ^{15}N nitrogen labeling of epiphytes has been used to determine the contribution of this source to the nutrition of *P. monodon* and *Litopenaeus vannamei* in pond and tank systems without substrates (Burford 2001, Ziemann and Schell 1999). However, the use of enrichment techniques had the advantage over natural abundance isotope techniques in that more accurate determinations of the contribution of food sources can be made. Natural abundance values can vary markedly within food sources due to a range of factors, increasing the error associated with this technique (Adams and Sterner 2000, Vander Zanden and Rasmussen 2001).

Previous studies have shown that the presence of substrates increased the production of the prawn, *Farfantepenaeus paulensis* and the freshwater prawn, *Macrobrachium rosenbergii* (Tidwell et al. 1999, 2002, Thompson et al. 2002). An additional potential benefit of substrates is the improvement in water quality as epiphytes utilize excess nitrogen and phosphorus in the water. The presence of substrates improved the water quality in intensive *Litopenaeus vannamei* culture systems (Bratvold and Browdy 2001).

In conclusion, this study has shown that the natural biota in high-density tank systems with substrates can contribute substantially to the carbon and nitrogen requirements of *P. esculentus* postlarvae. The epiphytes that grew in treatments with AquaMat™ substrates were a more important food source for prawns that those with the other structure. However, at the end of the 6 to 8 week experiment, there was no difference in the growth of prawns in treatments with the two substrates. This study suggests that there are benefits in promoting natural food in tanks with substrates, at least in the early stages of growth. It is not clear, however, whether the natural food continues to contribute to prawn nutrition throughout the nursery phase and this warrants further work. Both methods of determining the contribution of epiphytes to prawn nutrition gave similar results. However, enriched isotopes may be more useful when there is insufficient discrimination between the natural abundance isotope signatures of food sources.

3.8 Post-production behaviour of juveniles

3.8.1 Background

The MG Kailis Exmouth Hatchery, WA, successfully produced approximately 270,000 0.6 to 1.0 g *P. esculentus* juveniles in December 2000. Although releasing the prawns into the Gulf was not planned for this phase of the project, it was thought that valuable information could be gained by experimenting with harvest, transport and release techniques. On 5 separate days over a 1.5 week period approximately 200,000 prawns were harvested and released into the proposed release area about 8 nm south of Whalebone Island in Exmouth Gulf. The release was filmed underwater on two days, and this provided the opportunity to observe prawn behaviour during and shortly after the release. The footage showed that once they reached the bottom, the majority of the prawns did

not bury immediately, but either sat still on the sand, clung on to sea-grass or appeared to be foraging on the sea-grass. Prawns that did attempt to bury appeared to have some difficulty in doing so. For the last release a 100m long transect was laid directly in the path of the release vessel. At 1, 2 and 3 hours after the release the number of prawns observed 1 m either side of the transect were 20, 23 and 16 respectively. Of these only 25%, 26% and 12.5% were buried (Haywood pers. com. 2000). A sample of 26 prawns collected from the water column during the last release was examined for limb damage. From microscope examinations it was found that virtually all of the prawns were without antennae and most of them had some missing legs (Haywood pers. com. 2000). However, necrosis (the visible black scar formed around tissue damage) was observed on the tips of damaged extremities. This suggests it is old damage, and probably occurred during the production phase. These results raised concerns about the lack of burying behaviour of the prawns, and the following reasons were proposed:

- The prawns have no previous experience of sediment and as a consequence, don't know how to bury
- Reversed activity cycle – prawns are fed during the day and are therefore encouraged to be active during the day rather than night
- The prawns are hungry, as they would have missed two feeds on the morning of the release
- Limb damage – this maybe caused during handling from raceways to transporter tanks or the siphon hose used to release them into the Gulf. But, the more likely reason is an increase in events of cannibalism during moulting because of high stocking densities in the raceways.

3.8.2 Experiment 1: Burial behaviour of 1 g prawns released into a sandy-bottom environment during the day

3.8.2.1 Objectives

- To ascertain whether P. esculentus juveniles (mean weight of 1 g), grown at high densities in a raceway system, have the same burial behaviour during the day as juveniles grown at low densities in a sandy-bottom tank.
- To calculate the percentage of prawns which bury upon release into a sandy-bottom environment over a 3 hour period during the day.

3.8.2.2 Methods

The ideal approach to this experiment would be to compare the burial behaviour response of raceway reared prawns with prawns from wild caught populations of the same age. However, this was not possible due to a lack of time, so the next best option was to grow prawns at low densities in a sandy-bottom tank and compare these to high-density raceway-reared prawns. It was assumed that prawns grown to 1 g in the low-density sandy-bottom conditions will have minimal appendage damage because of reduced interactions with other prawns, and they will be more accustomed to burying than prawns grown in raceways.

Postlarvae from the same batch used to stock the raceway system were stocked into eight 2 tonne tanks with a sandy-bottom at a density of 75 m^{-3}, and grown for the same duration as prawns reared in the raceway system.

The four treatments tested in this experiment consisted of raceway and sandy-bottom tank reared prawns at different densities (Table 3.13). Each experimental tank was randomly allocated to one treatment. A total of sixty prawns (mean weight of 1 g), from each treatment were collected and divided into 6 lots of 10 and placed into buckets above the allocated experimental tanks. At the start of the experiment the prawns were released into the experimental tanks as simultaneously as possible. The number of prawns buried and unburied in each replicate was recorded 10, 20, 40, 90 and 180 minutes after the release.

Table 3.13 Experimental design for the comparison of burial behaviour.

Treatment (system and stocking density)	Replicates	No. prawns per replicate	Experimental tanks
Raceway 2860 m^{-3}	6	10	100 L circular tanks with a 20 mm deep sandy-bottom
Raceway 5720 m^{-3}	6	10	
Raceway 11430 m^{-3}	6	10	
Sandy-bottom tank 75 m^{-3}	6	10	

The results were expressed as a percentage of the prawns buried and unburied per treatment and time interval. Differences in the percentages among treatments and time intervals were compared by analysis of variance using the Generalised Linear Models procedure (SAS Institute software).

3.8.2.3 Results and discussion

The percentage of prawns, from all four treatments combined, that buried into the sand at 10, 20, 40, 90 and 180 minutes after release was 12.08%, 11.25%, 7.92%, 6.25% and 10.42%, respectively (Fig. 3.25). There was no significant difference (P>0.05), across all time intervals, which suggests that a prawn response of either burying or not burying occurs within 10 minutes after release.

The percentage of sandy-bottom tank-reared prawns that buried over a 3 hour period, was 17% (Fig. 3.26). This was significantly higher (P<0.05), than the percentage of prawns that buried from each of the three raceway densities, which ranged from 3% to 10%. Prawns reared at 5720 m^{-3} had a significantly lower (P<0.05) percentage of prawns that buried over a 3 hour period than prawns reared at 2860 m^{-3} and 11430 m^{-3}. However, there was no inverse relationship expressed between stocking density and percentage of prawns that buried.

When both treatments and time are combined, the percentage of prawns that buried was only 9.6% (Fig. 3.27). This was significantly less (P<0.05), than the 90.7% of prawns recorded as unburied.

The results suggest that prawns reared at high densities in a raceway system are less inclined to bury when released into a sandy-bottom environment during the day than prawns reared at low densities in a sandy-bottom tank. And, the overall percentage of prawns that respond by burying when released into a sandy-bottom environment during the day is considerably lower than the percentage that do not respond by burying.

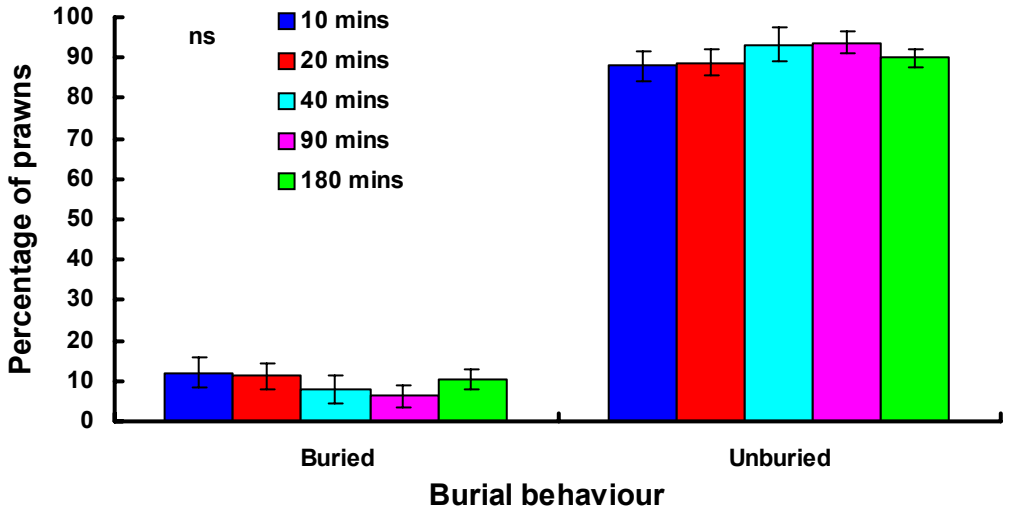


Figure 3.25 **Burial behaviour of *P. esculentus* juveniles (mean weight of 1 g), released into a sandy-bottom environment over a 3 hour period during the day. This graph represents the combined results of burial behaviour from prawns reared in raceways at stocking densities of 2860 m^{-3}, 5720 m^{-3} and 11430 m^{-3} and a sandy bottom tank system at a stocking density of 75 m^{-3}.**

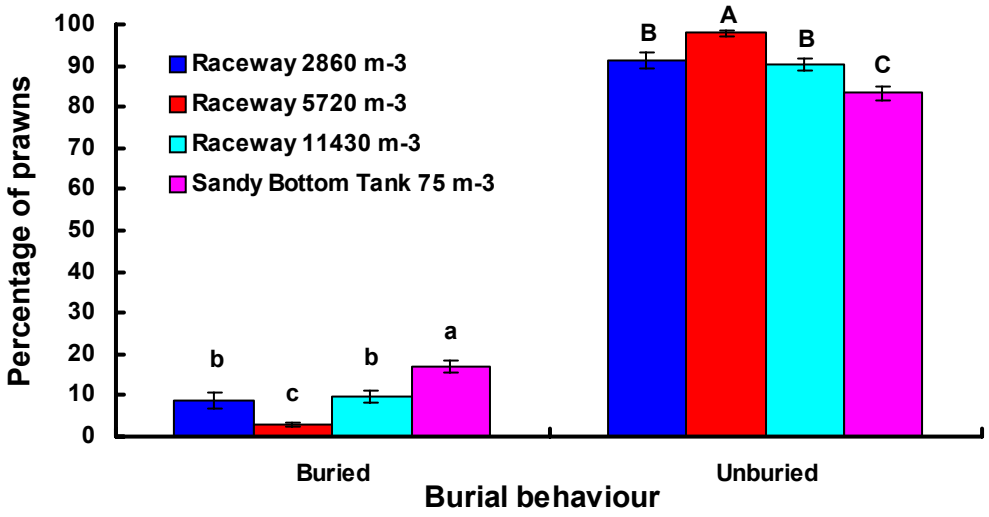


Figure 3.26 **Burial behaviour of *P. esculentus* juveniles (mean weight of 1 g), reared in different tank systems and stocking densities then released into a sandy-bottom environment during the day. This graph represents the combined results of burial behaviour recorded at five time intervals over a 3 hour period. Treatments with the same superscript are not significantly different (*P*>0.05).**

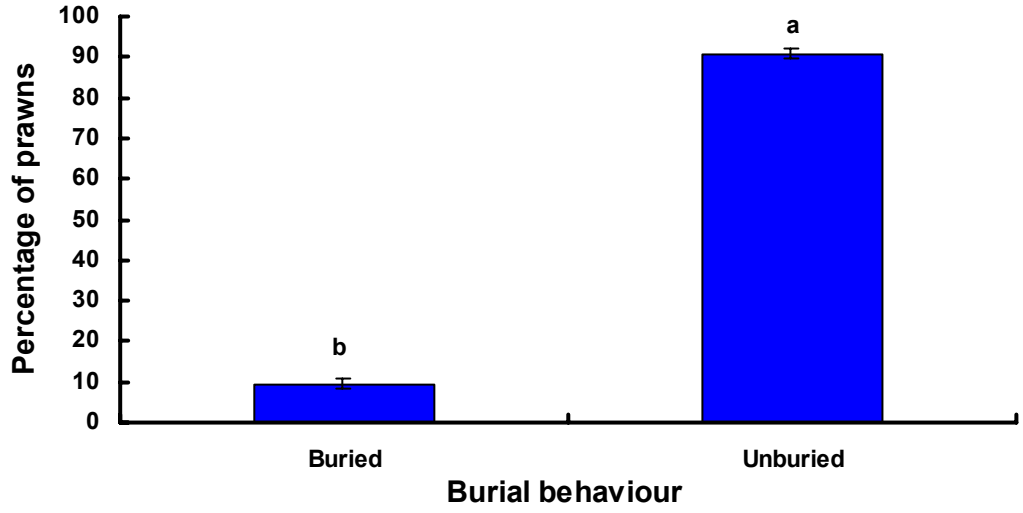


Figure 3.27 **Burial behaviour of *P. esculentus* juveniles (mean weight of 1 g), released into a sandy-bottom environment during the day. This graph represents the combined results from both, prawns reared in different tank systems and stocking densities, and also burial behaviour recorded at five time intervals over a 3 hour period. Treatments with the same superscript are not significantly different (*P*>0.05).**

3.8.3 Experiment 2: Burial behaviour of 1 g prawns released into a sandy-bottom environment during the night

3.8.3.1 Objectives

- To ascertain whether *P. esculentus* juveniles (mean weight of 1 g), grown at high densities in a raceway system, have the same burial behaviour during the night as juveniles grown at low densities in a sandy-bottom tank
- To calculate the percentage of prawns which bury upon release into a sandy-bottom environment over a 3 hour period during the night.

3.8.3.2 Methods

The same methods were used as those in experiment 1 except the prawns were captured and released into the 100 L sandy-bottom tanks during the night (7pm to 11pm). This experiment was also conducted on the same day as experiment 1.

3.8.3.3 Results and discussion

The percentage of prawns, from all four treatments combined, that buried into the sand at 10, 20, 40, 90 and 180 minutes after release was 2.08%, 1.25%, 0.83%, 0.42% and 0.00%, respectively (Fig. 3.28). There was no significant difference ($P>0.05$), across all time intervals, which suggests that a prawn response of either burying or not burying occurs within 10 minutes after release.

The percentage of sandy-bottom-tank reared prawns which buried over a 3 hour period, was 3% (Fig. 3.29). This was significantly higher ($P<0.05$), than the percentage of prawns that buried from each of the three densities in the raceway, which ranged from 0.0% to 0.7%.

When both treatment and time are combined, the percentage of prawns that buried is <1% (Fig. 3.30). This was significantly less ($P<0.05$), than the 99% of prawns recorded as unburied.

The results suggest that prawns reared at high densities in a raceway system are less inclined to bury when released into a sandy-bottom environment during the night than prawns reared at low densities in a sandy-bottom tank. And, the overall percentage of prawns that respond by burying when released into a sandy-bottom environment during the night is considerably lower than the percentage that do not respond by burying.

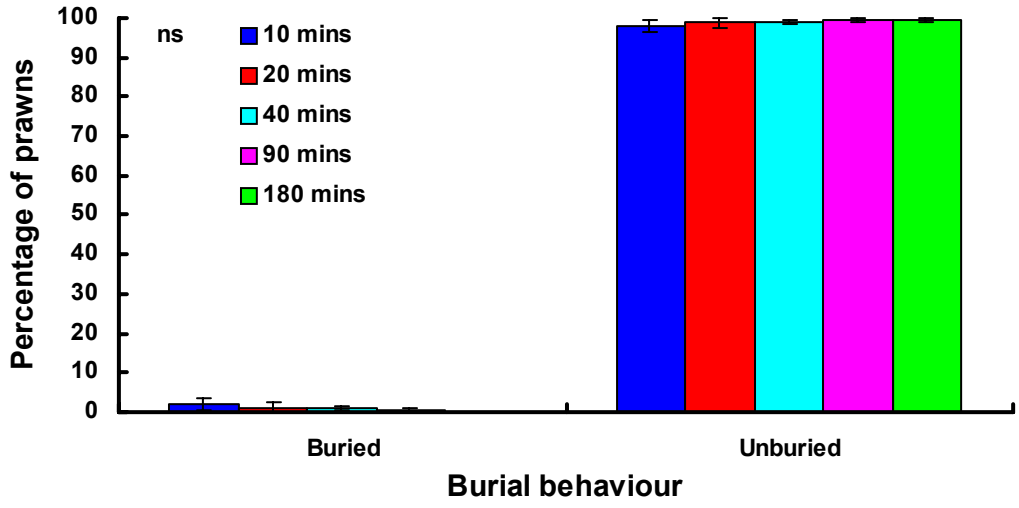


Figure 3.28 **Burial behaviour of *P. esculentus* juveniles (mean weight of 1 g), released into a sandy-bottom environment over a 3 hour period during the night. This graph represents the combined results of burial behaviour from prawns reared in raceways at stocking densities of 2860 m^{-3}, 5720 m^{-3} and 11430 m^{-3} and a sandy bottom tank system at a stocking density of 75 m^{-3}.**

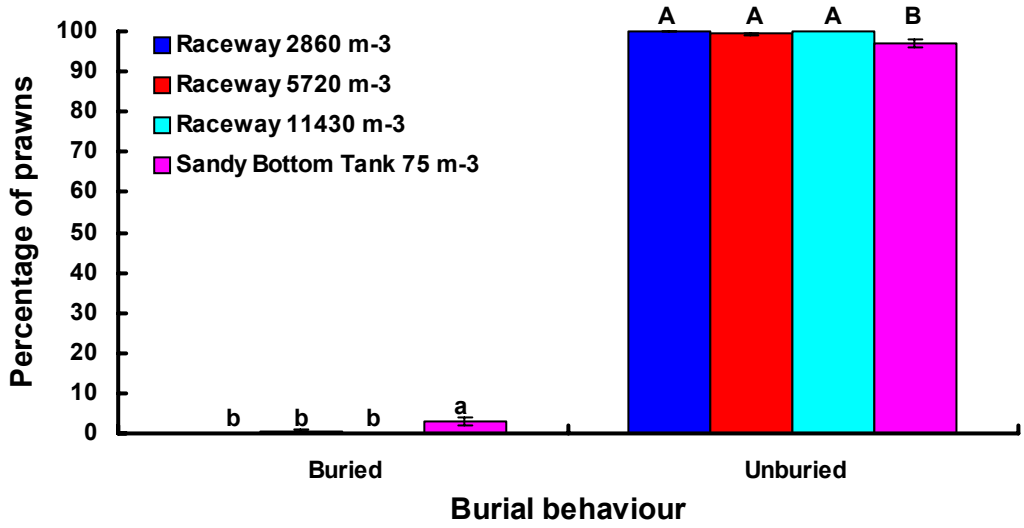


Figure 3.29 **Burial behaviour of P. esculentus juveniles (mean weight of 1 g), reared in different tank systems and stocking densities then released into a sandy-bottom environment during the night. This graph represents the combined results of burial behaviour recorded at five time intervals over a 3 hour period. Treatments with the same superscript are not significantly different (P>0.05).**

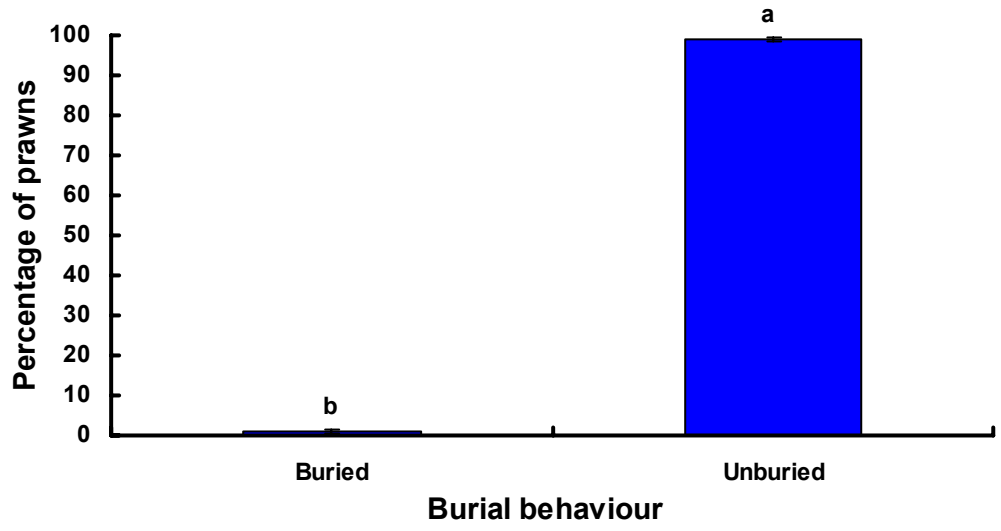


Figure 3.30 **Burial behaviour of *P. esculentus* juveniles (mean weight of 1 g), released into a sandy-bottom environment during the night. This graph represents the combined results from both, prawns reared in different tank systems and stocking densities, and also burial behaviour recorded at five time intervals over a 3 hour period. Treatments with the same superscript are not significantly different (*P*>0.05).**

3.8.4 Experiment 1 versus 2: Comparison of the burial behaviour of 1 g prawns released into a sandy-bottom environment during the day versus the night

3.8.4.1 Objectives

- To compare the burial behaviour of P. esculentus juveniles (mean weight of 1 g), reared in different tank systems, when released into a sandy-bottom environment during the day versus the night.

3.8.4.2 Methods

Differences in the percentage of prawns buried and unburied from experiment 1 and 2 were compared by analysis of variance using the Generalised Linear Models procedure (SAS Institute software). This was possible because the experiments were identical and carried out on the same day.

3.8.4.3 Results and discussion

The percentage of prawns that buried over a 3 hour period during the day was 9.6% (Fig. 3.31). This was significantly higher ($P<0.05$), than the 1% of prawns that buried over a 3 hour period during the night. This suggests that juvenile *P. esculentus* are more active at night.

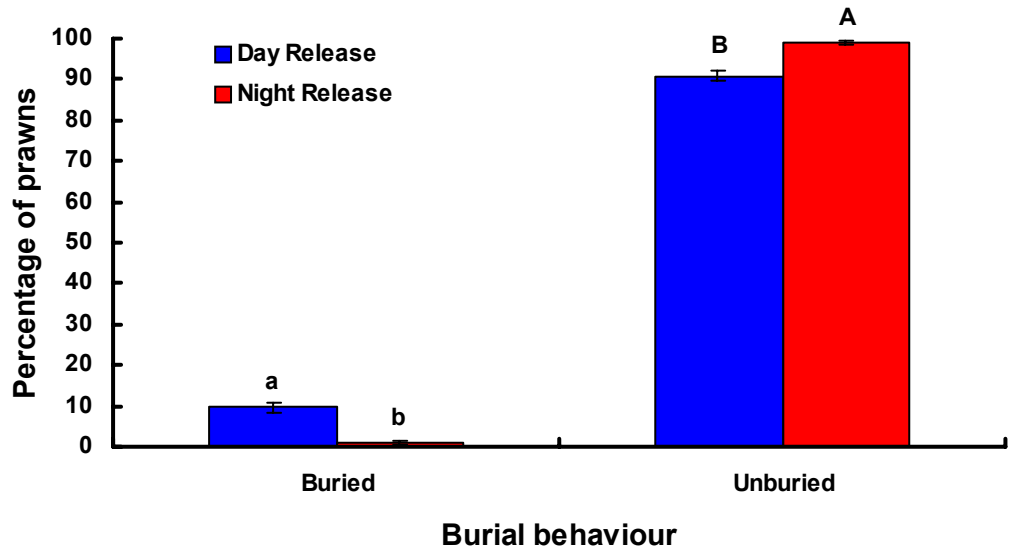


Figure 3.31 **Burial behaviour of *P. esculentus* juveniles (mean weight of 1 g), released into a sandy-bottom environment during the day versus the night. This graph represents the combined results from both, prawns reared in different tank systems and stocking densities, and also burial behaviour recorded at five time intervals over a 3 hour period. Treatments with the same superscript are not significantly different (*P*>0.05).**

3.8.5 Experiment 3: Burial behaviour between fed and unfed 1 g prawns released into a sandy-bottom environment during the day

3.8.5.1 Objective

- To ascertain whether feeding P. esculentus juveniles (mean weight of 1 g), grown at high densities in a raceway system and low densities in a sandy-bottom tank, prior to release into a sandy-bottom environment, will effect their burial behaviour.

3.8.5.2 Methods

The four treatments tested in this experiment consisted of raceway reared prawns at a stocking density of 2860 m^{-3} (fed and not fed prior to release), and sandy-bottom tank reared prawns at a stocking density of 75 m^{-3} (fed and not fed prior to release). The experiment was carried out in 24 x 100 L circular tanks with a 20mm deep sandy bottom. Each tank was randomly allocated to one treatment, with 6 replicates per treatment. A total of 60 prawns from each treatment were collected and held in 100 L tanks for 2 hours. After 1 hour the prawns in the fed treatments were given pellets (Higashi #9) and left to feed for another hour before the start of the experiment. Prawns from each treatment were then divided into 6 lots of 10 and placed into buckets above the allocated 100 L sandy-bottom tanks. At the start of the experiment the prawns were released into the 100 L tanks as simultaneously as possible. The number of prawns buried and unburied in each replicate was recorded 10, 20, 40, 90 and 180 minutes after the release.

The results were expressed as a percentage of the prawns buried and unburied per treatment and time interval. Differences in the percentages among treatments and time intervals were compared by analysis of variance using the Generalised Linear Models procedure (SAS Institute software).

3.8.5.3 Results and discussion

The percentage of prawns, from all four treatments combined, that buried into the sand at 10, 20, 40, 90 and 180 minutes after release was 22.08%, 22.50%, 20.83%, 15.42% and 25.00%, respectively (Fig. 3.32). There was no significant difference (P>0.05), across all time intervals, which suggests that a prawn response of either burying or not burying occurs within 10 minutes after release.

The percentage of prawns buried, was significantly higher (P<0.05), in the fed treatments for both the high-density raceway-reared prawns and the sandy-bottom tank-reared prawns (Fig. 3.33). This suggests that feeding the prawns prior to release affected their burial behaviour and increased the number of prawns that buried into the sand.

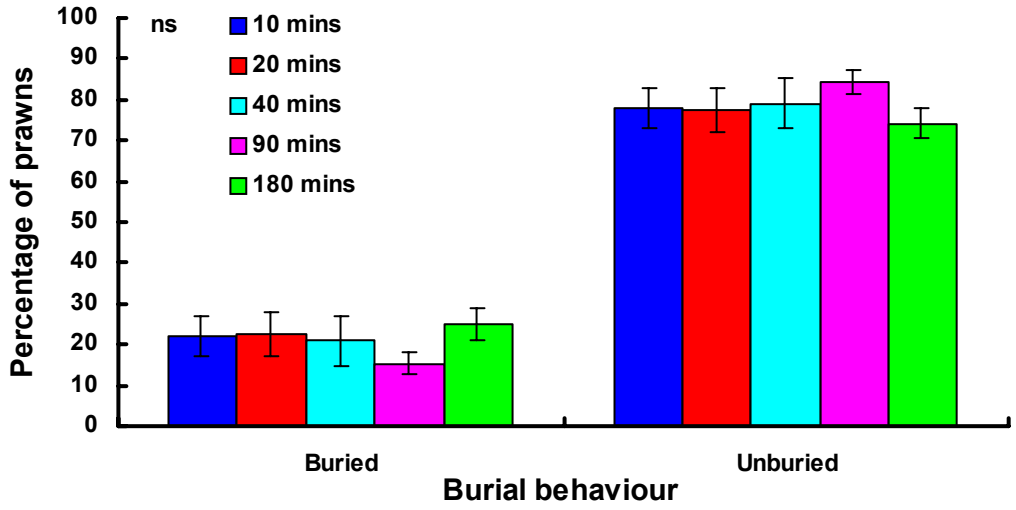


Figure 3.32 **Burial behaviour of *P. esculentus* juveniles (mean weight of 1 g), released into a sandy-bottom environment over a 3 hour period during the day. This graph represents the combined results of burial behaviour from prawns reared in raceways at a stocking density of 2860 m^{-3} and a sandy-bottom-tank system at a stocking density of 75 m^{-3}. Prawns were captured and kept in 100 L holding tanks prior to release into a sandy-bottom environment. Half of the prawns from each system were fed prior to release**.

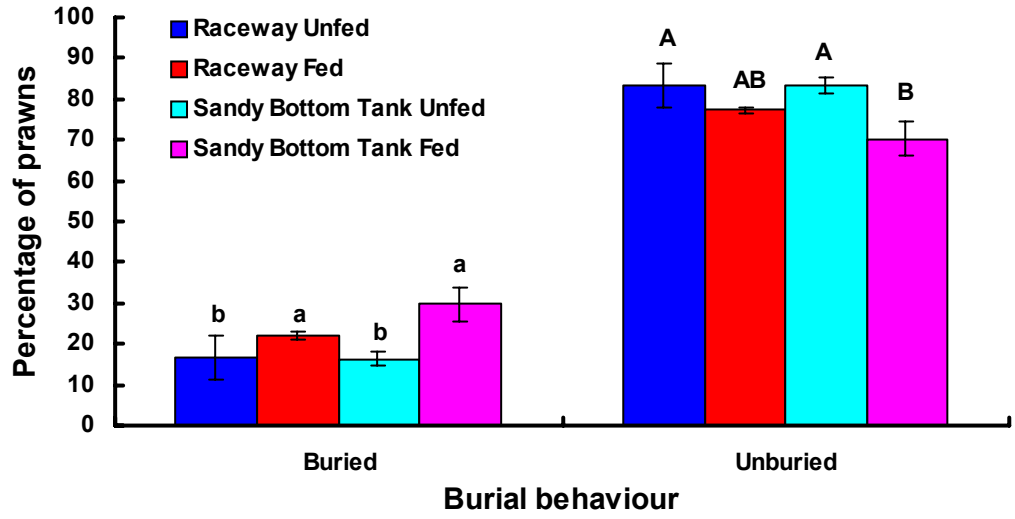


Figure 3.33 **Burial behaviour of *P. esculentus* juveniles (mean weight of 1 g), reared at 2860 m^{-3} in a raceway system and 75 m^{-3} in a sandy-bottom-tank system. The prawns were captured and kept in holding tanks for 2 hours prior to release and half of the prawns from each system were fed 1 hour prior to release. This graph represents the combined results of burial behaviour recorded at five time intervals over a 3 hour period. Treatments with the same superscript are not significantly different (*P*>0.05).**

3.8.6 Experiment 4: Behavioural response to habitat preference of 1 g prawns released into a tank with a sandy bottom and artificial sea-grass

3.8.6.1 Background

In wild populations juvenile *P. esculentus* show a strong habitat preference for sea-grass beds (Staples et. al. 1985, Haywood et. al. 1995). They are commonly most abundant on long bladed sea-grass, in water less than 2 m deep (Loneragan et. al. 1994, 1998). After *P. esculentus* postlarvae settle on sea-grass beds 3 to 4 weeks after spawning (Dall et. al. 1990, Haywood et. al. 1995), they remain there until they are <10 mm carapace length, for prawns in the Gulf of Carpentaria (Haywood et. al. 1995) and until they are <16 mm carapace length, for prawns in Moreton Bay (O'Brien 1994b), ie. approximately 10 weeks.

To gain a better appreciation of the likelihood of survival, the ability of high-density raceway-reared juvenile *P. esculentus* to select for their naturally preferred sea-grass habitat needs to be ascertained.

3.8.6.2 Objective

- To ascertain whether P. esculentus juveniles reared in raceways at stocking densities of 2860 m-3 (Run 1) and 5720 m-3 (Run 2) are capable of selecting artificial sea-grass habitats when released

3.8.6.3 Methods

This experiment was carried out using juveniles that were stocked at two different densities in a raceway system. The first run (Run 1) used juveniles that were stocked at 2860m^{-3}, and the second run (Run 2) used juveniles that were stocked at 5720 m^{-3}. The experiment was conducted in six 2 tonne circular tanks with a bottom surface area of 3 m^2 that were divided in two, with AquaMat™ fronds buried in sand creating an artificial sea-grass habitat on one half, and a bare sand habitat on the other half (Fig. 3.34). For both runs the prawns were released at three release sites:

- sand habitat;
- AquaMat™/sand interface (middle), and;
- AquaMat™ habitat.

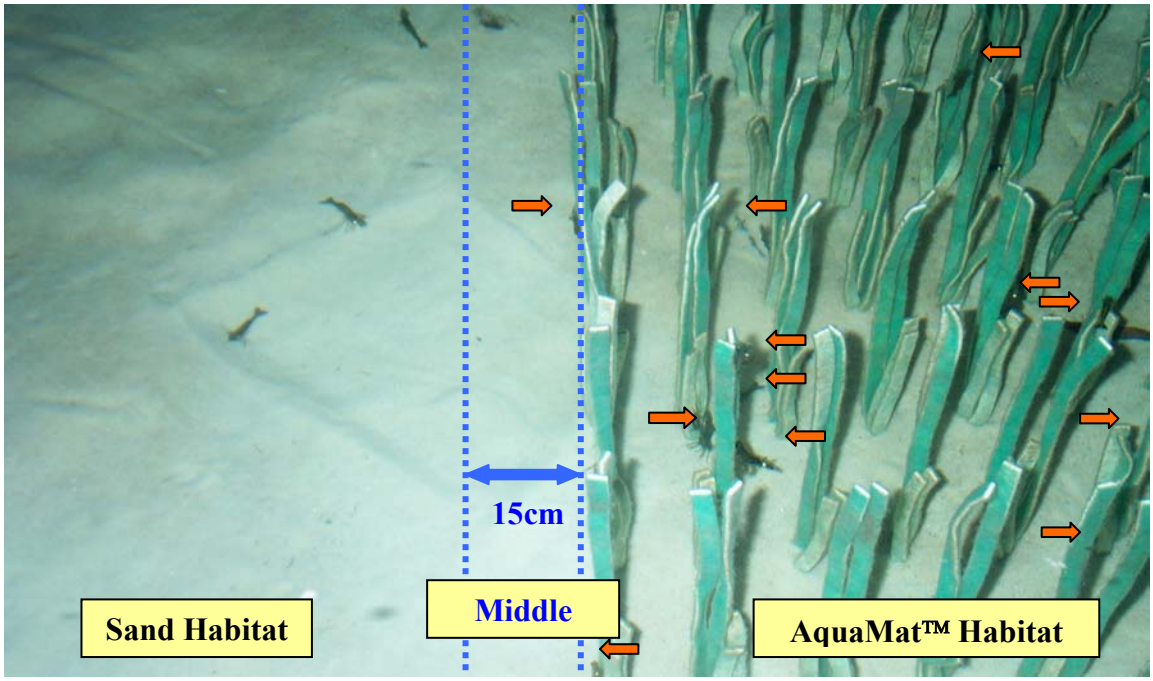


Figure 3.34 Tank set-up with the two habitat zones: AquaMat™ fronds (simulated sea-grass habitat) and bare sand. The arrows indicate prawns hiding amongst the AquaMat™.

Run 1

The six experimental tanks were randomly allocated a release site with two tanks per site.

A total of 180 prawns were then collected randomly from the six raceway tanks stocked at 2860 m^{-3} and divided into 6 lots of 30 and placed into buckets next to the allocated experimental tanks. At the start of the experiment the prawns were released into the experimental tanks as simultaneously as possible. The prawns were released within each site using a net that only released the prawns once they were at the bottom. The number of prawns within the AquaMat™ habitat and sand habitat were recorded 1, 2 and 24 hours after release. This procedure was then repeated on two more occasions resulting in 6 replicates per release site. The experimental tanks were allocated a different release site each time.

Run 2

The same methods were used as those in Run 1 except for the following alterations:

- the prawns used were collected from the raceway tanks stocked at a density of 5720 m^{-3};

– the number of prawns occupying the AquaMat™ habitat and sand habitat were recorded at 0.5, 1.5, 3 and 24 hours after release, and;
– the behaviour of the prawns occupying each habitat was recorded. Whether they were buried, unburied on the sand or clinging to the AquaMat™ fronds.

For both runs, differences in habitat preference between and within treatments were compared by analysis of variance using SAS (SAS Institute Systems). Differences in the number of prawns buried, unburied and on the AquaMat™ fronds for Run 2 were also compared by using analysis of variance.

3.8.6.4 Results and discussion

Run 1

The percentage of prawns, that occupied the AquaMat™ habitat over a 24 hour period was 75%, 68.7% and 62% for prawns released within the AquaMat™, middle and sand release sites respectively (Fig. 3.35). This difference was not significant (P>0.05), however it does show a slight tendency for prawns to stay within their allocated release site.

The percentage of prawns, from all three release sites combined, that occupied the AquaMat™ habitat 1, 2 and 24 hours after release was 66.3%, 68% and 71.5% respectively (Fig. 3.36). There were no significant differences (P>0.05), across all time intervals, which suggests that a preferred habitat was selected within 1 hour of release. However, there was a small increase over time in the percentage of prawns that occupied the AquaMat™ habitat, which may suggest that the prawns are still seeking out this habitat after 24 hours.

When both release site and time are combined, the percentage of prawns that occupied the AquaMat™ habitat was 68.6% (Fig. 3.37). This was significantly greater (P<0.05), than the 29.9% of prawns that occupied the sand habitat. This strongly suggests that the prawns preferred the AquaMat™ habitat over the bare sand habitat.

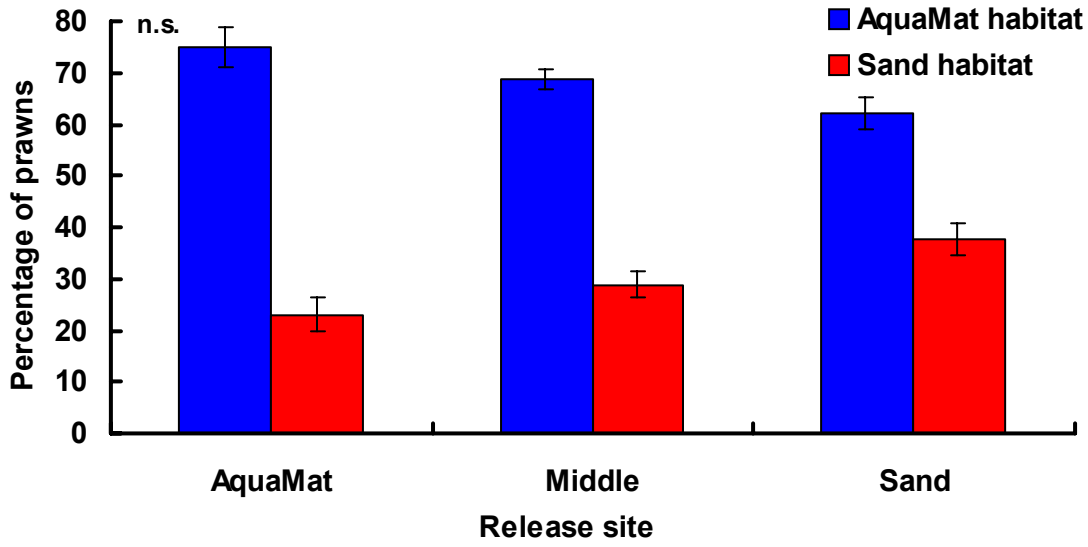


Figure 3.35 Habitat preference of high-density (2860 m^{-3}), reared juvenile *P. esculentus* (~1 g), after being released within three different sites in an environment with an AquaMat™ habitat and bare sand habitat. This graph represents the combined results of the percentage of prawns recorded within each habitat at three time intervals over 24 hours.

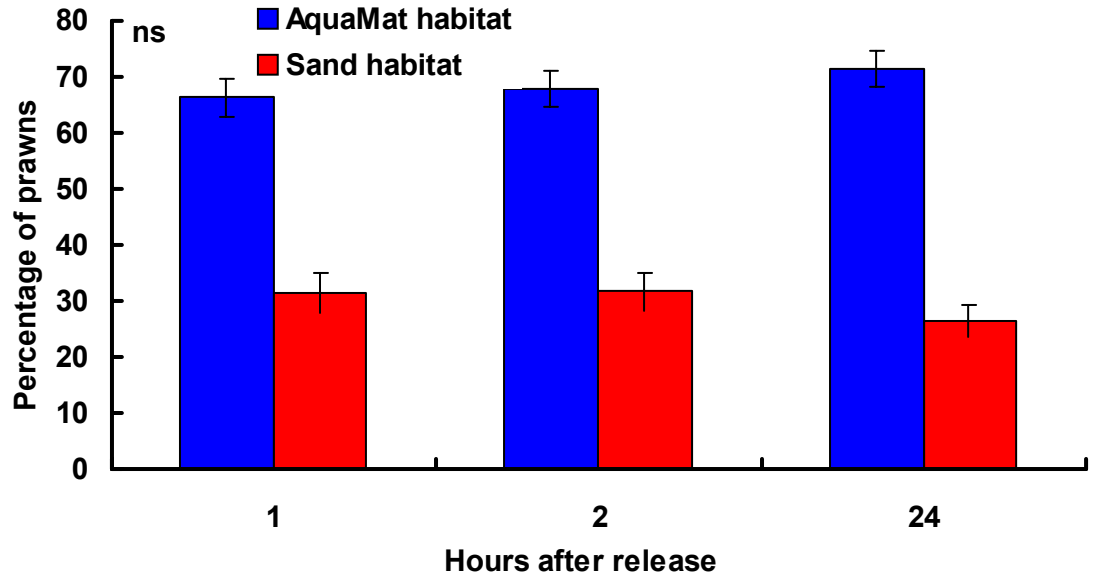


Figure 3.36 **Habitat preference of high-density (2860 m^{-3}), reared juvenile *P. esculentus* (~1 g), at three time intervals over 24 hours after being released into an environment with an AquaMat™ habitat and bare sand habitat. This graph represents the combined results of the percentage of prawns recorded within each habitat when prawns were released into three different sites.**

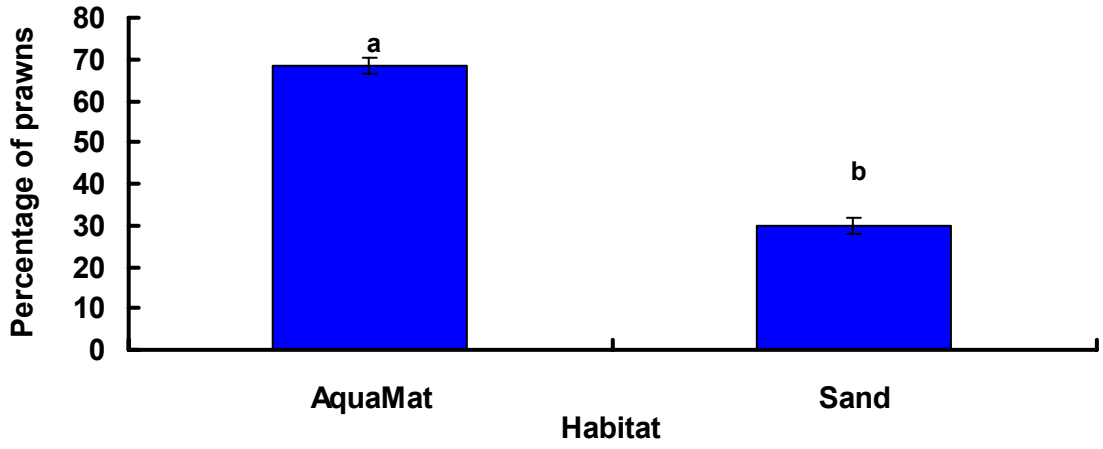


Figure 3.37 **Habitat preference of high-density (2860 m^{-3}), reared juvenile *P. esculentus* (~1 g), released into an environment with an AquaMat™ habitat and bare sand habitat. This graph represents the combined results of the percentage of prawns recorded within each habitat from both, prawns released into three different sites and also at three time intervals over a 24 hour period. Means with the same superscript are not significantly different.**

Run 2

The results of the second run are similar to the first in that after 24 hours the majority of the prawns were occupying the AquaMat™ habitat in all three release site treatments (Fig. 3.38). And again the number of prawns occupying the AquaMat™ habitat was greatest in the AquaMat™ release site treatment and lowest in the sand release site treatment, which shows a slight tendency for prawns to stay within their allocated release site. Also, the percentage of prawns clinging to the AquaMat™ fronds was greatest in the AquaMat™ release site treatment and lowest in the sand release site treatment. This suggests that releasing the prawns within or near to the AquaMat™ habitat increases the percentage of prawns that cling to the AquaMat™ fronds.

The results of habitat selection over time for Run 2 were similar to that of Run 1. A total of 68.4% of the prawns occupied the AquaMat™ habitat within the first half hour, which increased to 81% after 24 hours (Fig. 3.39). Again, this suggests that the majority of the prawns selected the AquaMat™ habitat within 30 minutes, but some were still seeking out this habitat after 24 hours.

Additionally, Run 2 also recorded the behaviour of the prawns within each habitat over time. Although there were no significant ($P>0.05$), differences in prawn behaviour within each habitat over time, the results expressed in Figure 3.40 show some noticeable changes. Firstly, the percentage of prawns unburied within the AquaMat™ habitat decreases from 43.9% in the first half hour to 27.8% after 24 hours, while the percentage of prawns clinging to the AquaMat™ fronds increased from 20.6% in the first half hour to 40% after 24 hours.

Secondly, the percentage of prawns unburied in the sand habitat decreased from 23.7% in the first half hour to 11.9% after 24 hours, while the percentage buried dropped only slightly from 7.8% to 6.3% after half an hour and 24 hours respectively.

These changes in behaviour support findings by Staples *et al*. (1985) and Haywood *et al*. (1995) who documented that wild populations of juvenile *P. esculentus* show a strong habitat preference for sea-grass beds, which they may use for a number of reasons including camouflage for protection against predators and a food source for grazing on biota that colonises the sea-grass. Since the AquaMat™ fronds had not been colonised with biota for this experiment, it is highly likely that the prawns prefer to cling to the fronds of the AquaMat™ as camouflage for protection against predators. And that this camouflage behaviour is the preferred response over burying into the sand or hiding unburied amongst the AquaMat™ fronds.

Further evidence that the prawns use the AquaMat™ habitat for protection is expressed by the combined results of the percentage of prawns that are buried within each habitat (Fig. 3.40). Of the total number of prawns that occupied that AquaMat™ habitat over a 24 hour period from three release sites, only 9.9% were recorded as buried. This was significantly less ($P<0.05$), than the 36.9% of prawns that were recorded as buried within the sand habitat. This suggests that prawns in an open sand habitat need to bury as a means of protection whereas prawns within an AquaMat™ habitat find protection amongst the AquaMa™ fronds.

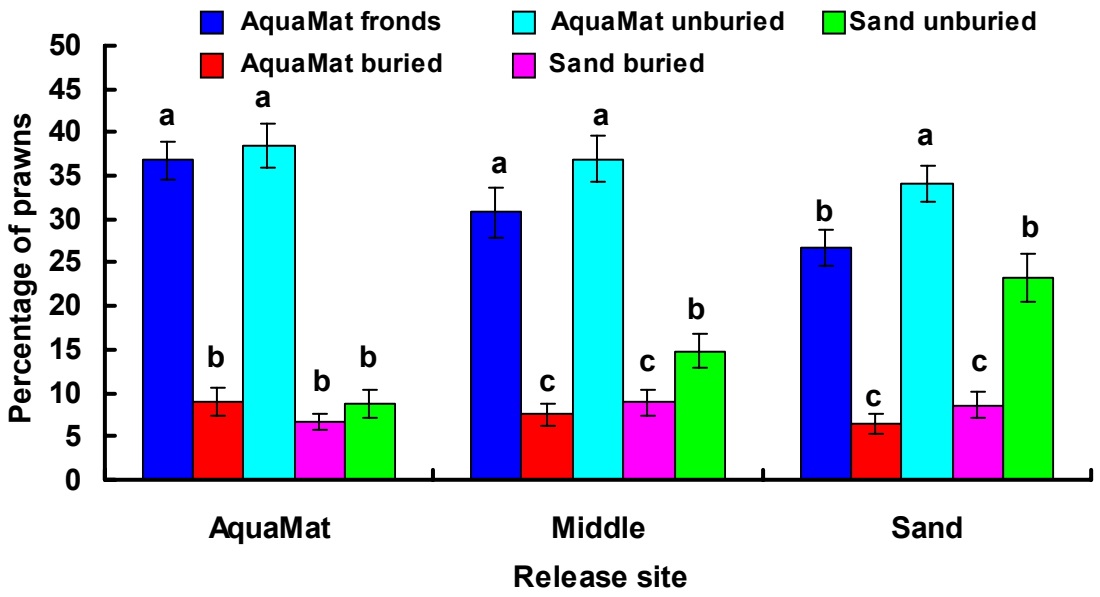


Figure 3.38 **Habitat preference and behaviour of high-density (5720 m^{-3}), reared juvenile *P. esculentus* (~1 g), after being released within three different sites in an environment with an AquaMat™ habitat and bare sand habitat. This graph represents the combined results of the percentage of prawns recorded within each habitat at three time intervals over 24 hours and their behaviour within each habitat. The behaviour responses with different superscripts within each release site are significantly different (P<0.05).**

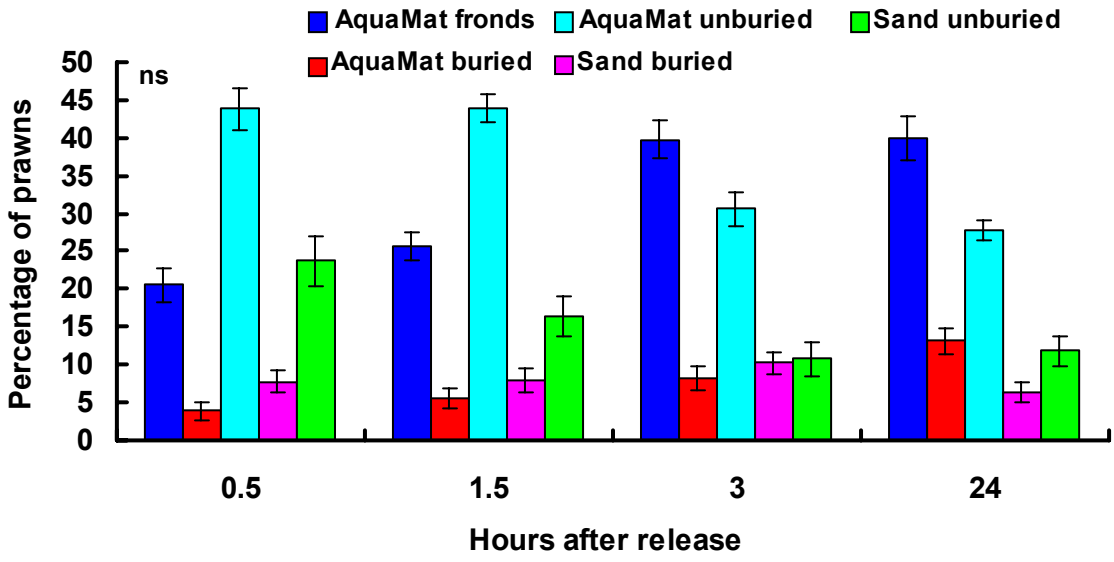


Figure 3.39 **Habitat preference and behaviour of high-density (5720 m^{-3}), reared juvenile *P. esculentus* (~1 g), at four time intervals over 24 hours after being released into an environment with an AquaMat™ habitat and bare sand habitat. This graph represents the combined results of the percentage of prawns recorded within each habitat when prawns were released into three different sites.**

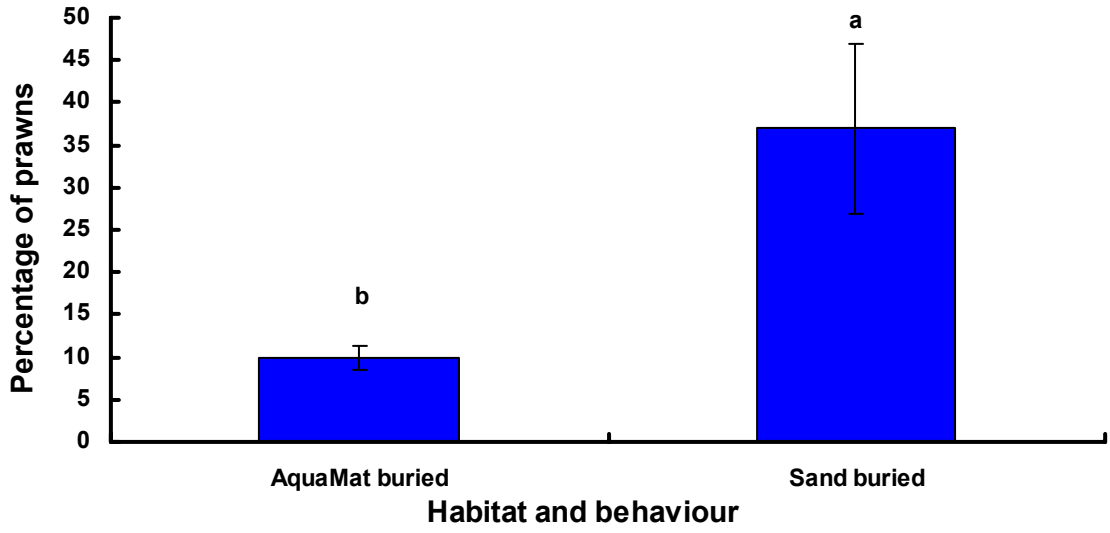


Figure 3.40 **Burial response of high-density (5720 m^{-3}), reared juvenile *P. esculentus* (~1 g), released into an environment with an AquaMat™ habitat and bare sand habitat. This graph represents the percentage of prawns that were buried within the AquaMat™ and sand habitats from the total number of prawns recorded occupying these habitats over a 24-hour period. This graph also represents the combined results from both, prawns released into three different sites and also at four time intervals over a 24-hour period. Burial responses with different superscripts are significantly (P<0.05), different.**

3.8.7 Conclusion

The primary concern that came from the observations of prawn behaviour during the trial release into Exmouth Gulf, was that certain aspects of high-density production affected the prawns' natural behaviour when released into the wild. The ideal method to test this theory would have been to compare the behaviour of juveniles cultured in a high-density raceway system to juveniles of the same age from wild populations. However, due to a lack of time, it was not possible to obtain juveniles from a wild population, so the next best option for a comparison was to culture juveniles at low densities in a sandy-bottom environment. Therefore, the experimental results can not be compared directly with the behaviour of juveniles from wild populations, but they can however, quantify the effect that high-density rearing has on the behaviour of juveniles when released into a simulated wild environment.

High-density, raceway-reared prawns were less inclined to bury

Juvenile *P. esculentus* cultured at high densities in a raceway system express an altered behavioural response when released during both the day and night into a sandy-bottom environment compared to juveniles cultured at low densities in a sandy-bottom tank. In each release, fewer prawns from the raceway system were inclined to bury over a 3-hour period. Possible reasons could be that the higher level of appendage damage suffered by raceway-reared prawns (see section 3.5.4.2), has slightly reduced their ability to bury, or prawns cultured in a sandy-bottom environment have become more accustomed to burying.

The majority of prawns didn't bury within 3 hours

Even though the burial response from raceway reared prawns is lower, this difference is relatively insignificant when comparisons are made with the percentage of prawns unburied. Only 17% of prawns cultured at low densities in a sandy-bottom tank buried over a 3 hour period during the

day, compared to 83% that remained unburied. This difference was even greater during the night when only 3% buried and 97% were unburied. This suggests that when prawns are released into a sandy environment, the overwhelming majority in a population do not respond by burying within the first 3 hours.

Recently fed prawns were more inclined to bury
Feeding prawns from both systems prior to release increased the percentage of prawns that buried into the sand. This could be because the need to forage for food is reduced when they have a full gut so they become less active. Other instincts such as avoiding predation take priority and they hide amongst sea-grass or bury into the sand. Again, this difference was relatively small when compared to the percentage of prawns unburied.

Day release and feeding prawns increases the percent that bury
The results suggest that releasing prawns during the day, and feeding them prior to release, effects the prawns behavioural response and increases the percentage that bury into a sandy bottom. Even though this increase is relatively small compared to the high percentage of prawns in a given population that don't bury into the sand, releasing the prawns during the day and feeding them prior to release would still be the best circumstance to release juvenile *P. esculentus*. Any methods that could increase the survival chance of the released prawns are well worth considering.

Raceway-reared prawns actively select sea-grass habitats
Juveniles cultured at high densities in a raceway system actively selected for an AquaMat™ habitat over a bare sand habitat when released. And they also actively selected to cling to the AquaMat™ fronds over a 24-hour period. This suggests that their habitat preference is similar to that documented for wild populations by Staples *et al*. (1985) and Haywood *et al*. (1995), who found that juvenile *P. esculentus* show a strong habitat preference for sea-grass beds. Juveniles may use the sea-grass habitat for a number of reasons including camouflage for protection from predators and a food source for grazing on biota which colonises the sea-grass. As the AquaMat™ was not colonised with biota, the results strongly suggest that juvenile *P. esculentus* selected the AquaMat™ habitat for protection against predators.

Methods to increase the survival chance of released prawns
A day release is more ideal than a night release, therefore the time of day will depend on factors that make the operation as cost effective as possible to the company. However, an early morning harvest is recommended because the level of dissolved oxygen in the water decreases as the water temperature increases. Because the prawns are being transported at high-densities it is important to get the prawns onto the boat with flow-through water before the temperature gets too high.

The prawns need to be released as close as possible to abundant sea-grass beds as this is their naturally preferred habitat and will increase their chance of survival.

Another method of ensuring maximum survival would be to feed the prawns prior to harvest while the prawns are still in the raceways. Enough time needs to be allowed for food to be ingested but not digested. Feeding the prawns in the transport containers would not be recommended because firstly, it's not practical, and secondly, left over feed could reduce the water quality in the tanks and also attract predators to the release site.

3.9 Economics of production – an analysis of the CSIRO Cleveland trial

3.9.1 Background

The cost of producing juvenile prawns for stock enhancement is a critical factor in the evaluation of the efficiency of a particular production system. In the consideration of an optimal stocking

density for a particular system, relative production costs are an important component. Economic modeling has been used to estimate an optimal raceway rearing density for juvenile *P. esculentus* in the CSIRO raceway system. The modeling exercise has allowed the optimal level of various inputs to be identified along with alternative cost-saving options. Overall the optimal rearing density was estimated at the point where yield and total income were greatest.

3.9.2 Objectives

- To estimate a stocking density that provides maximum yields at minimum costs for the Cleveland raceway system.

3.9.3 Methods

The economic performance of the CSIRO raceway production system was modelled by:

- tabulating variable and fixed costs, breaking each up into their individual components
- using costs, survival percentages and re-capture probability estimations to calculate revenues
- using dynamic programming to incorporate the different time periods it takes to produce prawns to 1 gram at the different densities, and
- considering risk by statistically simulating survivals at different densities using S-PLUS statistical modeling (survival was estimated by using the regression of log (p/(1-p))), and calculating confidence intervals at one standard deviation to show the possible range of survivals at each density.

3.9.4 Results and discussion

Variable and fixed costs

Both fixed and variable operating costs were found to increase with increased rearing density (Appendix 4). Fixed costs had a logarithmic increase from \$363.37 m^{-3} for the stocking density of 2860 m^{-3} to \$380.77 m^{-3} for stocking densities of 5720 and 11430 m^{-3}. Variable operating costs increased exponentially with increased rearing density (Fig. 3.41). This is an expected outcome as the higher the density the longer it takes for prawns to reach a mean weight of 1 gram, therefore the greater the food and labour inputs.

Labour is the greatest expense of high-density rearing (Fig. 3.41), suggesting that issues associated with time spent doing each individual task should be addressed and minimised by purchasing alternative equipment or adopting different techniques. For example, purchasing the correct sized pellets could alleviate food preparation costs and hours spent feeding could be substantially minimised by the use of automatic feeders. By addressing these costs the raceway rearing process could be substantially more feasible.

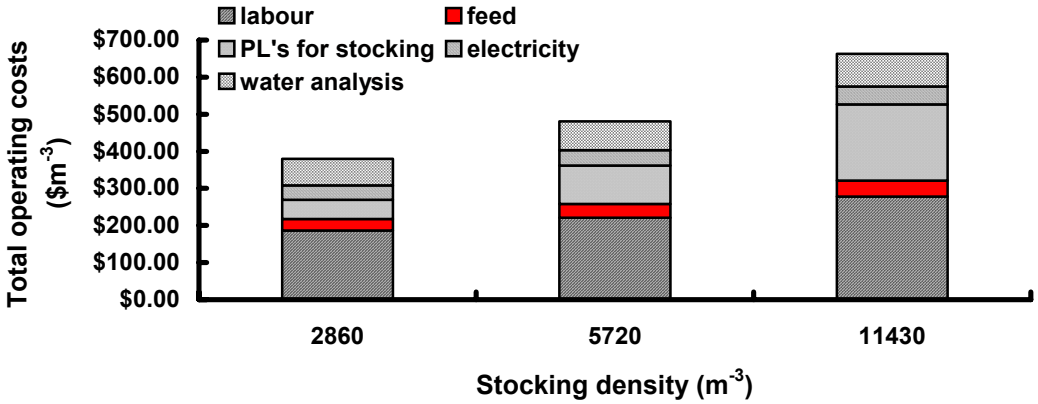


Figure 3.41 Variable operating costs of rearing prawns to a mean weight of 1 gram in a high-density raceway system at CSIRO.

Gross and net revenues

Revenues or incomes were estimated by using costs and survival rates form CSIRO's raceway rearing of prawns (Appendix 5). For this reason values are not directly comparable to the raceway system used by MG Kailis Exmouth Hatchery, Western Australia and should only be used as a guide. It would be most suitable to take figures from Exmouth and apply them to the model used here.

Revenues have been calculated for a range of re-capture probabilities based on results from the CMR modeling program. Gross revenue or total income, increases with increased density, even though survival rates decrease at a rapid rate (Fig. 3.42). This result is due to density being so high that even with a low survival the number of prawns which are harvested from the system, is still greater than for the lower densities. Net revenue or total income minus expenses, comes out extremely low for the CSIRO model. This is due to the experimental nature of CSIRO's work and related expenses. In the commercial situation at Exmouth this value should be much higher as operating costs will be less. For example, the commercial cost of PL's for stocking may be lower than used in the CSIRO model as they are produced 'on-farm'. Additionally, by addressing labour costs as mentioned earlier, net revenues can be easily improved.

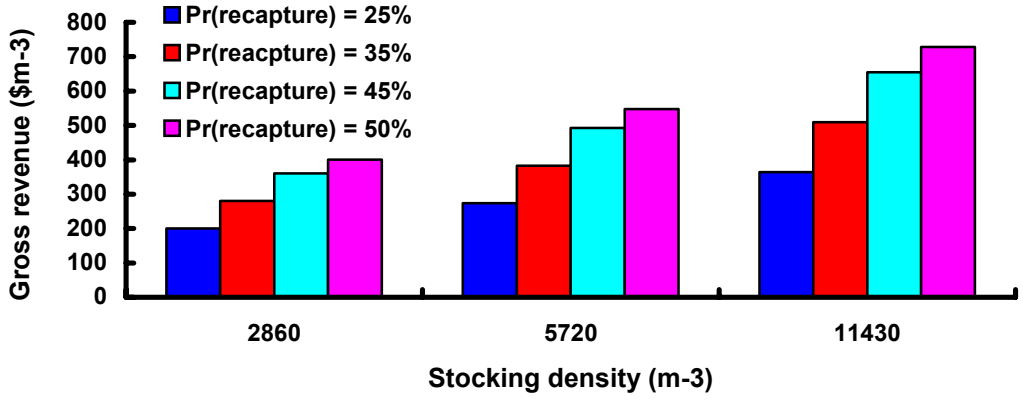


Figure 3.42 Gross revenues from CSIRO's raceway trial.

Dynamic programming

The effect of time on the raceway rearing process has been incorporated into this economic model by looking at the different time periods taken to rear prawns to 1 gram at different densities and different production season possibilities (Appendix 5). The number of runs that can be completed each year varies with rearing density as prawns reared at lower densities reach 1 gram in a shorter time period. These runs are as follows:

Scenario 1: one production season in November
Scenario 2: two production seasons in one year (November and April), and
Scenario 3: two production seasons in one year and two runs per production season (2 in Spring and 2 in Autumn).

Scenario three is more likely to be possible at lower densities as they take less time to reach 1 gram. For this reason, if scenario three is the preferred production season, it may be more feasible to grow at lower densities, as four low-density runs would produce more revenue than two high-density runs (Fig. 3.43).

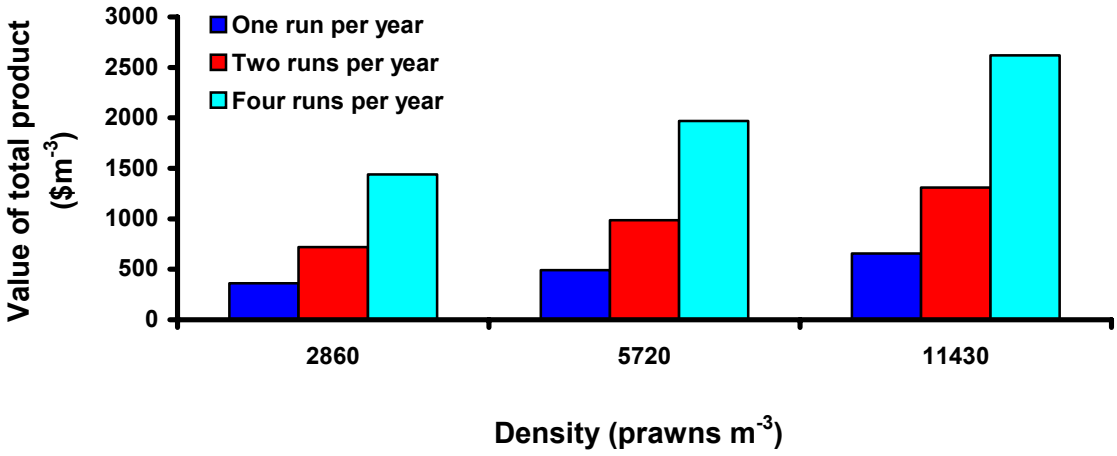


Figure 3.43 Gross revenues for the different production season possibilities at different densities with a re-capture probability of 45%.

Risk

The level of risk associated with successfully rearing prawns to one gram increases with increased density due to the increased length of time it takes to rear the prawns and the greater biomass within tanks. This level of risk can be assessed by looking at Fig. 3.44, which is a S-PLUS statistical simulation of survival at different densities. Survival was estimated by using the regression of log (p/(1-p)).

From the confidence intervals (Fig. 3.44) it can be seen that at lower densities there is greater variation in possible survival but the possibility of achieving a high survival is greater than at the higher rearing densities. In conclusion there is greater chance of high returns when rearing at lower densities and less risk associated with successfully rearing prawns to one gram.

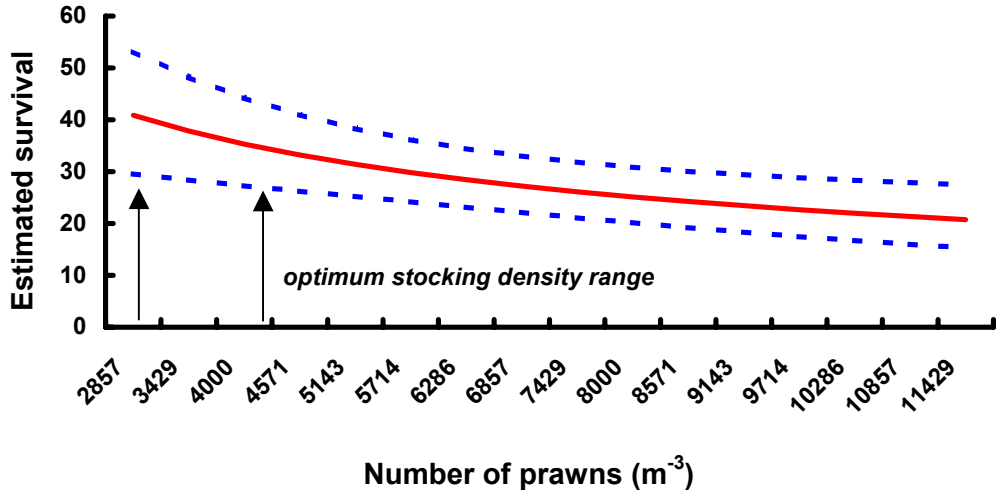


Figure 3.44 Estimated survival using S-PLUS statistical programming with confidence intervals at one standard deviation.

Optimal rearing density

From an analysis of the costs of production, revenues from production and risk associated with successfully rearing prawns to one gram at the different densities, the model suggests that the optimal stocking density is around 3000 m^{-3} to 4500 m^{-3}. Within this range production costs and risks are minimized, and revenues are maximized along with the probability of success. Previous estimations for optimal stocking density, which were based on production results, also fall within this range (section 3.2.4).

3.10 Further development of technology for the high-density production, transport and release of penaeus esculentus, for stock enhancement.

3.10.1 Objectives

- To further develop technology for high-density production of juvenile *P. esculentus* to 1 g.
- To understand the factors influencing the post harvest survival of juvenile prawns grown in ultra high density growout systems.
- To develop harvest, transport and release protocols to ensure maximum survival of juveniles released for stock enhancement.

3.10.2 Justification

CSIRO Marine Research and MG Kailis Pty Ltd have jointly developed and demonstrated a successful raceway system for the ultra high-density production (3,000 to 4,000 per m^3) of 1 g juvenile *P. esculentus*, and the system has successfully produced 1 g juveniles for trial release into Exmouth Gulf. Even though the target harvest weight of juveniles in the pilot trial was set at 1 g, the pilot trials demonstrated a complex dynamic relationship between stocking density, size, size range, survival, biomass and growout duration. These observations suggested that a strategy of partial harvests of particular size groups may result in higher overall production biomass for a particular growout run. We propose an experimental evaluation of this type of strategy in order to develop optimal production protocols.

In recognition of the need to achieve maximum survival in the post-harvest, transport, and release phase, as part of the initial production research CSIRO also conducted some pilot-scale post-production survival experiments to determine the suitability of high-density reared prawns for release into the wild. This preliminary work indicated the need to account for various factors affecting the survival of juveniles during the harvest, transport, release, and post-release phases.

In addition, in an external review of the project to date (Phase II), Ken Leber (Center for Fisheries Enhancement, Mote Marine Laboratory, Sarasota, Florida, USA) stressed the need in Phase III of the project for experiments to evaluate release strategies (e.g., size at release, release protocols, release habitat, release season, and release magnitude effects on growth and survival), based on the indication that to maximise the potential of prawn enhancement, an understanding of release strategy effects on post-release survival are critical.

CSIRO's preliminary post-harvest survival research indicated a number of areas needing further investigation in order to develop optimal release strategies:

- What effect does harvesting prawns at different sizes, and also partial harvests throughout production have on prawn survival, health and behaviour post release?
- What is the optimal harvest size protocol for maximising the efficiency of the production?
- What effect does transport density have on prawn survival, health and behavior post release?
- How quickly do prawns recover from damage sustained in the production, transport and release phases?
- Do prawns cultured at high densities in raceways express different behaviour responses to wild caught prawns when released into simulated seagrass environments?
- Which moult stage would produce the optimal chance of survival for released prawns and what would be an acceptable percentage of prawns within this stage to initiate harvest?
- How synchronous is moulting within the population of prawns in the raceway?
- Can feeding fresh feed (squid, pipis etc) prior to harvest increase the health of the prawns significantly enough to be included as a release strategy?
- Does appendage damage to prawns sustained in the high-density production effect foraging ability?
- Can prawns cultured at high densities be successfully grown to maturation and what is their reproductive performance?
- How does culture density affect predator evasion response?
- Investigation into effects of hydrogen sulfide levels on juvenile prawn growth and survival.

The proposed research will adopt a rigorous experimental approach to address these questions and to develop appropriate protocols to maximise post harvest and post-release survival.

3.10.3 Methods (preliminary only)

3.10.3.1 Production

Evaluate different parameters of production with prawns stocked at the optimal density of 3500/m^3 and harvested at different sizes and also a partial harvest. Experiments will be conducted in raceways at the Cleveland laboratories.

Treatments:

- Prawns reared at 3500/m^3 and harvested at 0.25 g
- Prawns reared at 3500/m^3 and harvested at 0.50 g
- Prawns reared at 3500/m^3 and harvested at 1.00 g
- Prawns reared at 3500/m^3 and partially harvested at 0.25 g and 0.50 g and completely harvested at 1.00 g.

Parameters measured:
- Time taken to reach harvest weight
- Survival and biomass at harvest
- Health status throughout production and at harvest
- Synchronisation of moult stage throughout production
- Water quality analysis (Ammonia, Nitrite, Nitrate, DO, pH)
- Feed rates and total feed amounts

3.10.3.2 Transport and release

Evaluate the effect of different harvest protocols and transport densities on various post harvest and post release parameters.

Treatments:
- 0.25 g prawns in a simulated transport situation at 5 kg/m^3, 10 kg/m^3 and 20 kg/m^3
- 0.50 g prawns in a simulated transport situation at 5 kg/m^3, 10 kg/m^3 and 20 kg/m^3
- 1.00 g prawns in a simulated transport situation at 5 kg/m^3, 10 kg/m^3 and 20 kg/m^3
- 1.00 g prawns (which have been partially harvested) in a simulated transport situation at 5 kg/m^3, 10 kg/m^3 and 20 kg/m^3

Parameters measured:
- Survival and behaviour of prawns after being transported at different densities then released into a simulated seagrass/sandy-bottom environment
- Health status prior to harvest and after release and recovery duration from damage sustained
- Post-release behaviour comparison of raceway reared prawns with prawns grown at low densities in sandy-bottom tanks and also wild caught prawns of a similar size
- Comparison of prawn health when fed fresh food (squid, pipis etc) prior to release
- Foraging ability of prawns with damaged appendages versus undamaged appendages
- Growth of prawns to maturation to assess broodstock performance of prawns harvested at different sizes
- Ascertain the moult stage that would produce the optimal chance of survival for released prawns
- Ascertain the acceptable percentage of prawns at the "desirable" moult stage so that survival is optimal during post harvest and release

3.11 References

Adams T.S. & Sterner R.W. (2000) The effect of dietary nitrogen content on trophic level ^{15}N enrichment. *Limnology and Oceanography* **45**: 601-607.

American Public Health Association, 1995. Standard Methods for the Examination of Water and Wastewater. 19th edition. Clesceri, L.S, Greenberg, A.E, Trussell, R.R. (Eds.), APHA, Washington D.C., USA.

Apud, F.D.; Gonzales K. and Deatras, N. (1981) Survival, growth and production of *Penaeus monodon* Fabricus at different stocking densities in earthen ponds with flow-through system and supplemental feeding. Fish. Res. J. Philipp. **6(2)**: 1-9.

Bratvold, D., Browdy, C.L. 2001. Effects of sand sediment and vertical surfaces (AquaMats™) on production, water quality, and microbial ecology in an intensive *Litopenaeus vannamei* culture system. Aquaculture 195: 81-95.

Burford, M.A. 2001. Fate and transformation of dietary nitrogen in penaeid prawn aquaculture ponds. PhD thesis. The University of Queensland, Australia.

Burford, M. A. & Williams, K. C. 2001. The fate of nitrogenous waste from shrimp feeding. Aquaculture 198: 79-93.

Burford, M. A., Preston, N.P., Glibert, P.M., Dennison, W.C. 2002. Tracing the fate of ^{15}N-enriched feed in an intensive shrimp system. Aquaculture, 206: 199-216.

Chen, J.C. and Lei, S.C. (1990) Toxicity of ammonia and nitrite to Penaeus monodon juveniles. *Journal of the World Aquaculture Society* **21(4):** 300-306

Chien, Y.H. (1992) Water quality requirements and management for marine shrimp culture. In: Proceedings of the special session on shrimp farming. World Aquaculture Society. (edited by Wyban, J.) Baton Rouge, LA USA, pp. 30-42.

Chin, T.S. and Chen, J.C. (1987) Acute toxicity of ammonia to larvae of the tiger prawn, *Penaeus monodon*. *Aquaculture* **66:** 247-253

Dall, W., Hill, B.J., Rothlisberg, P.C. and Staples, D.J. (1990) The biology of the Penaeidae. *Advanced Marine Biology* **27**: 1-489

Dittel A.I., Epifanio C.E., Cifuentes L.A. & Kirchman D.L. (1997) C and N sources for shrimp postlarvae fed natural diets from a tropical mangrove system. *Estuarine, Coastal and Shelf Science* **45**: 629-637.

Egusa, S. (1961) Studies on the respiration of the 'kuruma' prawn, *Penaeus japonicus* Bate – II Preminary experiments on its oxygen consumption. Bulletin of the Japanese Society of Scientific Fisheries **27(7)**: 650-659

Gearing, J.N. 1991. The study of diet and trophic relationships through natural abundance ^{13}C. In: Carbon Isotope Techniques, Coleman, D.C., Fry, B. (Eds.), Academic Press, San Diego, pp. 201-218.

Gu, B., Schelske, C.L., Hoyer, M.V. 1996. Stable isotopes of carbon and nitrogen as indicators of diet and trophic substrate of the fish community in a shallow hypereutrophic lake. Journal of Fish Biology **49**: 1233—1243.

Haywood, M.D.E., Vance, D.J. and Loneragan, N.R. (1995) Seagrass and algal beds as nursery habitats for tiger prawns (*Penaeus semilsulcatus* and *P. esculentus*) in a tropical estuary. *Marine Biology* **122:** 213-223.

Heales DS, Vance DJ, Loneragan NR (1996). Field observations of moult cycle, feeding behaviour, and diet of small juvenile tiger prawns *Penaeus semisulcatus* in a tropical seagrass bed in the Embley River, Australia. *Marine Ecology Progress Series* **145:** 43–51.

Law, A.T. (1988) Water quality requirements for *Penaues monodon* culture. pp. 53-65. In: Proceedings of the Seminar on Marine Prawn Farming in Malaysia. Serdang, malaysia, Malaysia Fisheries Society, 5th March 1998.

Leber K.M. & Pruder G.D. (1988) Using experimental microcosms in shrimp research: The growth-enhancing effect of shrimp pond water. *Journal of the World Aquaculture Society* **19**: 197-203.

Loneragan N.R., Bunn S.E. & Kellaway D.M. (1997) Are mangroves and seagrasses sources of organic carbon for penaeid prawns in a tropical Australian estuary? A multiple isotope study. *Marine Biology* **130**: 289-300.

Loneragan, N.R., Kenyon, R.A., Haywood, M.D.E. and Staples, D.J. (1994) Population dynamics of juvenile tiger prawns (*Penaues esculentus* and *Penaues semisulcatus*) in seagrass habitats of the Western Gulf of Carpentaria, Australia. *Marine Biology* **119**: 133-143.

Loneragan, N.R., Kenyon, R.A., Staples, D.J., Poiner, I.R. and Conacher, C.A. (1998) The influence of seagrass type on the distribution and abundance of postlarvae and juvenile tiger prawns (*Penaues esculentus* and *Penaues semisulcatus*) in the Eastern Gulf of Carpentaria, Australia. *Journal of Experimental Marine Biology and Ecology* **228**: 175-195.

Newell R.I.E., Marshall N., Sasekumar A. & Chong V.C. (1995) Relative importance of benthic microalgae, phytoplankton, and mangroves as sources of nutrition for penaeid prawns and other coastal invertebrates from Malaysia. *Marine Biology*, **123**: 595-606.

O'Brien, C.J. 1992. Some aspects of the production ecology of *Penaues esculentus* Haswell (Decapoda: Penaeidae) juveniles. Ph.D. thesis, University of New South Wales.

O'Brien, C.J. (1994a) The effects of temperature and salinity on growth and survival of juvenile tiger prawns *Penaeus esculentus* (Haswell). *Journal of experimental Marine Biology and Ecology* **183**: 133-145.

O'Brien, C.J. (1994b) Ontogenetic changes in the diet of juvenile tiger prawns *Penaues esculentus* (Decapoda: Penaeidae). *Marine Ecology Progress Series* **112(1-2):** 195–200.

O'Brien, C.J. 1994. Otogenetic changes in the diet of juvenile tiger prawns *Penaeus esculentus* (Decapoda: Penaeidae). *Marine Ecology Progress* Series 112(1-2), 195–200.

Otoshi C.A., Montgomery A.D., Look A.M. & Moss S.M. (2001) Effects of diet and water source on the nursery production of Pacific White Shrimp, *Litopenaeus vannamei. Journal of the World Aquaculture Society* **32**: 243-249.

Parker, P. L., Anderson, R. K. 1989. A $\delta^{13}C$ and $\delta^{15}N$ tracer study of nutrition in aquaculture: *Penaeus vannamei* in a pond growout system. In: Rundel, P. W., Ehleringer, J. R., Nagy, K. A. (Eds) Stable Isotopes in Ecological Research. New York: Springer-Verlag, pp. 288-303.

Parker, P. L., Anderson, R. K., Lawrence, A. L. 1991. Stable isotope methodology for evaluation of nutritional requirements of shrimp. In: De Loach, P. F., Dougherty, W. J., Davidson, M. A. (Eds) Frontiers of Shrimp Research. Amsterdam: Elsevier, pp. 157-171.

Preston, N.P. (1993) Fishmeal replacement in aquaculture feeds for prawns. Final report to FRCD, project 93/120-02.

Preston, N.P., Smith, D.M., Kellaway, D.M. and Bunn, S.E., 1996. The use of enriched ^{15}N as an indicator of the assimilation of individual protein sources from compound diets for juvenile Penaeus monodon. Aquaculture, 147: 249-259.

Shearer, G., Kohl, D.H. 1993. Natural abundance of 15N: Fractional contribution of tow sources to a common sink and use of isotope discrimination. In: Nitrogen Isotope Techniques, Eds. Knowles, R., Blackburn, T.H., Academic Press: San Diego, pp. 89-125.

Smith, D.M. and Dall, W. (1985) Moult staging the tiger prawn Penaeus esculentus. In: Rothlisberg, P.C., Hill, B.J. and Staples, D.J. (Eds), Second Australian National Prawn Seminar, NPS2, Cleveland, Australia. pp. 85-93.

Smith, D.S., Dall, W. and Moore, L.E. (1992) The natural food of some Australian Penaeids. In: *Proceedings of the Aquaculture Nutrition Workshop*, NSW Fisheries, Salamander Bay, NSW, Australia. pp. 95-96.

Staples, D.J., Vance, D.J. and Heales, D.S. (1985) Habitat requirements of juvenile penaeid prawns and their relationship to offshore fisheries. In: Second Australian National Prawn Seminar. NPS2, (edited by Rothlisberg, P.C., Hill, B.J. and Staples, D.J.) Cleveland, Australia, pp. 47-54.

Thompson, F.L., Abreu, P.C., Wasielesky, W. 2002. Importance of biofilm for water quality and nourishment in intensive shrimp culture. Aquaculture 203: 263-278.

Tidwell, J.H., Coyle, S., Weibel, C., Evans, J. 1999. Effects and interactions of stocking density and added substrate on production and population substrate of freshwater prawns *Macrobrachium rosenbergii*. Journal of the World Aquaculture Society 30: 174-179.

Tidwell, J.H., Coyle, S.D., VanArnum A., Weibel, C. 2002. Effects of substrate amount and orientation on production and population substrate of freshwater prawns *Macrobrachium rosenbergii* in ponds. Journal of the World Aquaculture Society 33: 63-69.

Vander Zanden M.J. & Rasmussen J.B. (2001) Variation in $\delta^{15}N$ and $\delta^{13}C$ trophic fractionation: Implications for aquatic food web studies. *Limnology and Oceanography* **26:**, 2061-2066.

Wickens, J.F. (1976) The tolerance of warm-water prawns to recirculated water. Aquaculture **9**: 19-37.

Williams, A.S.; Davis, D.A. and Arnold, C.R. (1996) Density-dependent growth and survival of *Penaeus setiferus* and *Penaeus vannamei* in a semi-closed recirculating system. *J. World Aquac. Soc.* **27(1)**: 107-112.

Ziemann, D.A., Schell, D.M. 1999. Tracking nitrogen and carbon flows and sinks in experimental shrimp culture systems using stable isotope additions. World Aquaculture Society Book of Abstracts, Sydney, Australia, p. 832.

3.12 Appendix

3.12.1 Appendix 1: ANOVA table of the results from the high-density production

Measure		Substrate comparison 2860 m^{-3} substrate A, 2860 m^{-3} substrate B			Density comparison 5720 m^{-3} substrate C, 11430 m^{-3} substrate C		
	Effects	d.f.	MS [a]	% MS [b]	d.f.	MS [a]	% MS [b]
(a) Growth	Treatment	1	0.006	0.1	1	0.221****	1.7
	Week	1	10.529**	99.9	1	12.670**	98.2
	Treatment x Week	1	0.000	0.0	1	0.001	0.0
	Error	38	0.009		44	0.007	
	R^2		0.97			0.98	
(b) Survival	Treatment	1	0.015	9.9	1	0.020	43.4
	Error	4	0.136		4	0.026	
	R^2		0.03			0.16	
(c) Biomass	Treatment	1	0.032	12.1	1	0.003	7.3
	Error	4	0.232		4	0.038	
	R^2		0.03			0.04	
(d) Size variation	Treatment	1	0.097	0.5	1	10.82	29.2
	Error	4	17.902		4	26.18	
	R^2		0.001			0.094	

[a] Type III Mean Squares; **** significant at P<0.0001
[b] Based on total Mean Squares of all effects

3.12.2 Appendix 2: Standard method for determination of ammonia concentrations

Part A- sample preparation and experimental procedure for colour development

1. Take pre-made standards out of the fridge and leave on the bench to warm up (standards should be made fresh every month). The oxidising solution is the only reagent which has to be made up fresh just prior to use. If you need to make fresh standards follow the recipes below.

Reagent recipes.

1. *Phenol solution: Dissolve 20 g of analytical grade phenol in 200 ml of 95% v/v ethyl alcohol.*
2. *Sodium nitroprusside solution: Dissolve 1.0 g of sodium nitroprusside (Na2[Fe(CN)5NO]H2O) in 200 ml of de-ionised water. This chemical is light sensitive and needs to be stored in a dark glass bottle or bottle covered with aluminium foil.*
3. *Alkaline reagent: Dissolve 100 g of sodium citrate and 5 g of sodium hydroxide in 500 ml of de-ionised water.*
4. *Sodium hypochlorite solution: Use commercially available hypochlorite (e.g. pool chlorine), which should be about 1.5 N.*
5. *Oxidising solution (make up fresh just prior to use): Mix 50 ml of reagent 3 with 10 ml of reagent 4 (use pipette) then swirl.*

2. Take 3 replicate samples from a standardised point within each tank. Wear a latex glove and take samples from as deep down as the glove will allow. Rinse containers and their lids with some of the water from each corresponding tank before taking samples. Ensure all containers are well labelled (ie. with tank #, experiment #, date, replicate # etc.).

3. Prepare samples by filtering through 5 μm filters into labelled tubes using a syringe. Use a new filter for each tank but the same filter for the 3 replicates of the same tank. Rinse the syringe with sample water between every sample being filtered.

4. Label acid washed and oven dried test-tubes. You will need at least 5 standards, two de-ionised water tubes (to ensure that there is no ammonia in the de-ionised water supply) and a tube for each tank and replicate. They should look like this:

    a) 0.0 (std)
    b) 0.10 (std)
    c) 0.25 (std)
    d) 0.5 (std)
    e) 1.0 (std)
    f) DI 1
    g) DI 2
    h) Tank 1 replicate 1
    i) Tank 1 replicate 2
    j) Tank 1 replicate 3
    k) Tank 2 replicate 1
    l) Tank 2 replicate 2 etc.

5. Use a fume cupboard making sure that all lights within the cupboard are turnt off. This will help prevent the overdevelopment of colour. It is also advisable to wear a pair of teflon gloves to complete the following procedures. Make up the oxidising reagent (the only one which has to be made up fresh) following the recipe outlined previously.

6. Pipette 5 ml of each standard into their corresponding test tubes using a fresh tip every time (ie. the 0.0 mg/l standard into the 0.0 mg/l test tube).

7. Pipette 5 ml of de-ionised water into each of the DI test tubes.

8. Pipette 5 ml of each filtered sample into their corresponding test tube using a fresh tip every time. If doing a dilution only add the appropriate amount of sample followed by the corresponding amount of de-ionised water.

9. To all test tubes add the colour reagents individually as follows (vortex each immediately after adding solution);
   - 0.2 ml of phenol solution
   - 0.2 ml of nitro-prusside solution, and
   - 0.5 ml of oxidising solution.

10. Seal the tops of all test tubes with parafilm and cover the bottom with aluminium foil. Leave for 3 hours to allow colour to develop.

Part B-spectrophotometer readings

1. Read the absorbance of standards and samples at 640 nm.

2. Using the standard values as x-values and absorbance readings for the standards as y-values, make a scatter plot or NH3-N calibration curve. By adding a trendline calculate the equation of the line from the expression;

$$y = mx + c$$

   where y is the absorbance, m is the slope, x is the ammonia concentration and c is the y-intercept. By re-arranging this equation to;

$$x = (y-c)/m$$

   ammonia concentrations of the samples can be calculated. This equation must also be multiplied by the dilution factor if samples were diluted.

3.12.3 Appendix 3: Scoring system used for health and post production recovery

Damage	Units of damage	Total score
Antennae		
81-100% remaining	1	1
61-80% remaining	2	2
41-60% remaining	3	3
21-40% remaining	4	4
0-20% remaining	5	5
Rostrum		
(1 case of necrosis = 1 unit of damage	0	1
tip broken = 1 unit of damage	1	2
for each tooth broken = 1 unit of damage)	2	3
	3	4
	4+	5
Periopods		
(1 case of necrosis = 1 unit of damage	0-1	1
for each segment missing = 1 unit of damage)	2-3	2
	3-5	3
	6-7	4
	8+	5
Pleopods		
(1 case of necrosis = 1 unit of damage	0	1
for each segment missing = 1 unit of damage	1	2
mattered hairs or parts nibbled = 1 unit of damage)	2	3
	3	4
	4+	5
Uropods		
(1 case of necrosis = 1 unit of damage	1	1
for each segment missing = 1 unit of damage	2	2
mattered hairs or parts nibbled = 1 unit of damage)	3	3
	4	4
	5	5
Gut content		
% fullness	81-100	1
% fullness	61-80	2
% fullness	41-60	3
% fullness	21-40	4
% fullness	0-20	5
Necrosis		
(1 case of necrosis = 1 unit of damage)	0	1
	1	2
	2	3
	3	4
	4+	5

3.12.4 Appendix 4: Variable and fixed costs

Variable and fixed costs of rearing prawns to a mean weight of 1 gram in a high density raceway system at CSIRO (all values are in $\$m^{-3}$ of tank area).

Components	Prawn density (m^3)		
	2860	5720	11430
Operating Costs			
Labour			
siphoning (hours)	1.86	2.80	4.57
food preparation (hours)	1.98	2.41	2.77
weighing food (hours)	1.08	1.17	1.33
water quality monitoring (hours)	1.11	1.20	1.37
feeding (hours)	2.64	2.84	3.24
other (hours)	1.55	1.67	1.90
Total labour costs ($\$m^{-3}$)	186.65	220.72	277.68
Feed ($\$m^{-3}$)	30.96	37.69	43.39
Post larvae for stocking ($\$m^{-3}$)	51.43	102.86	205.71
Electricity			
air pump ($\$m^{-3}$)	34.32	36.96	42.24
sump pump ($\$m^{-3}$)	4.68	5.04	5.76
HACH reagents ($)	71.91	77.44	88.50
Total operating costs ($\$m^{-3}$)	379.94	480.70	663.30
Fixed costs			
Diminishing infrasubstrate costs			
tank systems ($\$m^{-3}$)	119.05	119.05	119.05
HACH machine ($\$m^{-3}$)	14.29	14.29	14.29
pumps ($\$m^{-3}$)	10.32	10.32	10.32
other equipment (ie. nets, buckets etc.) ($\$m^{-3}$)	2.38	2.38	2.38
Opportunity costs of capital ($\$m^{-3}$)	1.46	1.46	1.46
Fixed labour costs (managerial) ($\$m^{-3}$)	226.20	243.60	243.60
Total fixed costs ($\$m^{-3}$)	363.37	380.77	380.77

3.12.5 Appendix 5: Revenues

Revenues from CSIRO raceway trial (all values are m^{-3} of tank area).

Components	Prawn density (m^3)		
	2860	5720	11430
Gross revenue			
# produced (m^{-3})	1333.7	1824.0	2426.3
# re-captured if Pr(recapture) = 25%	333.4	456.0	606.6
# re-captured if Pr(recapture) = 35%	466.8	638.4	849.2
# re-captured if Pr(recapture) = 45%	600.2	820.8	1091.8
# re-captured if Pr(recapture) = 50%	666.9	912.0	1213.1
Total value of re-captured harvest (m^{-3}) @ 25% recapture	200.06	273.60	363.94
Total value of re-captured harvest (m^{-3}) @ 35% recapture	280.08	383.04	509.52
Total value of re-captured harvest (m^{-3}) @ 45% recapture	360.10	492.48	655.10
Total value of re-captured harvest (m^{-3}) @ 50% recapture	400.11	547.20	727.89
Net revenue (Profit)			
Total value of re-captured harvest (m^{-3}) @ 25% recapture	-179.88	-207.10	-299.35
Total value of re-captured harvest (m^{-3}) @ 35% recapture	-99.86	-97.66	-153.78
Total value of re-captured harvest (m^{-3}) @ 45% recapture	-19.84	11.78	-8.20
Total value of re-captured harvest (m^{-3}) @ 50% recapture	20.18	66.50	64.59

Dynamic programming for CSIRO raceway trial (all values are m-3 of tank area).

Components	Prawn density (m^3)		
	2860	5720	11430
Dynamic programming *(see above text for scenario definitions)*			
# of prawns produced in scenario 1	1333.71	1824.00	2426.29
# of prawns produced in scenario 2	2667.43	3648.00	4852.57
# of prawns produced in scenario 3	10669.71	14592.00	19410.29
Operating costs in scenario 1	379.94	480.70	663.30
Operating costs in scenario 2	759.88	961.40	1326.59
Operating costs in scenario 3	1519.76	1922.80	2653.18
Fixed costs in scenario 1	1760.13	1865.26	1808.29
Fixed costs in scenario 2	3520.26	3730.52	3616.58
Fixed costs in scenario 3	7040.52	7461.03	7233.16
Gross revenue in scenario 1 @ $Pr_{(recapture)}$ = 45%	360.10	492.48	655.10
Gross revenue in scenario 2 @ $Pr_{(recapture)}$ = 45%	720.21	984.96	1310.19
Gross revenue in scenario 3 @ $Pr_{(recapture)}$ = 45%	1440.41	1969.92	2620.39
Gross revenue in scenario 1 @ $Pr_{(recapture)}$ = 50%	400.11	547.20	727.89
Gross revenue in scenario 2 @ $Pr_{(recapture)}$ = 50%	800.23	1094.40	1455.77
Gross revenue in scenario 3 @ $Pr_{(recapture)}$ = 50%	1600.46	2188.80	2911.54
Net revenue in scenario 1 @ $Pr_{(recapture)}$ = 45%	-19.84	11.78	-8.20
Net revenue in scenario 2 @ $Pr_{(recapture)}$ = 45%	-39.67	23.56	-16.40
Net revenue in scenario 3 @ $Pr_{(recapture)}$ = 45%	-79.35	47.12	-32.79
Net revenue in scenario 1 @ $Pr_{(recapture)}$ = 50%	20.18	66.50	64.59
Net revenue in scenario 2 @ $Pr_{(recapture)}$ = 50%	40.35	133.00	129.18
Net revenue in scenario 3 @ $Pr_{(recapture)}$ = 50%	80.70	266.00	258.36

CHAPTER 4

DEVELOPMENT AND DEPLOYMENT OF MICROSATELLITE MARKERS

Sigrid Lehnert (CSIRO Livestock Industry)
Jennifer Meadows (CSIRO Livestock Industry)
Bob Ward (CSIRO Marine Research)
Peter Grewe (CSIRO Marine Research)
Mark Bravington (CSIRO Mathematics and Information Sciences)

(Note that part of this Chapter has been accepted for publication:
Meadows JRS, Ward RD, Grewe PM, Dierens L, Lehnert, SA (2002). Characterisation of 23 tri- and tetranucleotide microsatellite loci in the brown tiger prawn, *Penaeus esculentus. Molecular Ecology Notes*, in press, and has been included in Appendix 4A.
Part is in draft form for publication in Molecular Ecology:
Bravington MV, Ward RD. Microsatellite DNA markers: evaluating their potential for estimating proportions of wild-born and hatchery-reared offspring in an enhancement program and the abstract of this manuscript is included in Appendix 4C.)

4.1 Summary

Two microsatellite loci developed in earlier work – one a dinucleotide repeat and one a trinucleotide repeat – were found to be both reliable and variable enough to form part of a panel of loci that could be used to discriminate stocked prawns from wild-born prawns. However, it was estimated that up to ten such loci would be required for effective discrimination of enhanced and wild prawns. Twenty three further microsatellite loci were developed (some were trinucleotide repeats, others tetranucleotide repeats), of which six proved to be reliable and variable. This gave a panel of eight reliable loci, six of which could be resolved on one gel, and two on another.

These eight loci showed no evidence of significant differentiation between two samples of *Penaeus esculentus* caught by different trawlers in different regions of Exmouth Gulf and two consecutive years (1999, 2000). Therefore, the null hypothesis that the Exmouth Gulf population is a single panmictic population cannot be rejected.

A statistical methodology was developed which showed that if the genotypes of both parents were known, these eight loci would enable us to monitor the success of any enhancement program with reasonable numbers of recaptures genotyped. However, because of the mating system of this species, mothers of the hatchery population would have already been fertilised in the wild, and it is therefore not feasible to directly genotype the fathers in a commercial production system. The paternal genotype could be deduced by genotyping a sample of progeny (possibly a homogenate) from mothers of known genotype. Further work is required to determine if this is feasible and how many progeny would need to be genotyped. If this system is not feasible, more loci would have to be developed and deployed or very large numbers of recaptured prawns would have to be genotyped. The number of additional loci needed would be determined by the variability shown at the loci: for example, three, hypervariable, microsatellite loci with high resolving power, would identify enhanced prawns by maternal-only genotyping, but three microsatellite loci with average resolving power would not be sufficient for maternal only identification.

The costs of analysing the present eight loci would be in the region of AUS $45 per individual for adults and $38 for larvae, but a two step approach (examining six loci on one gel in all individuals, and only the two remaining loci as required) could bring the costs down to closer to AUS$30 per individual.

4.2 Introduction

Evaluating the effectiveness of an enhancement program is a critical component of enhancement research. This objective requires a tag to identify the stocked individuals from those in the wild. For prawns, few physical tags can be used for animals less than 1 g or 12 mm in carapace length. In this study, molecular genetic methods were used to develop a tag for the brown tiger prawn. Microsatellites were chosen as the most accurate and informative genetic marker available, and because large numbers of individuals can be screened through developing high-throughput systems. During the planning phase of the project, it was thought that 10 microsatellites would be sufficient to assign the individuals caught in Exmouth Gulf to either the wild or the hatchery-reared population.

The mating system of penaeids differs from fish and imposes some constraints on using genetic techniques to identify enhanced prawns. In species of *Penaeus* with closed thyleca, such as *Penaeus esculentus*, the spermatophore is implanted in the female just after moulting and a plug forms over the thyleca. The females release the eggs (i.e. spawns) when the cuticle has hardened in early premoult (stage D), about 10 to 20 days after mating. In a commercial aquaculture production, mated females are taken from the wild and induced to release their eggs in the hatchery. The identity of the fathers of the resulting postlarvae are therefore unknown.

Microsatellites are short stretches of tandemly repeated short nucleotide motifs that are interspersed into the DNA of all organisms. They can consist of di-, tri- or tetranucleotide motifs. Among individuals, these sections of DNA may vary in the number of repeats they contain. The number of repeats can be determined and can serve as markers and indicators of genetic variation.

Previous studies have shown that microsatellites can be very informative when investigating fishery stock structures (O'Connell and Wright, 1997). A large number of microsatellites have been developed for *Penaeus monodon*, the most widely used prawn in aquaculture (Xu *et al.,* 1999). However, in contrast to mammals, microsatellite markers are not generally transferable between penaeid species (Moore et al*.,* 1999), and it was therefore necessary to isolate microsatellites specific for *Penaeus esculentus*.

A previous study had identified a number of dinucleotide microsatellites for *Penaeus esculentus* (Preston, 1997) and three of these, together with an additional seven new di-nucleotide markers, were evaluated in the first year of this study. Only one of these 10 di-nucleotide markers, Pe1.1, gave reproducible and clear genotyping results that would make it useful for genotyping applications. One further marker, Pmcd01, was discovered fortuitously when testing a *P. monodon* microsatellite primer on *P. esculentus*. This primer was designed within a prawn gene coding sequence, which probably contributed to its conservation across penaeid species.

After the first year of the project, the genetics team was therefore faced with the task of isolating up to eight novel microsatellite markers from the *Penaeus esculentus* genome. We now report the development of 23 novel microsatellite markers. These markers are based on tri- and tetranucleotide repeats rather than the previously used di- repeats. Nine of these new markers, together with Pe1.1 and Pmcd01, have been tested on a large sample of wild prawns collected from Exmouth Gulf.

The three main objectives of the genetic work were:

- to determine the genetic structure of the *P. esculentus* population in Exmouth Gulf and;
- to isolate genetic markers that will permit the success of the experimental stock-enhancement to be monitored
- to develop statistical methods to determine the probability of identifying enhanced prawns in the wild

4.3 Methods

4.3.1 Identification of 23 novel Penaeus esculentus tri- and tetranucleotide microsatellite markers

Four *Penaeus esculentus* genomic DNA libraries, enriched for four tri- and tetranucleotide motifs, were produced by Genetic Identification Services (Chatsworth, California 91311, USA) using DNA extracted from 1.5g of tail muscle (Moore et al. 1999). Recombinant plasmids were produced by ligating AAG, AAT, ATG and TAGA enriched restriction fragments into the HindIII site of pUC18. E.coli strain DH5α was then transformed with these plasmids and positive clones for sequencing were chosen using X-gal/IPTG/ampicillian selective plates.

The inserts were sized using PCR and M13-cloning site primers (Genetic Identification Services protocol, supplied with library) to ensure they fell within a 350-700 bp size range. Sequencing using ABI Prism™ Big Dye Terminator Mix (Applied Biosystems) was performed by the Australian Genome Research Facility (Brisbane, Queensland). 23 unique microsatellites were identified (Table 4.1) and Mac Vector™ 7.0 software was used to design primers to the flanking sequence (Meadows et al., 2002; see Appendix 4A). These oligonucleotides were submitted to Geneworks (Adelaide, South Australia) for synthesis. A nine bp tag was added to the 5' end of both the forward and reverse primer of each pair, except Pe1.1, to increase the GC content and thereby the annealing temperature. The forward primer of each pair was fluorescently labelled.

Table 4.1. Primer sequences, repeat type and GenBank accession numbers for the 23 isolated Penaeus esculentus microsatellites with tri and tetra-repeat motifs.

* Represents a 9bp proprietary sequence added to increase the GC content of the oligonucleotide.

Locus	Primer Sequence	Repeat Type	GenBank Accession #
CSGES045	F: 5'-* gag cgt tac tgg aaa gtg tcc-3'	$(TAGA)_9TA(TAGA)$	AF430380
	R: 5'-* tgc ctg aag ttg aaa agt gc-3'		
CSGES047	F: 5'-* tca tca gtt tct atc cat cca gcc-3'	$(CTAT)_{11}$	AF430381
	R: 5'-* ctt atc tga ttc tgt tcc acc g-3'		
CSGES027	F: 5'-* gac gac aac ttg atg aaa cgg-3'	$(TCT)(TTT)(TCT)_2(TTT)(TCT)_7$	AF430378
	R: 5'-* gaa tcg ggt aga aca aat ctg c-3'		
CSGES043	F: 5'-* cca gga gtt gta ttg aag gga g-3'	$(TTC)_{13}$	AF430379
	R: 5'-* cca gat tat cgt gac cgt gag-3'		
CSGES003	F: 5'-* tta tct ttt gag ggg gtt gc-3'	$(TATC)_8$	AF430398
	R: 5'-* gtt gat tag gaa ggg cat cc-3'		
CSGES015	F: 5'-* cgc tca cca aga aaa cta atg g-3'	$(ATG)_{15}$	AF430377
	R: 5'-* cgc tga gac aat gaa cac ttc g-3'		
CSGES120	F: 5'-* gga gaa gaa gga cga tag gaa g-3'	$(TGA)_7(TGG)(TGA)_7$	AF430385
	R: 5'-* tct tgg ggg ggt ctc ata tc-3'		
CSGES132	F: 5'-* gat ggt cgt aat agt ggt gag g-3'	$(TGA)_6$	AF430386
	R: 5'-* gtc aac atc gtc ctc tcc aac-3'		
CSGES110	F: 5'-* gcg ttc act ttg cct atg ttc-3'	$(CTT)_9$	AF430384
	R: 5'-* cga gcc tca aac aca cca ag-3'		

Locus	Primer Sequence	Repeat Type	GenBank Accession #
CSGES090	F: 5'-* ccg tgg aac aaa atc gca g-3'	$(GAA)_{14}$	AF430383
	R: 5'-* agg gtg tga tgt gcc gtt tc-3'		
CSGES078	F: 5'-* tgt aga cat aga cgg cag tgg-3'	$(GAA)_{9}$	AF430382
	R: 5'-* ggt ggc ttc ctg gat aag tc-3'		
CSGES215	F: 5'-* agg ggt ttc ctg cat tac c-3'	$(ATC)_{8}$	AF430391
	R: 5'-* aac gag att cca agg tgg g-3'		
CSGES217	F: 5'-* cac caa tca cca tca tct tca c-3'	$(TCA)_{6}(ACT)_{6}$	AF430392
	R: 5'-* aag gac atc gtt caa ggg c-3'		
CSGES218	F: 5'-* gga gtg cgt cgt att gag aag-3'	$(CAT)_{9}$	AF430393
	R: 5'-* ctg atg gtg ata aag gtg aaa gtg-3'		
CSGES189	F: 5'-* gga tta ttt ctc gtc cct tca c-3'	$(TTC)_{10}$	AF430389
	R: 5'-* cgg cga act tga ctt ttg g-3'		
CSGES190	F: 5'-* gga gga aga acc gaa cga ag-3'	$(TGA)_{11}$	AF430390
	R: 5'-* gga gag tca tta tca tca tcg cag-3'		
CSGES180	F: 5'-* cgt cag tca gac agc att gg-3'	$(TCT)_{7}$	AF430388
	R: 5'-* ctt gat ttc ttg cca cta cag g-3'		
CSGES176	F: 5'-* att gct ggc ata cgg tca cc-3'	$(GAG)_{9}(AAG)_{13}(GAG)_{4}$	AF430387
	R: 5'-* cgt ctt ctc cac aag tgt ttc g-3'		
CSGES257	F: 5'-* cca aac agc agc aaa caa aca cc-3'	$(CTT)_{3}(CAT)_{2}(CTT)_{18}$	AF430395
	R: 5'-* gga ttg aac gca ggt cag caa g-3'		
CSGES269	F: 5'-* aag aag agc aag gca agg aga tg-3'	$(ATG)_{20}$	AF430399
	R: 5'-* cca tta tca gca gca gca gta gc-3'		
CSGES268	F: 5'-* tgc caa taa gga gga gaa gg-3'	$(ATG)_{9}(GTG)(ATG)_{5}$	AF430396
	R: 5'-* ctt gaa cgg agc ctt gtt gt-3'		
CSGES256	F: 5'-* tga gct gcc gtc att aga caa-3'	$(CTT)_{17}$	AF430394
	R: 5'-* cct tcc cct tcg tca ttt tc-3'		
CSGES288	F: 5'-* ctc ccc ttc gtt gtt cca ctt ac-3'	$(GTAT)_{18}(GAA)_{2}(GATA)_{3}$	AF430397
	R: 5'-* ttg att ggg ttt gcc ttg act g-3'		

Total genomic DNA for microsatellite analysis was extracted from 40 mg of prawn pleopod tissue, (shattered after freezing in liquid nitrogen) using a modified DNeasy™ 96 Tissue Kit (QIAGEN®) protocol, in which a shortened 2 hour incubation for proteinase K digestion was used. Microsatellite amplifications were performed in 96-well plates using an MJ Research PTC-100 thermocycler. 20 µL reactions contained 67 mM Tris-HCl (pH 8.8); 16.6 mM $(NH_4)_2SO_4$; 0.45% Triton X-100; 0.2 mg/mL gelatin; 3mM $MgCl_2$; 0.2 mM forward primer (labelled); 0.2 mM reverse primer; 0.4 units *Tth* Plus DNA polymerase (Fisher Biotech); 125µM dNTP (Pharmacia Biotech) and approximately 50 ng genomic DNA template. The DNA template and enzyme were denatured at 94^{o}C for 3 min, followed by 30 cycles consisting of 94 ^{o}C for 30 sec, 60^{o}C for 2 min (exempting CSGES190 which anneals at 55^{o}C) and 72 ^{o}C for 1 min. A final extension at 72 ^{o}C for 30 min was used to ensure complete addition of adenine to the PCR product, essential for consistent allele calling during genotyping (Smith et al. 1995).

The PCR products were diluted, dried down and combined with sucrose-urea load dye and Genescan™-500 Tamra™ size standard (Applied Biosystems), before being denatured and visualised on a 6% denaturing polyacrylamide gel. Results were collected using an ABI 377 Prism DNA autosequencer and analysed using GeneScan® 3.1 and Genotyper® 2.5 software.

The 23 microsatellite loci were tested across a sample of 36 wild-caught individuals from the Exmouth Gulf. In these tests, 14 markers had to be discarded due to: 1) failure to amplify (CSGES015, CSGES132, CSGES180, CSGES269 and CSGES256), 2) monomorphism (CSGES027, CSGES110, CSGES215), 3) non-specific amplification (CSGES003), 4) a stuttery profile (CSGES043), and 5) one base pair allelic shifts which compromised accurate genotyping (CSGES045, CSGES078, CSGES257 and CSGES288).

4.3.2 Testing nine novel and two pre-existing markers

The nine new microsatellite loci together with the two pre-existing loci were used in a larger study, comprising two groups of 96 animals harvested from the Exmouth Gulf population in consecutive years (Table 4.2). The software package GENEPOP® 3.2 (Raymond and Roussett, 1995) was used for statistical analyses. Allele frequencies are given in Appendix 4B.

CSGES217 and CSGES090 were hyper-allelic, with apparent one base pair alleles, and were difficult to score consistently. They showed significant heterozygote deficiencies with respect to Hardy-Weinberg expectations (Table 4.2), which might indicate the presence of null or non-amplifying alleles. CSGES218 also showed a significant heterozygote deficiency in the sample collected in 2000; and could not be reliably scored in the 1999 sample. These three loci were considered too difficult to use in routine screening that required accurate genotyping. One of these three loci, CSGES090, showed evidence of statistically significant differentiation between the two samples, but the amount of differentiation was extremely small and may be an artefact (F_{ST} = 0.0002, Table 4.2, meaning that only 0.02% of all the variation at this locus could be ascribed to sample differences).

Based on the above analyses, the set of microsatellites to be taken forward for evaluation consisted of eight markers (Table 4.3), which could be resolved in two co-load systems (Fig. 4.1).

Table 4.2. Summary of variability for the eleven microsatellite loci in two samples from the Exmouth Gulf population of *Penaeus esculentus*.

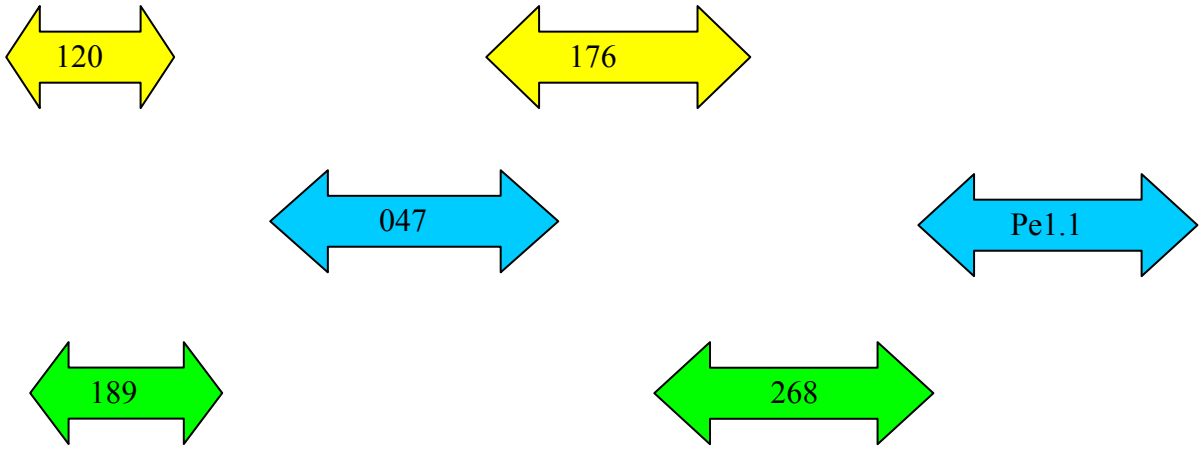
Total n = total number of individuals, Total alleles = total number of alleles, GM2000 = November 2000 sample from vessel George Michael, TUB1999 = November 1999 sample from trawler Tubridgi, Hob = observed heterozygosity, Hexp = Hardy-Weinberg expected heterozygosity, F_{ST} = amount of genetic differentiation between the two samples, * = $0.01 < P \leq 0.05$,** = $0.001 < P \leq 0.01$, *** = $P \leq 0.001$

	Total	Total	GM2000		Tub1999		
Locus	n	alleles	Hob	Hexp	Hob	Hexp	F_{ST}
CSGES120	183	11	0.500	0.524	0.396	0.472	-0.0030
CSGES189	186	11	0.800	0.769	0.681	0.705	0.0052
CSGES047	187	19	0.865	0.883	0.846	0.902	-0.0016
CSGES217	187	74	0.833	0.966***	0.868	0.963***	-0.0018
CSGES090	182	49	0.630	0.949***	0.622	0.954***	0.0002**
CSGES218	92	10	0.500	0.811***	-	-	-
CSGES176	186	15	0.833	0.849	0.800	0.845	-0.0040
CSGES268	183	16	0.719	0.708	0.644	0.723	-0.0025
CSGES190	180	8	0.495	0.489	0.494	0.549	0.0030
Pe1.1	186	27	0.936	0.907	0.859	0.920*	0.0060
Pmcd01	185	10	0.740	0.830	0.798	0.834	-0.0041

Table 4.3. The set of 8 microsatellite markers chosen * Represents a 9bp proprietary sequence added to increase the GC content of the oligonucleotides

Name	Type	Fluorescent dye	Primer sequence	Allele sizes (bp)
CSGES120	trinucleotide	yellow	F: 5'-* gga gaa gaa gga cga tag gaa g-3' R: 5'-* tct tgg ggg ggt ctc ata tc-3'	118-156
CSGES189	trinucleotide	green	F: 5'-* gga tta ttt ctc gtc cct tca c-3' R: 5'-* cgg cga act tga ctt ttg g-3'	123-157
CSGES047	tetranucleotide	blue	F: 5'-* tca tca gtt tct atc cat cca gcc-3' R: 5'-* ctt atc tga ttc tgt tcc acc g-3'	180-260
CSGES176	trinucleotide	yellow	F: 5'-* att gct ggc ata cgg tca cc-3' R: 5'-* cgt ctt ctc cac aag tgt ttc g-3'	224-272
CSGES268	trinucleotide	green	F: 5'-* tgc caa taa gga gga gaa gg-3' R: 5'-* ctt gaa cgg agc ctt gtt gt-3'	243-295
CSGES190	trinucleotide	blue	F: 5'-* gga gga aga acc gaa cga ag-3' R: 5'-* gga gag tca tta tca tca tcg cag-3'	261-282
Pe1.1	dinucleotide	blue	F: 5'-aaa tgc tct cat gaa taa cc-3' R: 5'- tct tgg acc act tgt aaa cga-3'	321-391
Pmcd01	trinucleotide	green	F: 5'- aga cag tca atc agt cc-3' R: 5'-gcc ata aac tct cta acg-3'	177-213

Coload system 1:



Coload system 2:

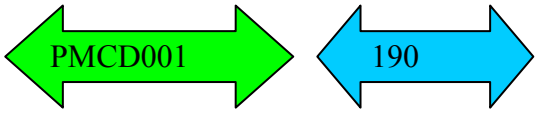


Figure 4.1 Penaeus esculentus microsatellite co-load systems. The colours and gel positions of the eight chosen loci. Left to right: low to high number of base pairs.

4.4 Results and Discussion

4.4.1 Population structure

The eight loci considered suitable for accurate genotyping all showed good fits to Hardy-Weinberg equilibrium (Table 4.2). They had average expected heterozygosities per locus (after pooling the two samples) of between about 0.5 and 0.9, and allele numbers ranging from 8 to 27 (Table 4.4). Estimates of disequilibrium between genotypes at different loci showed no significant evidence for association between loci, and the loci were taken as independent markers. There was no evidence of inter-sample heterogeneity (Table 4.2), despite the samples being collected in different areas and in different years. We therefore have no evidence to reject the null hypothesis that the Exmouth Gulf population of *P. esculentus* consists of a single panmictic population.

Table 4.4 Variability data for the set of 8 Penaeus esculentus microsatellite markers.

Locus	Number screened	Allele number	Expected heterozygosity	Probability of identity
CSGES120	183	11	0.496	0.290
CSGES189	186	11	0.737	0.112
CSGES047	187	19	0.889	0.022
CSGES176	186	15	0.843	0.040
CSGES268	183	16	0.713	0.099
CSGES190	180	8	0.518	0.270
Pe1.1	186	27	0.914	0.013
Pmcd01	185	10	0.828	0.051

4.4.2 Power of microsatellites to discriminate between individuals

Note that the analyses we have completed below assume that the enhanced and wild prawn populations are totally mixed and that the population of mixed prawns has been sampled randomly.

The probability of identity for a single locus (Table 4.4), that is, the probability that two prawns taken randomly from the wild have the same genotype for that locus, was estimated from the allele frequencies in the pooled sample as $I = \Sigma_i p_i^4 + \Sigma_i \Sigma_{j>I} (2p_i p_j)^2$, where p_i and p_j are the frequencies of the ith and jth alleles (Paetkau and Strobeck 1994). For example, the I for locus CSGES190 was 0.270, meaning that the probability that two randomly taken individuals have identical genotypes for this locus was 0.270, or nearly 1 in 4. On the other hand, for locus Pe1.1, with many more alleles and genotypes, I was 0.013, or nearly 1 in 80. The multilocus I value is simply the product of individual locus values, for independent markers. If all 8 loci were typed, the I would be 5.07×10^{-10}, or 1 in 2.0×10^9. There would only be a 1 in 2,000,000,000 chance that two prawns taken at random would share the same 8-locus genotype.

However, we are more interested in the probability that a randomly-chosen male-female pair, or randomly-chosen female, is compatible with being the parent of a recaptured individual. If this P value is high, we can have little confidence that any recaptures whose genotypes are consistent with the hatchery parents are the offspring of these parents. On the other hand, if this P value is low, then we can have increased confidence (dependent on population size) that any recaptures whose genotypes are consistent with the hatchery parents are offspring of these parents.

Mark Bravington (CMIS) kindly came up with analytical solutions to these questions. Note that p_i and p_j are the frequencies of the ith and jth alleles.

If we only know the maternal genotype, then

$$P_m = \Sigma_i p_i^2(1-(1-p_i)^2) + \Sigma_{i<j} 2p_ip_j(1-(1-p_i-p_j)^2)$$

If we know both paternal and maternal genotypes, then

$$P_{mp} = \Sigma_i p_i^2(1-(1-p_i)^2)^2 + \Sigma_{i<j} 2p_ip_j(2(1-(1-p_i)^2)(1-(1-p_j)^2)-(2p_ip_j)^2)$$

These are *P* values for a single locus, and are multiplied across loci to get the overall probability of a spurious match between a random recapture and a random mother or random parental pair. If the released offspring come from more than one mother, say *x* mothers, then the probability that a random offspring will spuriously match any of the *x* mothers is $1-(1-P_m)^x$. For a maternal-paternal pair, $P = 1-(1-P_{mp})^x$

Table 4.5 Chances of any given recapture randomly matching a randomly-chosen mother (Pm) or randomly-chosen maternal-paternal pair (Pmp), given a single hatchery mother or hatchery pair.

Locus	P_m	P_{mp}
CSGES120	0.8653	0.5377
CSGES189	0.6691	0.3093
CSGES047	0.3634	0.0760
CSGES176	0.4663	0.1260
CSGES268	0.6516	0.2388
CSGES190	0.8542	0.5229
Pe1.1	0.2922	0.0469
Pmcd01	0.5115	0.1630
Over all loci	0.0082	1.52×10^{-6}

P_m and P_{mp} values were estimated for each locus and over all loci (Table 4.5). Some features of these values are worth commenting on.

Firstly, the P_m values for individual loci are all quite high, but when multiplied together become a more reasonable 0.0082. There is quite a low probability that an individual chosen at random will be compatible with a mother chosen at random. Secondly, there is a much smaller probability, $P_{mp} = 1.52 \times 10^{-6}$, that an individual chosen at random will be compatible with a mother and father chosen at random. Thirdly, if there are say 10 mothers used to produce progeny in the hatchery, and we only know the maternal genotypes, then P_m increases from 0.0082 for a single mother to 0.079. Fourthly, if we know both maternal and paternal genotypes for the 10 hatchery crosses, then P_{mf} increases, but only to 1.52×10^{-5}. If additional reliable and variable markers were available, then of course these *P* values would be decreased.

However, all these apparently small *P* values must be assessed with respect to the number of likely parental pairs in the wild population. For example, even with the low $P_{mp} = 1.52 \times 10^{-6}$, if there are 10^6 wild parent pairs in the population, there is about a 78% chance that one will be compatible by chance, so finding compatibility wouldn't be a good basis for saying "this one is probably hatchery-raised".

Mark Bravington therefore also investigated how accurately the proportions of hatchery-released and wild-born prawns in a recaptured sample might be determined given genetic information on the hatchery parents and wild population, The proportion of wild-born individuals in the population is given by p_w, with its estimate being $\hat{p}_w$.

Then if n_H = number of hatchery born prawns, and n = number of recaptures,

$$\hat{p}_w = 1 - (n_H/n)$$

However, n_H is not known for certain because of accidental compatibilities with wild parents. This is taken into account in the formulation

$$\hat{p}_w = (n-n_c)/(p_o n)$$

where n_c = number of recaptures that are hatchery-compatible, and $p_o = (1-P)^h$, where P is the probability of accidental compatibility with a wild-parent pair, P_m or P_{mp} from the above depending on whether only maternal or maternal and paternal genotypes are known, and h = number of hatchery pairs.

The variance of our estimate of the proportion of wild-born prawns is:

$\hat{p}_w = p_w(1-p_o p_w)/(np_o)$, with the square root of this term being the standard error.

Table 4.6 gives estimates of the standard error of $\hat{p}_w$ for varying numbers of hatchery mothers, for varying numbers of recaptures, for varying enhancement levels, and for situations where only maternal genotypes are known and where both maternal and paternal genotypes are known. Note that these estimates are based on the allele frequencies at the eight microsatellite loci.

The data in Table 4.6 provide substantial assistance in determining how best to proceed with the genetic tag program. It shows, for example, that with 1,000 recaptures, if both maternal and paternal hatchery genotypes are known, then acceptable estimates of p_w will always be achieved regardless of the number of hatchery pairs used to produce the prawns for enhancement. It also indicates that if only the maternal genotype is known, 8 loci are unlikely to be powerful enough to estimate p_w with reasonable precision. This can only be achieved if the hatchery mothers produce huge numbers of released offspring. For example, if less than ten mothers can produce enough offspring to achieve a 3% stocking rate (p_w = 0.97), then a reasonable estimate of p_w can be derived from 1,000 genotyped recaptures. In many circumstances, such as knowing the genotypes of both parents and with supplementation of 3% or better, just 100 to 200 recaptures will provide sufficient power. Of course, with additional markers, and hence lowered P_m and/or P_{mp} estimates, our ability to determine the effectiveness of the enhancement project will be enhanced. However, the discrimination between enhanced and wild prawns would be more than adequate if we can determine the genotypes of both parents.

The statistical methodology also permits an assessment of certain ‘what-if’ situations. For example, what would be the consequences of adding further microsatellite loci to our current suite of eight – what impact would that have on our ability to assess enhancement success and, in particular, could a maternal-only genotyping system work?

Two ‘what-if’ scenarios were examined. The first included adding a further three microsatellite loci with average discriminatory power, the second included adding a further three loci with high discriminatory power. Thus both scenarios were based on a hypothetical suite of 11 loci.

For the first scenario, locus CSEG189 was used. This has a probability of identity of 0.112 (Table 4.4). Therefore in the statistical tests of 11 loci, this locus was represented four times.

Table 4.6 **Standard errors of the estimated proportions of wild-born prawns ($\hat{p}_w$) after enhancement, with p_w of 0.99, 0.97, 0.90 and 0.80, and where maternal genotypes only, or maternal and paternal genotypes, are known, and for varying numbers of hatchery mothers and recaptures, for the eight microsatellite markers.** (figures in **bold** are estimates of SE of p_w that are reasonably acceptable, i.e. less than about half the difference between p_w and 1)

		p_w = 0.99		p_w= 0.97		p_w = 0.90		p_w = 0.80	
Number of Hatchery mothers	Recaptures	SE of p_w Mother only	Mother Father	SE of p_w Mother only	Mother Father	SE of p_w Mother only	Mother Father	p_w Mother only	Mother Father
1	10	0.043	0.032	0.061	0.054	0.099	0.095	0.129	0.127
1	100	0.013	0.010	0.019	0.017	**0.031**	**0.030**	**0.041**	**0.040**
1	1000	**0.004**	**0.003**	**0.006**	**0.005**	**0.010**	**0.010**	**0.013**	**0.013**
5	10	0.072	0.032	0.084	0.054	0.113	0.095	0.139	0.127
5	100	0.023	0.010	0.026	0.017	**0.036**	**0.030**	**0.044**	**0.040**
5	1000	0.007	**0.003**	**0.008**	**0.005**	**0.011**	**0.010**	**0.014**	**0.013**
10	10	0.097	0.032	0.106	0.054	0.129	0.095	0.151	0.127
10	100	0.031	0.010	0.034	0.017	**0.041**	**0.030**	**0.048**	**0.040**
10	1000	0.010	**0.003**	**0.011**	**0.005**	**0.013**	**0.010**	**0.015**	**0.013**
50	10	0.227	0.032	0.229	0.054	0.234	0.095	0.238	0.127
50	100	0.072	0.010	0.072	0.017	0.074	**0.030**	**0.075**	**0.040**
50	1000	0.023	**0.003**	**0.023**	**0.005**	**0.023**	**0.010**	**0.024**	**0.013**
100	10	0.357	0.032	0.356	0.054	0.352	0.095	0.344	0.127
100	100	0.113	0.010	0.113	0.017	0.111	**0.030**	0.109	**0.040**
100	1000	0.036	**0.003**	0.036	**0.005**	**0.035**	**0.010**	**0.034**	**0.013**
1000	10	19.307	0.034	19.111	0.055	18.409	0.096	17.356	0.127
1000	100	6.105	0.011	6.043	0.018	5.821	**0.030**	5.488	**0.040**
1000	1000	1.931	**0.003**	1.911	**0.006**	1.841	**0.010**	1.736	**0.013**

The results of adding three additional copies of CES189 to our panel of loci are given in Table 4.7. It will be seen that this panel of 11 loci adds rather little additional power to the tests. Five more maternal-only tests become useful, at p_w = 0.99 with 5 hatchery mothers and 1000 recaptures, at p_w = 0.97 with 50 hatchery mothers and 1000 recaptures, at p_w = 0.90 with 50 hatchery mothers and 100, recaptures at p_w = 0.80 with 100 hatchery mothers and 100 recaptures, and at p_w = 0.80 with 1000 hatchery mothers and 1000 recaptures.

For the second scenario, locus Pe1.1 was used. This has a probability of identity of 0.013 (Table 4.4). In the statistical tests of 11 loci, this locus was represented four times. The results of adding three additional copies of Pe1.1 to our panel of loci are given in Table 4.8. Twelve more maternal-only tests become useful. These include, necessarily, the five from adding in CES189, together with seven new tests. Importantly, maternal-only genotyping becomes feasible in realistic situations. For example, with 1000 recaptures (and up to 1000 hatchery mothers), all possible combinations of numbers of hatchery mothers and p_w can be adequately assessed except for 1000 mothers at p_w = 0.99 and p_w = 0.97.

Table 4.7 Effect of adding in three extra copies of microsatellite locus CES189, which has average discriminatory power. Standard errors of the estimated proportions of wild-born prawns ($\hat{p}_w$) after enhancement, with p_w of 0.99, 0.97, 0.90 and 0.80, and where maternal genotypes only, or maternal and paternal genotypes, are known, and for varying numbers of hatchery mothers and recaptures, for the eleven microsatellite markers. (figures in **bold** are estimates of SE of p_w that are reasonably acceptable, i.e. less than about half the difference between p_w and 1)

		p_w = 0.99		p_w= 0.97		p_w = 0.90		p_w = 0.80	
Number of Hatchery mothers	Recaptures	SE of p_w Mother only	Mother Father	SE of p_w Mother only	Mother Father	SE of p_w Mother only	Mother Father	p_w Mother only	Mother Father
1	10	0.035	0.031	0.056	0.054	0.096	0.095	0.127	0.126
1	100	0.011	0.010	0.018	0.017	**0.030**	**0.030**	**0.040**	**0.040**
1	1000	**0.004**	**0.003**	**0.006**	**0.005**	**0.010**	**0.009**	**0.013**	**0.013**
5	10	0.047	0.031	0.064	0.054	0.100	0.095	0.130	0.126
5	100	0.015	0.010	0.020	0.017	**0.032**	**0.030**	**0.041**	**0.040**
5	1000	**0.005**	**0.003**	**0.006**	**0.005**	**0.010**	**0.009**	**0.013**	**0.013**
10	10	0.058	0.031	0.073	0.054	0.106	0.095	0.134	0.126
10	100	0.018	0.010	0.023	0.017	**0.033**	**0.030**	**0.042**	**0.040**
10	1000	0.006	**0.003**	**0.007**	**0.005**	**0.011**	**0.009**	**0.013**	**0.013**
50	10	0.117	0.031	0.124	0.054	0.143	0.095	0.162	0.126
50	100	0.037	0.010	0.039	0.017	**0.045**	**0.030**	**0.051**	**0.040**
50	1000	0.012	**0.003**	**0.012**	**0.005**	**0.014**	**0.009**	**0.016**	**0.013**
100	10	0.167	0.031	0.171	0.054	0.183	0.095	0.194	0.126
100	100	0.053	0.010	0.054	0.017	0.058	**0.030**	**0.061**	**0.040**
100	1000	0.017	**0.003**	0.017	**0.005**	**0.018**	**0.009**	**0.019**	**0.013**
1000	10	0.998	0.032	0.989	0.054	0.956	0.095	0.906	0.127
1000	100	0.316	0.010	0.313	0.017	0.302	**0.030**	0.286	0.040
1000	1000	0.100	**0.003**	0.099	**0.005**	0.096	**0.009**	**0.091**	**0.013**

Thus, if we able to develop three new hypervariable loci, each about as discriminatory as Pe1.1, then the new set of 11 loci would be able to provide the required power for assessing the success of any enhancement program with maternal-only genotyping. Of course, a reduced suite of loci would no doubt also provide adequate power if they all had similar discriminatory powers as Pe1.1. The problem is the time and expense of developing such loci, as it might be necessary to develop and test several dozen new loci before the desired ones are found.

Table 4.8 **Effect of adding in three extra copies of microsatellite locus Pe1.1, which has high discriminatory power. Standard errors of the estimated proportions of wild-born prawns ($\hat{p}_w$) after enhancement, with p_w of 0.99, 0.97, 0.90 and 0.80, and where maternal genotypes only, or maternal and paternal genotypes, are known, and for varying numbers of hatchery mothers and recaptures, for the eleven microsatellite markers. (**figures in **bold** are estimates of SE of p_w that are reasonably acceptable, i.e. less than about half the difference between p_w and 1)

		p_w = 0.99		p_w= 0.97		p_w = 0.90		p_w = 0.80	
Number of Hatchery mothers	Recaptures	SE of p_w Mother only	Mother Father	SE of p_w Mother only	Mother Father	SE of p_w Mother only	Mother Father	p_w Mother only	Mother Father
1	10	0.032	0.031	0.054	0.054	0.095	0.095	0.127	0.126
1	100	0.010	0.010	0.017	0.017	**0.030**	**0.030**	**0.040**	**0.040**
1	1000	**0.003**	**0.003**	**0.005**	**0.005**	0.009	0.009	0.013	0.013
5	10	0.033	0.031	0.055	0.054	0.095	0.095	0.127	0.126
5	100	0.010	0.010	0.017	0.017	**0.030**	**0.030**	**0.040**	**0.040**
5	1000	**0.003**	**0.003**	**0.005**	**0.005**	**0.010**	0.009	0.013	0.013
10	10	0.034	0.031	0.056	0.054	0.096	0.095	0.127	0.126
10	100	0.011	0.010	0.018	0.017	**0.030**	**0.030**	**0.040**	**0.040**
10	1000	**0.003**	**0.003**	**0.006**	**0.005**	**0.010**	0.009	0.013	0.013
50	10	0.045	0.031	0.062	0.054	0.100	0.095	0.130	0.126
50	100	0.014	0.010	0.020	0.017	**0.031**	**0.030**	**0.041**	**0.040**
50	1000	**0.004**	**0.003**	**0.006**	**0.005**	**0.010**	0.009	0.013	0.013
100	10	0.055	0.031	0.070	0.054	0.104	0.095	0.133	0.126
100	100	0.017	0.010	0.022	0.017	**0.033**	**0.030**	**0.042**	**0.040**
100	1000	**0.005**	**0.003**	**0.007**	**0.005**	**0.010**	0.009	0.013	0.013
1000	10	0.151	0.031	0.156	0.054	0.170	0.095	0.184	0.126
1000	100	0.048	0.010	0.049	0.017	0.054	**0.030**	**0.058**	**0.040**
1000	1000	0.015	**0.003**	0.016	**0.005**	**0.017**	0.009	0.018	0.013

4.4.3 Costs of using markers and ways to improve cost-effectiveness of markers

We have estimated that the cost (labour plus consumables, about 50% for each component) for genotyping the eight chosen loci in each individual would be about AUS$45 per adult and AUS$38 per larval offspring. This covers the costs of extracting DNA, amplifying it with the chosen primers, running the products on two gel lanes, and determining genotypes from the results. As six of the markers are resolved on one gel (Fig. 4.1), a two-step strategy to genotyping could be employed, where all individuals are initially genotyped at six loci, at a cost of AUS$30 per adult (AUS$23 per post larva). The second gel, with an additional two markers (CSGES190 and Pmcd01) would only be used for those animals that remain compatible with hatchery genotypes after the first six markers. The second gel would cost an additional AUS$15 per individual.

For the genetic tag to be effective, we have to ascertain whether wild-caught individuals are progeny of hatchery broodstock or progeny of wild spawnings. Our initial expectation was that we would be able to determine the genotype of both the hatchery mother and her mate. However, because the females used for the broodstock have been fertilised in the wild, we planned to genotype the mother and 20-30 of her progeny at the PL15 stage to deduce the father's genotype. This approach however is

both expensive (requiring 20-30 progeny to be typed per broodstock female) and difficult for the hatchery to implement. The hatchery would find it difficult to maintain progeny from different broodstock separately until the PL15 stage. Another option is to use a homogenate of 20-30 early stage larvae from each spawning female. This approach would be much simpler for the hatchery to implement because larvae would not have to be held separately for more than a few days, and requires only a single typing per broodstock female. An initial statistical investigation showed that this approach is likely to be feasible for hypervariable markers, but this needs to be confirmed with both lab-based genotyping experiments and further simulations.

We also looked at the feasibility of relying only on knowledge of the mother's genotype in determining the proportion of wild-caught prawns. With the current set of loci, this does not appear to be possible unless very large numbers of recaptures are genotyped. We need additional hypervariable loci or we need paternal genotyping. Which option is selected depends on the scale of the enhancement.

The 'what-if' scenarios presented in Tables 4.7 and 4.8 show that maternal-only genotyping would be possible if more hypervariable loci were added to the panel of loci. Indeed, if some of the existing less-variable loci were replaced by more-variable loci, then it is likely that a suite of eight loci would still permit maternal-only genotyping. The problem, of course, is the time and expense required to develop the required hypervariable loci. These are essentially unknowns, and would have to be weighed against the pragmatic approach of using the current suite of loci plus the additional expense of larval genotyping to deduce the paternal genotype.

Some of the early simulations of the revised model estimated that about 75 broodstock mothers would be needed for a pilot release of about 1.75 million juvenile prawns, potentially leading to a catch of 10 tonnes, a 3% supplementation (p_W = 0.97). They also estimated that about 750 mothers would be needed for a commercial scale release of about 100 tonnes of catch from 17.5 million juveniles, a 30% supplementation (p_W = 0.70). We have used these numbers here, although it may be that somewhat fewer mothers will be needed - about 50 and 500 respectively for the pilot and commercial scale releases (See Chapter 8). This would have the effect of slightly improving the power of the method for detecting released prawns.

Table 4.9 indicates how well we will be able to estimate the effectiveness of the planned supplementations using the early simulation results. As already indicated, with the current set of eight loci and 3% supplementation, both maternal and paternal genotypes will be required if 1,000 recaptures are genotyped. However, if about 4,000 recaptures are genotyped, then a reasonably precise estimate of the effectiveness of supplementation can be achieved if only the maternal genotype is known. Increasing the recaptured sample size and using maternal-only genotyping is a possible alternative to having to resort to larval typing to identify the father. The difference in total numbers of animals that need to be genotyped may not be very great. With 75 mothers and p_w = 0.97 with a standard error of 0.015, maternal-only genotyping will require about 4075 animals (the 75 mothers plus 4000 recaptures), while maternal plus paternal genotyping will require about 2090 animals (the 75 mothers, 25 offspring per mother, and 140 recaptures – the latter data not in Table 4.9 – more than 140 recaptures will reduce the standard error substantially). Maternal plus paternal genotyping would therefore be the more cost-efficient option. On the other hand, with 750 mothers and p_w = 0.70 with a standard error of 0.15, maternal-only genotyping will require about 15,750 animals (the 750 mothers plus 15,000 recaptures), while maternal plus paternal genotyping will require about 19,520 animals (the 750 mothers, 25 offspring per mother, and only 20 recaptures – the latter data not in Table 4.9 – again, more recaptures will substantially reduce SE). In this circumstance, maternal-only genotyping would be the preferred route. As already indicated, with extra hypervariable loci, maternal-only genotyping becomes increasingly effective and the difference between this approach and maternal and paternal genotyping becomes smaller (Table 4.9). Clearly, the addition of three more hypervariable loci like Pe1.1 makes maternal-only genotyping very feasible; the difficulty is finding those loci.

Table 4.9 **Tests at 3% (pw = 0.97) and 30% (pw = 0.70) supplementation, with 75 and 750 mothers respectively. Standard errors of the estimated proportions of wild-born prawns ($\hat{p}_w$) after enhancement are given, where maternal genotypes only, or maternal and paternal genotypes, are known, and for varying numbers of microsatellite markers.** (figures in **bold** are estimates of SE of p_w that are reasonably acceptable, i.e. less than about half the difference between p_w and 1)

Number of		current 8 loci		8 loci + 3 x CES189		8 loci + 3 x Pe1.1	
Hatchery mothers	Recaptures	Mother only	Mother Father	Mother only	Mother Father	Mother only	Mother Father
Pw = 0.97, 3% suppl.							
75	10	0.293	0.054	0.149	0.054	0.066	0.054
75	100	0.093	0.017	0.047	0.017	0.021	0.017
75	1000	0.029	**0.005**	**0.015**	**0.005**	**0.007**	**0.005**
75	4000	**0.015**	**0.003**	**0.007**	**0.003**	**0.003**	**0.003**
Pw = 0.70, 30% suppl.							
750	10	5.797	**0.145**	0.613	**0.145**	0.180	**0.145**
750	100	1.833	**0.046**	0.194	**0.046**	**0.057**	**0.046**
750	1000	0.580	**0.015**	**0.061**	**0.014**	**0.018**	**0.014**
750	15000	**0.150**	**0.003**	**0.016**	**0.004**	**0.005**	**0.004**

4.4.4 Feasibility of paternal genotyping

We tested whether it was possible to determine the paternal genotype by extracting DNA from the spermatophore left in place after mating. We tested the feasibility of this with a number of freshly-mated *Penaeus japonicus* females. The spermatophore was carefully dissected out of the female opening of a freshly-killed female, disrupted in liquid nitrogen and the DNA extracted. A number of females had lost the spermatophores and in a number of individuals it was very difficult to retrieve the spermatophore without contaminating female tissues. Even though the team used freshly-mated females, with the highest probability of large numbers of sperm still contained in the spermatophore, it proved impossible to obtain DNA suitable as a PCR template from this tissue. This is possibly due to the high proportion of crustacean cuticle in the sample and it may be possible to further optimise the extraction technique to access the sperm DNA. However, we are pessimistic about the prospect of obtaining spermatophores from females at the end of spawning, considering the difficulties that were encountered in reliably retrieving spermatophores from freshly mated females. As one of the objectives of the hatchery program in an enhancement project will be to maximise the number of offspring per female, there will be no opportunity to remove a spermatophore until the female has spawned multiple times.

It therefore seems likely that the genotyping of a sample of offspring will be the only reliable way to derive the paternal genotype. However, the feasibility of this approach must be assessed. However, we have successfully used microsatellites to genotype 9-day old Pacific oyster larvae (*Crassostrea gigas*), which suggests that it could be possible to genotype very young prawn larvae.

4.4.5 Sampling strategies for genetics of wild populations and revised risk assessment of genetic impacts of enhancement in Exmouth Gulf

We assessed the genotypes of prawns caught by two trawlers in different regions of Exmouth Gulf in two consecutive years (1999 and 2000): no detectable differences in genotype frequencies were recorded for the eight reliable and sensitive microsatellite loci (Table 4.2). We therefore cannot the reject the null hypothesis of a single panmictic population in the Gulf. However, the sampling strategies for determining the success or otherwise of a release program would have to be carefully considered. For example, if all released animals are released in one area, then any subsequent sampling of that area would be expected to yield more released prawns than sampling a distant area. The amount of dispersal and homogenisation across the Gulf would be expected to depend on the time since release, substrate, and weather conditions. Release and recapture strategies clearly need to be carefully thought through prior to any releases.

The genetic impacts of any releases will be minimal. Most gene tag assessments in the past have involved the release of large numbers of offspring from parents that have been selected to carry an otherwise rare gene. Such selection programs have frequently involved one or more generations of captive breeding and the release of large numbers of otherwise extremely rare homozygotes. This can have an effect on the genetic structure of the population by changing genotype frequencies. The release strategy we are planning involves taking already-mated females from the wild and releasing their progeny back into the wild. It is only possible because the hypervariable nature of microsatellites means that we can still detect released offspring without making them in any way genetically unusual – these sorts of approaches were not available in the earlier days of gene tagging. The hatchery-rearing and juvenile production of prawns to the stage when they can be released does expose them to an unnatural environment for about seven to nine weeks (Chapter 3). It is possible that natural selection during this time could enable the survival (or death) of genotypes that might otherwise not have survived (or died). Further, each broodstock female will clearly be more represented in the wild population in the release generation than she would otherwise be, or the program would not work. This is achieved by reducing mortality levels in the juvenile stages in the hatchery compared with the wild population.

4.5 General Discussion

Most monitored enhancement programs appear to rely on physical/chemical tags (such as coded wire tags or tetracycline-induced marking of hard parts) rather than genetic tags. A literature search found few clear examples of the use of genetic approaches for monitoring the success of enhancement programs, although several are being developed. The success of a restocking program for red abalone (*Haliotis rufescens*) in a previously depleted area has been monitored by allozyme and mtDNA analyses (Gaffney et al. 1996, Burton and Tegner 2000). In earlier fish stocking programs, selected fish with genotypes rare in the stocked population have been used as broodstock and ensuing allele frequency changes monitored (e.g. Crozier and Moffett 1995, Kristiansen et al. 1997); in the prawn project, we wished to avoid selection of broodstock in order to minimise any genetic effects from stocking.

Genetic monitoring of the success of an enhancement program is not straight-forward, and in prawns it is more difficult than for most other species. Usually, it is possible to carry out controlled matings in a hatchery, mating known females with known males. For example, male and female finfish can be stripped of sperm and eggs, which can be fertilised in beakers. Therefore both parents can be easily genotyped. If this were possible with prawns, we would have no problems applying the suite of loci we developed and using these loci to identify enhanced from wild prawns. However, since we cannot directly genotype the male parent, we either have to work without the paternal genotype (by developing further loci or by having very large numbers of recaptures genotyped), or deduce it by larval typing.

Are there any alternatives? Since paternal genotype cannot be identified directly, would it make more sense to be working with mitochondrial DNA variation? Mitochondrial DNA is inherited directly from mothers to all offspring. The problem with this approach is that we do not know how variable the mtDNA genome is in *Penaeus esculentus*. If we found a hypervariable region of mtDNA, it might be possible to genotype (for example) 1,000 female prawns, select 50 with rare genotypes, and use these 50 as mothers in the hatchery. However, this approach does not use randomly chosen females as parents, and would change mtDNA gene frequencies in the enhanced population. Whether this would matter or not is debatable; nuclear DNA gene frequencies probably would not change as nuclear genes are expected to be randomly associated with mtDNA. In the fish red drum (*Sciaenops ocellatus*), mtDNA has been developed as a tag for monitoring enhancement (Bert et al. 2001), but it is not yet clear how effective it will be. Bert et al. (2001) state that the hatchery genotypes are "naturally very rare in red drum, and (hatchery) offspring can be distinguished with confidence from wild red drum without the need for selective breeding"; it is possible the authors have not considered that even very rare mtDNA genotypes might be present in many individuals in a large wild population. The data simply do not exist to know whether a mtDNA approach would have been a feasible alternative in prawns to the use of microsatellite markers.

The microsatellite approach adopted here has not yet been fully deployed in any other enhancement program that we are aware of, with the possible exception of red sea bream in Japan. However, microsatellite-based methods are certainly being developed for other species. This probably reflects the novelty of microsatellites as a marker class coupled with the general rarity of genetically-monitored enhancement programs. A microsatellite approach is being developed for red sea bream (*Pagrus major*) enhancement off Kochi, Japan (Perez-Enriquez and Taniguchi 1999; Perez-Enriquez et al. 1999; Perez-Enriquez et al. 2001; Doyle et al. 2001). However, a first trial using microsatellitec tags and parentage tests indicated that no released fish were found among the recaptured wild animals (Perez-Enriquez and Taniguchi 1999). Possible explanations were a low mixing rate of released and wild fish combined with a probably high mortality of fish after release. The potential of microsatellites for genetic monitoring of Atlantic salmon enhancement programs has also been flagged (Martinez et al. 2000).

In conclusion, we are seeking to deploy innovative genetic approaches to monitoring enhancement in what must be considered a 'difficult' species to so monitor. We have developed a suite of eight reliable microsatellite together with a robust statistical approach that permits an assessment of the power of such loci for monitoring enhancement success. The panel of microsatellite loci already developed is certainly adequate if paternal genotypes can be assessed, or if very large numbers of recaptured animals are genotyped; if neither of these is feasible, attention must be given to developing further hypervariable loci.

Acknowledgements

We thank Jennifer Meadows for her expert laboratory work and Richard McCulloch and MG Kailis for obtaining the wild caught prawns.

4.6 References

Bert TM, Tringali MD, Seyoum S (2001) The uses of a genetic tag in a stock enhancement experiment. World Aquaculture Society 2001, Abstracts, p. 59.

Burton RS, Tegner MJ (2000) Enhancement of red abalone *Haliotis rufescens* stocks at San Miguel Island: reassessing a success story. *Marine Ecology Progress Series*, 202: 303-308.

Crozier WW, Moffett IJJ (1995) Application of low frequency genetic marking at GPI-3* and MDH-B1,2* loci to assess supplementary stocking of Atlantic salmon, *Salmo salar* L., in a Northern Irish stream. *Fish Management and Ecology*, 2: 27-36.

Doyle RW, Perez-Enriquez R, Takagi M, Taniguchi N (2001) Selective recovery of founder genetic diversity in aquacultural broodstocks and captive, endangered fish populations. *Genetica* 111: 291-304.

Gaffney PM, Rubin VP, Hedgecock D, Powers DA, Morris G, Hereford L (1996) Genetic effects of artificial propagation – signals from wild and hatchery populations of red abalone in California. *Aquaculture* 143: 257-266.

Kristiansen TS, Jorstad KE, Ottera H, Paulsen OI, Svasand T. (1997) Estimates of larval survival of cod by releases of genetically marked yolk-sac larvae. *Journal of Fish Biology* 51 (Suppl. A): 264-283.

Martinez JL, Gephard S, Juanes F, Garcia-Vasquez, E. (2000) The use of microsatellite DNA loci for genetic monitoring of Atlantic salmon populations. *North American Journal of Aquaculture* 62: 285-289.

Meadows JRS, Ward RD, Grewe PM, Dierens L, Lehnert, SA (2002). Characterisation of 23 tri- and tetranucleotide microsatellite loci in the brown tiger prawn, *Penaeus esculentus. Molecular Ecology Notes*, in press.

Moore SS, Whan V, Davis GP, Byrne K, Hetzel DJS, Preston N (1999). The development and application of genetic markers for the Kuruma prawn *Penaeus japonicus*. *Aquaculture* **173**: 19-32.

O'Connell M, Wright JM (1997). Microsatellite DNA in fishes. *Reviews in Fish Biology and Fisheries* **7**: 331-363.

Paetkau D, Strobeck C (1994). Microsatellite analysis of genetic variation in brown bear populations. *Molecular Ecology* **3**: 489-495.

Perez-Enriquez R, Taniguchi N (1999) Use of microsatellite DNA as genetic tags for the assessment of a stock enhancement program of red sea bream. *Fisheries Science* 65: 374-379.

Perez-Enriquez R, Takagi M, Taniguchi N (1999) Genetic variability and pedigree tracing of a hatchery-reared stock of red sea bream (Pagrus major) used for stock enhancement, based on microsatellite markers. *Aquaculture* 173: 413-423.

Perez-Enriquez R, Takemura M, Tabata K, Taniguchi N (2001) Genetic diversity of red sea bream *Pagrus major* in western Japan in relation to stock enhancement. *Fisheries Science* 67: 71-78.

Preston N (1997). *The development of biological tagging techniques for penaeid prawns* Final Report for FRDC project 93/093

Raymond M, Rousset F (1995). GENEPOP (v. 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity* **86**: 248-249.

Smith J, Carpten J, Brownstien M, Ghosh S, Magnuson V, Gilbert D, Trent J, Collins F (1995). An approach to genotyping errors caused by non-templated nucleotide addition by *Taq* DNA polymerase. *Genome Research* **5**:312-317.

Xu Z, Dhar AK, Wyrzykowski J, Alcivar-Warren A. (1999). Identification of abundant and informative microsatellites from shrimp (*Penaeus monodon*) genome. *Animal Genetics* **30**:150-156.

4.7 Appendices

APPENDIX 4A. CHARACTERISATION OF 23 TRI- AND TETRANUCLEOTIDE MICROSATELLITE LOCI IN THE BROWN TIGER PRAWN, *PENAEUS ESCULENTUS*

J. R. S. Meadows[a], R. D. Ward[b], P.M. Grewe[b], L. M. Dierens[a] and S. A. Lehnert[a]
In press in Molecular Ecology Notes

[a]*CSIRO Livestock Industries, Molecular Animal Genetic Centre, Level 3 Gehrmann Laboratories, University of Queensland 4072, Queensland, Australia*
[b]*CSIRO Marine Research, GPO Box 1538 Hobart 7001, Tasmania, Australia*

Keywords: Microsatellites, Genetic markers, Shrimp, Penaeidae,
Correspondence: J. R. S. Meadows, CSIRO Livestock Industries, Level 3, Gehrmann Laboratories, University of Queensland, St Lucia 4072, Australia; fax + 61 7 3214 2480; Jennifer.Meadows@csiro.au
Running Title: Penaeus esculentus microsatellites

Abstract
Twenty-three unique tri and tetra-nucleotide microsatellites were isolated and characterized from the genome of the Brown Tiger Prawn *Penaeus esculentus*. Nine of the 23 microsatellite loci were found to be polymorphic and were tested across a sample of wild caught *Penaeus esculentus*. Good fits to Hardy-Weinberg expectations were observed for six of the markers when tested in a population of at least 180 individuals. The total number of alleles for these six markers ranged from 8 to 19, with an average total of 13. These values will make them useful for future stock identification and population genetics studies.

PRIMER NOTE:
The brown tiger prawn (*Penaeus esculentus*) is endemic to the marine waters of Australia and can be consistently found above the 29^{o}S line of latitude (Grey *et al*. 1983). A well managed brown tiger prawn fishery exists in the Exmouth Gulf (Western Australia), however it is nevertheless subject to high annual catch variation, ranging from 205-682 tonnes/annum since 1987 (Sporer and Kangas, 2000). This area could commercially benefit from the supplementation of juvenile prawns to its wild nurseries in years where a below standard catch is expected. It is therefore an ideal system in which to investigate the feasibility of *P. esculentus* stock enhancement.

In order to understand the impact such a venture might have on the existing ecosystem and stocks, and to assist with the continued sustainability of the fishery, molecular genetic tools for population studies and stock identification had to be developed for this species. Previous aquaculture studies have shown that microsatellites (highly variable genetic markers) can be very informative when investigating fishery stock structures (O'Connell and Wright, 1997). Microsatellite markers are not generally useful between penaeid species (Moore *et al.* 1999), and as such it was necessary to isolate microsatellites specific for *Penaeus esculentus*. We report the development of 23 novel microsatellite markers, nine of which have been tested on wild prawn samples in the Exmouth Gulf region.

Four *P. esculentus* genomic DNA libraries, enriched for four tri- and tetranucleotide motifs were produced using a magnetic bead capture method by Genetic Identification Services (Chatsworth, California, USA) using DNA extracted from 1.5g of tail muscle (Moore *et al*. 1999). Biotin-AAG(12), Biotin-AAT(15), Biotin-ATG(12) and Biotin-TAGA(8) were the capture molecules used to construct the libraries in a variation of the method described by Peacock *et al*. 2002.

The inserts of positive clones from the enriched libraries were sized using PCR and M13-cloning site primers to ensure they fell within a 350-700 bp size range. Sequencing using ABI Prism™ Big Dye Terminator Mix (Applied Biosystems, Scoresby, Victoria, Australia) was performed by the Australian Genome Research Facility (Brisbane, Queensland, Australia). Twenty-three unique microsatellites were identified (Table 1) and Mac Vector™ 7.0 software was used to design primers to the flanking sequence. These oligonucleotides were submitted to Geneworks (Adelaide, South Australia, Australia) for synthesis. The forward primer of each pair was fluorescently labelled.

Table 1. Primer sequences, repeat type and GenBank accession numbers for the isolated *Penaeus esculentus* microsatellites.

Locus	Primer Sequence	Repeat Type	Optimal Annealing Temperature (°C)	GenBank Accession #
CSGES045	F' 5'- gag cgt tac tgg aaa gtg tcc-3'	$(TAGA)_9TA(TAGA)$	50	AF430380
	R' 5'- tgc ctg aag ttg aaa agt gc-3'			
CSGES047	F' 5'- tca tca gtt tct atc cat cca gcc-3'	$(CTAT)_{11}$	54	AF430381
	R' 5'- ctt atc tga ttc tgt tcc acc g-3'			
CSGES027	F' 5'- gac gac aac ttg atg aaa cgg-3'	$(TCT)(TTT)(TCT)_2(TTT)(TCT)_7$	50	AF430378
	R' 5'- gaa tcg ggt aga aca aat ctg c-3'			
CSGES043	F' 5'- cca gga gtt gta ttg aag gga g-3'	$(TTC)_{13}$	52	AF430379
	R' 5'- cca gat tat cgt gac cgt gag-3'			
CSGES003	F' 5'- tta tct ttt gag ggg gtt gc-3'	$(TATC)_8$	52	AF430398
	R' 5'- gtt gat tag gaa ggg cat cc-3'			
CSGES015	F' 5'- cgc tca cca aga aaa cta atg g-3'	$(ATG)_{15}$	53	AF430377
	R' 5'- cgc tga gac aat gaa cac ttc g-3'			
CSGES120	F' 5'- gga gaa gaa gga cga tag gaa g-3'	$(TGA)_7(TGG)(TGA)_7$	51	AF430385
	R' 5'- tct tgg ggg ggt ctc ata tc-3'			
CSGES132	F' 5'- gat ggt cgt aat agt ggt gag g-3'	$(TGA)_6$	51	AF430386
	R' 5'- gtc aac atc gtc ctc tcc aac-3'			
CSGES110	F' 5'- gcg ttc act ttg cct atg ttc-3'	$(CTT)_9$	52	AF430384
	R' 5'- cga gcc tca aac aca cca ag-3'			
CSGES090	F' 5'- ccg tgg aac aaa atc gca g-3'	$(GAA)_{14}$	52	AF430383
	R' 5'- agg gtg tga tgt gcc gtt tc-3'			
CSGES078	F' 5'- tgt aga cat aga cgg cag tgg-3'	$(GAA)_9$	52	AF430382
	R' 5'- ggt ggc ttc ctg gat aag tc-3'			
CSGES215	F' 5'- agg ggt ttc ctg cat tac c-3'	$(ATC)_8$	51	AF430391
	R' 5'- aac gag att cca agg tgg g-3'			
CSGES217	F' 5'- cac caa tca cca tca tct tca c-3'	$(TCA)_6(ACT)_6$	51	AF430392
	R' 5'- aag gac atc gtt caa ggg c-3'			
CSGES218	F' 5'- gga gtg cgt cgt att gag aag-3'	$(CAT)_9$	48	AF430393
	R' 5'- ctg atg gtg ata aag gtg aaa gtg-3'			
CSGES189	F' 5'- gga tta ttt ctc gtc cct tca c-3'	$(TTC)_{10}$	50	AF430389
	R' 5'- cgg cga act tga ctt ttg g-3'			
CSGES190	F' 5'- gga gga aga acc gaa cga ag-3'	$(TGA)_{11}$	54	AF430390
	R' 5'- gga gag tca tta tca tca tcg cag-3'			
CSGES180	F' 5'- cgt cag tca gac agc att gg-3'	$(TCT)_7$	50	AF430388
	F' 5'- ctt gat ttc ttg cca cta cag g-3'			
CSGES176	F' 5'- att gct ggc ata cgg tca cc-3'	$(GAG)_9(AAG)_{13}(GAG)_4$	56	AF430387
	F' 5'- cgt ctt ctc cac aag tgt ttc g-3'			
CSGES257	F' 5'- cca aac agc agc aaa caa aca cc-3'	$(CTT)_3(CAT)_2(CTT)_{18}$	54	AF430395
	F' 5'- gga ttg aac gca ggt cag caa g-3'			
CSGES269	F' 5'- aag aag agc aag gca agg aga tg-3'	$(ATG)_{20}$	55	AF430399
	F' 5'- cca tta tca gca gca gca gta gc-3'			

CSGES268	F' 5'- tgc caa taa gga gga gaa gg-3'	$(ATG)_9(GTG)(ATG)_5$	50	AF430396
	F' 5'- ctt gaa cgg agc ctt gtt gt-3'			
CSGES256	F' 5'- tga gct gcc gtc att aga caa-3'	$(CTT)_{17}$	55	AF430394
	F' 5'- cct tcc cct tcg tca ttt tc-3'			
CSGES288	F' 5'- ctc ccc ttc gtt gtt cca ctt ac-3'	$(GTAT)_{18}(GAA)_2(GATA)_3$	51	AF430397
	F' 5'- ttg att ggg ttt gcc ttg act g-3'			

Total genomic DNA for microsatellite analysis was extracted from 40mg of prawn pleopod tissue, (shattered after freezing in liquid nitrogen) using a modified DNeasy™ 96 Tissue Kit (QIAGEN®) protocol, in which a shortened 2 hour incubation for proteinase K digestion was used. Microsatellite amplifications were performed in 96-well plates using an MJ Research PTC-100 thermocycler. 20µL reactions contained 67 mM Tris-HCl (pH 8.8); 16.6 mM $(NH_4)_2SO_4$; 0.45% Triton X-100; 0.2 mg/mL gelatin; 3mM $MgCl_2$; 0.2mM forward primer (labelled); 0.2mM reverse primer; 0.4 units *Tth* Plus DNA polymerase (Fisher Biotech, Subiaco, Western Australia, Australia); 125µM dNTP (Pharmacia Biotech) and approximately 50ng genomic DNA template. The DNA template and enzyme were denatured at 94^{o}C for 3 min, followed by 30 cycles consisting of 94 ^{o}C for 30 sec, annealing temperature (See Table 1) for 2 min and 72 ^{o}C for 1 min. A final extension at 72 ^{o}C for 30 min was used to ensure complete addition of adenine to the PCR product, essential for consistent allele calling during genotyping (Smith *et al*. 1995).

The PCR products were diluted, dried down and combined with sucrose-urea load dye and Genescan™-500 Tamra™ size standard (Applied Biosystems), before being denatured and visualised on a 6% denaturing polyacrylamide gel. Results were collected using an ABI 377 Prism DNA autosequencer and analysed using GeneScan® 3.1 and Genotyper® 2.5 software.

The 23 microsatellite loci were tested across a sample of 36 wild-caught individuals from the Exmouth Gulf. In these tests, 14 markers had to be discarded due to: 1) failure to amplify (CSGES015, CSGES132, CSGES180, CSGES269 and CSGES256), 2) monomorphism (CSGES027, CSGES110, CSGES215), 3) non-specific amplification (CSGES003), 4) a stuttery profile (CSGES043), and 5) one base pair allelic shifts which compromised accurate genotyping (CSGES045, CSGES078, CSGES257 and CSGES288).

Nine of the 23 microsatellite loci were used in a larger study, comprising two groups of 96 animals harvested from the Exmouth Gulf population in consecutive years (Table 2). The software package GENEPOP® 3.2 (Raymond and Roussett, 1995) was used for statistical analyses.

Table 2. **Summary of variability for the nine microsatellite loci in two samples from the Exmouth Gulf population of *Penaeus esculentus*.**

Total n = total number of individuals, Total alleles = total number of alleles, GM2000 = November 2000 sample from vessel George Michael, TUB1999 = November 1999 sample from trawler Tubridgi, Hob = observed heterozygosity, Hexp = Hardy-Weinberg expected heterozygosity, F_{ST} = amount of genetic differentiation between the two catches, **0.01>*P*>0.001, ***P<0.001, all other P>0.05

	Total	Total	GM2000		Tub1999		
Locus	n	alleles	Hob	Hexp	Hob	Hexp	F_{ST}
CSGES120	183	11	0.500	0.524	0.396	0.472	-0.0030
CSGES189	186	11	0.800	0.769	0.681	0.705	0.0052
CSGES047	187	19	0.865	0.883	0.846	0.902	-0.0016
CSGES217	187	74	0.833	0.966***	0.868	0.963***	-0.0018
CSGES090	182	49	0.630	0.949***	0.622	0.954***	0.0002**
CSGES218	92	10	0.500	0.811***	-	-	-
CSGES176	186	15	0.833	0.849	0.800	0.845	-0.0040
CSGES268	183	16	0.719	0.708	0.644	0.723	-0.0025
CSGES190	180	8	0.495	0.489	0.494	0.549	0.0030

CSGES217 and CSGES090 were hyper-allelic, with apparent one base pair alleles, and were difficult to score consistently. They showed significant heterozygote deficiencies, indicating the possible presence of null alleles. CSGES218 also showed a significant heterozygote deficiency in the GM2000 sample; and could not be reliably scored in the TUB1999 sample. The remaining six loci showed good fits to Hardy-Weinberg expectations. No evidence of significant sample differentiation could be found between the two catches, an expected result since both prawn groups were harvested from the same putative population.

ACKNOWLEDGEMENTS

The research was supported by the Fisheries Research and Development Corporation (project 1999/222) and by the MG Kailis Group.

References:

Grey DL, Dall W, Baker AA (1983) A guide to the Australian penaeid prawns. NT Government Printing Office, Darwin, Australia. pp. 66-67.

Moore SS, Whan V, Davis GP, Byrne K, Hetzel DJS, Preston N (1999) The development and application of genetic markers for the Kuruma prawn Penaeus japonicus. *Aquaculture*, **173**: 19-32.

O'Connell M & Wright JM (1997) Microsatellite DNA in fishes. *Reviews in Fish Biology and Fisheries*, **7**: 331-363.

Peacock MM, Kirchoff VS, Merideth SJ (2002) Identification and characterisation of nine polymorphic microsatellite loci in the North American pika, Ochotona princeps. *Molecular Ecology Notes* 2: 360-362.

Raymond M, Rousset F (1995) GENEPOP (v. 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity* **86**: 248-249.

Smith J, Carpten J, Brownstien M, Ghosh S, Magnuson V, Gilbert D, Trent J, Collins F (1995) An approach to genotyping errors caused by non-templated nucleotide addition by *Taq* DNA polymerase. *Genome Research*, **5**: 312-317.

Sporer E and Kangas M (2000) Exmouth Gulf Prawn Managed Fishery Status report. *In* State of the Fisheries Report 1999/2000, JW Penn (Ed.) Fisheries WA., pp. 35-38.

APPENDIX 4B. Microsatellite allele frequencies at 11 loci, sample sizes (numbers of genes), numbers of alleles, observed and expected heterozygosity, and *P* of fit to Hardy-Weinberg equilibrium.

Allele	GM 2000	TUB 1999
CSGES 120		
118	0.005	-
121	0.005	0.016
127	-	0.005
130	0.179	0.181
133	0.033	0.016
136	0.005	0.011
139	0.663	0.703
144	0.076	0.049
147	0.016	0.016
150	0.005	-
156	0.011	-
N	184	182
n. alleles	10	8
Obs. Het.	0.500	0.396
Exp. Het.	0.524	0.472
P (HW)	0.518	0.131
CSGES 189		
123	-	0.005
126	0.026	0.016
129	0.021	0.005
132	0.263	0.297
135	0.321	0.418
138	0.111	0.055
141	0.232	0.181
144	0.021	0.016
150	0.005	-
156	-	0.005
N	190	182
n. alleles	9	9
Obs. Het.	0.800	0.681
Exp. Het.	0.769	0.705
P (HW)	0.084	0.879
CSGES 047		
180	-	0.005
184	0.010	0.005
188	0.021	0.016
192	0.057	0.099
196	0.026	0.027
200	0.021	0.038
204	0.182	0.159
208	0.198	0.154
212	0.089	0.077
216	0.063	0.082
220	0.068	0.082
224	0.156	0.132
228	0.057	0.049
232	0.016	0.044
236	0.010	0.005
240	-	0.011
244	0.010	0.005
252	0.010	0.005
260	0.005	-
N	192	182
n. alleles	17	18
Obs. Het.	0.865	0.846
Exp. Het.	0.883	0.902
P (HW)	0.490	0.334
CSGES 217		
187	0.016	0.011
193	0.026	0.022
196	0.005	-
198	0.016	0.011
200	0.005	-
202	-	0.022
203	0.130	0.126
204	0.010	0.005
205	0.016	0.005
206	0.047	0.066
207	-	0.005
209	0.010	0.011
210	0.063	0.071
211	-	0.016
212	0.005	0.038
213	0.042	0.060
215	0.021	0.016
216	0.047	0.044
217	0.005	0.005
218	0.026	0.011
219	0.005	0.038
220	0.010	0.011
221	0.005	0.005
222	-	0.005
223	-	0.005
224	0.010	0.016
226	0.031	-
227	0.010	0.011
228	0.031	0.022
229	0.010	0.027
230	0.005	-
231	0.005	0.011
232	0.021	0.011
233	0.005	0.011
234	0.010	0.005
235	0.026	0.011
236	0.021	0.011
237	0.010	0.005
238	0.010	0.011
239	0.021	0.016
240	0.005	-
241	-	0.005
242	0.021	0.011
244	0.005	-
245	0.021	0.016
246	0.016	0.016
247	0.010	0.011
248	0.031	0.022
249	0.010	-
250	0.005	0.005
251	0.021	0.005
252	-	0.005
253	-	0.022
254	0.021	0.016
255	0.005	0.016
256	0.010	0.005
257	-	0.005
260	0.005	0.005
261	0.005	-
262	-	0.005
264	0.005	0.005
266	0.005	-
267	0.005	0.005
269	0.005	-
270	0.005	-
271	-	0.005
272	0.005	-
273	-	0.005
276	0.005	0.005
278	0.005	0.005
284	0.005	-
292	0.010	-
301	-	0.005
327	0.010	-
N	192	182
n. alleles	61	59
Obs. Het.	0.833	0.868
Exp. Het.	0.966	0.964
P (HW)	<0.001	<0.001

CSGES 090

238	-	0.006
239	0.016	0.011
243	-	0.033
244	0.005	-
245	-	0.028
246	0.027	-
247	-	0.006
248	0.022	0.033
249	0.027	0.006
250	0.022	0.017
251	0.022	0.033
252	0.087	0.106
253	0.005	0.006
254	0.130	0.122
255	0.033	0.011
256	0.027	0.044
257	0.033	0.017
258	0.033	0.050
259	0.011	0.033
260	0.082	0.078
261	0.027	0.028
262	0.038	0.039
263	0.011	0.011
264	0.005	0.011
265	-	0.028
266	-	0.006
271	0.005	0.006
272	-	0.011
273	0.038	0.022
274	0.005	0.006
275	0.022	0.022
276	0.011	0.006
277	0.011	0.006
278	0.033	0.050
279	-	0.011
280	0.011	-
281	0.098	0.022
282	-	0.006
283	-	0.017
284	0.033	0.017
285	0.005	0.006
286	0.011	-
287	0.027	0.017
288	0.005	-
291	0.005	-
293	0.011	-
295	-	0.006
298	-	0.011
302	0.005	-
N	184	180
n. alleles	37	41
Obs. Het.	0.630	0.622
Exp. Het.	0.949	0.954
P (HW)	<0.001	<0.001

Pe1.1

321	-	0.016
323	-	0.005
333	0.005	-
335	0.223	0.125
337	0.096	0.054
339	0.053	0.076
341	0.085	0.103
343	0.053	0.043
345	0.048	0.027
347	0.021	0.027
349	0.112	0.179
351	0.043	0.054
353	0.043	0.011
355	0.021	0.011
357	0.005	0.005
359	0.037	0.038
361	0.043	0.043
363	0.043	0.054
365	0.027	0.049
367	0.016	0.049
369	0.005	0.005
371	0.011	-
375	-	0.011
377	-	0.005
381	-	0.005
385	0.005	-
391	0.005	-
N	188	184
n. alleles	22	23
Obs. Het.	0.936	0.859
Exp. Het.	0.907	0.920
P (HW)	0.710	0.013

Pmcd01

177	-	0.006
186	0.036	0.034
189	0.083	0.107
192	0.109	0.084
195	0.141	0.174
198	0.229	0.225
201	0.266	0.247
204	0.104	0.090
207	0.026	0.034
213	0.005	-
N	192	178
n. alleles	9	9
Obs. Het.	0.740	0.798
Exp. Het.	0.830	0.834
P (HW)	0.184	0.708

CSGES 218

173	0.022	-
176	0.370	0.103
179	0.087	0.103
182	0.125	0.071
185	0.120	0.198
188	0.087	0.111
191	0.071	0.135
194	0.065	0.175
197	-	0.016
200	0.049	0.063
203	0.005	0.008
209	-	0.016
N	184	126
n. alleles	10	11
Obs. Het.	0.500	0.238
Exp. Het.	0.811	0.876
P (HW)	<0.001	<0.001

CSGES 176

223	0.068	0.061
226	0.052	0.078
229	0.125	0.117
232	0.302	0.311
235	0.115	0.122
238	0.104	0.117
241	0.109	0.072
244	0.068	0.067
247	0.026	0.033
250	0.010	0.006
256	0.005	-
259	0.005	0.006
262	0.005	0.006
268	0.005	-
272	-	0.006
N	192	180
n. alleles	14	13
Obs. Het.	0.833	0.800
Exp. het.	0.849	0.845
P (HW)	0.901	0.138

CSGES 268

243	0.016	0.006
249	0.120	0.086
255	0.005	-
258	0.010	-
261	0.042	0.034
264	0.021	0.017
267	0.047	0.040

270	0.010	0.046
273	0.063	0.063
276	0.073	0.109
279	0.516	0.500
282	0.005	0.011
285	0.026	0.034
288	0.026	0.017
291	0.021	0.029
295	-	0.006
N	192	174
n. alleles	15	14
Obs. het.	0.719	0.644
Exp. het.	0.708	0.723
P (HW)	0.357	0.204

CSGES 190

261	-	0.024
264	0.005	-
267	0.026	0.024
270	-	0.006
273	0.684	0.641
276	0.068	0.135
279	0.016	0.018
282	0.200	0.153
N	190	170
n. alleles	6	7
Obs. het.	0.495	0.494
Exp. het.	0.489	0.549
P (HW)	0.745	0.181

APPENDIX 4C DRAFT ABSTRACT FOR MANUSCRIPT TO BE SUBMITTED TO MOLECULAR ECOLOGY: MICROSATELLITE DNA MARKERS: EVALUATING THEIR POTENTIAL FOR ESTIMATING PROPORTIONS OF WILD-BORN AND HATCHERY-REARED OFFSPRING IN AN ENHANCEMENT PROGRAM.

Authors: Bravington[1], M.V and Ward[2], R.D.

CSIRO Mathematics and Information Science[1] and CSIRO Marine Research[2], GPO Box 1538, Hobart, Tasmania 7001, Australia.

Abstract

We describe a statistical method for estimating the effectiveness of a stock enhancement program using nuclear DNA loci. It is based on knowing the population allele frequencies and the genotypes of the hatchery parents (mother only, or mother and father), and determining the probability that a wild-born animal will by chance be compatible with a hatchery origin. It allows the proportion of released animals, and its standard error, in the wild population to be estimated. The method is applied to a dataset of eight microsatellite loci in brown tiger prawns (*Penaeus esculentus*), prior to the start of a possible enhancement program. It is concluded that, for this particular dataset, the effectiveness of such an enhancement program could be quantified accurately if both maternal and paternal genotypes are known, but not if maternal genotypes only are known. However, the latter would be feasible if three further microsatellite loci were added to the existing eight locus panel. The methods detailed should be of interest to any enhancement project that relies on nuclear DNA markers to provide tags.

CHAPTER 5

BENTHIC HABITATS IN EXMOUTH GULF

Rob Kenyon
Mick Haywood
Neil Loneragan
Fiona Manson
Peter Toscas

Summary

In March 1999, Cyclone Vance devastated the benthic habitats of Exmouth Gulf. We began studying the Exmouth Gulf seagrass community in June 1999, after severe cyclonic disturbance, with no direct pre-1999 knowledge of Exmouth seagrasses.

A survey of the whole of Exmouth Gulf in June 1999, showed that the dominant benthic habitat was bare substrate; sand, silt, coral rubble or hard-pavement. The mean seagrass cover was only 0.15% and seventy percent of the 119 sites were devoid of seagrass. *Halodule uninervis* and *Cymodocea serrulata* were found as remnant shoots or seedlings. Following this survey and from the results of previous surveys (McCook et al. 1995), most seagrass was found along the eastern side of Exmouth Gulf. Thus, further sampling (qualitative estimates of seagrass cover and quantitative samples) was concentrated along the southern and eastern sides of Exmouth Gulf. By November 1999, the seagrass cover remained sparse (1.2%) and none of the 137 sites had > 30% cover. The dominant seagrasses were *Halodule uninervis* and *Halophila ovalis*; recolonising seagrasses. By November 2000, seagrass cover had increased to 10.3%, though in some areas 14% of sites had > 60% cover. *Halodule uninervis* and *Halophila ovalis* remained the dominant seagrasses. *Halophila spinulosa* was common at sites > 4-5 m depth and *Halophila decipiens* was also recorded.

The areal extent and species richness of the seagrass community in Exmouth Gulf increased greatly between 2000 and 2001. The average seagrass cover increased to 41.9%. In the east, a diverse seagrass community dominated the benthos. Nineteen percent of sites had > 80% cover. *Halophila spinulosa* (about 80% of biomass) *Cymodocea serrulata* and *Syringodium isoetifolium* dominated the seagrasses, in addition to those species common in 1999.

In Exmouth Gulf, seagrass habitats were most abundant in the eastern and south-eastern sections of the gulf; south of Whalebone Island and in Gales Bay. Disregarding the effects of cyclonic disturbance, high-biomass seagrasses were recorded in areas where they had been recorded in the mid 1990's and where the recruitment paths of sub-adult prawns suggest that their juvenile habitats should be located. The areas of seagrass likely to support the enhancement of juvenile tiger prawns (biomass greater than 5 gm^{-2}) was 2.92 km^{-2} in 1999 and 24.84 km^{-2} in 2000. Qualitative data suggest that the area of high-biomass seagrass increased markedly by 2001 and that the area or type of seagrass will continue to change. The seagrass community of Exmouth Gulf needs to be quantitatively re-surveyed prior to any experimental release of prawns as part of an enhancement project.

The seagrasses have recolonised the bare substrates of Exmouth Gulf very rapidly in terms of areal extent. Further change in species composition may occur. The rate of re-establishment of the seagrass community is much faster than that documented for other tropical seagrass communities in Australia. For example, seagrasses in the Gulf of Carpentaria took ten years to recover from Cyclone Sandy, while in Hervey Bay, Queensland, partial recovery of sparse seagrasses had occurred after 20 months, a similar time frame.

5.1 Introduction

Aquatic vegetation provides the critical nursery habitat for juvenile brown shrimp *Penaeus aztecus* (saltmarsh) and tiger prawns *Penaeus esculentus* and *P. semisulcatus* (seagrass and algae), which contribute to valuable commercial fisheries in respectively, the northern and southern hemispheres (Minello *et al*. 1989, Somers 1994, Loneragan *et al*. 1998). In Australia, tiger prawns spawn in offshore waters and the planktonic larvae are transported to shallow coastal areas where as postlarvae, they settle on beds of seagrass and algae, three to four weeks after the eggs are released from the females (Dall *et al*. 1990, Haywood *et al*. 1995). In fact, the extent of seagrass in northern Australia limits the distribution of tiger prawns in the Northern Prawn Fishery (Australia's largest prawn fishery), which extends around northern Australia from Cape Londonderry in the west to Cape York in the east, a distance of about 6 000 km (Staples *et al*. 1985, Somers 1994).

Although the general importance of seagrasses to juvenile tiger prawns is well known in northern Australia, the relationship and mechanism of how the abundance of juveniles varies between seagrass beds of different types has only recently been studied (Loneragan *et al*. 1998, 2001). This knowledge is particularly valuable in tropical Australia, where ten or more species of seagrass are found in seven main communities (Poiner *et al*. 1987, 1989). The seagrasses in this region vary widely in their morphology: some are short species with small surface areas, and high shoot densities (e.g. *Halodule uninervis* and *Halophila ovalis*); some are of medium height with larger surface area and moderate shoot density (e.g. *Cymodocea serrulata* and *Thalassia hemprichii*); and one (*Enhalus acoroides*) has very long, broad leaves, with a large surface area and low shoot density (Lanyon 1986, Poiner *et al*. 1987). Laboratory experiments have shown that the amount and structure of the vegetation affects the behaviour of juvenile tiger prawns and brown shrimp, and the success rates of visual fish predators (Minello and Zimmerman 1985, Laprise and Blaber 1992, Kenyon *et al*. 1995). Predation rates on juvenile tiger prawns are lower in seagrasses with tall, wide leaves than in those with short, narrow leaves (Kenyon *et al*. 1995).

The characteristics of the release site can determine the movement and survival of the hatchery-reared stock after they are released during a stock enhancement (Schiel 1993, Munro & Bell 1997, Leber 1999). In seagrass habitats, shoot density and leaf morphology define facets of microhabitat that probably have a major impact on the ability of a site (habitat) to support the successful post-release recruitment of reared stock to the harvested fishery (Loneragan *et al*. 2001). Characteristics of the release site (e.g. seagrass density and morphology) would determine processes such as movement, behaviour, predation, growth and feeding, as well as competition with con-specific and other individuals, post-release. These processes contribute to the 'stock enhancement effect' of Leber (1999).

Little was known of the benthic habitats in Exmouth Gulf, except for a survey of seagrasses and algae in September 1994 (McCook et al. 1995) which found <20% cover of seagrasses at any site they investigated within the Gulf. As the distribution and abundance of seagrasses in Exmouth Gulf were poorly understood, the prime habitats for the juvenile *P. esculentus* that contribute to the Gulf fishery were unknown. They were determined by monitoring the regions where the natural abundance of wild sub-adult *P. esculentus* was high, then using their abundance as a corollary for optimal habitat (see Leber 1999).

The aim of our study was to describe the benthic habitats that might provide optimal nursery habitats for juvenile tiger prawns in Exmouth Gulf. This Chapter reports on the results from sampling the benthic habitats themselves, while the distribution of juvenile prawns and potential fish predators is reported in Chapter 6. We have also used the data from point sampling to estimate and model the extent and biomass of seagrass within the areas identified as potential release sites.

5.2 Methods

5.2.1 Mapping benthic habitats – whole of Exmouth Gulf

In 1999, we conducted two surveys of the whole of Exmouth Gulf, to determine the distribution of benthic habitats. As the critical nursery habitats for juvenile tiger prawns are shallow and intertidal macrophyte beds (Loneragan *et al*. 1994, 1998, Staples *et al*. 1985), we limited our survey to areas within the 0-5 m depth zone in the littoral and sub-littoral zone of Exmouth Gulf. We digitized an Admiralty chart of the region (AUS 744; 1:150 000) and converted this to an ArcView shapefile. Initially, we divided the Gulf into 4 sections; two were on the east coast, one on the south and one on the west coast. Each was stratified into two depth zones, < 1 m and 1-5 m. We spent more effort on the east coast because previous work suggested there was less seagrass or algae on the west coast and that juvenile tiger prawns seemed to enter the fishery from the south and east coasts (Western Australian Fisheries, pers. comm.). A 1 km grid of points was superimposed on the area where the depth was <5 m. Forty sites from each of the 4 sections were randomly selected from the 1 km grid. In addition to the random grid-sites, we sampled sites along a transect to the south of Whalebone Island that was previously sampled by McCook *et al*. (1995) in September 1994.

During June 1999, qualitative estimates of habitat type were made at each of the 119 sites that could be sampled (Table 5.1, Figure 5.2). During the second qualitative survey (November 1999), we reallocated sites within the 0-2.5 m depth zone as there was little evidence of vegetated substrates at depths > 2.5 m. The November survey did not visit sites on the north-west coast of Exmouth Gulf. No sign of seagrass was detected in this region during the June survey. The seafloor habitats had a steep profile and were exposed to wave action; most likely unsuitable for seagrass. Instead, more sites were sampled on the eastern and southern coasts, including surveying further north on the east Gulf coast (137 sites in total, Table 5.1, Figure 5.2).

The qualitative estimates of habitat cover were made by diving along a 30 m transect and recording the percentage cover of different benthic habitats 1 m each side of the transect. At each site, we recorded the following measurements:- depth, visibility, sediment type/vertical structure, patchiness, hard coral cover and percent composition of each species, soft coral cover and percent composition of each species, algae cover and seagrass cover and percent composition of each species.

Table 5.1 Summary of surveys of benthic habitats in Exmouth Gulf between June 1999 and December 2001. — = not sampled.

	Number of Samples	
	Qualitative	Quantitative
June 1999	119	—
November 1999	137	60
December 1999	—	75
October 2000	—	243
November 2000	—	151
December 2001	12 video transects	120
Total	256	649

5.2.2 Quantitative sampling of the extent of seagrass and marcoalgae in Exmouth Gulf

The Gulf-wide surveys, to determine the distribution of benthic habitats, identified areas within Exmouth Gulf where benthic macrophytes that may support juvenile prawns were recorded. We chose five 'regions' where macrophytes were most abundant (although their abundance was very low, presumably due to cyclonic disturbance). The regions were:-

Simpson - an area behind Simpson and Burnside Islands;
Whalebone North; and
Whalebone South - two regions within an extensive shallow subtidal zone to the south of Whalebone Island (Whalebone North, the northern most of the two; and Whalebone South, the southern most);
Giralia Bay - a region in the north east of Giralia Bay; and
Gales Bay - a region in the north east of Gales bay, including extensive subtidal shallows adjacent to the Sandalwood Peninsula.

Quantitative samples of benthic macrophytes were taken in each of these five regions to determine more precisely the extent and abundance of seagrass and algal habitats within Exmouth Gulf (Table 5.2). Quantitative samples were made by taking a shovel of seagrass (area = 0.07 m^{-2}), together with a sediment sample (following Poiner *et al*. 1987). We also noted the percentage cover of different benthic habitats at the point where the quantitative sample was taken, as we had done during the qualitative surveys.

Table 5.2 **A summary of the quantitative habitat samples that were taken in the main regions where sampling was carried out in Exmouth Gulf in June 1999, October-December 1999, October-November 2000 and December 2001. In June and October 1999, 22 and 6 samples (respectively) were taken from regions other than those listed.**

Region	June 1999	Late 1999	2000	2001
Tent Island	16	23	13	0
Simpson/ Burnside Island	6	13	42	0
Whalebone North	27	20	58	23
Whalebone South	16	26	96	27
Giralia Bay	19	33	78	0
Table 5.2 continued Sandalwood/ Gales	13	16	54	25
Total	97	131	341	75

In November and December 1999, 135 sites (concentrated around each region at about 25 sites each) were sampled quantitatively as part of the investigation of the extent of macrophyte habitats (Table 5.2, Figure 5.3). In 2000, 394 quantitative samples (243 sites in

October and 151 in November) (Table 5.1, Figure 5.3) were taken to improve the precision of the sampling of the extent of macrophyte habitats and to improve our ability to model their distribution. These samples were taken at previously sampled sites and new sites, including the same sites in the five regions where seagrasses were quantitatively sampled during 1999. In addition, sites that had been only sampled qualitatively during 1999 were sampled, to increase the overall area of the Exmouth Gulf benthic habitat that was sampled quantitatively. Further sampling (at 49 new sites, Figure 5.3) was undertaken in the area from Whalebone Island to Giralia Bay, the area where seagrasses had the greatest cover and where in some places, the density of sample sites was low. These extra samples ensured that a continuous band of quantitative samples was taken from Whalebone Island in the north, to Giralia Bay in the south.

Following discussions at the project Review Workshop in Perth (October, 2000), we also took quantitative samples at 54 deeper sites (to 8 m deep), adjacent to, and offshore from the sites that had been sampled quantitatively in 1999 (Figure 5.3). These samples were taken to investigate the benthic habitats on deeper substrates where additional trawls were to be made with the 1.5/12 mm net. Following the same recommendations, we also took quantitative habitat samples to the east of Tent Island in preparation for trawling non-seagrass inshore habitats, looking for tiger prawns in all habitat types.

No sampling was planned for 2001. However, because of the rapid recovery of seagrass, quantitative habitat samples were taken in three of the 'regions' and at the same sites where seagrasses were quantitatively sampled during 1999 and 2000 (i.e. Whalebone Island, Whalebone South and Gales Bay; about 25 sites per region) (Table 5.2). The sites were a sub-set of those sampled qualitatively during 2000. We also sampled three extra sites adjacent to five core sites in each region (at distances of 10 m, 100m and 500m from the five sites) to investigate the small-scale variation in the seagrass community. The biomass samples were not processed for 2001. However, the samples are being processed opportunistically at the time of writing, so biomass data for a few sites are available.

5.2.3 Modelling the extent of seagrass

Spatial smoothing of the seagrass above ground biomass (AGB) data was done in two stages. In the first stage, the data were de-trended using *loess*, a local regression modeling technique (Cleveland *et. al.* 1992). The purpose of this was to remove the large scale spatial structure in the data. The small scale spatial structure in the data, could then be modeled from the residuals from the *loess* fit. The small scale spatial structure was estimated by fitting the exponential variogram to the residuals from the *loess* fit. Using these estimates for the variogram, the residuals from the *loess* fit were then kriged (Cressie 1993). Kriging is a linear interpolation technique that uses observations at known spatial locations to make predictions of a variable at these or new locations. The final biomass estimates were calculated as the sum of the estimated fitted values from the *loess* analysis and the estimates from the kriging analysis of the *loess* residuals.

The total above-ground biomass of seagrass (i.e. all species combined) was predicted at points on a regular grid at 200 m intervals. These data were loaded into a Geographical Information System (GIS; ArcInfo) and were used to generate a surface having a 200 x 200 m cell size. The area of seagrass of a particular biomass category within the study area was estimated by summing the number of cells having the appropriate estimated value of seagrass biomass and multiplying by the area of a cell (40,000 m^2).

Seagrass biomass data for the November and December 1999 cruises were combined for these analyses, because the time between the two cruises was relatively short and the total amount of seagrass recovered was small. However, the data from the October and

November/December 2000 cruises was analysed separately because there was significantly more seagrass present during the November/December cruise than the October cruise.

5.2.3.1 Estimation of the area of habitat suitable for Penaeus esculentus

The overall aim of this estimate was to determine whether there was enough seagrass of sufficient quality to support an experimental release of 20 million juvenile *Penaeus esculentus*. To gauge the biomass of seagrass that would constitute a level suitable for sustaining juvenile *P. esculentus* of the planned release size (10 mm CL), we obtained values from the scientific literature. In cases where the catch rates of *P. esculentus* were reported, we converted these to density estimates by assuming a net efficiency of 0.49 (Loneragan *et al*. 1995). We then calculated the area of suitable seagrass habitat available during both 1999 and 2000 in Exmouth Gulf. The increase in the amount of seagrass between October and late November 2000 led us to suspect that there may have been significant seasonal changes in the above ground biomass of these seagrass; so we mosaiced the interpolated seagrass maps for the two periods. At points where the seagrass biomass was estimated for both sampling periods, the mosaicing routine calculated average biomass.

5.3 Results

5.3.1 Benthic habitats of the whole of Exmouth Gulf – June and November 1999

In mid-1999, the benthic habitats of littoral and sub-littoral Exmouth Gulf were dominated by bare sand and silt sediments with little-to-no epiflora or epifauna (96% of substrates were sand, silt or mud bare-habitats). Forty four of the 119 sites (37%) were completely devoid of hard- or soft-corals, algae or seagrass, and 85 sites (71%) had 1% or less cover of epibiota. In June 1999, brown algae or hard- and soft-corals were the most abundant epibiota and they were found at a few sites (e.g. 10 to 15% cover of brown algae was present at 5 or 6 sites) (Figure 5.4). Along the west coast of the Gulf (north of the Bay of Rest), the most abundant substrate type was bare sand and coral rubble. In the north, the coral rubble seemed to be recently-destroyed live coral, presumably destroyed by Cyclone Vance. In southern Exmouth Gulf (Giralia and Gales Bays) the most abundant substrate type was bare sand or silt (>99%). Along the eastern coast of the Gulf, the most abundant substrate type was also bare sand, sometimes covered in fine mud which seemed to have settled from the water column following Cyclone Vance. In the shallow bay behind Tent Island, the bottom was covered with a very fine silt/floc and visibility was very poor (0.3 m).

The cover of macroalgae was very low (107 out of 119 sites had < 2% cover, Figure 5.4). The small amount of the dominant *Sargassum* that we found had very little above-ground biomass, usually the plant was comprised of just the holdfast and a small amount of 'stem'. Most of the macroalgae was restricted to the shallow stratum (0-2 m). The highest coverage of macroalgae (15-100%) occurred at a few sites on the western side of the Gulf and a couple of sites about halfway up the eastern coast (Figure 5.4).

Living reef-building corals were found mainly on the western and southern sections of Exmouth Gulf, particularly the northwestern side of Exmouth Gulf (Figure 5.5). The most common forms here were: *Acropora, Porites, Turbinaria* and various Favids. Many colonies were broken, damaged or reduced to rubble, particularly those around the northwestern Gulf. At deeper sites in Exmouth Gulf (e.g. to the northwest of Whalebone Island) we found live corals, unturned sponges and some sparse *Sargassum*.

In June 1999, the seagrass cover throughout the Gulf was very sparse (average cover 0.15 ± 0.03%), with a maximum cover of only 2% at any site (Figure 5.6). One hundred and seven of the 119 sites (90%) had less than 1% seagrass cover, while 70% of sites were devoid of

seagrass. Seagrasses were restricted to the southern and south-eastern sections of the Gulf and in these areas, most seagrass was found in the shallows (<3 m depth). Seagrass were most abundant between Gales and Giralia Bays, adjacent to the Sandalwood Peninsula, and the inshore region south of Whalebone Island, towards Giralia Bay (Figure 5.6). Here, large areas of shallow sub-tidal banks supported *Halodule uninervis* (probably seedlings) growing at a very low shoot density.

At those few sites where seagrass was present, the species recorded were *Cymodocea serrulata*, *Cymodocea rotundata*, *Halodule uninervis* (broad and thin morphs), *Halophila ovalis*, *Syringodium isoetifolium* and *Thallasodendron ciliatum* (Table 5.3). *Halodule uninervis* and *Cymodocea serrulata* were the most common seagrasses that were found; usually as a few scattered seedlings or remnant shoots only.

During November 1999, bare substrate remained the dominant benthic habitat in Exmouth Gulf (average seagrass cover about 1.2%). No seagrass was found at the sites on the north-eastern coast of the Gulf that were visited for the first time, although 5-10% cover of brown algae and hard corals was found at a few sites. In general, very little epiflora (including seagrass) was found in the western and northern parts of the Gulf.

In some regions in the south-eastern Gulf, some of the bare substrate was replaced by seagrass in the five months since June (Figure 5.7). Some sites, from Whalebone Island south to Giralia Bay and adjacent to the Sandalwood Peninsula, had up to 20% cover of seagrass. The abundant seagrass species were *Halophila ovalis*, *H. dicipiens* and *H. uninervis*. Only a couple of shoots of *Cymodocea serrulata* were found at one or two sites during November 1999. One shoot of S. *isoetifolium* was also found.

5.3.2 The extent of seagrass and macro algae in Exmouth Gulf, and change in extent over 1999-2001

5.3.2.1 Seagrass cover

Despite bare substrate being the dominant benthic habitat in June, November and December 1999, over the next two years the percent cover of epibenthos, particularly seagrasses, steadily increased (see Figures 5.6 to 5.9). The mean percent cover of seagrass (± 1 SE) in late 1999, 2000 and 2001 was 1.17 ± 0.28%; 10.28 ± 1.69%; and 41.93 ± 3.50%, respectively.

In October and November 2000 in the Whalebone South region, the seagrass cover at 15% of sites was estimated between 31 and 60%, and a few sites had a cover of 100%. In other regions in the south-east, Whalebone North and Gales Bay, it had increased significantly also (8-9% of sites with 11-30% cover, Table 5.4, Figure 5.8). As well as seagrass, the benthic habitats of Exmouth Gulf were being recolonised by macroalgae, sponges and other fauna.

However, by late 2000, over most of Exmouth Gulf the seagrass cover remained relatively sparse; about 60-90% of sites per region had seagrass cover < 10% (Table 5.4), while 56% of over 300 sites had less than 5% cover (Figure 5.8). There had been no seagrass recolonisation of the extensive areas of sandflat in the southern shallows of Giralia or Gales Bays. However, very sparse *Halodule uninervis* was found on the northeast extremities of the sandflats of Gales Bay; it seems to have extended west from the Sandalwood Peninsula region. The seagrasses behind Simpson and Burnside Islands had not regenerated to any extent since 1999.

The dominant seagrass species were high shoot density, low biomass, short-leaved species – *Halodule uninervis* and *Halophila ovalis* (Table 5.3, Figure 5.11 a). The height of the seagrass canopy of these dominant species is only 2-3 cm. The longer leaved *H. spinulosa* was the dominant species at some deeper sites (Figure 5.1 b).

In December 2001, the areal extent and percent cover of seagrasses had increased greatly and the species composition changed (Tables 5.4, 5.5, 5.3, Figure 5.9). For example, the average percent cover of seagrass at Whalebone North increased from 2% in 1999, to 6% in 2000 and 57% in 2001. The greatest cover of seagrass was found from Whalebone Island south to Giralia Bay and Gales Bay where 60-80% cover was recorded at many sites (Table 5.4, Figure 5.9). The seagrass cover at Gales Bay was the least of the regions surveyed in 2001; only 28% of sites had 60% or more cover (Table 5.4). In general, inshore sites that were exposed at low tide had less that 60% cover.

In 2001, the extent of recolonisation reached well beyond the area covered by our previously chosen regions and the limited number of sites that were sampled in each region that year. Our best guess is that there is a continuous 'seagrass meadow' in Exmouth Gulf that extends from Whalebone Island, south 20 km or so to Giralia Bay, and is about 3-4 km wide. Seagrass distribution probably extends to the north of Whalebone Island in the eastern-central section of Exmouth Gulf, and into Gales Bay and the Roberts Island area in the south.

A total of five species were commonly recorded, compared with only two previously. The dominant seagrass species in December 2001 were high biomass, medium shoot density species such as *Halophila spinulosa*, *Syringodium isoetifolium* and *Cymodocea serrulata* in the 1-2 m depth strata (Table 5.3). These species created a 20-30 cm high seagrass canopy. *Halophila spinulosa* dominated the seagrass community and made up 60-80% of the seagrass species mix at most sites, while 10-20% cover of *Syringodium isoetifolium* or *Cymodocea serrulata* was common. The *H. spinulosa* was laden with seed.

Table 5.3 The percentage of sites with different species of seagrass (the 2001 data from qualitative samples are indicative only and may change).

Seagrass Species	Survey				
	Nov-99	Dec-99	Oct-00	Nov-00	Dec-01
Cymodocea serrulata	-	-	0.4	4.6	35.8
Halodule uninervis (b)	-	-	0.8	1.3	2.5
Halodule uninervis (t)	28.0	35.0	20.6	33.1	70.0
Halophila ovalis	18.7	21.7	14.8	27.8	8.3
Halophila spinulosa	-	1.7	5.3	9.3	68.3
Halophila descipens	-	1.7	5.8	9.9	0
Syringodium isoetifolium	-	-	-	1.3	14.2
Total number of species	2	4	5	6	6
Total number of sites	60	75	243	151	120

Table 5.4 **Percent of sites with different categories of seagrass cover in different regions sampled in Exmouth Gulf. Simpson Island and Giralia Bay were not sampled in 2001. The rows will not necessarily add to 100%, as the >31-60% and ≥60% categories overlap.**

Region	Cover of Seagrass				Number of sites
	≤ 10%	10-30%	>31-60%	≥60%	
1999					
Simpson Island	100	0	0	0	19
Whalebone North	98	2	0	0	47
Whalebone South	93	7	0	0	42
Sandalwood/ Gales	100	0	0	0	29
Giralia Bay	100	0	0	0	52
2000					
Simpson Island	100	0	0	0	47
Whalebone North	89	9	2	0	76
Whalebone South	67	9	15	14	106
Sandalwood/ Gales	90	8	0	2	59
Giralia Bay	92	5	2	2	88
2001					
Whalebone North	16	0	18	82	38
Whalebone South	14	19	33	45	42
Sandalwood/ Gales	32	38	18	28	40

5.3.2.2 Seagrass biomass and shoot density

Reflecting the increase in percent cover of seagrass, the above-ground biomass (AGB) of seagrasses in most regions increased from October 1999 to November 2000 (Figures 5.10, 5.12). The exception was the seagrasses in the Simpson region (behind Simpson and Burnside Islands), where both percent cover and biomass have declined over the duration of the study. In 1999 in areas where seagrass was abundant, the maximum seagrass above-ground biomass that was found was 16 g m^{-2}, whereas by 2000 maximum biomass found had increased to 26 g m^{-2} (the average biomass was much lower in both 1999 and 2000, Figure 5.10).

However, the above-ground biomass from some samples collected in 2001 are greater than 70 g m^{-2}, much greater than the maximum of 26 g m^{-2} found in 2000. Photographs of the seagrasses taken in 2000 and 2001 support these preliminary data showing an increase in

biomass (cf Figure 5.11a, b). The photographs show a change in community composition from high shoot density, small-leaved species, to medium shoot density, long-leaved species.

Table 5.5 Mean biomass (g m-2) (a) and percent cover (b) of seagrass within regions of Exmouth Gulf in 1999, 2000 and 2001. Simpson Island and Giralia Bay were not sampled in 2001 (ns).

Year of	Region				
Sampling	Simpson Island	Whalebone North	Whalebone South	Giralia Bay	Gales Bay
(a) mean biomass	(± 1 SE)				
Dec, 1999	0.14±0.05	0.41±0.14	0.74±0.34	0.02±0.02	0.95±0.60
Oct, 2000	0.03±0.01	0.35±0.11	1.22±0.28	0.55±0.17	0.48±0.18
Nov, 2000	0	2.02±0.56	3.10±0.76	1.77±0.76	1.83±0.40
(b) mean percent cover	(± 1 SE)				
1999	0.17±0.10	1.70±0.80	3.43±1.18	0.41±0.17	1.07±0.38
2000	0	6.04±1.47	22.22±4.54	5.14±1.97	4.42±1.15
2001	ns	56.78±6.57	36.22±4.89	ns	34.44±6.03

5.3.3 Modeling the extent of seagrass

For November and December 1999, we restricted our prediction of seagrass distribution to the five regions sampled (Table 5.5), as the technique becomes unreliable when data are sparse. The total area of seagrass at this time was estimated to be approximately 75 km^2, but it was mostly of very low biomass with only 2.9 km^2 being > 5 g m^{-2} (Table 5.6). The higher-biomass seagrass was located between the 1 and 2 m depth contours (MLWS) to the south of Whalebone Island and at the mouth of Gales Bay (Figure 5.13).

Table 5.6 Area of seagrass as estimated from kriging of the above-ground biomass of all seagrass species collected within five regions on field surveys in Exmouth Gulf during November/December 1999, October 2000 and November 2000

Category of above-ground biomass (g m^{-2})	Nov/Dec 1999		Oct 2000		Nov 2000	
	Area (km^2)	%	Area (km^2)	%	Area (km^2)	%
No seagrass	29.6	28.2	64.44	25.7	13.28	11.3
0.001 to 5	72.56	69.1	181.00	72.1	79.56	67.6
5.001 to 10	2.36	2.2	5.48	2.2	18.96	16.1
10.001 to 20	0.56	0.5	0	0.0	5.04	4.3
> 20	0	0.0	0	0.0	0.84	0.7
Total area	105.08		250.92		117.68	

In October 2000, the seagrass sampling was extended along the eastern coast of Exmouth to resample sites visited the previous year, but also to sample the areas between 3 of the south eastern regions sampled the previous year (Whalebone north, Whalebone south and Giralia Bay) (Figure 5.3). The area of relatively high biomass seagrass to the south of Whalebone Island had increased, but the high biomass seagrass patch at the mouth of Gales Bay was no longer predicted. The majority of the area sampled either had no seagrass (25.7%) or low biomass seagrass (<5 g m^{-2}, 72.1%) (Table 5.6). Only a relatively small area (5.48 km^2) had an above ground biomass of seagrass >5 g m^2 (2.2%, Table 5.6).

By late November 2000 the estimated area of benthic habitat with an above ground biomass of seagrass >5 g m^2 had increased to 24.84 km^2 (Table 5.6). The increases occurred in all areas sampled, except for Simpson Island (Figure 5.14). The significant increase in the extent and percent cover of seagrass within Exmouth Gulf in 2001 (with indications of AGB up to 70 g m^2), suggests that area of seagrass > 5 g m^2 was much greater in 2001 than in 2000. When the samples collected in 2001 are sorted and the data are analysed, we will have a better estimate of areas that would be suitable for release sites during future enhancement experiments.

5.3.3.1 Estimation of the area of habitat suitable for Penaeus esculentus

From studies of seagrass nursery habitats for juvenile *Penaeus esculentus* in the Gulf of Carpentaria and in south eastern Queensland, the total seagrass above-ground biomasses ranged from 6 to 123 g m^{-2} (Table 5.7). Natural densities of juvenile brown tiger prawns ranged from 0.3 to 4.1 prawns m^{-2}, although during a caging experiment, prawns grew at densities as high as 32 prawns m^{-2} over 3 weeks (Loneragan et al. 2001) (Table 5.7). Based on these values, we can assume that for a seagrass bed to act as successful nursery habitat for juvenile *P. esculentus*, the cover of seagrass needs to be at least 5 g m^{-2} (AGB).

The estimated area of seagrass meeting this assumption in Exmouth Gulf was 2.92 km^2 in 1999 and 24.84 km^2 in November 2000 (Table 5.6). A hypothetical release of 20 million juvenile *P. esculentus* would have resulted in densities of 6.85 prawns.m^{-2} and 1.29 prawns m^{-2} in 1999 and 2000 respectively (Table 5.7).

Table 5.7 Natural densities of juvenile brown tiger prawns (Penaeus esculentus) and seagrass above ground biomasses at a number of areas in northern Australia compared to those at Whalebone South (6.85 and 1.29 prawns.m-2) following a theoretical release of 20 million juvenile P. esculentus.

	Locality				
Dominant seagrass	Embley River, *Halophila ovalis*	Groote Eylandt, Angurugu Creek	Mornington Island, Dugong River	Moreton Bay, Toondah Harbour	Embley River, Enclosures on *Enhalus*
Seagrass AGB ($g\ m^{-2}$)	~6	18.1 - 110	>500 (wet)	50 – 125	~70
Juvenile *P. esculentus* density ($n.m^{-2}$)	0.3	0.37	0.34 – 4.1	0.82	4 – 32
	Enhalus acoroides, Halophila ovalis, Halodule uninervis	*Halodule uninervis, Halophila ovalis, H. spinulosa, Cymodocea serrulata*	*Halodule uninervis, Halophila ovalis, H. spinulosa, Cymodocea serrulata*	*Zostera capricorni*	*Halophila ovalis, Halodule uninervis*
Reference	Haywood et al. 1995	Loneragan et al. 1994	Coles & Lee Long 1985	O'Brien 1994	Loneragan et al. 2001

5.4 Discussion

5.4.1 Dominant benthic habitat in Exmouth Gulf

During our first broad-scale survey of Exmouth Gulf in June 1999 we found very little seagrass or algae. The dominant benthic habitat was bare substrate; either, sand, silt or mud, and at several isolated sites in the north-west a consolidated 'pavement' surface. Coral reefs in north-western Exmouth Gulf were reduced to rubble, and solitary corals and sponges were 'up-rooted' and upturned. Mangrove communities on the eastern coast of the Gulf were dead and all trees were stripped of leaves. Sediment loads in the water column were high, and they had been much higher immediately following the cyclone (Mark Longhurst, Manager, Exmouth Pearls, pers. comm.), reducing visibility to less than 0.5 m. An intense cyclone (Category Five Vance, 267 kph wind speeds) had passed through Exmouth Gulf during March 1999 (Figure 5.15) and caused massive destruction to the terrestrial environment (including the built-environment). Based on experience following Cyclone Sandy in the Gulf of Carpentaria (Poiner *et al*. 1989, 1993), the evidence described above pointed to massive destruction of the inter- and sub-tidal benthic habitats throughout Exmouth Gulf.

It is impossible for us to provide a precise description of the degree of impact of Cyclone Vance on the benthic macrophytes of Exmouth Gulf because the cyclone occurred just prior to our first planned survey. Not all severe cyclones have a destructive effect on benthic habitats (Poiner *et al*. 1989), so the degree of destruction from Vance cannot be estimated with precision. However, many factors suggest that the destruction of littoral habitats in Exmouth Gulf was severe. The most compelling of these factors can be listed under four broad categories:-

1. no benthic macrophyte communities were found in Exmouth Gulf, yet for over 30 years the Gulf has supported a tiger prawn fishery (Penn *et al*. 1995, Caputi *et al*. 1998, Chapter 7), the juveniles of which are obligate users of seagrass and algal habitats as nursery areas (Haywood *et al*. 1995, Loneragan *et al*. 1998);
2. one previous study of the benthic communities in Exmouth Gulf identified a seagrass community comprised of 4 species (McCook *et al*. 1995) including 'climax' species (*Cymodocea serrulata*, *Syringodium isoetifolium*) that characterise well developed tropical seagrass communities (Poiner *et al*. 1987); yet seagrasses were not found in June 1999;
3. the track of Cyclone Vance travelled up the centre of Exmouth Gulf, effectively parallel to both the east and west coasts (Figure 5.15); past studies have shown that cyclones that travel parallel to the coast are the most destructive for littoral seagrass habitats (Poiner *et al*. 1989);
4. in the three years since the Cyclone, the seagrass and algae have recolonised the benthic sand substrates of Exmouth Gulf to create a complex macrophyte community and a nursery habitat for juvenile tiger prawns; probably similar to seagrass and algal habitats that existed prior to cyclonic disturbance.

In the 10 years before the McCook *et al*. (1995) survey of seagrass in Exmouth Gulf (September 1994), five cyclones passed in the vicinity of the Gulf (Figure 5.16). None of these cyclones travelled close enough to Exmouth Gulf, or parallel to the coast, to have had a severe impact on its seagrass community (see Poiner *et al*. 1989). It therefore seems unlikely that the seagrass community in September 1994 had been impacted by a cyclone in the previous 10 years. Consequently, the seagrass community found by McCook in 1994 was probably a relatively stable community. However, community biomass would have varied with season and its variation would be consistent from year to year (Lanyon & Marsh 1995). We have shown a significant increase in seagrass biomass from October to November/December 2000 (with season). Thus, the seagrasses found by McCook *et al*. (1995) in September (towards the end of the dry season) were probably less abundant than the community that we found one to two months later at the beginning of the wet season. We might expect the biomass and shoot density found by McCook to be less that that found by ourselves in hotter months.

Consequently, we have presumed that the seagrasses and algal communities of Exmouth Gulf had been destroyed during the cyclone, both through mechanical disturbance and water column sediments restricting the light available for surviving macrophytes. By late 1999, colonising seagrasses (*Halodule uninervis* and *Halophila ovalis*) had re-established in the Gulf, mostly at a very low biomass, with some sites supporting 20% cover. These seagrasses were settled by postlarval tiger prawns, however, few of them survived to grow to juveniles (see Chapter 6). Between 1999 and 2001 (33 months since the cyclone), the seagrasses of Exmouth Gulf increased in both areal extent (a mean of 1%, 10% and 42% cover at surveyed sites each year, respectively) and species diversity (Table 5.3). By 2001, seagrasses created a complex habitat that had supported low densities of juvenile tiger prawns in 2000 and 2001 (see Chapter 6). In 2000, the biomass of seagrass in Exmouth Gulf remained low compared with seagrass communities elsewhere that support abundant populations of juvenile tiger prawns (e.g. Loneragan *et al*. 1998). In 2001, *Halophila spinulosa*, *Cymodocea serrulata* and *Syringodium isoetifolium* were the most common seagrasses that were found in Exmouth Gulf. Experience with tropical seagrass recolonisation at other areas suggests that the latter

two species may come to dominate the seagrass community at 2-4 m depth zones as it reaches a climax community (Poiner *et al*. 1987, 1989, Kenyon *et al*. 1999). Preliminary data from ongoing sorting of the 2001 samples show seagrass biomass values of up to 70 gm^{-2}; a biomass capable of supporting a high abundance of juvenile tiger prawns (Loneragan *et al*. 1998).

The re-establishment of seagrasses has been accompanied by increased sightings of dugong in Exmouth Gulf. In 1999, the only dugong seen were in the shallows behind Simpson and Burnside Islands, grazing on remnant *H. uninervis* rhizomes found there. No dugongs were seen in the eastern open Gulf. By 2000, a few dugongs were seen in Whalebone North, Whalebone South and Gales Bay regions. However, during 2001 they were seen in the same regions as 2000, yet the sightings were noticeably more frequent (R. Kenyon, pers. obs.).

The re-establishment of the seagrass community seems to be one of the most rapid recovery events documented for seagrasses (Poiner *et al*. 1989), although the partial recovery 20 months after a cyclone/flood event has been documented in Hervey Bay, Queensland (Preen *et al*., 1995). Some of the first species to recolonise Hervey Bay were the same species that we found recovering in Exmouth Gulf, *H. ovalis* and *H.* spinulosa; together with *H. uninervis* in Exmouth Gulf. However in Hervey Bay, *H. spinulosa* only recolonised sand habitats > 10 m depth; in Exmouth Gulf in 2001 it was the dominant recoloniser at all depths, except intertidal areas. In Hervey Bay, *H. decipiens* seemed to initiate recolonisation at all depths (Preen *et al*. 1995), while in Exmouth Gulf it was initiated by *H. uninervis* and *H. ovalis*. The recovery at both Exmouth Gulf and Hervey Bay would have been facilitated by the growth rate of *H. ovalis* which is the fastest growing tropical seagrass; with growth rates of up to 8.8 mm per rhizome per day (Nakaoka & Aioi 1999). They found that it takes two months for a patch within a seagrass bed that was denuded of *H. ovalis* to reach the same state of colonisation as that prior to impact.

In September 1994, the percent cover of seagrass at most sites in the inshore areas of the eastern and southern Gulf was estimated to be < 5% (McCook *et al*. 1995), with a maximum of 20% (Schaffelke *et al*. 1996). These values are less than those recorded in 2000, and much less than those in 2001. McCook *et al*. (1995) measured the wet-weight of seagrass, so direct comparisons cannot be made with our dry-weights. However they also measured the shoot density of the seagrass species. In 1994 the shoot density of *Cymodocea serrulata* was 10-20 times greater than the 1-2 shoots m^{-2} (maximum), that we found in 1999/2000. In 1994, it grew at about 40 shoots m^{-2} offshore from Simpson and Burnside islands, while in the vicinity of Whalebone Island and to the north of Giralia Bay it grew at about 10-80 shoots m^{-2}. *Cymodocea serrulata* is a climax species, not common in recolonising seagrass beds (such as we found in 1999), but common in beds that have not suffered disturbance in over 10 years. In contrast in 2000, we found shoot densities of colonising seagrasses- *Halophila ovalis* and *H. uninervis* -of over 2000 shoots m^{-2}, much higher than those recorded in 1994 (about 100 shoots m^{-2} and 1-20 shoots m^{-2}; McCook *et al*., 1995).

5.4.2 Exmouth Gulf as a site for an experimental enhancement project

When assessing the habitats of Exmouth Gulf as potential release sites for hatchery-reared prawns, the sparse distribution and low abundance of seagrasses that had been impacted by a catastrophic cyclonic disturbance did not provide us with a clear picture. Exmouth Gulf seagrasses will continue to change, and have done so between 2000 and 2001, resulting in underestimates of seagrass habitat with >5 g m^{-2} (as estimated from our most comprehensive survey in 2000).

The results of the kriging show that in 1999 only a relatively small portion of potential seagrass habitat (about 3 km^{-2}) in Exmouth Gulf supports a seagrass community capable of

providing critical habitat for juvenile tiger prawns (AGB >5 g m^{-2}). Had a release of 20 million prawns occurred in 1999, the density of juvenile prawns (about 7 m^{-2}) on these seagrass habitats would have been relatively high compared to those observed under natural conditions elsewhere. By 2000, this area has increased to about 15.5 km^{-2} and released-prawn density would have been comparable with natural densities in many other seagrass habitats (about 1.3 m^{-2}). However, our results from 2001 show that the area of seagrass habitat with > 5 g m^{-2} of seagrass would have increased dramatically. As the quantitative data for 2001 are not available to be modelled, no reliable estimate can be made. However, a seagrass community 'guesstimated' to be roughly 20 km x 3 km with biomass per site reaching 70 g m^{-2} was found in 2001, suggesting that a much larger area of seagrass would have been suitable for release sites.

The habitat at a release site has a crucial effect on the survivorship of released stock (Schiel 1993, Leber *et al*. 1996, 1998, Wahl 1995). Among seagrass habitats, the higher the seagrass biomass the higher the abundance and growth of juvenile tiger prawns (Loneragan *et al*. 1998, 2001). Post settlement mortality of tiger prawn postlarvae appears to be much higher in seagrass beds with low biomass compared with those with a high biomass (Loneragan *et al*. 1998). In low biomass seagrass beds, the abundance of tiger prawn postlarvae declines markedly between two size classes. Recently-settled, highly-mobile postlarvae (1-1.9 mm CL) are more abundant than larger postlarvae that are benthic orientated (2-2.9 mm CL), and juvenile prawns, as well. The decline in abundance between sizes is probably due to migration and mortality, removing the smaller size class from the habitat before they can grow (Haywood *et al*. 1998, Jenkins & Sutherland 1997, Loneragan *et al*. 1998). In contrast, high biomass seagrass beds (AGB > 100 g m^{-2}) support a higher abundance of benthic postlarvae and juveniles than recently-settled postlarvae (Loneragan *et al*. 1998), the reverse case. High biomass, broad-leaved seagrasses reduce fish predation on postlarval and juvenile prawns (Kenyon *et al*. 1995), probably enhancing the survival of the small size classes in high biomass seagrass, resulting in more juveniles in these habitats. Denuded substrates have no potential as release sites as the lack of vegetation would likely result in high mortalities of released stock.

We have monitored the seagrasses of Exmouth Gulf during a period when they are undergoing seral change, recolonising following cyclonic disturbance. Were an experimental release to go ahead in Exmouth Gulf, knowledge of the distribution and abundance of a less dynamic 'climax' seagrass community (with high AGB and a complex physical structure) is crucial to be able to select potential release sites for the long term. Although it is valuable to document the recolonisation process, currently the Exmouth Gulf seagrass community is in flux. The seagrasses of Exmouth Gulf would need to be re-surveyed prior to any experimental releases as part of future Stages of this project. Furthermore, the distribution of juvenile tiger prawns within the seagrass community at that future time, used as a corollary for optimal habitat (Munro & Bell 1997), and the distribution of their potential predators, will help to determine the selection of release sites.

5.4.3 Predicted optimal release sites for Penaeus esculentus hatchery-reared stocks

However, in 2000, the last year for which we have a widespread quantitative data on seagrass distribution in Exmouth Gulf, the areas most suited for release sites (high AGB) were to the south of Whalebone Island and in Gales Bay. The predicted (kriged) seagrass distribution in 2000 suggested that the region with the greatest area of high-biomass and percent-cover seagrass was Whalebone South. Whalebone South had the highest mean biomass and was probably the best release location, followed by Whalebone North and Gales Bay, which also supported high biomass seagrasses. The trend in seagrass abundance between regions had not been consistent from 1999, either from the actual data or kriging predictions. In late 2000, the

trend in percent cover matched the trend in biomass between regions. Whalebone South had the highest biomass and cover. By 2001, the greatest percent cover of seagrass had changed and now was found at Whalebone North (57%), followed by Whalebone South (36%) and Gales Bay (34%). Significantly greater seagrass abundance at Whalebone North in 2000 was predicted compared with 1999 (see Figures 5.13 and 5.14), and the increase seems to have continued in 2001, making it a likely release site.

The change in seagrass abundance between regions highlights the need to re-survey the Gulf to establish optimal release sites prior to an experimental release. As well, in the process of these areas being assessed and selected as release sites for a future enhancement project, the distribution of tiger prawns and their predators among all five regions must be considered (Munro & Bell 1995, Leber 1999).

5.4.4 Short- and long-term changes in the seagrass community in Exmouth Gulf

There have been both short- and long-term changes in the seagrass community of Exmouth Gulf during this project. Long-term increases in the areal extent (average seagrass cover per site changed from 1% to 42%) and species richness (two to seven species) have been a function of successional processes, as the seagrass community of Exmouth Gulf re-establishes itself following cyclonic disturbance. These patterns may reflect earlier changes in the seagrass communities which have been historically subject to cyclonic influences of varying severity. Short-term changes have been part of the seasonal flux of seagrass community abundance, which normally occurs in any seagrass habitat. Seasonal change in seagrass abundance during this project has been influenced by the fast-growing species that were present in Exmouth Gulf in 1999/2000.
Seasonal change in biomass was detected between surveys carried out in the same year. For example, during October 2000, the abundance of low biomass, high shoot density seagrass had increased, yet it remained < 10 g m^{-2} in all areas. One month later in late November 2000, 14 of the 151 sites sampled had an above ground biomass of seagrass > 10 g m^{-2} and overall, seagrass was present at more sites.

The dramatic increase in seagrass biomass from October to November 2000 suggests that the seagrasses in Exmouth Gulf may exhibit a seasonal cycle in production of above ground biomass. Elsewhere in Australia, tropical seagrass communities have shown two to four fold differences in seagrass abundance depending on season, with a minimum in August to September and a maximum in November to March (Lanyon & Marsh 1995). Their abundance has been correlated with day-length, temperature and rainfall. High-biomass seagrasses that are known to support an abundant population of tiger prawn juveniles in the early- to late-summer also show seasonal fluctuations in biomass, growth rate and the production of new leaves, with a peak in spring and summer (Kenyon *et al*. 1997). In other geographic regions, seasonal patterns also have been demonstrated in the typically high biomass temperate seagrasses e.g. *Posidonia oceanica* (Alcoverro *et al*. 1995) and *Zostera noltii* (Philippart 1995), as well as in various tropical species e.g. *Halodule wrightii* (Creed 1999), *H. uninervis* (de Iongh *et al*. 1995, Jupp *et al*. 1996) and *Halophila ovalis* (Jupp *et al*. 1996). In all probability, the abundance of seagrasses in Exmouth Gulf would have been increasing throughout the early summer of 2000.

In future, estimates of the extent and distribution of seagrass in Exmouth Gulf should be undertaken in December, following rapid spring growth. Seagrass mapping in Exmouth Gulf may be facilitated by using methods such as remote sensing together with ground truthing; or a combination of remote sensing and rapid assessment methods. To begin to investigate these methods, in 2001 we made 12 video transects at seagrass sites representing a range of seagrass abundance. At each site, we also took two qualitative and quantitative samples (as

described in the Methods). The data for each site will be ranked and compared with that site's video image which will be categorised in an attempt to match field data with an image 'category' recorded by video. This technique is a variation on the visual census developed by Mellors (1991) and offers the advantage of being able to remotely sample large areas of seagrass habitat. The video data can then be analysed in the laboratory and seagrass habitats categorised according to a ranking of the above-ground structure of the vegetation.

5.5 References

Alcoverro T, Duarte CM, Romero J (1995). Annual growth dynamics of Posidonia oceanica: contribution of large-scale versus local factors to seasonality. *Marine Ecology Progress Series*. 120: 203-210.

Caputi N., Penn J.W., Joll L.M. and Chubb C.F. (1998). Stock-recruitment-environment relationships for invertebrate species of Western Australia. In: Proceedings of the North Pacific Symposium on Invertebrate Stock Assessment and Management. Jamieson G.S. and Campbell A. (Eds.) *Can.Spec.Publ.Fish.Aquat.Sci.* 125: 247-255.

Cleveland WS, Grosse E & Shyu WM (1992). Local regression models. In Chambers, J.M and Hastie, T.J., editors, Statistical Models in S, pp. 309-376. Wadsworth and Brooks, Pacific Grove, California.

Coles RG, Lee Long WJ (1985). Juvenile prawn biology and the distribution of seagrass prawn nursery grounds in the southeastern Gulf of Carpentaria. *In* PC Rothlisberg, BJ Hill and DJ Staples (Editors) Second Australian National Prawn Seminar, NPS2. Cleveland, Australia. pp 55-60.

Creed, J. C. (1999). Distribution, seasonal abundance and shoot size of the seagrass *Halodule wrightii* near its southern limit at Rio de Janeiro state, Brazil. *Aquatic Botany*. 65(1-4): 47-58.

Cressie NAC (1993). Statistics for Spatial Data. Wiley, New York.

Dall W, Hill BJ, Rothlisberg PC, Staples DJ (1990). The Biology of the Penaeidae. *Adv. Mar. Biol.*, Vol. 27, pp. 1–489.

de Iongh HH, Wenno BJ, Meelis E (1995). Seagrass distribution and seasonal biomass changes in relation to dugong grazing in the Moluccas, East Indonesia. *Aquatic Botany* 50: 1-19

Haywood MDE, Vance DJ & Loneragan NR (1995). Seagrass and algal beds as nursery habitats for tiger prawns (*Penaeus semisulcatus* and *P. esculentus*) in a tropical Australian estuary. *Marine Biology* 122, pp. 213–223.

Haywood MD, Heales DS, Kenyon RA, Loneragan NR, Vance DJ (1998). Predation of juvenile tiger prawns in a tropical Australian estuary. *Marine Ecology Progress Series* 162: 201-214.

Jenkins GP & Sutherland CR (1997). The influence of habitat structure on nearshore fish assemblages in a southern Australian embayment: colonisation and turnover rate of fishes associated with artificial macrophyte beds of varying physical, structure. *Journal of Experimental Biology and Ecology* 218: 103-125.

Jupp BP, Durako MJ, Kenworthy WJ, Thayer GW, Schillak L (1996). Distribution, abundance, and species composition of seagrasses at several sites in Oman. *Aquatic Botany*. 53: 199-213.

Kenyon R, Burridge C, Poiner I, Hopkins E (1999). Impact of the Macarthur River Project Mine Transhipment Facility on the Marine Environment: Final Report. *CSIRO Marine Laboratories Report*. CSIRO Marine Research. PO. Box 120 Cleveland, Queensland, 4163.

Kenyon RA, Conacher CA, Poiner IR (1997). Seasonal growth and reproduction of *Enhalus acoroides* (L.f.) Royle in a shallow water embayment in the western Gulf of Carpentaria, Australia. *Marine and Freshwater Research.* 48: 335–342.

Kenyon RA, Loneragan NR, Hughes J (1995). Habitat type and light affect sheltering behaviour of juvenile tiger prawns (*Penaeus esculentus* Haswell) and success rates of

their fish predators. *Journal of Experimental Marine Biology and Ecology*. 192: 87–105.

Lanyon J (1986). *Guide to the identification of seagrasses in the Great Barrier Reef region*. Great Barrier Reef Marine Park Authority, Townsville, Australia.

Lanyon JM & Marsh H (1995). Temporal changes in the abundance of some tropical intertidal seagrasses in North Queensland. *Aquatic Botany*. 49: 217-237.

Laprise R. & Blaber SJM (1992). Predation by moses perch, *Lutjanus russelli*, and blue spotted trevally, *Caranx bucculentus*, on juvenile brown tiger prawn, *Penaeus esculentus*: effects of habitat structure and time of day. *Journal of Fisheries Biology*. 40: 627–635.

Leber KM (1999). Rationale for an experimental approach to stock enhancement. pp. 63-75 *In:* Stock Enhancement and Sea Ranching (eds. BR Howell, E Moksness, T Svasand). Chapter 5. Fishing News Books, Blackwell Science Ltd., Oxford.

Leber KM, Arce SM, Sterritt DA, Brennan NP (1996). Marine stock-enhancement potential in nursery habitats of striped mullet, *Mugil cephalus*, in a Hawaii. *Fishery Bulletin*. 94: 452-471.

Leber KM, Brennan NP, Arce SM (1998). Recruitment patterns of cultured juvenile pacific threadfin, *Polydactylus sexfilis* (Polynemidae), released along sandy marine shores in Hawaii. *Bulletin of Marine Science*. 62(2): 389-408.

Loneragan NR, Kenyon RA, Haywood MDE, Staples DJ (1994). Population dynamics of juvenile tiger prawns in seagrass habitats of the north-western Gulf of Carpentaria, Australia. *Marine Biology*. 119: 133-143.

Loneragan NR, Kenyon RA, Staples DJ, Poiner IR, Conacher CA (1998). The influence of seagrass type on the distribution and abundance of postlarval and juvenile tiger prawns in the western Gulf of Carpentaria, Australia. *Journal of Experimental Marine Biology and Ecology*. 228: 175-196.

Loneragan NR, Wang Y-G, Kenyon RA, Staples DJ, Vance DJ (1995). Estimating the efficiency of a small beam trawl for capturing small tiger prawns (*Penaeus esculentus* and *Penaeus semisulcatus*) by removal methods in seagrass beds of northern Australia. *Marine Ecology Progress Series*. 118: 139-148.

Loneragan NR, Heales DS, Haywood MDE, Kenyon RA, Pendrey RC, Vance DJ (2001). Estimating the carrying capacity of seagrass for juvenile tiger prawns (*Penaeus semisulcatus*): enclosure experiments in high and low biomass seagrass beds. *Marine Biology*. 139: 343-354.

McCook LJ, Klumpp DW, McKinnon AD (1995). Seagrass communities in Exmouth Gulf, Western Australia: a preliminary survey. *Journal of the Royal Society of Western Australia*. 78: 57-63.

Merrors JE (1991). An evaluation of a rapid visual technique for estimating seagrass biomass. *Aquatic Botany*. 42: 67-73.

Minello TJ & Zimmerman RJ (1985). Differential selection for vegetative structure between juvenile brown shrimp (*Penaeus aztecus*) and white shrimp (*P. setiferus*), and implications in predator-prey relationships. *Estuarine Coastal and Shelf Science.* 20: 707–716.

Minello TJ, Zimmerman RJ, Martinez EX (1989). Mortality of young brown shrimp *Penaeus aztecus* in estuarine nurseries. *Transactions of the American Fisheries Society.* 118: 693–708.

Munro JL & Bell JD (1997). Enhancement of marine fisheries resources. *Reviews in Fisheries Science*. 5: 185-222.

Nakaoka M & Aioi K (1999). Growth of seagrass *Halophila ovalis* at dugong trails compared to existing within-patch variation in a Thailand intertidal flat. *Marine Ecology Progress Series* 184: 97-103.

O'Brien CJ (1994). Population dynamics of juvenile tiger prawns *Penaeus esculentus* in south Queensland, Australia. *Marine Ecology Progress Series* 104: 247-256.

Penn JW, Caputi N, Hall NG (1995) Stock-recruitment relationships for the tiger prawn *(Penaeus esculentus)* stocks in Western Australia. *ICES Marine Science Symposium* 199: 320-333.

Philippart CJM (1995). Seasonal variation in the growth and biomass of an intertidal *Zostera noltii* stand in the Dutch Wadden Sea. *Netherlands Journal of Sea Research* 33: 205 - 218.

Poiner IR, Conacher CA, Loneragan NR, Kenyon, RA, Somers I (1993). Effect of cyclones on seagrass communities and penaeid prawn stocks of the Gulf of Carpentaria. *CSIRO Marine Laboratories Report*. CSIRO, Division of Fisheries, Marine Laboratories PO. Box 120 Cleveland, Queensland, 4163.

Poiner IR, Staples DJ, Kenyon RA (1987). Seagrass communities of the Gulf of Carpentaria, Australia. *Australian Journal of Marine and Freshwater Research* 38: 121–131.

Poiner IR, Walker DI, Coles RG (1989). Regional studies — seagrasses of tropical Australia. In, *Biology of seagrasses: A treatise on the biology of seagrasses with special reference to the Australian region*. edited by ADW Larkum, AJ McComb & S Shepherd, pp. 279–303.

Schiel DR (1993). Experimental evaluation of commercial-scale enhancement of abalone *Haliotis iris* populations in New Zealand. *Marine Ecology Progress Series* 97: 167-181.

Schaffelke B, McCook L, Klumpp D, McKinnon D (1996). Seagrasses and seaweeds (macroalgae) of Exmouth Gulf: their distribution and importance in primary production. *Western Australian Research Activities*. 1994-1996: 27-29.

Somers I (1994). No resource, no fishery: prawns and their habitat. In, *Australia's Northern Prawn Fishery — the first 25 years*. edited by P.C. Pownall, NPF 25, Cleveland, Australia, pp. 49–65.

Staples DJ, Vance DJ, Heales DS 1985. Habitat requirements of juvenile penaeid prawns and their offshore fisheries. *In* Rothlisberg PC Hill BJ Staples DJ (eds). Second Australian National Prawn Seminar, NPS2, Cleveland Australia, pp. 47–54.

Wahl DH 1995. Effect of habitat selection and behaviour on vulnerability to predation of introduced fish. *Canadian Journal of Fisheries and Aquatic Science*. 52: 2312-2319.

a)

b)

Figure 5.1 **(a) Brown tiger prawn on seagrass Halophila spinulosa in the laboratory and (b) baler shell on sparse bed of the seagrasses H. spinulosa and Halodule uninervis.**

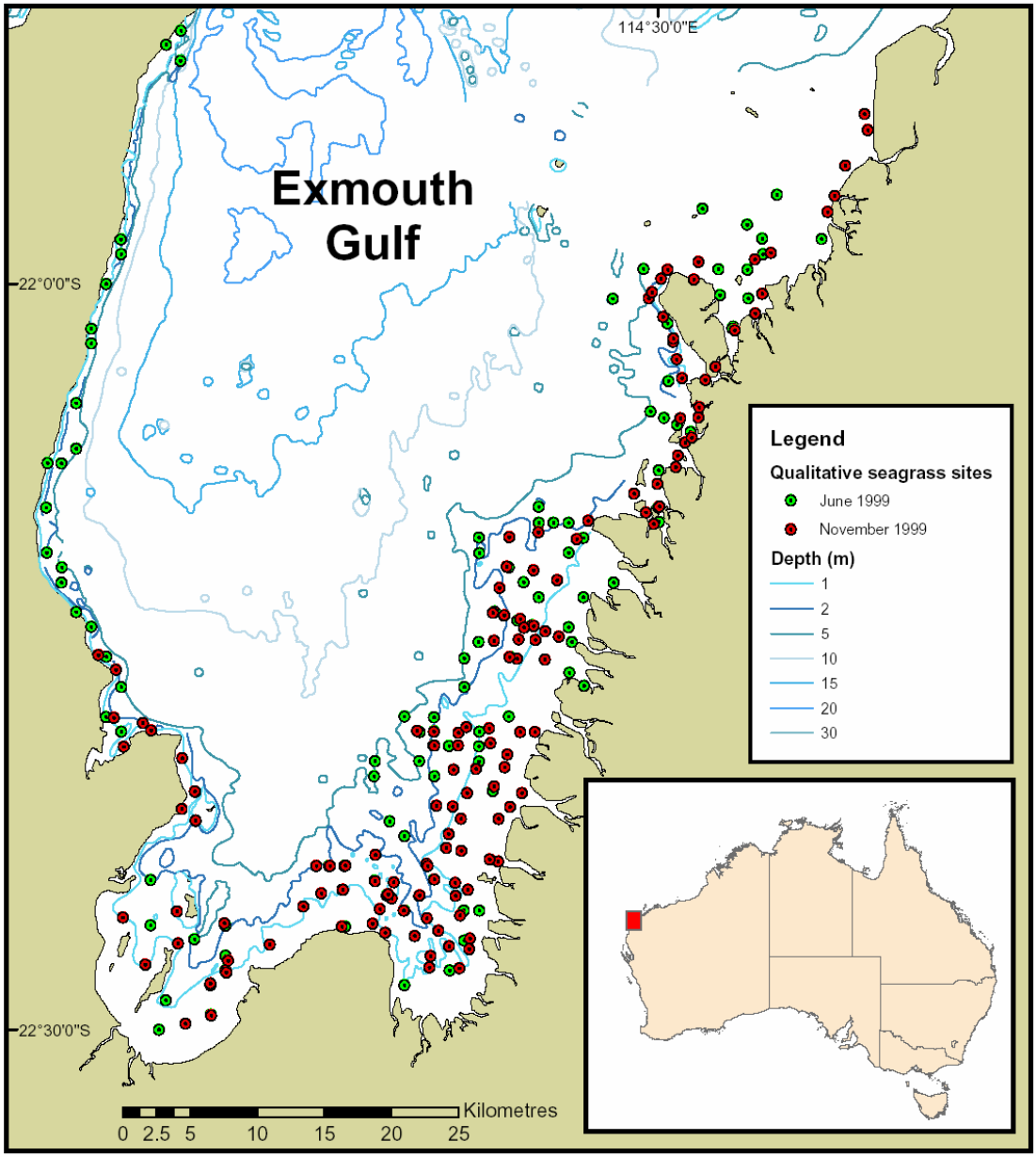


Figure 5.2 **Map of the location of habitat qualitative sampling sites (30 m swim transect) during the Gulf-wide habitat assessment in June and November in Exmouth Gulf.**

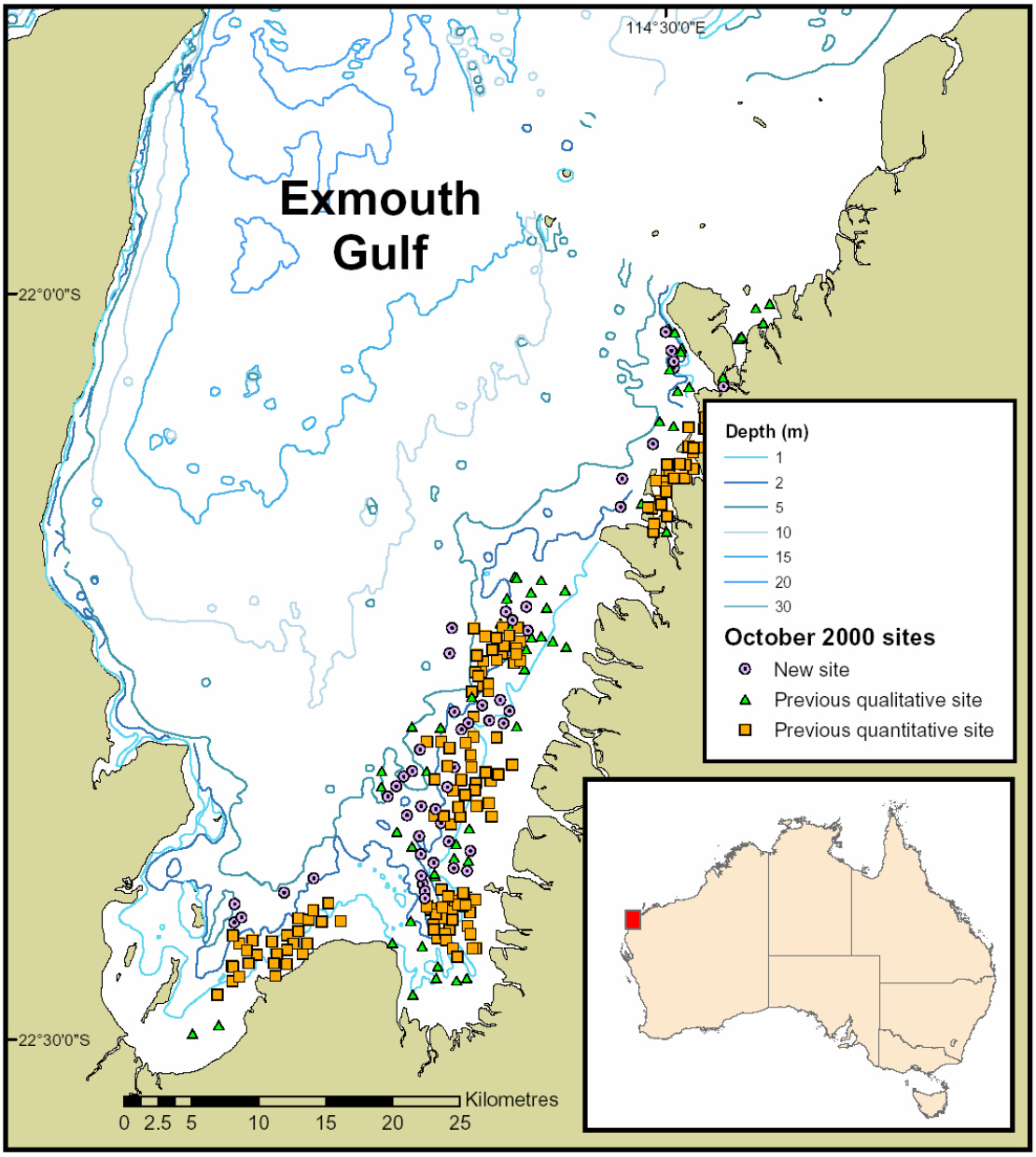


Figure 5.3 **Sample sites where quantitative habitat samples were taken in Exmouth Gulf during December 1999 (blue sites only) and October 2000 (all sites). Sites where quantitative samples were taken during 1999; sites where qualitative samples only had been taken previously; and new sites where no sampling had been undertaken, are shown in different colours.**

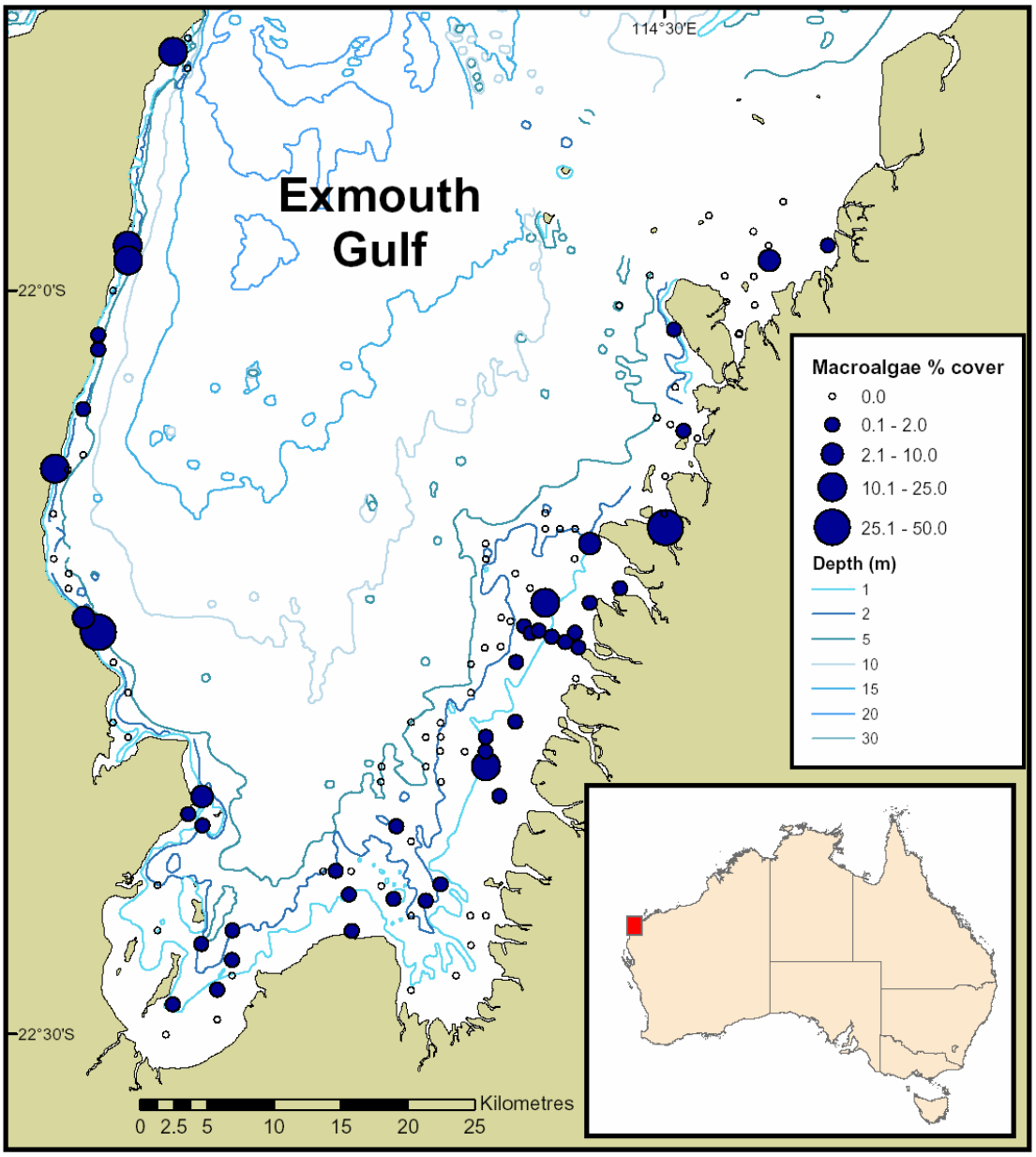


Figure 5.4 Percent cover of macroalgae found in Exmouth Gulf in June 1999.

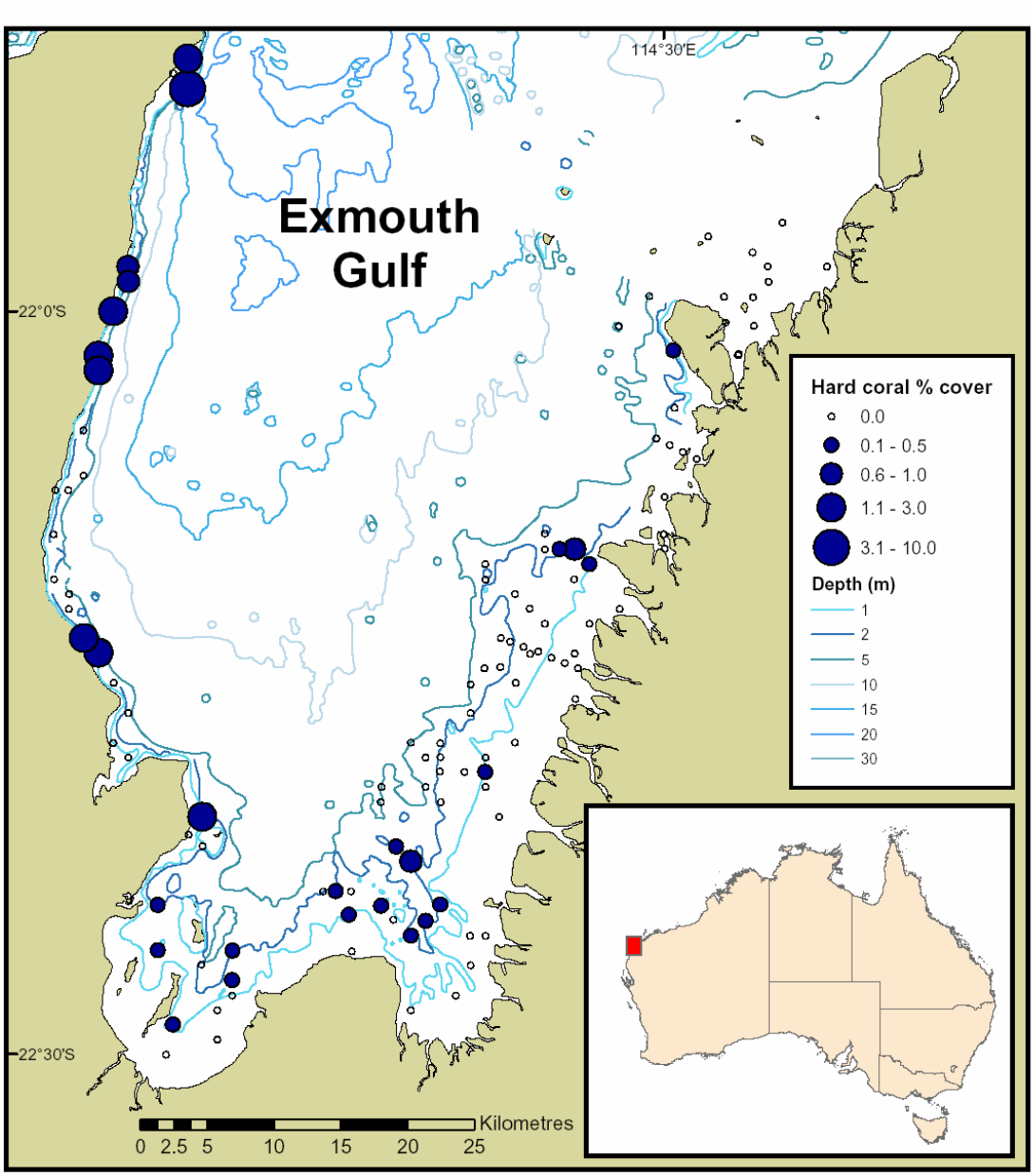


Figure 5.5 Percent cover of living hard coral found in Exmouth Gulf in June 1999.

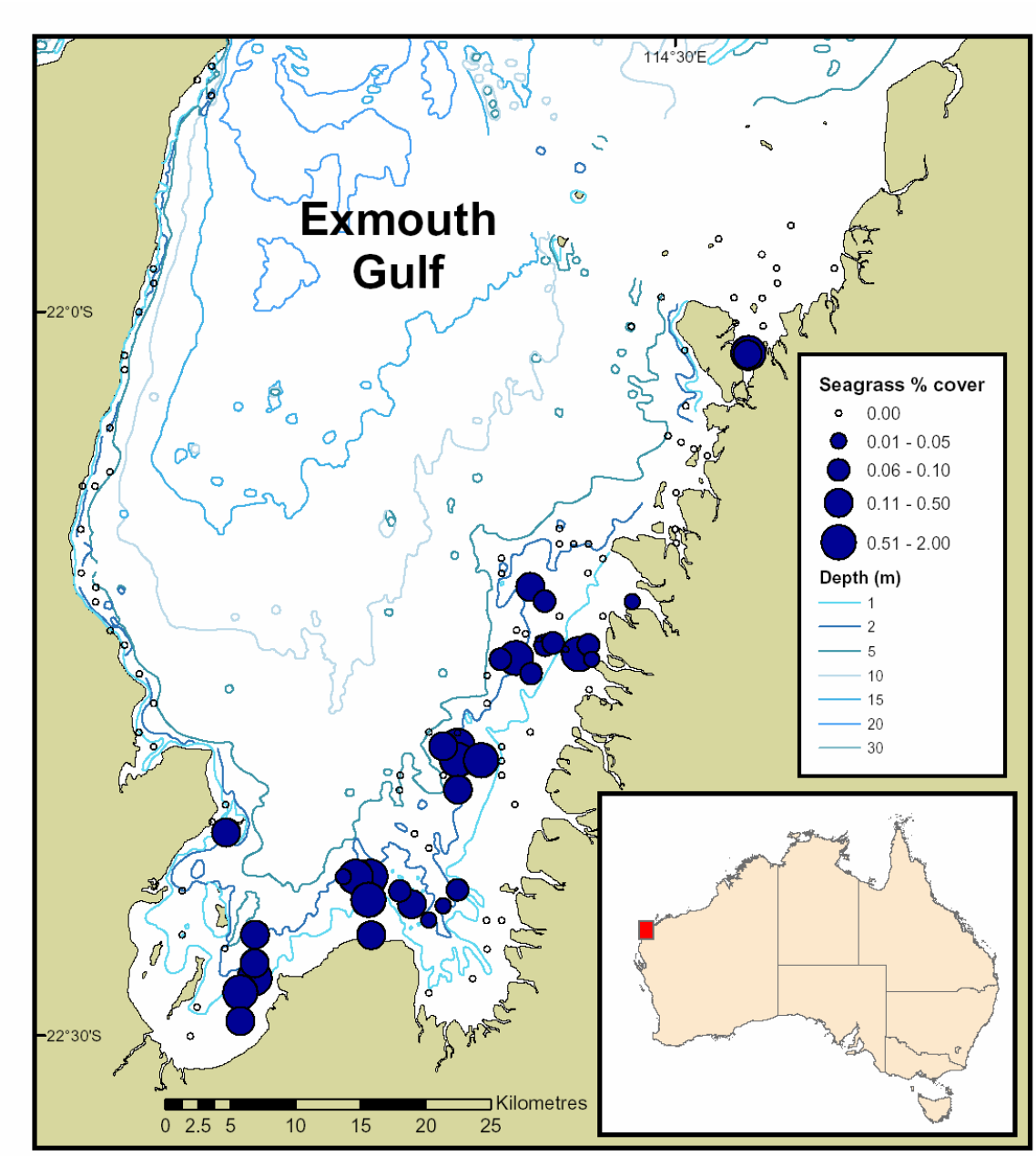


Figure 5.6 Percent cover of seagrass found in Exmouth Gulf in June 1999.

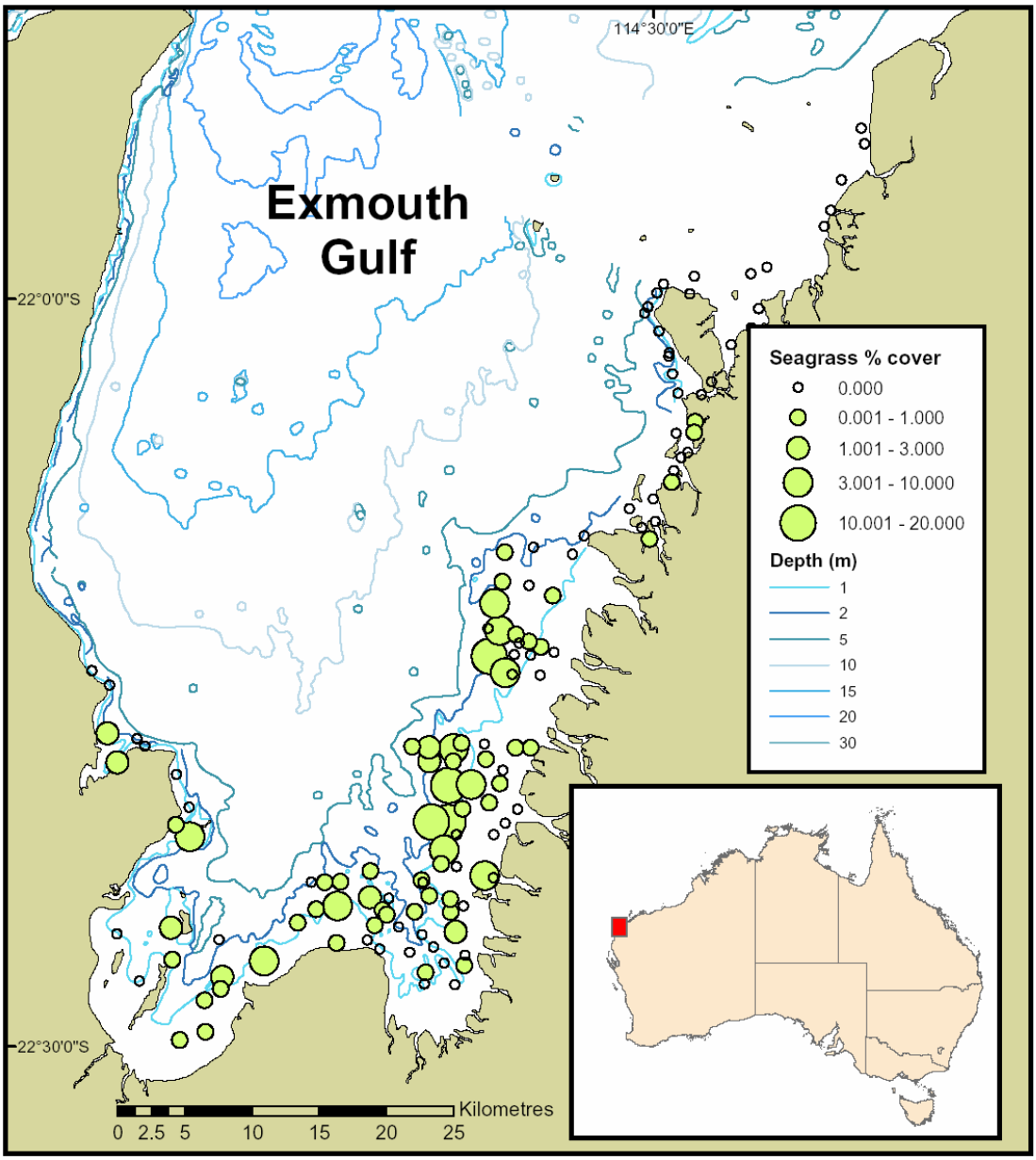


Figure 5.7 Percent cover of seagrass found in Exmouth Gulf in November 1999.

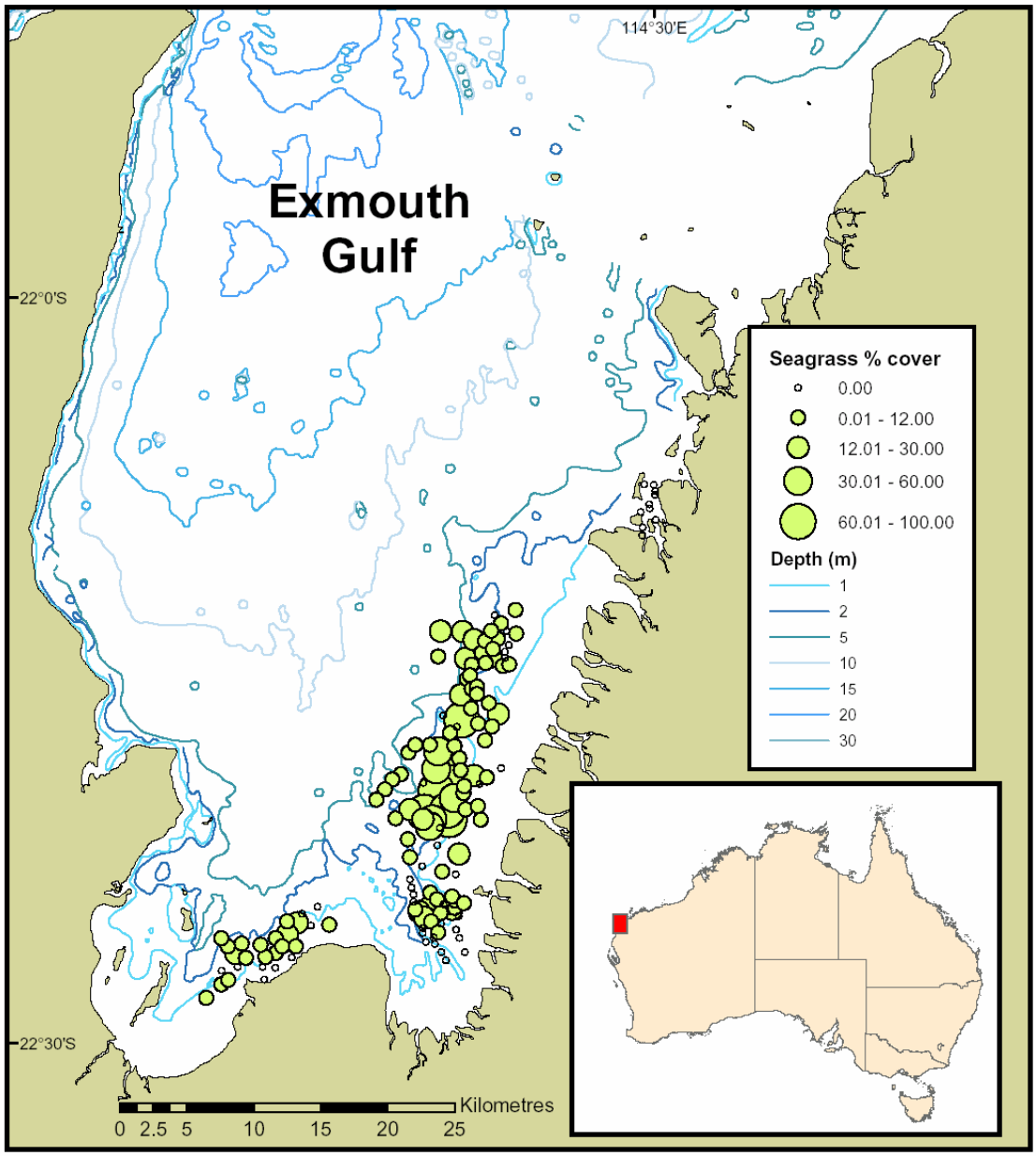


Figure 5.8 Percent cover of seagrass found in Exmouth gulf in November 2000.

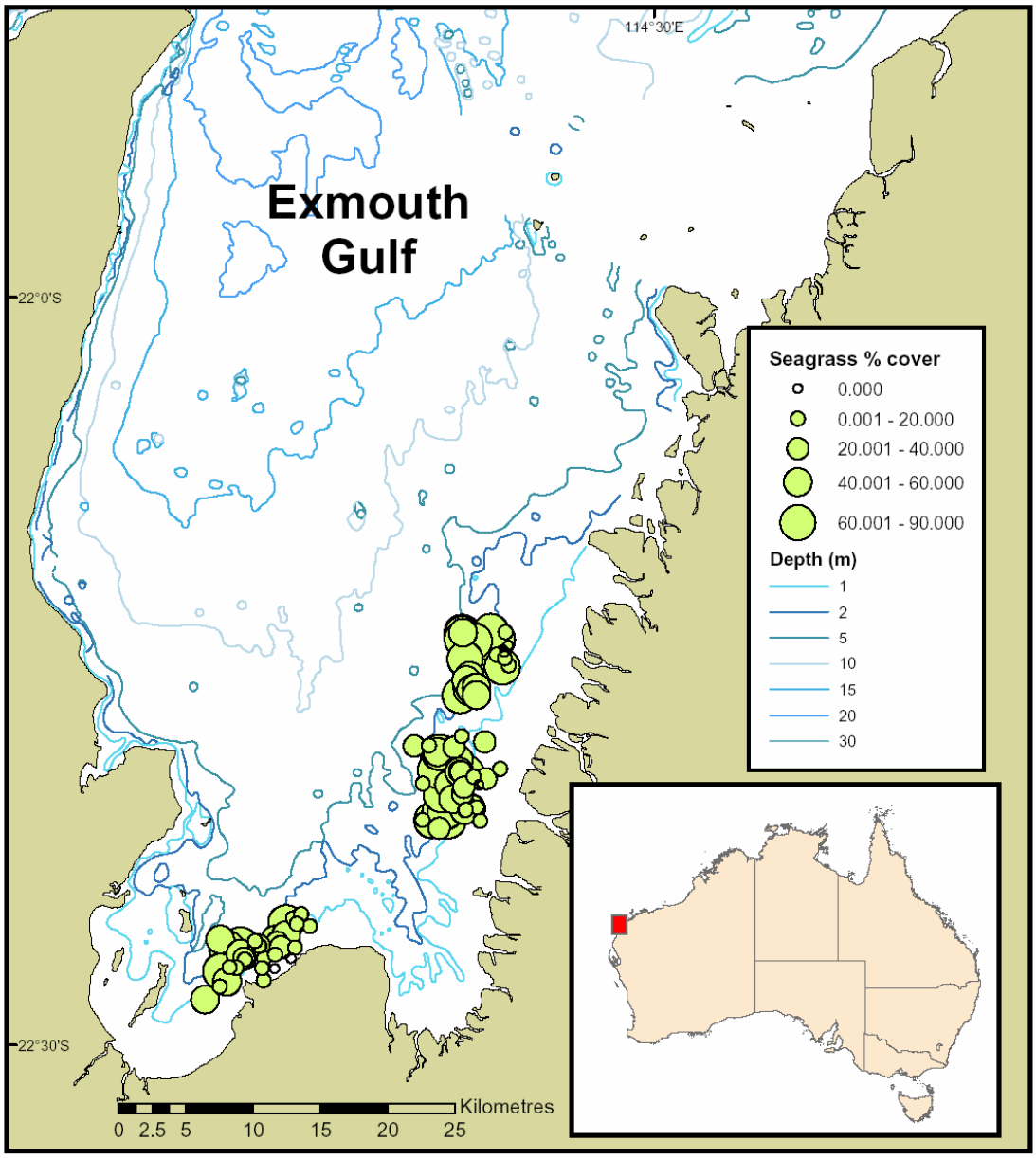


Figure 5.9 Percent cover of seagrass found in Exmouth Gulf in December 2001.

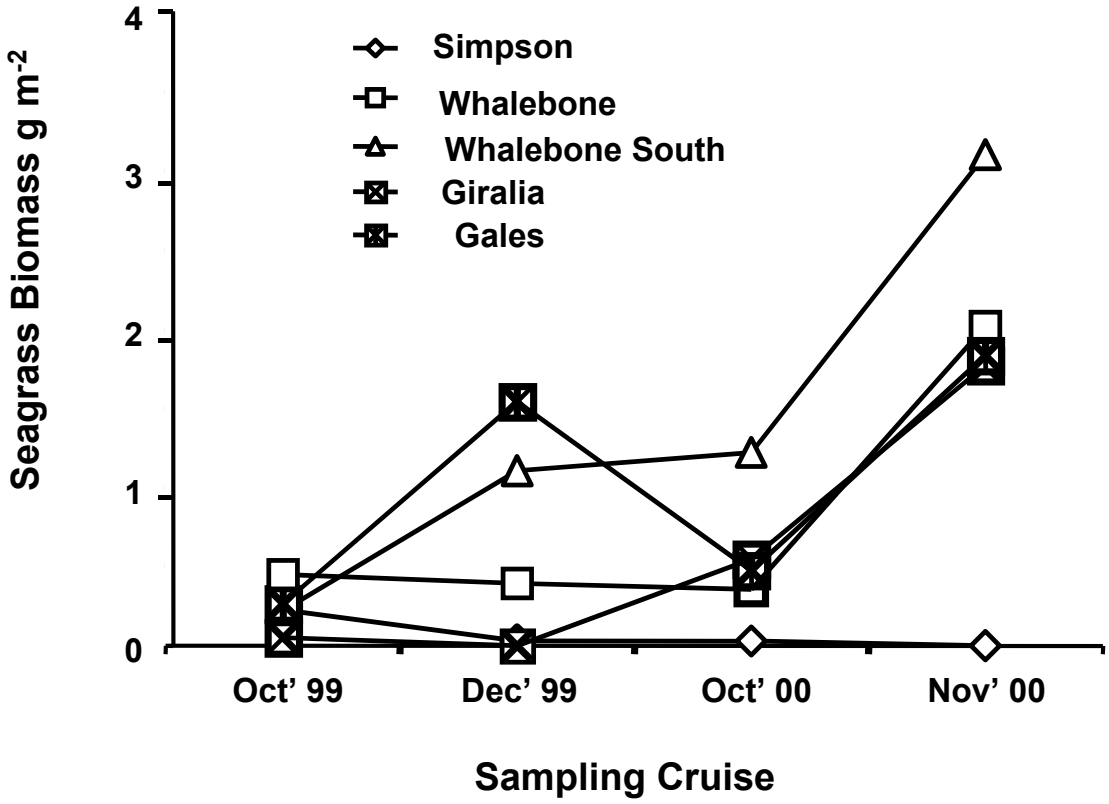
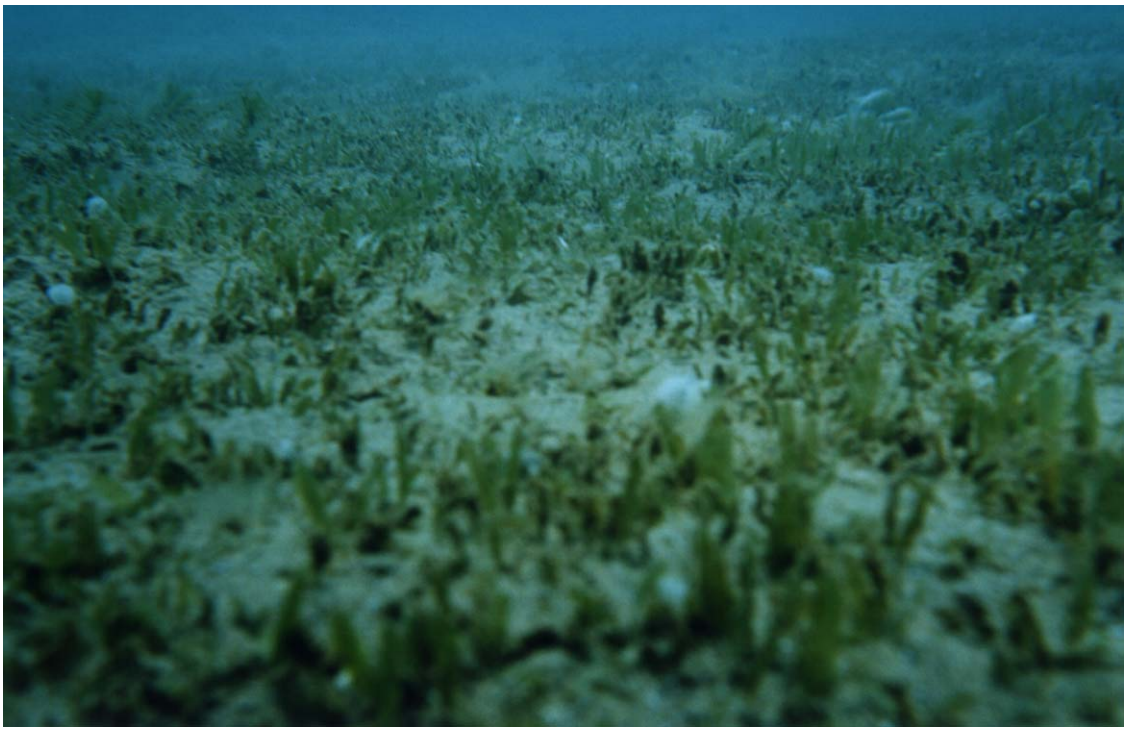


Figure 5.10 **Change in seagrass biomass at five 'regions' in Exmouth Gulf from October 1999 to December 2000**



a)

b)

Figure 5.11 (a) The seagrasses in Exmouth Gulf in late 2000 and (b) the seagrass in Exmouth Gulf in late 2001.

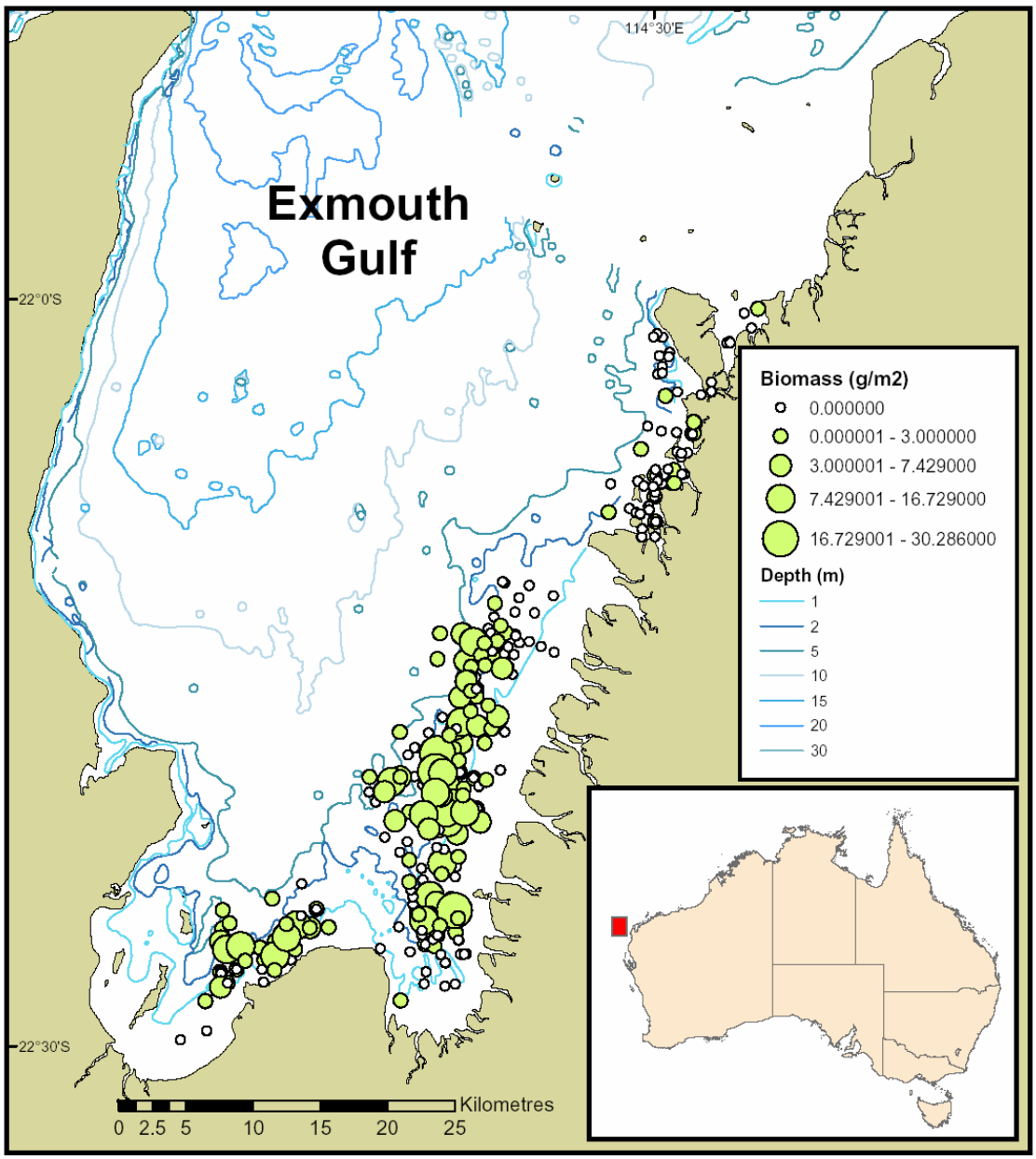


Figure 5.12 **Above-ground-biomass of seagrasses in Exmouth Gulf during November 2000.**

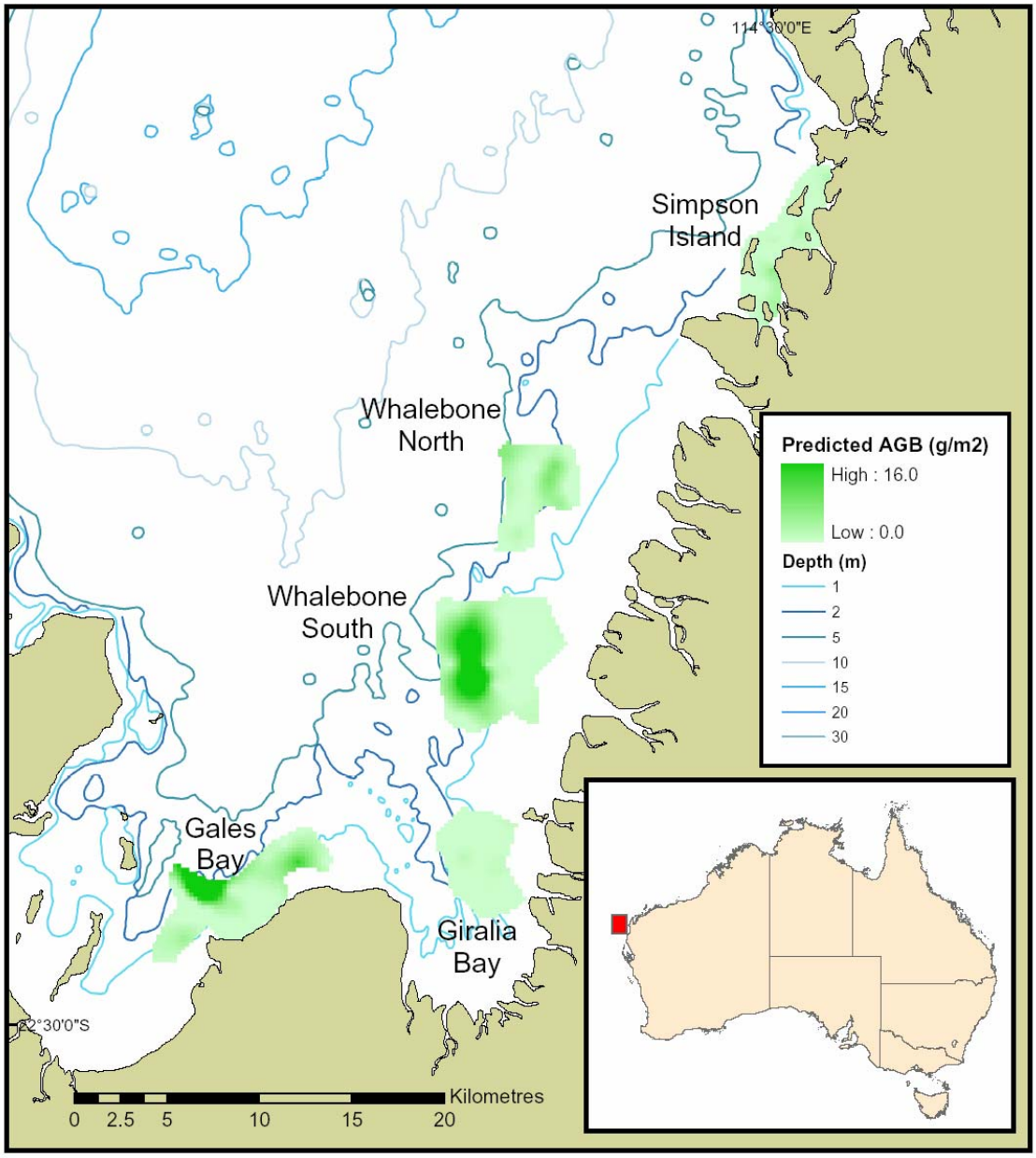


Figure 5.13 **Kriged interpolation of the distribution and above-ground biomass (AGB) of all seagrass species as sampled during November and December 1999.**

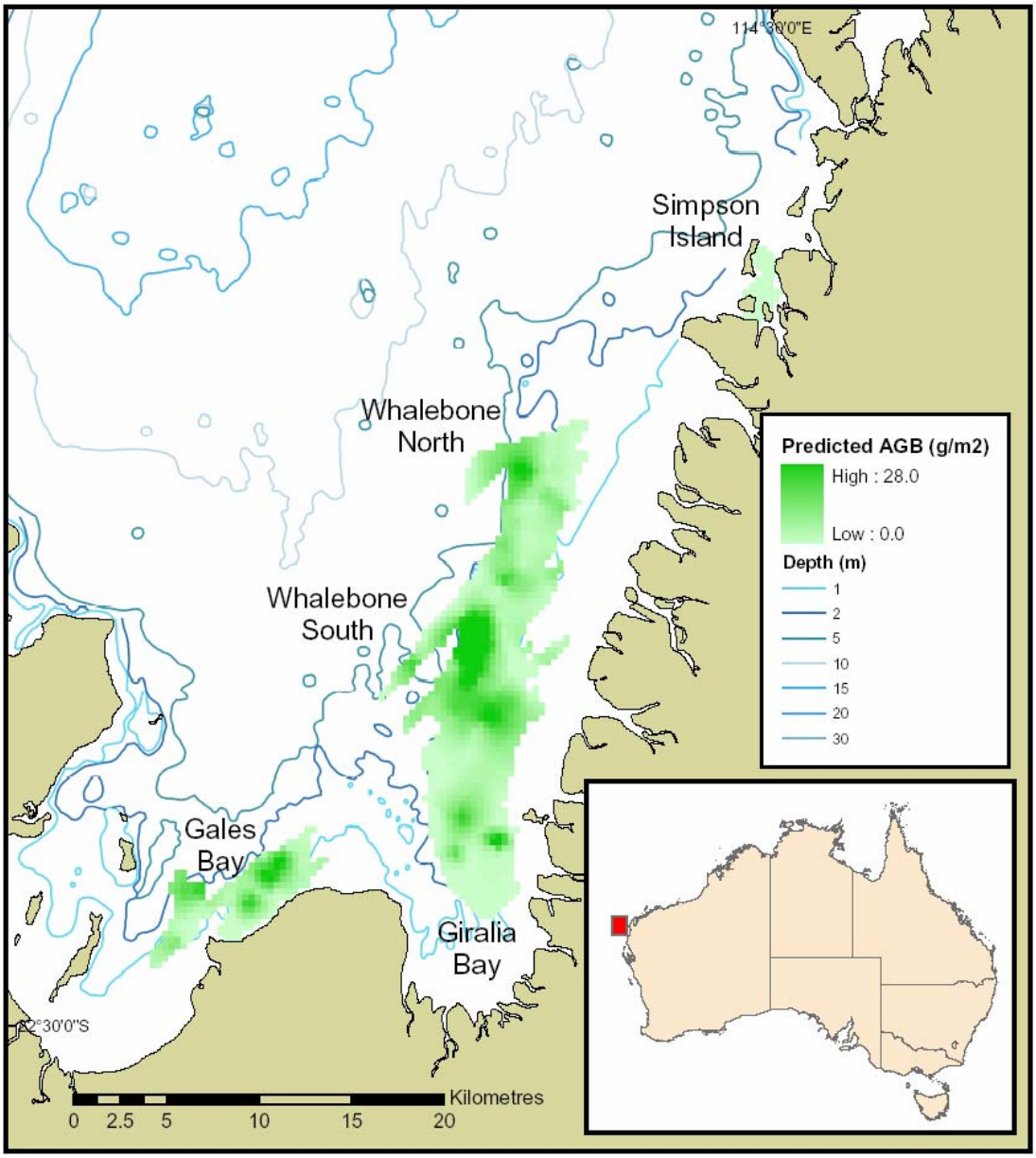


Figure 5.14 **Kriged interpolation of the distribution and above-ground biomass (AGB) of all seagrass species as sampled during November 2000.**

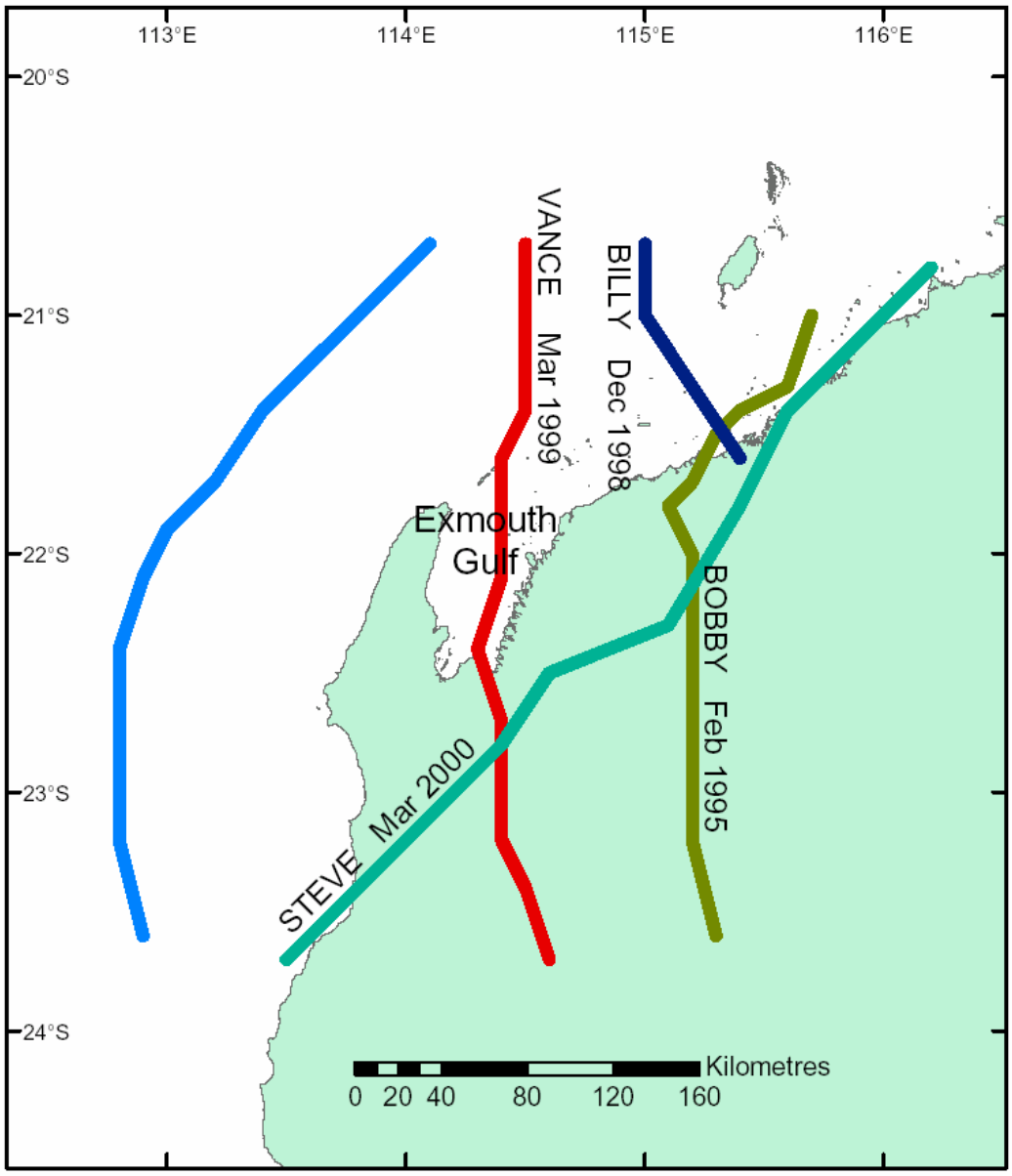


Figure 5.15 **Cyclone tracks in the region around Exmouth Gulf for the years between the McCook et al. (1995) seagrass and algae survey and our survey in June 1999.**

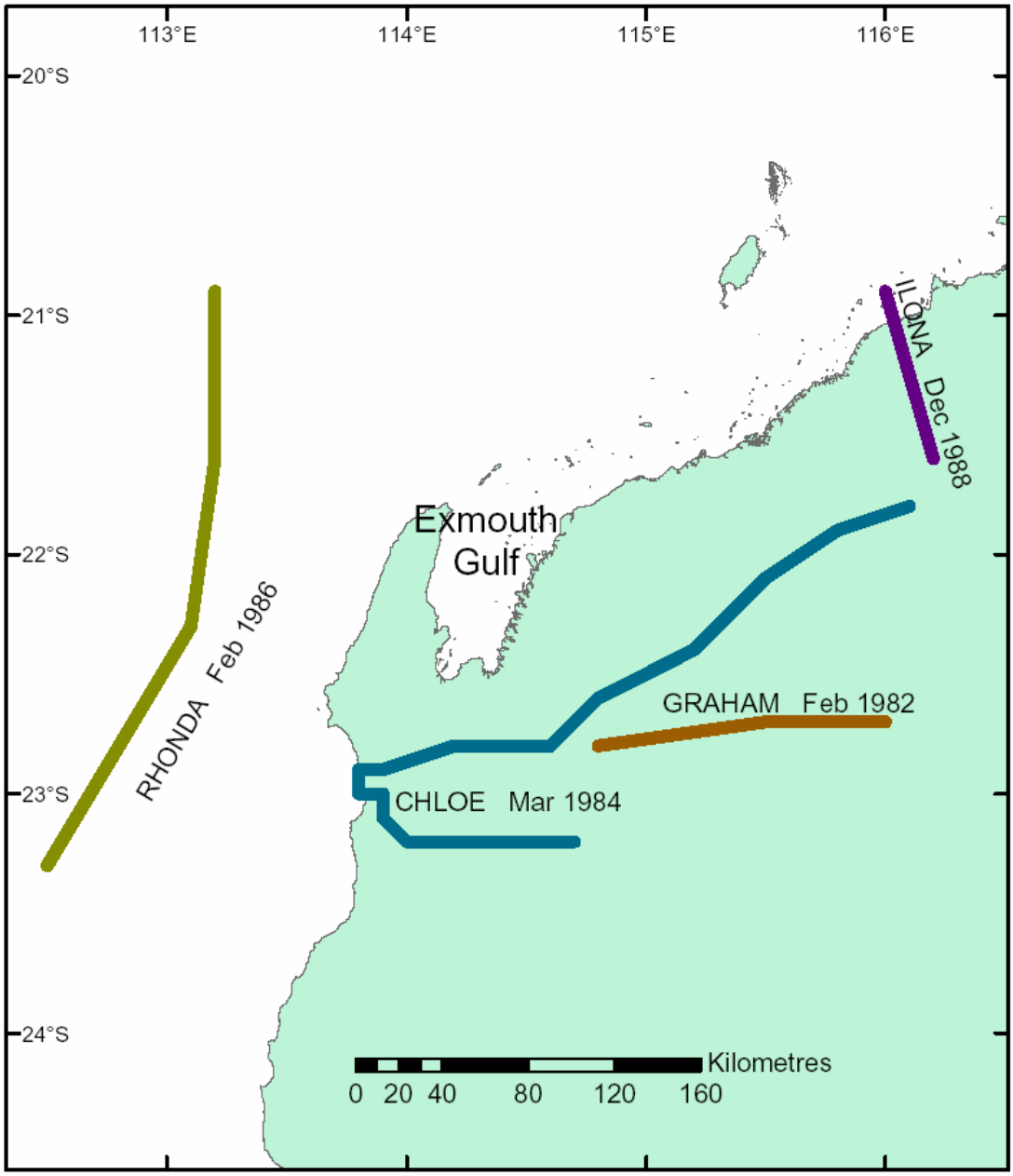


Figure 5.16 **Cyclone tracks in the region around Exmouth Gulf for the 12 years prior to the seagrass and algae survey in September 1994 (McCook et al. 1995).**

CHAPTER 6

ASSESSING POTENTIAL RELEASE SITES FOR THE ENHANCEMENT OF JUVENILE PRAWNS IN EXMOUTH GULF: SURVEYS OF JUVENILE PRAWNS AND POTENTIAL FISH PREDATORS

Rob Kenyon
Mick Haywood
Neil Loneragan
Fiona Manson

Summary

In March 1999, Cyclone Vance devastated the seagrasses (tiger prawn nursery habitat) of Exmouth Gulf. We began studying the distribution and abundance of juvenile tiger prawns in Exmouth Gulf benthic habitats in June 1999 to help identify potential release sites for an experimental fishery enhancement. These studies commenced just after cyclonic disturbance, when our studies suggest that seagrass habitats had declined. Studies of the benthic habitats in Exmouth Gulf, including the seagrass communities, are reported in Chapter 5.

In October and December 1999, juvenile prawns were sampled using small beam trawls (2 and 12 mm mesh nets). The abundance of juvenile tiger prawns was very low (<1 prawn 100 m^{-2}) which seemed to reflect lack of suitable seagrass habitat along eastern Exmouth Gulf (average seagrass cover = 1.2%) to support them. In October and November 2000, juvenile tiger prawns were more abundant than in 1999, reflecting an increase in seagrass cover (average cover = 10.3%). However, mean catch rates remained low (< 3 prawns 100 m^{-2}) relative to their abundance on undisturbed seagrasses elsewhere in tropical and sub-tropical Australia. No juvenile tiger prawns were found on un-vegetated habitats.

Despite a significant increase in the abundance and extent of seagrass habitat by December 2001 (average cover = 41.9%), juvenile tiger prawn catches continued to be low (< 2 prawns 100 m^{-2}). Their abundance was low compared to observed abundance in other high-biomass seagrasses e.g. 'embayment' seagrass communities. However, catch rates were similar to those in some other undisturbed high-biomass 'open coastline' seagrasses elsewhere (e.g. 3.5 prawns 100 m^{-2}).

Juvenile tiger prawns were most abundant in the east and south-east of Exmouth Gulf, to the south of Whalebone Island and adjacent to Sandalwood Peninsula in Gales Bay. In both 1999 and 2000, prawn fish-predators were least abundant at Whalebone South. The most likely sites for the experimental release of prawns during enhancement are among the most dense, broad-leaved seagrasses in eastern Exmouth Gulf, where the natural abundance of tiger prawns is high and the abundance of their predators is low. Due to ongoing ecological changes in Exmouth Gulf following cyclonic disturbance, tiger prawn and fish-predator populations need to be re-surveyed prior to a future release.

Commercial tiger prawn landings in 2000, the year after the cyclone, were 82 tonnes; well below the 200 to 680 t range in the previous 10 years of the fishery, and one of the lowest catches on record (see Chapter 7). The low catch in 2000 matched the very low abundance of juvenile prawns the previous year (1999) and came despite the above average spawning index for the fishery in 1999. The commercial landings of tiger prawns in 2001 increased to 208 t, and the commercial landings are predicted to increase to around 330 t in 2002.

In three years, the tiger prawn habitats and juvenile recruitment have recovered to a significant extent, supporting the increase in commercial landings in Exmouth Gulf. The cyclonic destruction of the Exmouth Gulf seagrass community, followed by a decline in the juvenile tiger prawn population and fishery landings, and then by an increase in juvenile prawns and catch as the seagrasses recolonise, reflects a similar response to cyclonic disturbance in the Gulf of Carpentaria in the 1980's. However, in the latter case the recovery of seagrass took over 10 years, compared with only three years for Exmouth Gulf.

6.1 Introduction

6.1.1 Tiger prawns in Exmouth Gulf

Our approach to identifying suitable release sites for juvenile brown tiger prawns (*Penaeus esculentus*) was conducted in three stages: a broad scale survey of benthic habitats; a survey of the distribution of juvenile tigers prawns; and a survey of potential prawn predators. The initial broad-scale study was completed to describe the distribution and quality of benthic habitats in Exmouth Gulf (Chapter 5). We then focused on investigating the distribution of small tiger prawns within the main macrophyte habitats that were found (this Chapter). This was done because the distribution of the wild population can be used as the first step in finding the optimal release sites for a species during stock enhancement (Munro and Bell 1995, Leber 1999). The assessment of the potential predation threats to juvenile tiger prawns was carried out by sampling fish in selected regions because high abundances of predators can lead to high post-release mortality.

Research in tropical and sub-tropical Australia has shown that inshore seagrass and marco-algal communities form critical nursery habitat for juvenile tiger prawns (Young & Carpenter 1977, Young 1978, Staples *et al*. 1985, Loneragan *et al*. 1994, 1998). Furthermore, studies on the effects of increasing prawn density on growth, suggest that in the absence of predators, high biomass seagrass beds may be able to support larger densities of prawns than are typically found on them i.e. they may be recruitment limited, rather than limited by habitat 'carrying capacity' (Loneragan *et al*. 2001).

6.1.2 Fish in Exmouth Gulf

Predation by fish has been identified as a major determinant of prawn abundance in tropical inshore habitats (Salini *et al*. 1990, Brewer *et al*. 1995, Haywood *et al*. 1998), and is also an important factor responsible for determining the success or failure of many stock enhancement programs (Furuta 1996, Murai *et al*. 1998). In many cases, it seems that hatchery-reared juveniles of many species are behaviourally naïve or have physical deformities that render them less able to avoid fish predators than their wild counterparts (Stoner and Davis 1994, Furuta 1996). In addition, stress induced by transport and release has been implicated in the short-term inability to avoid predators (Schreck *et al*. 1989, Olla and Davis 1989, Olla *et al*. 1994, Olla *et al.* 1995).

In this Chapter, we describe the distribution of juvenile tiger prawns in Exmouth Gulf. We also characterise the distribution of the potential fish predators in each region at the time of year when releases are most likely to occur.

6.2 Methods

6.2.1 Prawn samples

6.2.1.1 Distribution of juvenile tiger prawns in Exmouth Gulf

In 1999 and 2000, juvenile brown tiger prawns were sampled in each of the five regions where benthic macrophytes were sampled (Figure 6.1). In each region, we sampled prawns

using a 1 x 0.5 m beam trawl (2 mm mesh net with a 1 mm mesh codend) and a 1.5 x 0.5 m beam trawl (12 mm mesh net) (Tables 6.1, 6.2). Samples were taken on the spring tides during two cruises in both 1999 and 2000 in the following five regions:–

- Behind Simpson/Burnside Islands (three sites in 1999 and six sites in 2000);
- At Whalebone North (two sites in1999 and four sites in 2000);
- At Whalebone South (two sites in 1999 and four sites in 2000)
- At Giralia Bay (two sites in 1999 and four sites in 2000);
- At Gales Bay (two sites in 1999 and four sites in 2000).

During the first cruise each year, trawl samples were taken in all five regions. However, during the second cruise, trawl samples were only taken at three regions (Simpson Island, Whalebone South and Gales Bay) to reduce effort and enable the gill netting to sample fish predators.

Table 6.1 **Summary of surveys of juvenile prawns and fish completed in Exmouth Gulf between June 1999 and December 2001. In December 1999, two trawls were made in the southern-central Exmouth Gulf and one was made on a *Sargassum* bed. In December 2001, 4 trawls were made with the trawler the FV *Jurabi* K, outside our sampling area. — = not sampled.**

Month and Year	Number of samples	
	Trawls	Fish
1999		
June	8 (Pilot)	—
November	48	—
December	31 *	48 net sets
2000		
October	57	—
November	52	48 net sets
2001		—
December	45	
Total	241 Trawls	96 net sets

* 5 scoop net samples were also taken in *Sargassum* beds

At each site, two trawls in 1999 (at each of two sites) and one trawl in 2000 (at each of four sites), were made with both the 1 m beam trawl and the 1.5 m beam trawl. Samples were sorted for prawns on deck (12 mm mesh trawl) or bagged (2 mm mesh trawl), placed on ice and taken to the laboratory to identify and measure. The salinity and temperature of the surface water, secchi depth and latitude/longitude were recorded at the start of each trawl. Latitude and longitude were recorded again at the conclusion of the trawl. Trawls were 200 m long (about 6 min duration) in all areas, except behind Simpson and Burnside Islands where 100 m trawls were completed.

The exception to the sample design described above, was the June 1999 cruise where we made eight pilot trawls only. Four trawls were made at each of two sites, one near Whalebone Island (eastern Exmouth Gulf) and one in north-east Gales Bay, adjacent to the Sandalwood Peninsula (southern Exmouth Gulf). These sites were selected because very sparse seagrass was found in the vicinity (the highest cover of seagrass we found at the time). We used both the 1.5 m and 1 m beam trawls, with two trawls of each net per site.

In December 1999, we also trawled a site in the deeper central zone of Exmouth Gulf, south of Whalebone Island (12 m deep; 12° 19.0’ S, 114° 17.0’ E) to investigate juvenile prawn

abundance in deeper water. Two trawls were done at this location, one with the 1 x 0.5 m beam trawl and one with the 1.5 m x 0.5 m beam trawl with tickler chains (12 min duration, length of trawl ~400 m). To search for tiger prawns in habitats other than seagrass, we made one trawl and scoop-netted macroalgae habitats (dominated by *Sargassum*) in an attempt to catch postlarvae and juveniles. Scoop net samples were collected by a diver dragging a hand-held scoop net (25 x 18 cm, 2 mm mesh) through clumps of *Sargassum* for approximately 15 min at each site.

In 2000, following discussions at the Project Review Workshop in Perth, new trawl sites were selected in a deeper zone adjacent to, and offshore from, the five original trawl-regions, as well as in the shallow depth zone behind Tent Island (Table 6.2, Figure 6.1). At the 'offshore' sites, one trawl was made with the 1.5 m/12 mm net beam trawl. Our decision to trawl four sites in each region in 2000 (instead of two as in 1999) was also made following the Workshop; three new sites were chosen behind Simpson and Burnside Islands and two new sites were chosen in the other four regions.

Table 6.2 **A summary of the number of inshore and offshore trawls and gill net sets that were taken in the main regions of Exmouth Gulf in June, October and December 1999, and October/November 2000. Some trawls at some sites and times were not made due to poor weather conditions or the loss of trawl-gear. Forty one trawls made in 2001 are documented in the text.**

Region	Trawls			Gill nets	
	1999	2000		1999	2000
		Inshore	Offshore		
Tent Island	Nil	4 sites x 1.5/12 net	Nil	Nil	Nil
Simpson/ Burnside Island	3 sites x 2 nets x 2 trawls x 2 occasions	6 sites x 2 nets x 2 occasions	4 sites x 1.5/12 net	2 sets of 3",4",5",6" nets on 2 occasions	2 sets of 3",4",5",6" nets x 2 occasions
Whalebone North	2 sites x 2 nets x 2 trawls	4 sites x 2 nets	4 sites x 1.5/12 net	Nil	Nil
Whalebone South	2 sites x 2 nets x 2 trawls x 2 occasions	4 sites x 2 (or 1) nets x 3 occasions	4 sites x 1.5/12 net x 2 occasions	2 sets of 3",4",5",6" nets x 2 occasions	2 sets of 3",4",5",6" nets x 2 occasions
Giralia Bay	2 sites x 3 (or 2) nets x 2 trawls	4 sites x 2 nets	4 sites x 1.5/12 net	Nil	Nil
Gales Bay	2 sites x 3 (or 2) nets x 2 trawls x 2 occasions	4 sites x 2 nets x 2 occasions	4 sites x 1.5/12 net x 2 occasions	2 sets of 3",4",5",6" nets x 2 occasions	2 sets of 3",4",5",6" nets x 2 occasions
Total	76 trawls	80 trawls	28 trawls	48 sets	48 sets

6.2.1.2 Comparing effectiveness of trawl nets for large juvenile tiger prawns

Additional sampling was completed in 2001, to compare the catches of juveniles tiger prawns from the 1.5 m beam trawl with those from a small otter trawl (as used by the Department of Fisheries WA) and a commercial try-net. The dimensions of the three nets were:

- The 1.5m CSIRO beam trawl with 12 mm mesh and the 'rope-tickler' configuration (used in 1999/2000);
- The 'Fisheries WA' otter trawl - an otter trawl with a 5 m foot-rope length (2.5 m sweep) and a 12 mm mesh codend; and
- The 5 m foot-rope otter trawl (3 m sweep) used as the try-gear on the Kailis' vessel FV JURABI-K (with a 12 mm internal codend insert).

Unfortunately, the Kailis' try-gear was impossible to deploy by hand, so it could only be used from the *JURABI-K* in water about 6 m deep. Thus, we used the try-gear trawl on or about one to four of the 'offshore' sites per region that were trawled in 2000 (see below). The other two nets were used from the *Advance-K* and we were able to trawl at the 'inshore' sites that were trawled in 1999/2000.

The sites chosen for the comparison of catches from the three nets were located in the following regions:

- **Whalebone North** — two inshore sites with both the WA otter and 1.5/12 beam; four offshore sites with the Kailis' try-gear;
- **Whalebone South** — two inshore sites with both the WA otter and the 1.5/12 beam; four offshore sites with the Kailis' try-gear (with day/night comparisons);
- **Gales Bay** — one inshore site with both the WA otter and the 1.5/12 beam; one offshore site with the Kailis' try-gear.

Trawls were made for 500 m (0.28 nautical mile) which took about 15 minutes. We collected salinity, temperature and start-and-finish latitude/longitude at each trawl site.

The *JURABI-K* also made four trawls at some sites that are sampled by the Department of Fisheries WA each year as part of their recruitment surveys, adjacent to and further offshore from our CSIRO juvenile prawn sites. These trawls were 1500 m long and the start and finish latitude and longitude were recorded.

6.2.1.3 Analysis of prawn abundance data

We calculated the mean catches of *P. esculentus* postlarvae and juveniles ($100\ m^{-2}$) for each year and region. We used a two-way ANOVA in SAS (SAS Institute, 2000) to test for differences in the abundance of tiger prawns between 1999 and 2000 and among the five regions. The data were log-transformed (x+1.5) to normalise the data and ensure that the variances were homogeneous.

6.2.2 Gill net samples

Potential predators of prawns were sampled in three regions identified from the initial habitat surveys (Chapter 5) as having the greatest potential as release areas. The sites were shallow (< 2.5 m depth), inshore areas at Simpson Island, an area to the south of

Whalebone Island (Whalebone South) and the eastern side of Gales Bay (Figure 6.2). Fish were sampled by setting a suite of gill nets: 3", 4", 5", and 6" stretch mesh nets, each 60 m long with a drop ranging from 2 to 3 m (and therefore fished most of the water column).

The nets fished during the night as brown tiger prawns are active at night and so are more likely to be caught by predators at this time (Haywood *et al*. 1998). Gill nets were set at dusk and retrieved just after dawn. Fish sampling was conducted on two occasions during the project: in December 1999 and November 2000 (Tables 6.1, 6.2). During each of these months, fish were sampled twice in each area; once on the neap-spring tides and once on the spring-neap tides. Fishing effort was equal at all sites and sampling periods. The nets were cleared after dark, about 3 h after setting, and after dawn each day. All fish were identified to species, measured and weighed. Guts were removed from all fish that were not herbivores, bagged and frozen for laboratory analysis. In the laboratory, the gut contents were sorted under a binocular microscope and the components were identified as far as possible. The gut contents were then dried to a constant weight in an oven at 60 °C.

Catch rates for each species and night were calculated and expressed as g/h/200 m of gill net. The fish species assemblages caught at each site each year were characterised by displaying their relative similarity to each other in a Non-Metric Multidimensional Scaling (MDS) plot. This was constructed by square-root transforming the fish species catch rates, calculating the Bray-Curtis similarities between each sample. The MDS plot was produced using PRIMER software (PRIMER-E 2001).

6.2.3 Prawn Predation Index

In selecting a site for releasing juvenile penaeids, one of the important considerations is the impact of predators, but merely identifying sites having the lowest catches of potential predators is not sufficient. The proportion of a fish's diet that is penaeid prawns will depend upon the species of fish, so the impact of the predator assemblage at a particular site will be determined by a combination of the catch rates of the different predator species and the proportion of their diet comprised of penaeids. For each of the three sites, we calculated a Prawn Predation Index (PPI) to incorporate these two aspects.

The PPI is simply the product of the catch rate of each predator species (psp) at each site and the proportion of that species' diet that is penaeid (csp, taken from the literature) summed (Equation 1).

$$PPI_{site} = \sum_{sp_1}^{sp_n} p_{sp_1} \times c_{sp_1} \quad \text{.....Equation 1}$$

6.2.4 Fish from prawn recruitment surveys

Samples of fish were obtained opportunistically from the prawn recruitment surveys conducted by the Department of Fisheries. These fish were caught using commercial trawls (4.5 fathom Florida Flyer; 45 mm mesh) from areas offshore from our gill net

sites. Samples were obtained from 15 trawls done between October 2000 and March 2001. These fish and the contents of their guts were processed as described previously.

The recruitment surveys in March and April each year are a part of a series of annual trawl-surveys made by the Department of Fisheries WA as part of their management measures for the Exmouth Gulf commercial fishery. As well as recruitment to the fishery, the Department of Fisheries measures a spawning index during August to October each year to estimate the level of spawning stock in Exmouth Gulf (see Chapter 7). These indices have been compared and contrasted with commercial landings and data collected during this project (seagrass percent cover and juvenile tiger prawn catch) to gain further understanding of the dynamics of the Exmouth Gulf ecosystem.

6.3 Results

6.3.1 Prawn abundance

6.3.1.1 Distribution and abundance of tiger prawns in Exmouth Gulf

During June 1999, about three months after the passage of Cyclone Vance through Exmouth Gulf, no juvenile tiger prawns were caught in the beam trawls at either the Whalebone Island or the Gales Bay sites. Two postlarval tiger prawns (< 2.9 mm carapace length (CL)) were caught at the Gales Bay site. A few large king prawns (*Penaeus latisulcatus*), as well as several *Metapenaeus*, *Parapenaeopsis* and *Metapenaeopsis* prawns were caught at both sites.

During both November and December 1999, about nine months after Cyclone Vance, postlarval and juvenile tiger prawns were caught in all five regions in the eastern and southern Exmouth Gulf (Figure 6.3). However, the catches of postlarval (< 6 prawns 100 m^{-2}) and juvenile (< 1 prawns 100 m^{-2}) brown tiger prawns were very low at all sites (Figure 6.3). They made up less than 7% of the catch of juvenile penaeid prawns (Table 6.3). Most of the tiger prawns caught were 1-2 mm CL postlarvae (113 out of 129 prawns). Sixteen juveniles were caught – 11 on the Sandalwood Peninsula, three at Whalebone South and two at Whalebone North. The highest catches of the postlarvae were made at sites behind Simpson and Burnside Islands, whereas the highest catches of juvenile tiger prawns were made at Gales Bay (Figure 6.3). Three tiger prawn postlarvae were scoop-netted from the *Sargassum* habitats in December 1999.

During October and November 2000, about 21 months after Cyclone Vance, tiger prawns remained patchily distributed among the regions in Exmouth Gulf. Catches of postlarval (<5 prawns 100 m^{-2}) and juvenile (<3 prawns 100 m^{-2}) tiger prawns remained low at all sites. However, the proportion of the catch made up of juvenile tiger prawns had increased to about 20% (Table 6.3). The highest catches of juvenile tiger prawns were found at Simpson Island and Gales Bay and the lowest catches at Whalebone South (Figure 6.3, 6.4). No tiger prawns (or king prawns) were caught in the bay behind Tent Island. Behind Tent Island (< 1 m deep), several greasyback prawns of the genus *Metapenaeus* were caught.

During the additional sampling in 2001, we caught 34 juvenile tiger prawns in the three regions (Whalebone North, Whalebone South, Gales Bay) that were trawled with the two 12 mm mesh nets (11 trawls each with both the WA otter trawl and the 12 mm mesh beam trawl). In 2001, the catches of juvenile tiger prawns were greater at Whalebone South (0.23 ± 0.12 (mean ± 1 SE) prawns 100 m^{-2}) than at Gales Bay (0.16 ± 0.09 prawns 100 m^{-2}) or Whalebone North (0.06 ± 0.04 prawns 100 m^{-2}) (Figure 6.5). In past years, catches were greatest at Gales Bay and least at both regions near Whalebone Island.

Table 6.3 **Percent composition by species of the juvenile prawn catch in Exmouth Gulf in a) the 2 mm mesh beam trawl, b) the 12 mm mesh beam trawl and the 12 mm mesh beam and otter trawls combined for 2001 (last column). Oct. = October; Nov. = November; Dec. = December. - = not sampled**

Species	Year and Month					
	1999		2000		2001	
	Oct.	Dec.	Oct.	Nov.	Dec.	Combined nets (Dec.)
a) 2 mm mesh trawl						
P. esculentus	2.6	6.7	15.6	19.8	-	-
P. latisulcatus	58.8	50.0	46.5	42.0	-	-
Metapenaeus endeavouri	7.9	1.7	17.0	14.7	-	-
Metapenaeus sp.	30.7	41.7	20.8	23.6	-	-
Total number of prawns	**114**	**120**	**288**	**157**		
#Trawls	22	15	23	18		
b) 12 mm mesh trawl						
P. esculentus	0.8	2.8	0.6	12.2	6.8	16.5
P. latisulcatus	48.9	51.4	21.9	52.4	75.7	58.7
M. endeavouri	1.9	0	20.4	4.9	8.7	20.2
Metapenaeus sp.	48.5	45.8	57.1	30.5	8.7	4.6
Total number of prawns	**260**	**107**	**338**	**164**	**103**	**218**
# Trawls	26	13	34	34	11	22

A between-year comparison of catches from the trawl that was used during both years (the 12 mm mesh beam trawl) suggests that the density of juvenile tiger prawns remains low in 2001, despite the increase in areal extent, biomass and vertical structure of the seagrass habitat. Less than 0.1 juvenile tiger prawns 100 m^{-2} (seven prawns in total, 0.08 ± 0.05 prawns 100 m^{-2}) were caught in eleven 500 m trawls with the beam trawl in 2001, compared with 0.30 ± 0.22 tiger prawns 100 m^{-2} in the same regions in 2000. Despite the relatively low catch, the proportion of the total prawn catch that was made up of tiger prawns was higher in 2001 than that in 2000 (Table 6.3).

6.3.1.2 King prawns

In 1999, the catches of king prawns (159 postlarvae and 287 juveniles) were much higher than those of tiger prawns (Figure 6.6). Juvenile king prawns were caught in all five regions (~1 – 3 prawns·100 m^{-2}) (Figure 6.6). Three sub-adult king prawns (> 15 mm CL) were caught at the 'mid-gulf' site where the water depth was over 10 m.

In 2000, the catches of king prawns (249 postlarvae and 293 juveniles) were similar to those in 1999 and again much higher than those of tiger prawns. The proportion of the catch made up of king prawns was about 50% in both 1999 and 2000 (Table 6.3). Juvenile king prawns were most abundant in Giralia Bay (~12 prawns·100 m^{-2}) and least abundant behind Simpson/Burnside Islands (<0.5 prawns·100 m^{-2}, Figure 6.6). The catches of postlarval and juvenile king prawns were considerably greater than those of tiger prawns in all regions, except Simpson/Burnside Islands (juveniles only) and Gales Bay, where catches of tiger prawns were about double those of king prawns.

In 2000, the abundance of both juvenile tiger and king prawns was greater in the shallow benthic habitats (about 2-3 m deep at the inshore sites) than in deeper water (5-8 m deep at the offshore sites). A total of 19 tiger prawns (0.19±0.12 prawns $100 \cdot m^{-2}$) and 147 king prawn (1.25±0.27 prawns·100 m^{-2}) were caught in the 36 trawls made with the 12 mm net at the 'inshore' sites. At the 'offshore' sites, only three tiger prawns (0.04±0.03 prawns·100 m^{-2}) and 28 king prawns (0.33±0.09 prawns·100 m^{-2}) were caught in the 28 trawls made.

In 1999, few tiger prawns (9%) were larger than postlarvae (i.e ≥ 3 mm CL) and few other size classes of juveniles (3 - 14 mm CL) were represented (Figure 6.7 a). In 2000, the proportion of the population that were juveniles (33%) was greater than in 1999 and all size classes up to 14 mm CL were represented. The larger size range of individuals in 2000 represents greater survival of each size class in 2000 (in recolonising seagrass habitats) than in 1999 (on mainly bare habitats).

6.3.1.3 Change in abundance of tiger and king prawns in Exmouth Gulf over 1999, 2000 and 2001

The abundance of juvenile tiger prawns (and their proportion in the catch) has increased from 1999 to 2000 and then remained the same or perhaps declined per unit area of seagrass from 2000 to 2001, depending on the locations and nets used to estimate yearly catch.

In the shallow benthic habitats that were trawled in both 1999 and 2000, the mean number of tiger prawn postlarvae did not differ significantly between years, but did differ between regions (postlarvae were not sampled in 2001) (Table 6.4). Most tiger prawn postlarvae were caught at Simpson Island and Gales Bay and the least were caught at Whalebone North and South (significantly fewer than Simpson Island). However, the catch in Gales Bay was not significantly greater than Giralia Bay, Whalebone North or Whalebone South. In contrast, the mean catch of juvenile tiger prawns caught with the 2 mm mesh net differed significantly between years, and between regions (Table 6.4). In 2000, we caught significantly more juvenile tiger prawns throughout Exmouth Gulf (17 per trawl at most, few zeros) than we caught in

1999 (2 per trawl at most, many zeros) (Figure 6.3, Table 6.4). The highest catches of juveniles were made at Gales Bay and significantly fewer were caught at Whalebone North and South in both 1999 and 2000. The catches at Gales and Giralia Bays and Simpson Island were not significantly different. The use of different nets in 2001 and the low catches in the net that was used in all years, precluded an analysis of catch between 2001 and other years.

Table 6.4 Mean squares and significance levels for two-way Analyses of Variance of the densities of postlarval and juvenile tiger prawns (log((n/100 m-2)+1.5)) over five regions in 1999 and 2000.

Factor	d.f	Mean squares and probability	
		Postlarvae	Juveniles
a) postlarvae			
Year	1	0.01 $^{0.92}$	1.39 **
Region	4	2.89 ***	0.59 **
Year * Region	4	0.20 $^{0.73}$	0.22 $^{0.26}$
Error	67	0.39	0.16

** = $0.001 < p \leq 0.01$; *** = $p \leq 0.001$

The catches of king prawn postlarvae and juveniles caught in the 2 mm mesh net did not differ significantly between years, but differed significantly between regions (Table 6.5). Twice as many king prawn postlarvae were caught at Simpson Island and in Giralia Bay than in any other region (Figure 6.6). Significantly more juvenile king prawns were caught in Giralia Bay than in all other regions, except Whalebone South (high catches in 1999) (Figure 6.6, Table 6.5). The lowest catches of juvenile king prawns were recorded at Simpson Island. Catches of juveniles did not differ significantly between Simpson Island and Whalebone North and Gales Bay.

Table 6.5 Mean squares and significance levels for two-way Analyses of Variance of the densities of postlarval and juvenile king prawns (log((n/100 m-2)+1.5)) over five regions in 1999 and 2000.

Factor	d.f	Mean square and probability	
		Postlarvae	Juveniles
Year	1	0.02 $^{0.85}$	0.04 $^{0.74}$
Region	4	5.78 ***	2.12 ***
Year * Region	4	0.68 $^{0.37}$	0.32 $^{0.42}$
Error	67	0.62	0.33

*** = $p \leq 0.001$

At the inshore sites in the three regions that were trawled during 1999, 2000 and 2001, the catches of juvenile tiger prawns in the 12 mm mesh beam trawl (used in all years) increased from 0.08 ± 0.04 prawns 100 m^{-2} in 1999, to 0.30 ± 0.22 prawns 100m^{-2} in 2000 (18 prawns in total), then declining to 0.08 ± 0.05 prawns 100 m^{-2} in 2001 (7 prawns in total). If we include all sites trawled in each year, the densities in 2000

were less (0.17 ± 0.11 prawns 100 m^{-2}). The king prawn population in the inshore zone also changed from 1999 to 2001. The densities of juvenile king prawns in 1999 and 2000 were about the same, 1.86 ± 0.32 and 1.60 ± 0.23, respectively, declining to 0.95 ± 0.49 prawns 100 m^{-2} in 2001.

Although we cannot compare catch rates for all nets directly between years because of different sampling gear, our data suggest that catches on a regional and site scale support the contention that the catches of juvenile king prawns were lowest in 2001. No king prawns were caught at sites with a high cover of seagrass, e.g. sites with 80% *H. spinulosa* (seagrass cover data as found in Chapter 5). Whereas in 2000, few sites had 80% seagrass cover and trawls at almost all sites caught king prawns. For example, at Whalebone North, one tiger prawn and 18 king prawns were caught in the 12 mm nets in 2000 when the habitat was mainly bare sands and silts (no sites with > 60% seagrass cover). Six tiger prawns and only seven king prawns were caught at Whalebone North in 2001 when the habitat was mainly seagrass (81% of sites with > 60% cover). The king prawns from Whalebone North were only caught at one site were the abundance of seagrass was low to medium (< 50% cover). Likewise at Whalebone South, as seagrass cover increased from 2000 to 2001, one tiger prawn and 42 king prawns were caught in 2000 compared to 21 tiger prawns and no king prawns in 2001. At Whalebone South, all trawl sites had about 80% seagrass cover in 2001.

In contrast, at Gales Bay, a region where the seagrass cover remains lower (27% of sites with > 60% cover), king prawns remained the dominant species in 2001. Eight tiger prawns and 111 king prawns were caught at Gales Bay in 2001 compared to 17 tiger prawns and 38 king prawns in 2000.

6.3.1.4 Tiger and king prawn catch in different nets in 2001

The three nets used in 2001 caught different size classes of juvenile and sub-adult prawns. The 12 mm mesh beam trawl caught the smallest size classes of juvenile tiger prawns, 8-14 mm CL, while the WA otter trawl caught larger size classes and a broader size range of juveniles and sub-adults, 10-24 mm CL (Figure 6.7 b). The try-gear caught the largest size classes of tiger prawns, 24-32 mm CL. The three different trawl nets also had different catch rates and differing net selectivity or densities of the different size classes of prawns (juvenile and sub-adults) may account for some of that variation.

In 2001, the mean catches of juvenile tiger prawns in the otter trawls (0.21 ± 0.09 prawns 100 m^{-2}) were higher, but not significantly higher, than those in the 1.5 m beam trawl (mean catch = 0.08 ± 0.05 prawns 100 m^{-2}) in the inshore zone in Exmouth Gulf (ANOVA, $p > 0.05$). The trawls with each net were made sequentially at the same sites, or nearby sites, in 2001, so a direct comparison can be made for that year. The mean catch in the Jurabi-K try-gear was the least (0.03 ± 0.02 prawns 100 m^{-2}). However, the try-gear trawls were made in the deeper zone, offshore from the trawls with the other two nets.

The mean catches of king prawns were higher, but not significantly higher ($p > 0.05$), in the 1.5 m beam trawl (0.95 ± 0.49 prawns 100 m^{-2}) than the otter trawl (0.36 ± 0.19

prawns 100 m^{-2}). The try-gear catch (in the deeper zone) (0.04 ± 0.02 prawns 100 m^{-2}) was very low and similar to the tiger prawn catch in that zone.

In 2001, the otter trawl seemed to catch more prawns on the high-biomass seagrass found at Whalebone North and South than on low-biomass seagrass, while the beam trawl was more efficient on the low-biomass seagrass found at Gales Bay. The comparison is not strong as the numbers of trawls and prawns caught were low. At Whalebone North and South, the otter trawls caught more tiger and king prawns (31 prawns; 0.27 ± 0.12 tiger prawns 100 m^{-2}, 0.04 ± 0.04 king prawns 100 m^{-2}) than the beam trawl (4 prawns; 0.02 ± 0.02 tiger prawns 100 m^{-2}, 0.05 ± 0.04 king prawns 100 m^{-2}), while at Gales Bay, the beam trawl caught more prawns (81 in total; 0.27 ± 0.15 tiger prawns 100 m^{-2}, 3.33 ± 0.73 king prawns 100 m^{-2}) that the otter trawl (48 in total; 0.05 ± 0.05 tiger prawns 100 m^{-2}, 1.23 ± 0.32 king prawns 100 m^{-2}).

6.3.2 Potential fish predators of tiger prawns

6.3.2.1 All fish in gill nets

The total catches of fish in gill nets were higher during December 1999 (453) than in November 2000 (128) (Figure 6.8). This was despite the fact that fishing effort was equivalent during both surveys (Table 6.6). In terms of numbers of fish caught and catch rates (g/h/200 m of net), catches were about 75% lower at the Gales Bay and Simpson Island sites in 2000 compared with 1999, whereas catches at Whalebone South were similar in both years (Table 6.6; Figure 6.8). The numbers of species caught at Gales Bay (6) and Simpson Island (7) during 2000 were much less than in 1999 (17 and 12 species respectively; Table 6.6).

On the basis of presence/absence of the various species, the fish species assemblages were quite variable among sites and years (Figure 6.9 a; Table 6.6). Only three species were found at all three sites and between two and four species were found at the same site during both years (Table 6.6). However, when catch rates are considered, Whalebone South contrasts strongly with the other two sites, both because of the very low numbers of fish caught there during both years and because several species were only caught there e.g. *Carcharhinus falciformes*, *C. brevipinna*, *Chanos chanos* and *Tylourus crocodiles* (Figure 6.9 b).

6.3.2.2 Penaeid predators

Of the 29 species of fish caught during 1999 and 2000, 19 were species that have been identified as penaeid predators, either during this study or in previous studies in tropical Australian waters (Table 6.6). In general the catch rates of penaeid predator species were higher in 1999 than 2000 (Figure 6.8). Catches of most species were highly variable, many were zero and in several cases they were up to 1.5 times outside the interquartile range and could be considered to be outliers (Figure 6.10). For example, nightly catch rates of one of the most commonly caught species, *Rhizoprionodon acutus* ranged from 0 to 8,582 g/h/200 m of net during November 1999 and from 269 to 1,885 g/h/200 m of net during December 2000. The median catch rates of *Scomberoides commersonianus*, *Carcharhinus cautus*, *C. limbatus* and *C. sorrah* were higher in 1999 than 2000, whereas the median catch rate of *Arius proximus* was higher in 2000 than in 1999.

Over the two years, the median catch rate of all penaeid predators combined was highest at Gales Bay (7,306 g/h/200 m of net) followed by Simpson Island (3,878 g/h/200m of net) and Whalebone south (1,674 g/h/200 m of net) (Figure 6.11). The dominant predators in terms of catch rates were sharks; *Carcharhinus limbatus* which were found mainly at Gales Bay, *C. cautus* which were caught mostly at Simpson Island and Gales Bay and *Rhizoprionodon acutus* which were caught at all three sites (Figure 6.11). The other widespread penaeid predator was the queenfish (*Scomberoides commersonianus*) was also caught at all three sites at relatively high catch rates.

Table 6.6 **Total numbers of fish caught at Gales Bay (GLBY), Simpson Island (SIMP) and Whalebone South (WBSH) using 3, 4, 5 and 6" mesh gillnets during December 1999 and November 2000. Species marked with an asterisk are known penaeid predators. '.' = not caught.**

Species	December 1999			November 2000		
	GLBY	SIMP	WBSH	GLBY	SIMP	WBSH
*Arius proximus**	.	31	.	5	9	.
Carcharhinus falciformes	.	.	.	.	.	2
*Caranx ignobilis**	.	.	.	.	1	.
Caranx tille	.	1	.	.	.	.
Carcharhinus brevipinna	.	.	.	.	.	1
*Carcharhinus cautus**	17	35	1	2	19	.
*Carcharhinus limbatus**	18	1	.	19	.	.
*Carcharhinus melanopterus**	.	4	.	.	.	.
*Carcharhinus sorrah**	30	.	1	.	1	3
Chanos chanos	.	.	.	.	.	1
*Echeneis naucrates**	3	.	.	.	.	.
Galeocerdo cuvier	.	.	1	.	.	.
Hemigaleus microstoma	1	2	.	.	.	.
Mugil cephalus	.	1	.	.	.	.
Nematolosa come	142	1	.	.	.	.
*Platycephalus endrachtensis**	1	.	.	.	.	.
*Polydactylus plebius**	11	.	.	.	.	.
*Rachycentron canadum**	1	.	2	.	.	1
*Rhizoprionodon acutus**	19	97	5	24	18	9
*Saurida undosquamis**	1	.	.	.	.	.
*Scomberoides commersonianus**	2	8	2	4	5	1
*Scomberoides tol**	1	.	.	.	.	.
*Scomberomorous queenslandicus**	1	1	.	.	.	.
*Scomberomorus munroi**	5	.	1	1	.	.
Selenotoca multifasciata	.	2	.	.	.	.
*Sillago analis**	1	.	.	.	.	.
*Thryssa hamiltoni**	2	.	.	.	.	.
*Tylosurus crocodiles**	.	.	.	.	.	1
Valamugil buchanani	.	.	.	.	1	.
Total number of fish	257	183	13	55	54	19
Number of species	17	12	7	6	7	8

Table 6.7 Total number of penaeids found in guts of fish caught at Gales Bay (GLBY), Simpson Island (SIMP) and Whalebone South (WBSH) using 3, 4, 5 and 6" mesh gillnets during December 1999 and November 2000.

Penaeid prawn	Region						Total
Species	December 1999			November 2000			
	GLBY	SIMP	WBSH	GLBY	SIMP	WBSH	
Metapenaeopsis palmensis	0	0	1	0	0	0	1
Metapenaeopsis sp.	1	0	0	0	2	0	3
Metapenaeus moyebi	1	2	0	2	0	0	5
Metapenaeus ensis	1	0	0	0	0	0	1
Metapenaeus sp.	3	1	0	0	0	0	4
Parapenaeopsis cornuta	0	0	0	1	0	0	1
Unidentified penaeid	0	0	1	0	1	0	2
Penaeus esculentus	1	0	0	0	1	0	2
Penaeus latisulcatus	8	1	0	3	0	0	12
Total penaeids eaten	15	4	2	6	4	0	31
Total number of guts	113	180	12	55	54	17	431

6.3.2.3 Penaeids found in fish guts

During 1999 and 2000 a total of 431 fish guts were examined for penaeids. Thirty-one penaeids were found, with 21 from fish that were caught in Gales Bay (Table 6.7). More prawns were found in guts in 1999 (21) than in 2000 (10). Only two of the penaeids were *Penaeus esculentus*, one from a fish (*Scomberomorus munroi*) caught in Gales Bay in December 1999 and the second from a fish (*Carcharhinus cautus*) caught at the Simpson Island site in November 2000.

Carcharhinus cautus had the highest number of penaeids in their guts (8), followed by *Rhizoprionodon acutus* (6) and *Arius proximus* (5) (Table 6.8).

Table 6.8 Numbers of prawns eaten by fish caught at Gales Bay (GLBY), Simpson Island (SIMP) and Whalebone South (WBSH) using 3, 4, 5 and 6" mesh gillnets during December 1999 and November 2000.

Fish species	Region						Total
	December 1999			November 2000			
	GLBY	SIMP	WBSH	GLBY	SIMP	WBSH	
Arius proximus	.	2	.	1	2	.	5
Carcharhinus cautus	5	.	.	1	2	.	8
Carcharhinus melanopterus	.	1	.	.	.	.	1
Carcharhinus sorrah	2	.	.	.	.	.	2
Echeneis naucrates	4	.	.	.	.	.	4
Polydactylus plebius	1	.	.	.	.	.	1
Rachycentron canadum	.	.	1	.	.	.	1
Rhizoprionodon acutus	1	1	.	4	.	.	6
Scomberomorus munroi	2	.	1	.	.	.	3
Total penaeids eaten	15	4	2	6	4	0	31
Total number of guts	113	180	12	55	54	17	431

6.3.2.4 Prawn Predation Index

The Prawn Predation Indices at Simpson Island and Gales Bay are very much greater than that at Whalebone South (Table 6.9) suggesting that this site may provide the lowest threat from predation to juvenile prawns.

Table 6.9 Prawn Predation Index calculated for each site and pooled for the December 1999 and November 2000 sampling trips.

Site	Prawn Predation Index
Gales Bay	820
Simpson Island	953
Whalebone South	255

6.3.2.5 Fish in prawn recruitment surveys

Seventy-nine guts from 38 different species of fish were examined from the 15 trawls done between October 2000 and March 2001. Only six species of fish were found with penaeids in their guts and only one contained *Penaeus esculentus* (found in a *Lagocephalus spadiceus* gut) (Table 6.10). Sixteen of the 38 species caught in the trawls have previously been recorded as consuming prawns. Only four of the species caught in the recruitment-survey trawls were also caught in the inshore gill nets (*C. sorrah*, *H. microstoma*, *S. undosquamis* and *S. tol*), and only one (*S. undosquamis*) had prawns in their guts (Table 6.10).

Table 6.10 Numbers and species of penaeids found in the guts of fish species caught in trawls by the Department of Fisheries WA during prawn recruitment surveys between October 2000 and March 2001. * = also caught in gill nets, - = not caught in gill nets.

Fish Species	Number of Prawns	Prawn Species
Saurida undosquamis *	4	Metapenaeopsis novaeguinae, Metapenaeopsis sp., Metapenaeus endevouri, unidentified penaeid
Polydactylus multiradiatus -	3	Metapenaeopsis palmensis, Trachypenaeus sp., Metapenaeopsis sp.
Caranx bucculentus -	2	Metapenaeus ensis, Metapenaeopsis palmensis
Lagocephalus spadiceus -	1	Penaeus esculentus
Lutjanus russelli -	1	unidentified penaeid
Johnius vogleri -	1	Metapenaeus ensis

6.4 Discussion

6.4.1 Abundance of tiger prawns

In Exmouth Gulf, the catches of postlarval (<6 prawns 100 m^{-2}) and juvenile (<3 prawns 100 m^{-2}) tiger prawns were much lower than those for tropical and sub-tropical seagrass nursery habitats elsewhere (see Loneragan *et al.* 1994, 1998). Our sampling of the distribution and abundance of tiger prawns in Exmouth Gulf was limited to two occasions during October to

December each year (once in 2001). We chose this period as the recruitment surveys carried out by the Department of Fisheries WA, and the timing of the commercial fishery, suggested that catches of postlarvae and juveniles should be high from September to December. In general, spring and early summer are periods of high juvenile recruitment in sub-tropical and tropical seagrass communities (O'Brien 1994, Loneragan *et al*. 1994). However, during periods of juvenile recruitment in the Gulf of Carpentaria, the catch of postlarval and juvenile tiger prawns can vary greatly; fortnightly at one location (Vance *et al*. 1996) and seasonally between nursery locations separated by about 400 km (see Coles and Lee Long 1985, Loneragan *et al*. 1994).

As the abundance of tiger prawns in Exmouth Gulf from 1999 to 2001 was low, we cannot make strong comparisons of the distribution of juvenile tiger prawns between regions (different seagrass communities) or years. Our ability to use the results of our trawls as a proxy for optimal release habitat is weak. Until more comprehensive further studies are carried out on the distribution and abundance of juvenile tiger prawns in Exmouth Gulf, the distribution of seagrasses (see Chapter 5) provides the best indicator of potential release sites.

Seagrass communities with low above-ground biomass have been shown to support low abundances of juvenile tiger prawns. Both stable seagrass habitats with low biomass (Loneragan *et al*. 1998) and seagrass habitats that have been impacted by cyclones, reducing seagrass biomass (Poiner *et al*. 1989, 1993), do not support a dense population of juvenile tiger prawns. Thus, the low catches of juvenile tiger prawns in Exmouth Gulf during 1999 and 2000 are not unexpected on the low above-ground biomass of seagrass habitat found during the first two years of the study.

The lack of large juvenile prawns in 1999 suggests that the postlarvae that settled on the sparse seagrasses that were available following the cyclone did not survive. In 2000, the greater percentage of juvenile prawns (≥3 mm CL) in the tiger prawn population, particularly large juvenile prawns, suggests that the survival of postlarvae in 2000 was greater than that in 1999. Improved survival to grow to a larger size would have been facilitated by the shelter and food provided by the seagrass habitat in 2000 (Kenyon *et al*. 1995, 1997, Loneragan *et al*. 2001).

In 2001, despite seagrass cover increasing to > 60% over large areas of the shallow sub-tidal substrates and seagrass above-ground biomass reaching 70 g m^{-2} at some sites (preliminary data - Chapter 5), our catches of juvenile prawns remained low compared with seagrass communities in the Gulf of Carpentaria, for example. At sites in both the eastern and western Gulf of Carpentaria about 50-200 postlarval tiger prawns 100 m^{-2} are common, as are about 20-50 juvenile tiger prawns 100 m^{-2}, particularly on seagrass habitats with > 100 gm^{-2} above-ground biomass (Loneragan *et al*. 1994, 1998, Haywood *et al*. 1995, Vance *et al*. 1996). However, while catches in 2001 are much lower than those for some high biomass seagrasses in the Gulf of Carpentaria, they were similar to those in an 'open coastline' seagrass community in the south-western Gulf of Carpentaria. On the open-coastline habitats, similar habitats to those sampled in Exmouth Gulf, catches of 3.5-10.0 juvenile tiger prawns 100 m^{-2} were made at sites within a *C. serrulata*/*S. isoetifolium*/*H. uninervis* dominated seagrass community with 30-95 g m^{-2} above ground biomass (Kenyon *et al*. 1999).

6.4.2 Potential predation on prawns

Very few *Penaeus esculentus* were found in the guts of fish caught in gill nets. However, this is probably because the densities of juvenile *P. esculentus* were very low in Exmouth Gulf following cyclone Vance. It is likely that once the number of juvenile prawns increases, then the number eaten by predatory fish will also increase (Salini *et al*. 1990). Although the catch rates of fish species known to eat prawns were highly variable, in general they were lowest in

the Whalebone South region. The main juvenile penaeid predators in Exmouth Gulf were sharks (*Carcharhinus cautus* and *Rhizoprionodon acutus*) and a species of catfish (*Arius proximus*). A predation index generated for each region, using a combination of predator catch rate and proportion of penaeid prawn in the diet (PPI), indicated that Whalebone South would provide the lowest threat of predation to juvenile *Penaeus esculentus* that were released during any enhancement trials.

Gill nets tend to be selective in terms of the fish species that they capture. Highly mobile pelagic species are most vulnerable to capture in gill nets, with less mobile benthic species like rays rarely being caught. This problem was recognised at the beginning of the project and other techniques such as beach seines were discussed. However, because of the very wide (up to several km) intertidal zone in the eastern part of Exmouth Gulf beach seines are not effective. A small otter trawl was tested at Roberts Island, but the vessel was not large enough to tow it efficiently and no fish were caught. It is interesting (although not unexpected) that, of the 29 fish species caught inshore with the gill nets and the 38 species caught offshore by the recruitment-survey trawls, only four species were caught in both areas. The habitats and depths in the areas sampled by each method (gill nets and trawls) are different, as is the selectivity of each of the gears used, resulting in few common species caught.

6.4.3 Prawn distribution as an indicator of optimal release sites

We have identified the regions of Exmouth Gulf where the natural abundance of juvenile tiger prawn is highest. The regions of highest abundance changed between 2000 and 2001, probably as the abundance of seagrass habitat has changed between regions. The natural abundance of prawns may be a good proxy to indicate optimal regions and habitats for the release of tiger prawns during enhancement trials (Munro and Bell 1997, Leber 1999). However, the distribution of penaeid prawn predators will also affect survival post-release, and thus, areas of optimal habitat.

In 2000, Gales Bay had the highest abundance of juveniles caught with both the 2 mm and 12 mm mesh nets. However, it also had a high prawn predation index, suggesting that it may not be an optimal site for release, as post-release mortality may be high. In 2001, Whalebone South had the highest catches of juvenile prawns and, though the distribution of predators was not assessed in 2001, the prawn predation index was lowest there in both 1999 and 2000. Thus, Whalebone South probably was the optimal release region in 2001, as prawn abundance was high and predation probably remained low. These conditions may offer the best chance of survival and recruitment for hatchery-reared prawn stock in Exmouth Gulf. However, as the seagrass and prawn community within Exmouth Gulf remains very dynamic, the distribution of seagrass habitats, prawns and prawn predators will need to be re-surveyed prior to any enhancement trials, to re-estimate the location of optimal release habitats.

6.4.4 Comparing effectiveness of trawl nets for large juvenile tiger prawns

Restricting the nets used to the 12 mm mesh trawls in 2001 was effective at investigating better ways to catch > 10 mm CL animals; the target size for future enhancement work. However, the change in sampling strategy in 2001 has limited our ability to compare catches year-to-year. As well, the 2 mm mesh net caught at least 4-5 times the number of juvenile prawns (> 3 mm CL juveniles) than the 12 mm mesh nets (> 10 mm CL juveniles) resulting in a better estimate of juvenile tiger prawn abundance. The small mesh nets may be best used to estimate the natural distribution and abundance of juvenile tiger prawns, and thus, the best estimate of a proxy for optimal release sites.

Comparison of catches between different nets showed that the WA otter trawl caught most prawns on the re-established seagrasses. It may be that the density of 10-24 mm CL tiger prawns on the seagrass beds was higher than those of other large-juvenile size classes, resulting in the otter trawls catching more prawns. It is also possible that the otter trawl is more efficient at catching larger size classes, particularly in high above-ground biomass seagrasses, than the beam trawl configuration.

The otter trawl caught the target size range of prawns that would be in the seagrass habitats following a release of hatchery-reared stock (10-20 mm CL). Enhanced prawns would probably be about 10 mm CL at release and they would grow in the ensuing weeks. The trawler try-gear caught only sub-adult and adult tiger prawns (≥ 23 mm CL) which are larger than the target size range of released juveniles. Small prawns may have escaped through the larger mesh net, despite the 12 mm codend insert. However, as the trawler could not access shallow waters, the lack of small prawns may have been due to their absence from the deeper habitats. Larger sized tiger prawns are often caught in deeper water as they move offshore from shallow nursery habitats (O'Brien 1994).

6.4.5 Effect of Cyclone Vance on the Exmouth Gulf penaeid prawn community

The increase in the catch of tiger prawns, and their percent composition of the juvenile penaeid prawn community, has reflected an increase in seagrass abundance during 1999 to 2001; from an average of 1% cover in 1999, to 10% cover in 2000 and 42% cover in 2001, as the seagrass community of Exmouth Gulf regenerates. The abundance of juvenile tiger prawns in Exmouth Gulf has increased as the benthic habitats changed from mostly bare sand substrates in 1999, to mostly tall, dense seagrass in 2001. As well, a shift in the proportions of species in the prawn population has occurred, from predominantly king prawns on bare habitats to predominantly tiger prawns on seagrass habitats. The change in benthic habitats, from un-vegetated to vegetated, has driven the change in the prawn community. A similar shift in species composition was documented following Cyclone Sandy in the Gulf of Carpentaria. Immediately after the cyclone, king prawns dominated the penaeid prawn community on shallow bare habitats where the seagrass had been removed (Poiner *et al*. 1989, 1993). Tiger prawns replaced the king prawns as the seagrass community recovered.

A significant problem in interpreting the effect of Cyclone Vance on Exmouth Gulf nursery habitats and tiger prawns is the lack of pre-cyclone data on the extent of benthic vegetated habitat, particularly seagrass, or data on the abundance of juvenile tiger prawns. However, the path of the cyclone (see Chapter 5), as well as trends in commercial catch and prawn recruitment- and spawning-indices for the offshore fishery (Department of Fisheries WA, unpublished data) before and after the cyclone, provide strong indicators that the nursery habitat for juvenile tiger prawns was removed by the cyclone; and support the hypothesis that the destruction of the seagrass beds was the cause of the decline in catch in 2000 (Figure 6.12).

From 1995 to 1999, the recruitment indices and commercial catch for Exmouth Gulf tiger prawns were mostly within the 10 year historical range - 250-550 tonnes catch per annum (Figure 6.12). Abundant seagrasses would have supported juvenile prawns and the commercial tiger prawn fishery prior to 2000. The high catch from the 1999 season (April to November, the year of the cyclone) resulted from juvenile tiger prawns that moved onto nursery habitats in late 1998 and early 1999, prior to Cyclone Vance in March 1999. These juveniles had access to un-impacted nursery habitats for most or all of their inshore phase, resulting in successful recruitment to the offshore fishery. As well, the spawning index estimated from the commercial catch in 1999 was above average (Figure 6.12, see 1999/2000), suggesting that the subsequent catch should have been high; yet the 82 t catch in 2000 was the lowest in the last 17 years. In 2000, the indices of offshore recruitment also

decreased (Figure 6.12, see 1999/2000) and remained low in 2001. The commercial recruitment index in 2002 has increased to past levels, the spawning index increased from 7.4 in 2000 to 18.9 in 2001, and the 2002 predicted catch (330 t) is again within the 1980/90's range (Figure 6.12).

Thus, it seems beyond doubt that both the recruitment of commercial tiger prawns to the Exmouth Gulf fishery and fishery catch declined following cyclone impacts to the Exmouth Gulf ecosystem in March 1999. The recovery of recruitment and catch (lagged by a year as they contribute to the next years catch) is mirrored by the increase in both tiger prawn nursery habitat (seagrass) and juvenile prawn abundance over 1999-2001; four indices increasing in unison after the cyclone. Importantly, the miss-match between the spawning index in 1999 (very high) and the following years catch (very low) shows recruitment to the fishery failed, probably because of the removal of the seagrass community that supports juvenile tiger prawns during the cyclone.

6.4.6 Contribution to a rigorous evaluation of stock enhancement effects

The results from this phase (Phase Two) of the enhancement program have identified potential release sites for hatchery-reared prawn stock (i.e. Whalebone South), using the distribution of seagrass, the natural distribution of juvenile tiger prawns and the lowest abundances of potential *P. esculentus* predators in Exmouth Gulf. Whalebone South and Gales Bay both support higher catches of juvenile tiger prawns than other areas, although the region where they were most abundant has changed over time with the seagrass recolonisation of Exmouth Gulf. This is also the region where most recruits to the offshore fishery are thought to originate. Actual releases of hatchery reared stock are needed to test release sites for density dependence in the nursery habitats.

In the past, stock enhancement has focussed on 'aquaculture production and release magnitude' with no rigorous scientific investigation of the 'stock enhancement effect' on fish populations (wild and stocked) or fishery landings (see Leber 1999 for a review of stock enhancement). Internationally, large-scale releases of more than 100 million juvenile commercial prawns have been made over many years in Japan (*Penaeus japonicus*, Kurata 1981, Fushimi 1999) and China (*Penaeus orientalis*, Liu 1990, Xu *et al*. 1997, Wang *et al*. 2002). During these releases, it is not apparent that optimal release sites or strategies were investigated, and the only evidence of success may be an increase in catch that correlates with the period of enhancement (Liu 1990). Leber (1999) points out that comprehensive scientific study of enhancement is in its infancy and that rigorous experimental investigation of post-release processes (his 'stock enhancement effect') has only been undertaken in the last 10 years.

In practice, the lessons learnt from rigorous stock enhancement science are still to be employed in most situations. For example, *P. monodon* postlarvae were stocked in an estuarine lagoon which had natural recruitment of both *P. indicus* and *P. monodon* (Davenport *et al*. 1999). They were stocked six months out of synchrony with the natural abundance of *P. monodon* in the wild to reduce competition and provide a definite "signal" of the success of enhancement. Prior to enhancement *P. monodon* comprised only 2.5% of the catch by weight, while post-enhancement they made up about 11% of the catch. Enhancement seemed to work as both fishery yield and the economic impact improved (Davenport *et al*. 1999). However, the increase in the catch of *P. monodon* represented only 3% of the 55,000-70,000 postlarvae released, suggesting a high juvenile mortality rate (Davenport *et al*. 1999). During the Sri Lankan study, no assessment of the juvenile habitat of the prawns or monitoring of processes affecting the juvenile phase of the released-stock ('enhancement effect') was undertaken. Neither was an assessment made of the potential

impact of the released prawns on the wild population. Thus, no knowledge to contribute to an understanding of why the *P. monodon* juveniles suffered such high mortality was gained.

6.4.7 Further research

Following the August 2001 Project Meeting and Independent Review, the Steering Committee agreed that Stage Three of the Enhancement Program should focus on assessing different release strategies. Ultimately, we will identify the optimal release strategies, in terms of site (habitat), size-at-release, season and presence/absence of predators, which would support a successful enhancement project in Exmouth Gulf. Enhancement of finfish and abalone has shown that releases at some sites and times may suffer 100% mortality (Schiel 1993, Leber and Arce 1996). Despite these setbacks, rigorous experimentation during enhancement trials has investigated post-release processes and shown that refining release protocols can increase the proportions of cultured stock that survive long-term in nursery habitats from 3% to 50% of the total cultured and wild catch (Leber 1999). If hatchery-reared stock make up over 50% of the population, three possible scenarios dominate, either:-

- wild stocks were severely depleted, or
- that the carrying capacity of the habitat can support wild and released stock, or
- that enhanced stocks may displace wild stock (Leber *et al*. 1998).

In Exmouth Gulf, we have the opportunity to undertake controlled trials to investigate release strategies that ensure that both wild and enhanced stock survive to contribute to the commercial landings. We would also investigate risk factors inherent in environmental, economic and genetic aspects of enhancement, thus employing the 'responsible approach to stock enhancement' (Blankenship and Leber 1995, Leber 1999).

A future enhancement program in Exmouth Gulf would use a rigorous experimental approach to determine habitats with the carrying capacity to support the natural and enhanced stocks. Initially, such an approach would require the identification of probable optimal release sites for juvenile tiger prawns. Their selection would integrate the mapping of habitats that are used by juvenile tiger prawns with the estimation of the distribution and abundance of the wild population of juvenile prawns to better determine optimal habitat as has been achieved during Stage Two.

Prior to any future experimental releases, we would need to re-survey the benthic habitats of Exmouth Gulf and the distribution and abundance of juvenile tiger prawns. At present, both the habitats and the prawn population that they support are in a state of flux. As well, we need to test further the otter-trawl net configuration as a method of sampling large juvenile tiger prawns, particularly if juvenile prawns are more abundant and able to provide a better catch-comparison between the nets.

6.5 References

Blankenship HL, Leber KM (1995). A responsible approach to marine stock enhancement. *American Fisheries Society Symposium*. 15: 167-175.

Brewer DJ, Blaber SJM, Salini JP, Farner MJ (1995). Feeding ecology of predatory fishes from Groote Eylandt, Australia; with special reference to predation on penaeid prawns. *Estuarine, Coastal and Shelf Science*. 40: 577-600.

Coles RG, Lee Long WJ (1985). Juvenile prawn biology and the distribution of seagrass prawn nursery grounds in the southeastern Gulf of Carpentaria. *In*: PC Rothlisberg, BJ Hill and DJ Staples (Editors) Second Australian National Prawn Seminar, NPS2. Cleveland, Australia. pp 55-60.

Davenport J, Ekaratne SUK, Walgama SA, Lee D, Hills, JM (1999) Successful stock enhancement of a lagoon prawn fishery at Rekawa, Sri Lanka using cultured postlarvae of penaeid shrimp. *Aquaculture*. 180: 65-78.

Fushimi H (1999). How to detect the effect in releasing operation of hatchery raised Kuruma prawn postlarvae? – case study of the operation in the Hamana Lake. *Bulletin Tohoku National Fisheries Research Institute*. 62: 1-12.

Furuta S (1996). Predation on juvenile Japanese flounder (*Paralichthys olivaceus*) by diurnal piscivorous fish: Field observations and laboratory Experiments. Int. Workshop: Survival Strategies in Early Life Stages of Marine Resources, Yokohama (Japan), 11-14 Oct 1994. In: Watanabe, Yoshiro; Yamashita, Yoh; Oozeki, Yoshioki (eds). *Proceedings of an international workshop: survival strategies in early life stages of marine resources*. A.A. Balkema publishers, Brookfield, VT (USA), pp. 285-296.

Haywood MDE, Vance DJ, Loneragan NR (1995). Seagrass and algal beds as nursery habitats for tiger prawns (*Penaeus semisulcatus* and *P. esculentus*) in a tropical Australian estuary. *Marine Biology*. 122: 213-223.

Haywood MDE, Heales DS, Kenyon RA, Loneragan NR, Vance DJ (1998). Predation of juvenile tiger prawns in a tropical Australian estuary. *Marine Ecology Progress Series*. 162: 201-214.

Kenyon RA, Loneragan NR, Hughes JM (1995). Habitat type and light affect the sheltering behaviour of juvenile tiger prawns (*Penaeus esculentus* Haswell) and the success rates of their fish predators. *Journal of Experimental Biology and Ecology*. 192: 87-105.

Kenyon RA, Loneragan NR, Hughes J, Staples DJ (1997). Habitat type influences the microhabitat preference of juvenile tiger prawns (*Penaeus esculentus* Haswell amd *Penaeus semisulcatus* de Haan). *Estuarine, Coastal and Shelf Science*. 45: 393–403.

Kenyon R, Burridge C, Poiner I, Hopkins E (1999). Impact of the Macarthur River Project Mine Transhipment Facility on the Marine Environment: Final Report. *CSIRO Marine Laboratories Report*. CSIRO Marine Research. PO. Box 120 Cleveland, Queensland, 4163.

Kurata H (1981). Shrimp fry releasing techniques in Japan, with special reference to the artificial tideland. *Kuwait Bulletin of Marine Science*. 2: 117-147. (International Shrimp Releasing, Marking and Recruitment Workshop, Kuwait 1978).

Leber KM (1999). Rationale for an experimental approach to stock enhancement. pp. 63-75. *In:* Stock Enhancement and Sea Ranching (eds. BR Howell, E Moksness, T Svasand). Chapter 5. Fishing News Books, Blackwell Science Ltd., Oxford. 606pp.

Leber KM, Arce SM (1996). Stock enhancement in a commercial mullet, *Mugil cephalus* L., fishery in Hawaii. *Fisheries Management and Ecology*. 3: 261-278.

Leber KM, Brennan NP, Arce SM (1998). Recruitment patterns of cultured juvenile pacific threadfin, *Polydactylus sexfilis* (Polynemidae), released along sandy marine shores in Hawaii. *Bulletin of Marine Science*. 62: 389-408.

Liu JY (1990). Resource enhancement of Chinese Shrimp, *Penaeus orientalis*. *Bulletin of Marine Science*. 47: 124-133.

Loneragan NR, Kenyon RA, Haywood MDE, Staples DJ (1994). Population dynamics of juvenile tiger prawns in seagrass habitats of the north-western Gulf of Carpentaria, Australia. *Marine Biology*. 119: 133–143.

Loneragan NR, Kenyon RA, Staples DJ, Poiner IR, Conacher CA (1998). The influence of seagrass type on the distribution and abundance of postlarval and juvenile tiger prawns in the western Gulf of Carpentaria, Australia. *Journal of Experimental Marine Biology and Ecology*. 228: 175-196.

Loneragan NR, Heales DS, Haywood MDE, Kenyon RA, Pendrey RC, Vance DJ (2001). Estimating the carrying capacity of seagrass for juvenile tiger prawns (*Penaeus semisulcatus*): enclosure experiments in high and low biomass seagrass beds. *Marine Biology*. 139: 343-354.

Munro JL, Bell JD (1997). Enhancement of marine fisheries resources. *Reviews in Fisheries Science*. 5: 185-222.

Murai T, Koshiishi Y (1998). Prospects in stock enhancement of Japanese flounder. 26. U.S.-Japan Aquaculture Symp., Durham, New Hampshire (USA), 16-18 Sep 1997 Howell, WH; Keller, BJ; Park, PK; McVey, JP; Takayanagi, K; Uekita, Y (eds). Nutrition and Technical Development of Aquaculture. Nov 1998, pp. 115-124.

O'Brien CJ (1994). Population dynamics of juvenile tiger prawns *Penaeus esculentus* in south Queensland, Australia. *Marine Ecology Progress Series*. 104: 247-256.

Olla BL, Davis MW (1989). The role of learning and stress in predator avoidance of hatchery-reared coho salmon (*Oncorhynchus kisutch*) juveniles. *Aquaculture*. 76: 209-214.

Olla BL, Davis MW, Ryer, CH (1994). Behavioural deficits in hatchery-reared fish: Potential effects on survival following release. Int. Symp. on Sea Ranching of Cod and Other Marine Fish Species, Arendal (Norway), 15-18 Jun 1993. Danielssen, DS; Howell, BR; Moksness, E (eds). An international symposium. Sea ranching of cod and other marine fish species., pp. 19-34. *Aquaculture and Fisheries Management*. 25, Suppl.

Olla, B. L., Davis, M. W., and Schreck, C. B. (1995). Stress-induced impairment of predator evasion and non-predator mortality in Pacific salmon. *Aquaculture Research*. 26: 393-398.

Poiner IR, Walker DI, Coles RG (1989). Regional studies: Seagrasses of tropical Australia. *In*: *The Biology of Seagrass – An Australian Perspective*. Larkum AWD, McComb AJ, Sheppard SA (eds). Elsevier, Amsterdam, pp. 279-303.

Poiner IR, Conacher C A, Loneragan NR, Kenyon RA, Somers I (1993). Effect of cyclones on seagrass communities and penaeid prawn stocks of the Gulf of Carpentaria. *CSIRO Marine Laboratories Report*. CSIRO, Division of Fisheries, Marine Laboratories PO. Box 120 Cleveland, Queensland, 4163.

PRIMER-E (2001). PRIMER 5 for Windows, Multivariate statistics for ecologists. PRIMER-E Ltd., 6 Hedingham Gardens, Roborough, Plymouth, PL67DX, United Kingdom.

Salini JP, Blaber SJM, Brewer DT (1990). Diets of piscivorous fishes in a tropical Australian estuary; with special reference to predation on penaeid prawns. *Marine Biology*. 105: 363-374.

SAS Institute Inc. (2000). SAS/STAT Users Guide, 8.1 Edition. SAS Institute Inc., Cary, NC, USA.

Schiel DR (1993). Experimental evaluation of commercial-scale enhancement of abalone *Haliotis iris* populations in New Zealand. *Marine Ecology Progress Series*. 97: 167-181.

Schreck, C. B., Solazzi, M. F., Johnson, S. L., and Nickelson, T. E. (1989). Transportation stress affects performance of coho salmon, *Oncorhynchus kisutch*. Hansen, LP, Clarke, WC, Saunders, RL, and Thorpe, JE *eds*. Salmonid Smoltification III Workshop, Trondheim (Norway), 27 Jun - 1 Jul 1988: Salmonid Smoltification Iii. Proceedings of a Workshop Sponsored by The Directorate For Nature Management, Norwegian Fisheries Research Council, Norwegian Smolt Producers Association and Statkraft, held at the University Of Trondheim, Norway, 27 June - 1 July 1988. *Aquaculture*. 82: 15-20.

Staples DJ, Vance DJ, Heales DS (1985). Habitat requirements of juvenile penaeid prawns and their relationship to offshore fisheries. *In* PC Rothlisberg, BJ Hill and DJ Staples (Editors) Second Australian National Prawn Seminar, NPS2. Cleveland, Australia. pp. 47-54.

Stoner AW, Davis M (1994). Experimental outplanting of juvenile queen conch, *Strombus gigas*: Comparison of wild and hatchery-reared stocks. *Fisheries Bulletin of the United States*. 92: 390-411.

Vance DJ, Haywood MDE, Heales DS, Staples DJ (1996). Seasonal and annual variation in abundance of postlarval and juvenile grooved tiger prawns, *Penaeus semisulcatus*, and environmental variation in the Embley River, Australia: a six-year study. *Marine Ecology Progress Series*. 135: 43-55.

Wang Q, Zhuang Z, Deng J (2002). The development and prospects of stock enhancement and sea ranching industry China. Paper for *Second International Symposium on Stock Enhancement and Sea Ranching*, Kobe, Japan 28th January – 1st February 2002.

Xu J, Xia M, Ning X, Mathews CP (1997). Stocking, enhancement, and mariculture of *Penaeus orientalis* and other species in Shanghai and Zhejiang Provinces, China. *Marine Fisheries Review*. 59: 8-14.

Young PC, Carpenter SM (1977). Recruitment of postlarval penaeid prawns to nursery areas in Moreton Bay, Queensland. *Australian Journal of Marine and Freshwater Research*. 28: 745-773.

Young P C (1978). Moreton Bay, Queensland: a nursery area for juvenile penaeid prawns. *Australian Journal of Marine and Freshwater Research*. 29: 55-79.

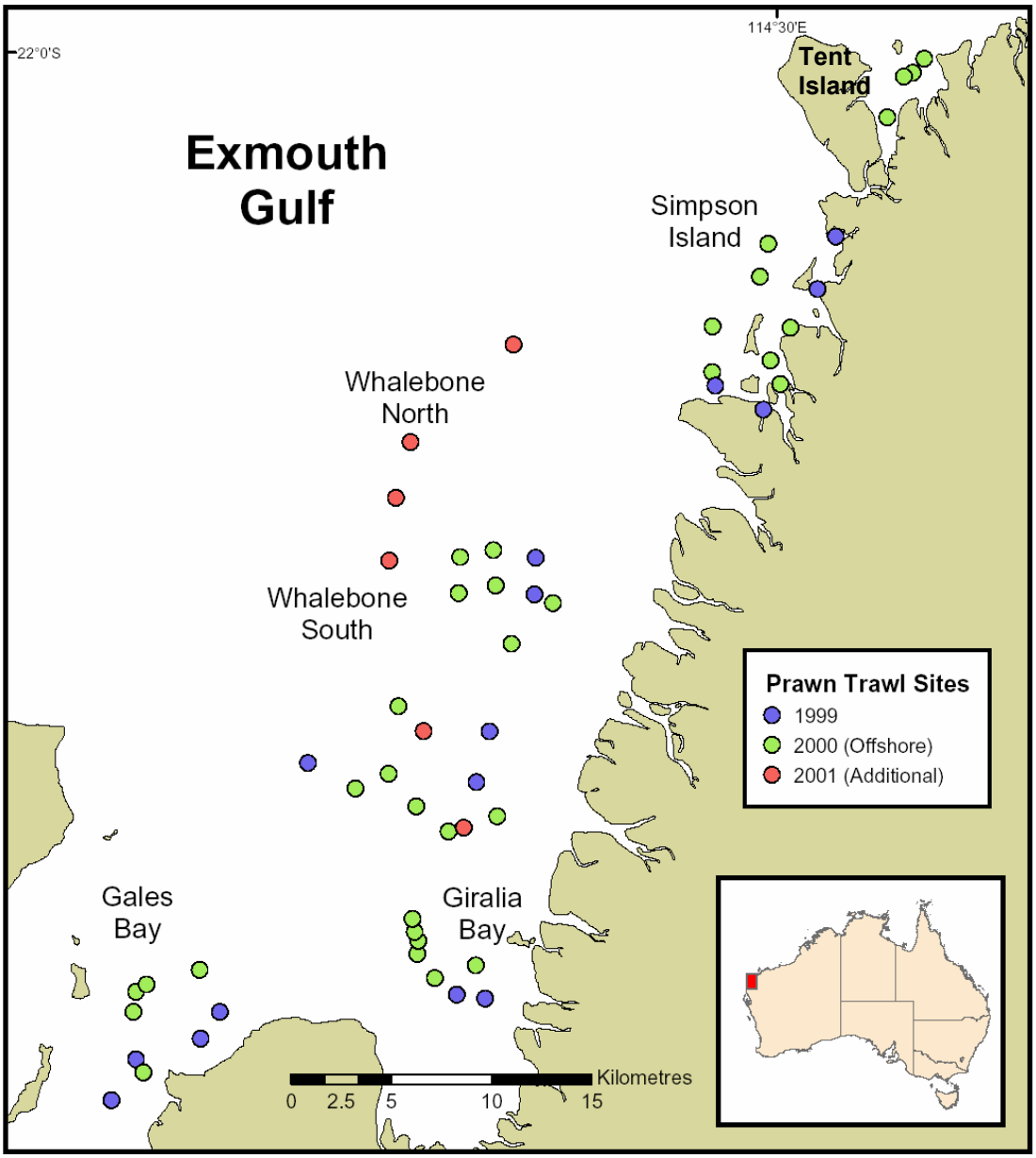


Figure 6.1 **Prawn sample sites (beam trawls) during November and December 1999 (red) and October and November 2000 (red and blue) and gill net sites during November 2000. Note that beam trawl sites were selected offshore (5-8 m deep) from the 1-2 m deep sites that were sampled during 1999. Some sites were not sampled during October 2000 due to bad weather and gear failure.**

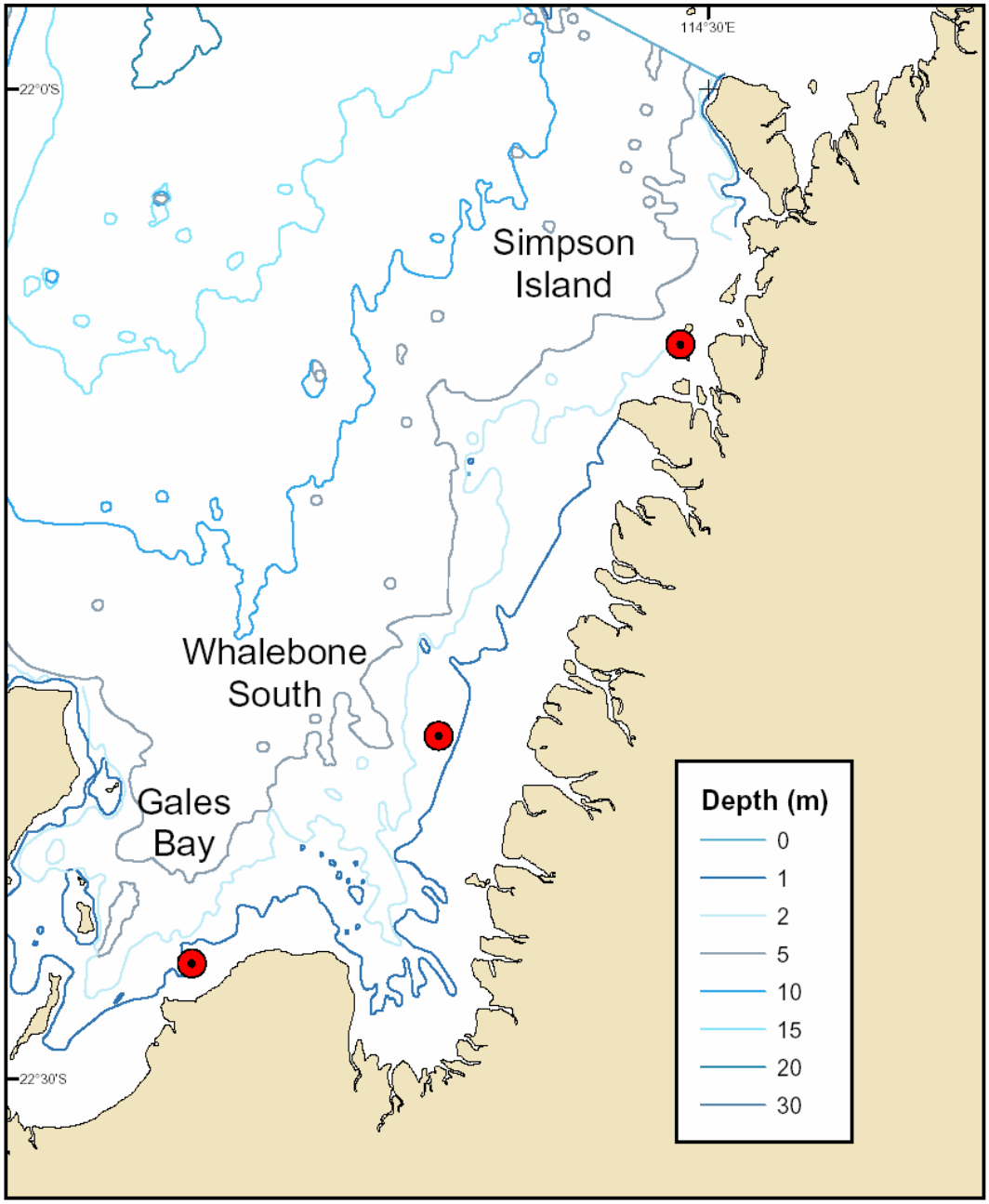


Figure 6.2 **Map of the eastern side of Exmouth Gulf showing the locations of the sites sampled for penaeid predators.**

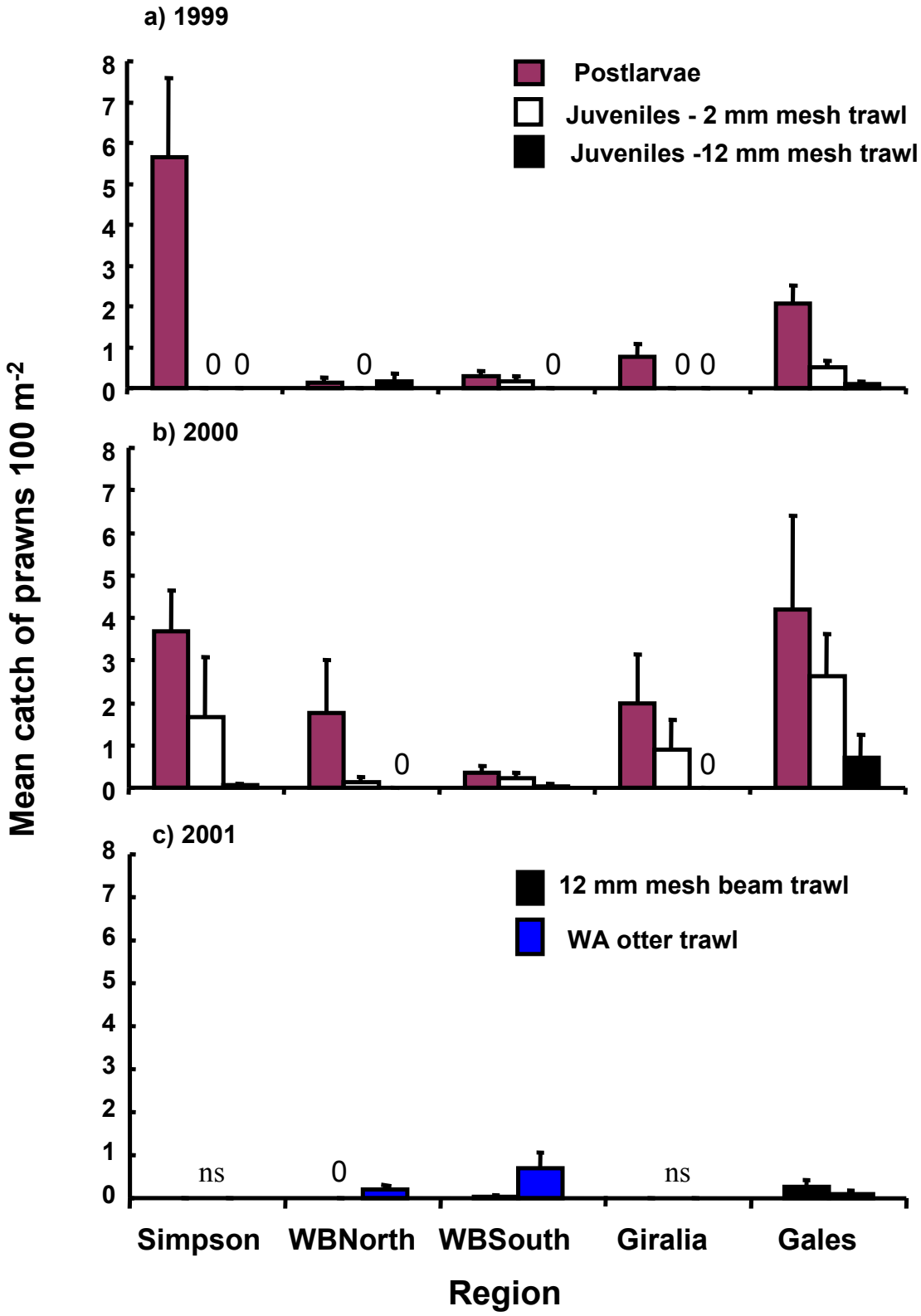


Figure 6.3 **Mean catches of Penaeus esculentus at five regions within Exmouth Gulf in a) October/December 1999, b) October/November 2000 and c) December 2001.**

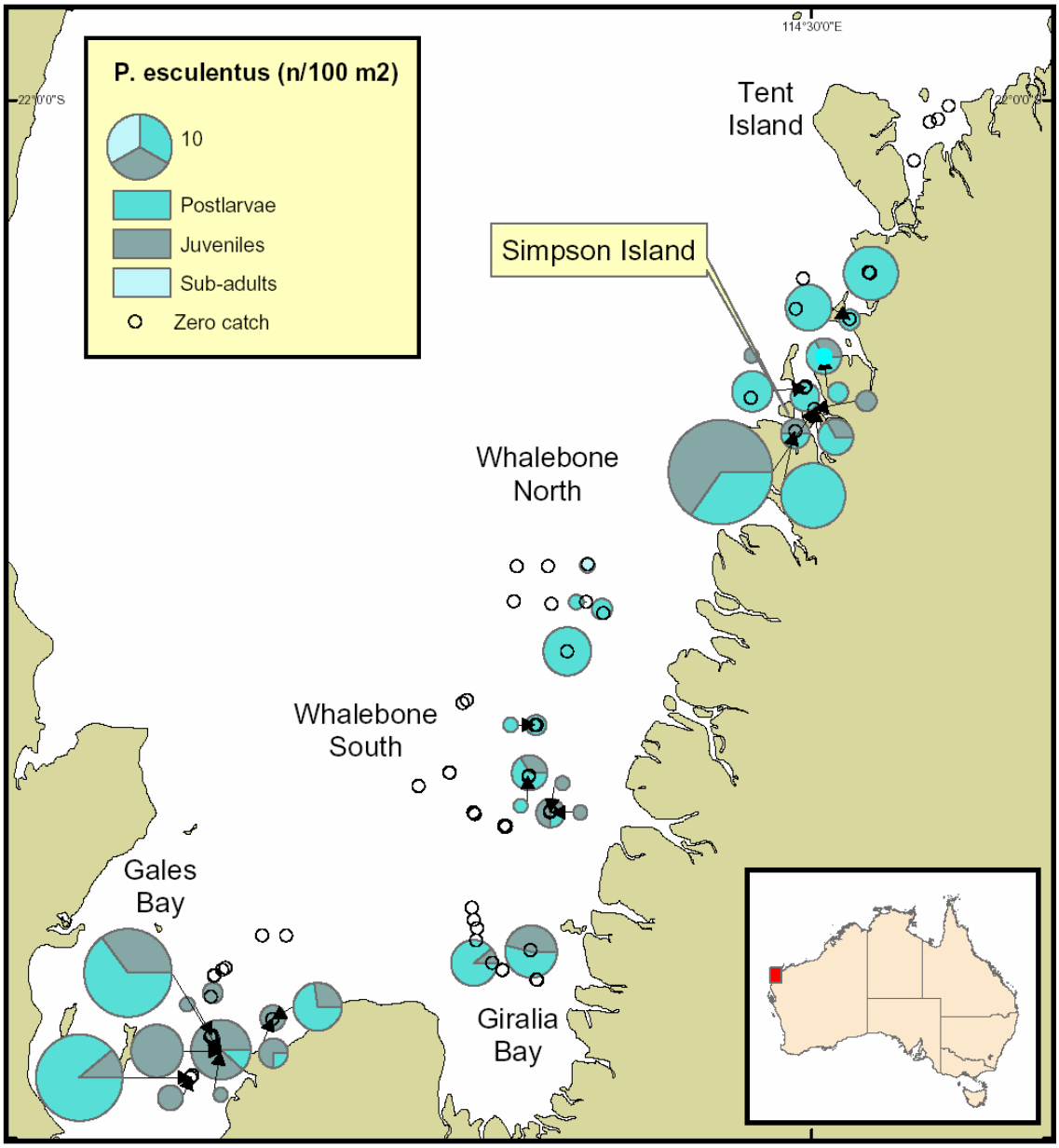


Figure 6.4 **Postlarval, juvenile and sub-adult Penaeus esculentus distribution October and November 2000.**

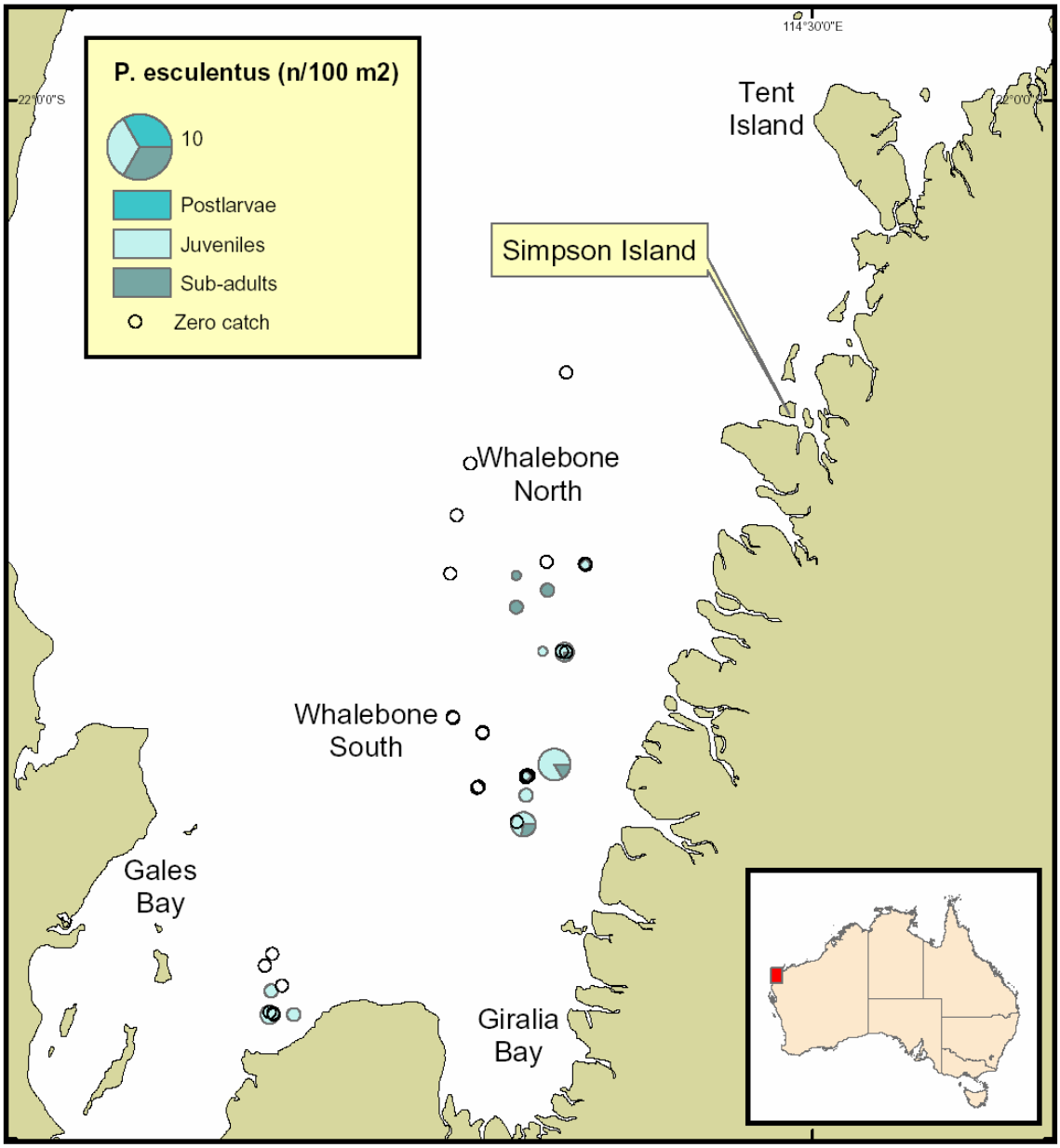


Figure 6.5 Juvenile and sub-adult Penaeus esculentus distribution December 2001.

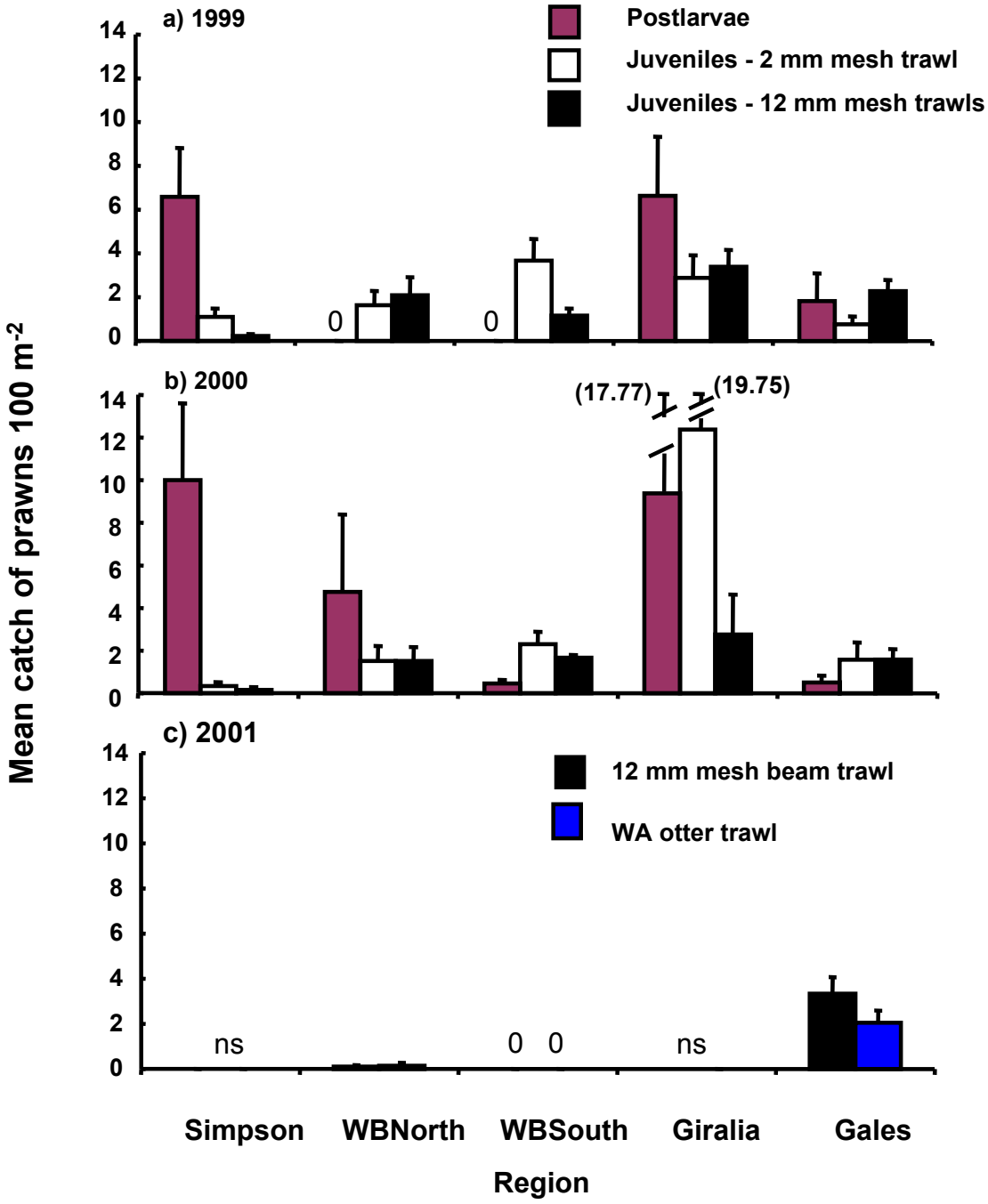


Figure 6.6 **Mean catches of Penaeus *latisulcatus* at five regions within Exmouth Gulf in a) October/December 1999, b) October/November 2000 and c) December 2001.**

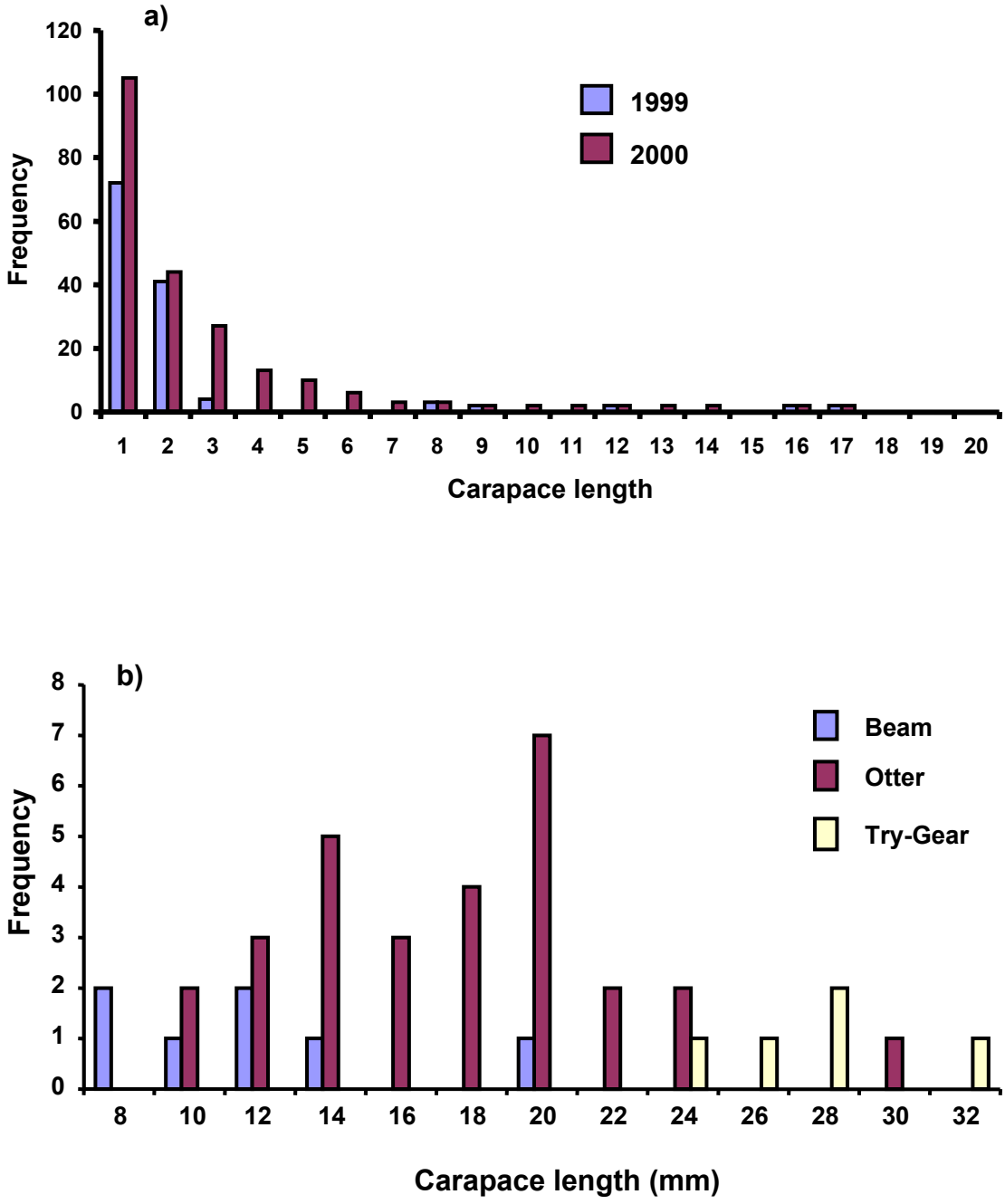


Figure 6.7 **Size classes of *Penaeus esculentus* in Exmouth Gulf in a) October/ December 1999 and October/November 2000, caught with the 2 mm mesh net; b) December 2001, caught with the 12 mm mesh beam trawl, the 12 mm mesh otter trawl and the Jurabi K try gear.**

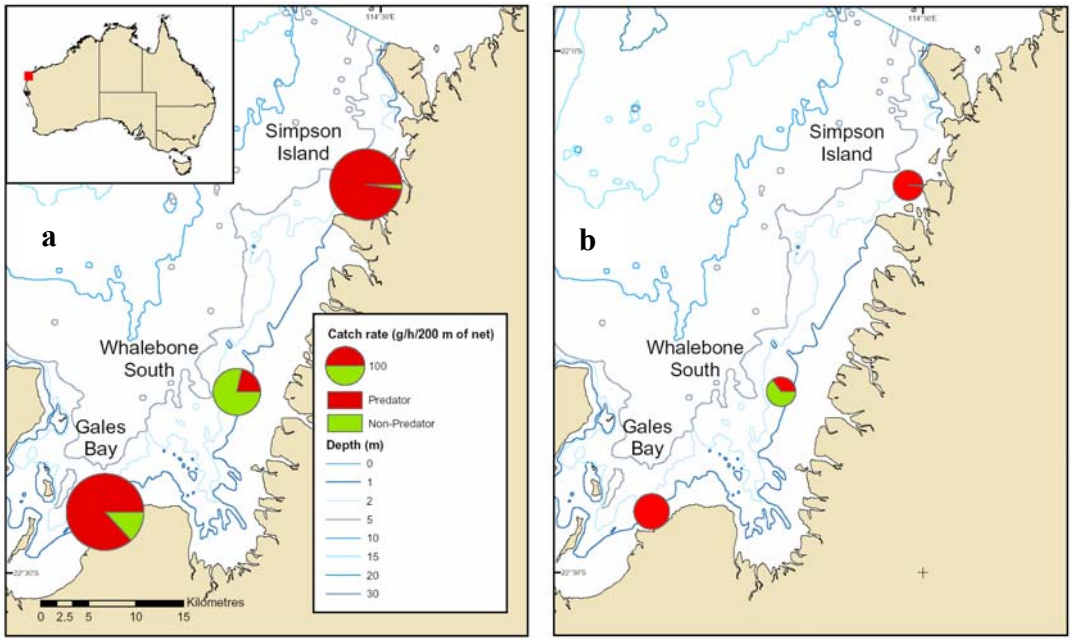


Figure 6.8 **Gill net catch rates of all fish species pooled. The area of the bubbles is proportional to the catch rate. The proportion of the catch that was comprised of species known to eat prawns is shown as red; the proportion of non-predators is coloured green. Sites were at Gales Bay, Simpson Island and south of Whalebone Island and were sampled during December 1999 (a) and November 2000 (b).**

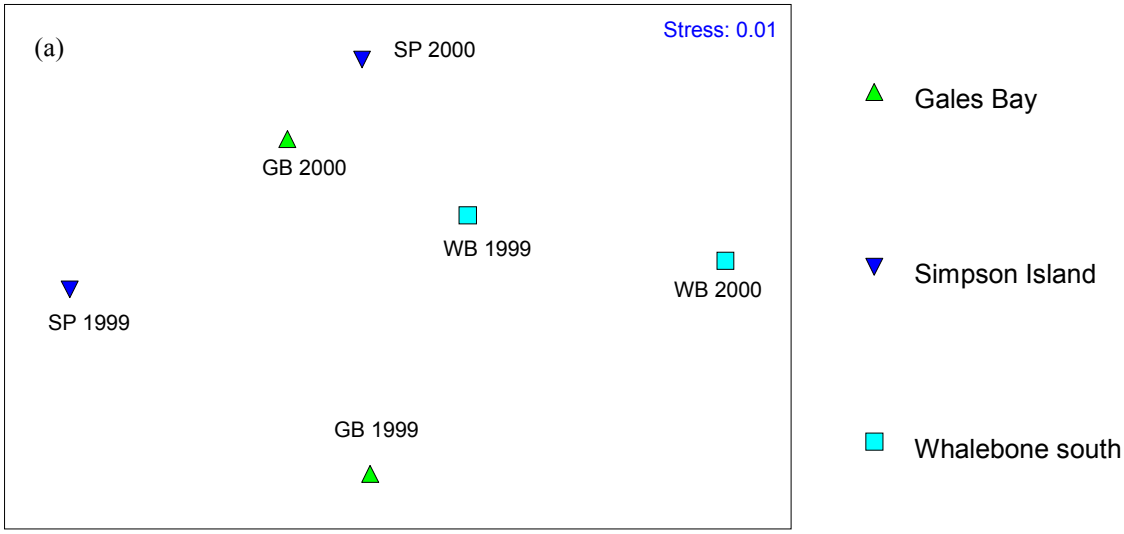


Figure 6.9 a **Non-metric Multidimensional Scaling plot of the presence/absence of the fish species sampled with 3, 4, 5 and 6" gill nets at Gales Bay, Simpson Island and Whalebone South during December 1999 and November 2000.**

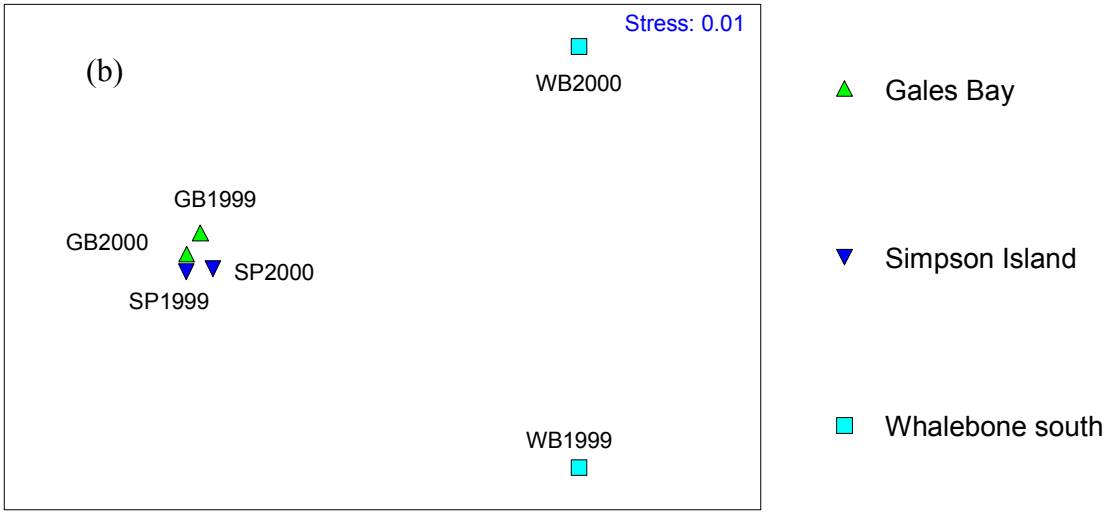


Figure 6.9 b **Non-metric Multidimensional Scaling plot of the square-root transformed catch rates, of the fish species sampled with 3, 4, 5 and 6" gill nets at Gales Bay, Simpson Island and Whalebone South during December 1999 and November 2000.**

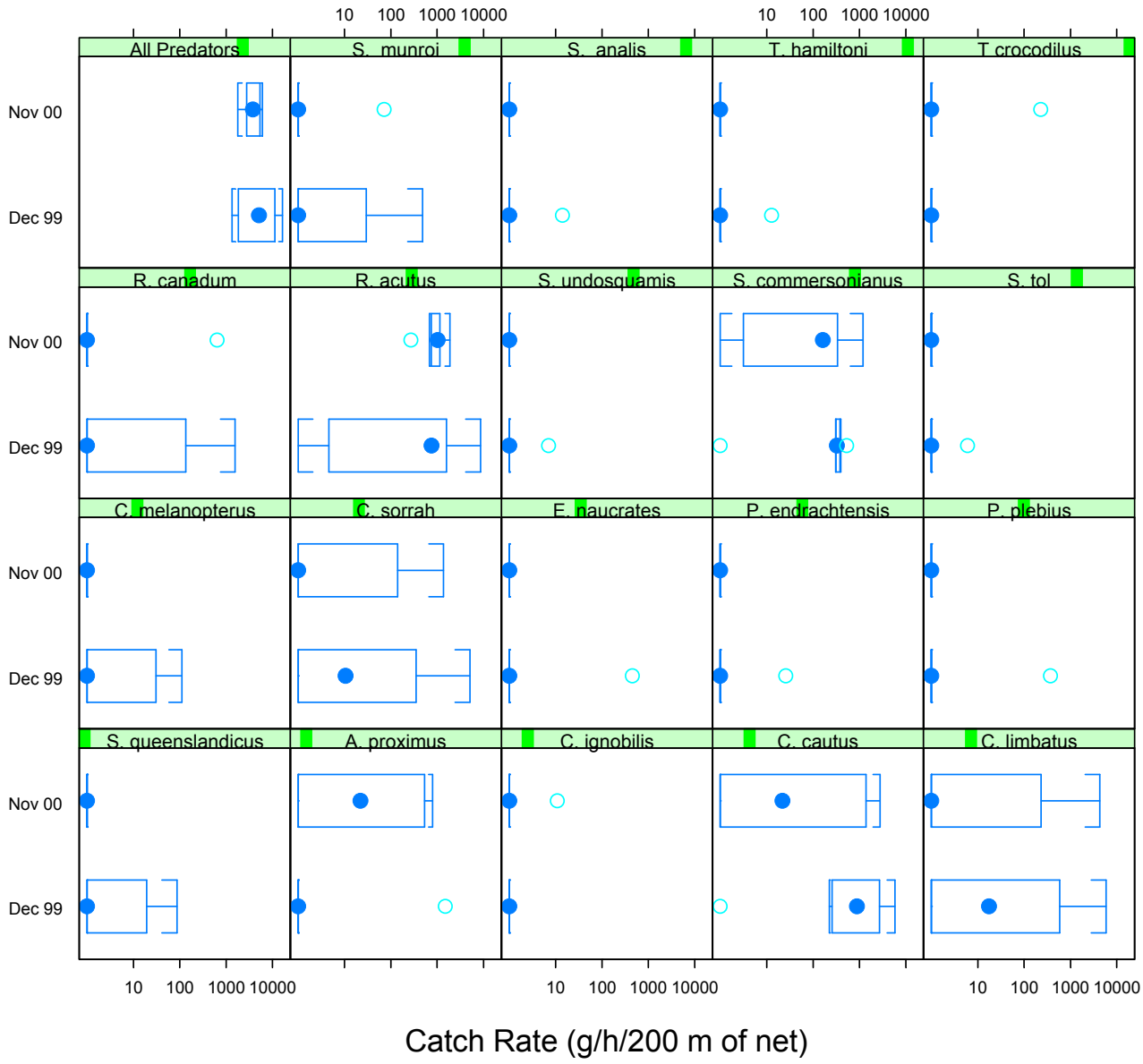


Figure 6.10 Box and whisker plots of catch rates of all fish caught in Exmouth Gulf that are known to eat prawns. Note that the catch rate axis is on a logarithmic scale. Samples are from gill nets set at Gales Bay, Simpson Island and south of Whalebone Island during December 1999 and November 2000. The nets were set 4 times at each site each sampling season i.e. 24 sets in total. Median (dark blue closed circles), inter-quartile ranges (dark blue open boxes), 1.5 times the interquartile range (outer fences) except the outliers (light blue open circles). We have displayed the median rather than mean catch rates because the mean is more affected by extreme scores than the median and is therefore not a good measure of central tendency for extremely skewed distributions. Species names are abbreviated as follows:
***Arius proximus* = *A. proximus*, *Caranx ignobilis* = *C. ignobilis*, *Carcharhinus cautus* = *C. cautus*, *Carcharhinus limbatus* = *C. limbatus*, *Carcharhinus melanopterus* = *C. melanopterus*, *Carcharhinus sorrah* = *C. sorrah*, *Echeneis naucrates* = *E. naucrates*, *Platycephalus endrachtensis* = *P. endrachtensis*, *Polydactylus plebius* = *P. plebius*, *Rachycentron canadum* = *R. canadum*, *Rhizoprionodon acutus* = *R. acutus*, *Saurida undosquamis* = *S. undosquamis*, *Scomberoides commersonianus* = *S. commersonianus*, *Scomberoides tol* = *S. tol*, *Scomberomorous queenslandicus* = *S. queenslandicus*, *Scomberomorus munroi* = *S. munroi*, *Sillago analis* = *S. analis*, *Thryssa hamiltoni* = *T. hamiltoni*, *Tylosurus crocodiles* = *T crocodiles*.**

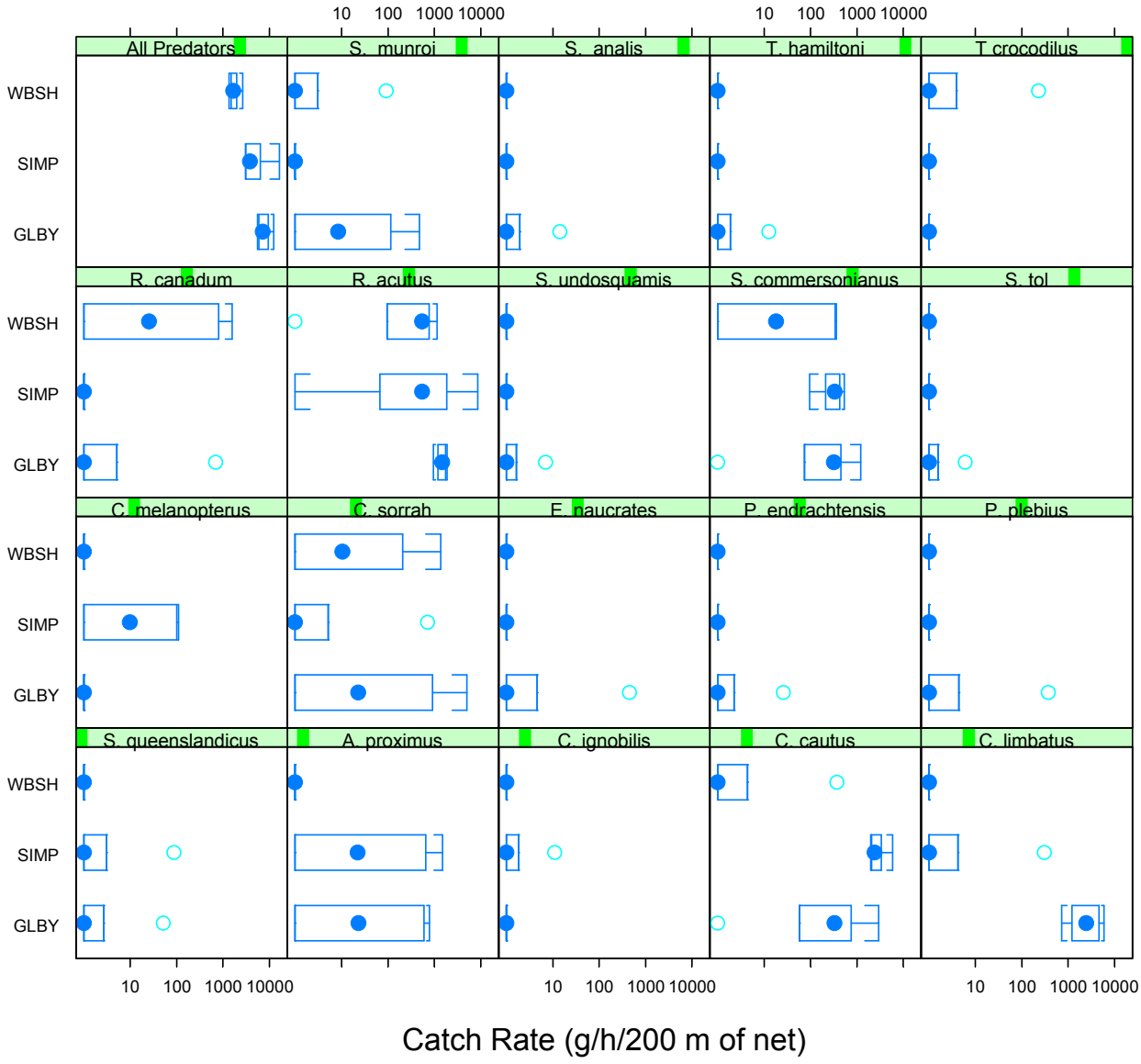


Figure 6.11 **Gill-net catch rates of prawn predators during December 1999 and November 2000 at 3 sites in Exmouth Gulf. Note that the catch rate axis is on a logarithmic scale. The sites were South of Whalebone Island (WBSH), Simpson Island (SIMP) and in Gales Bay (GLBY). Species names abbreviated as for Figure 6.10.**

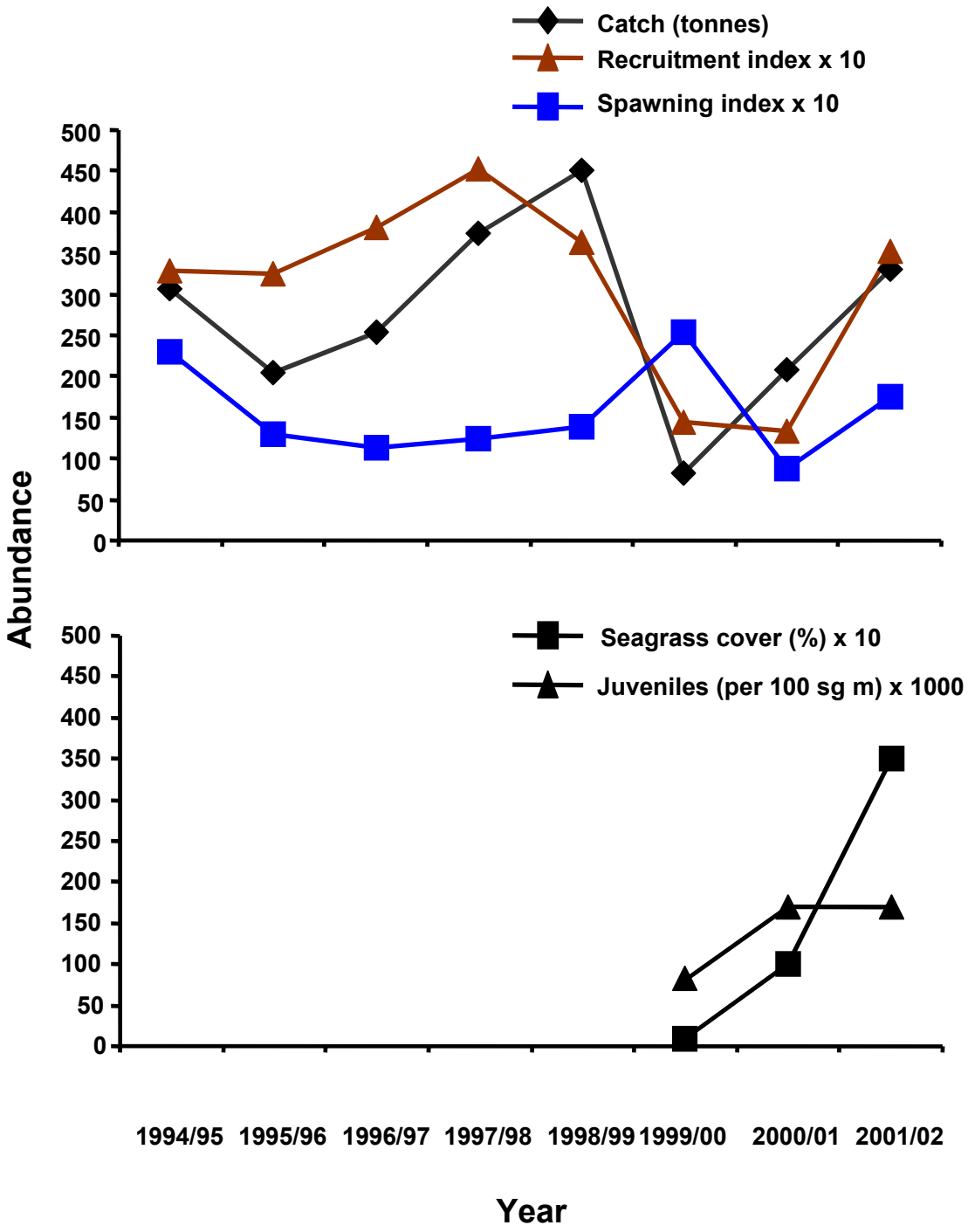


Figure 6.12 **Trends in seagrass percent cover, juvenile tiger prawns abundance (1999/2000-2001/02), recruitment indices and commercial catch (1995/96-2001/02) in Exmouth Gulf.**

CHAPTER 7

EXMOUTH GULF PRAWN FISHERY 2001

Mervi Kangas
Nic Caputi
Jim Penn

7.1 Tiger prawn catch history

The Exmouth Gulf fishery began targeting banana prawns in 1963 but by 1966, night trawling, which targeted tiger prawns had become the major fishing activity. In 1975, catches of tiger prawns had reached 1,239 tonnes. Until 1980, tiger prawns were the dominant catch and during these years the effort in fishery increased (Penn et al., 1997). In 1981 and 1982, there was a decline in recruitment and subsequent catch of tiger prawns because of overfishing of this tiger prawn stock. This resulted in a catch of only 77 tonnes in 1983 (Figure 7.1). Tight management restrictions were introduced at this time in order to rebuild the tiger prawn stocks. Variable closures of the main tiger prawn fishing grounds and extension of permanent closure areas (Figure 7.2) were introduced into the fishery to reduce effort levels (Penn et al., 1997) to allow a constant escapement of tiger prawn. The objective was to provide an optimal level of spawning stock irrespective of annual recruit strength.

Since the introduction of these additional management measures, tiger prawn stocks improved as breeding stock increased. This improvement is reflected in the tiger prawn catches returning to levels achieved in the 1970s (400 – 600 tonnes). Management strategies are continually being reviewed to ensure adequate spawning stock levels (for normal environmental conditions) remain.

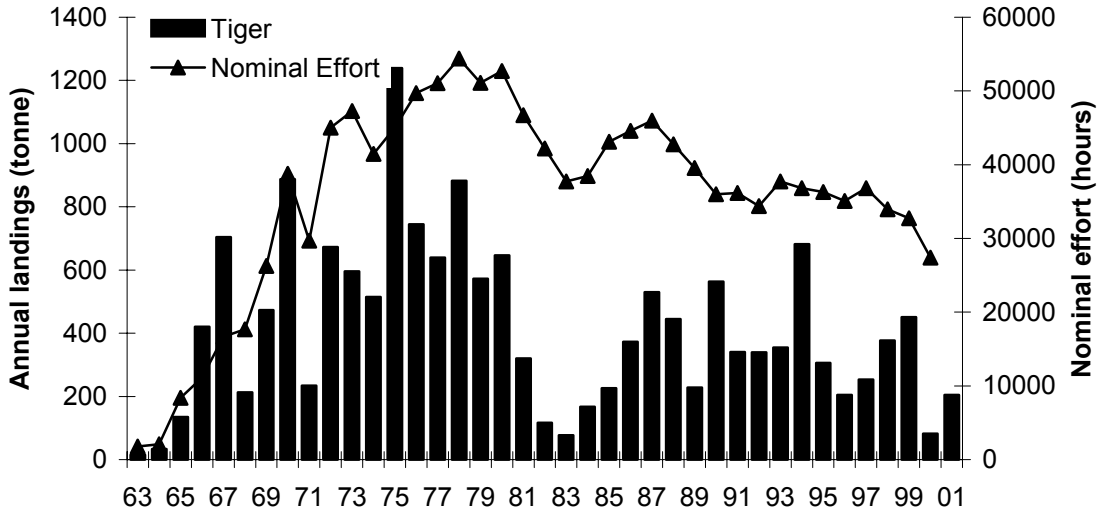


Figure 7.1 Historical landings of tiger prawns in the Exmouth Gulf prawn fishery.

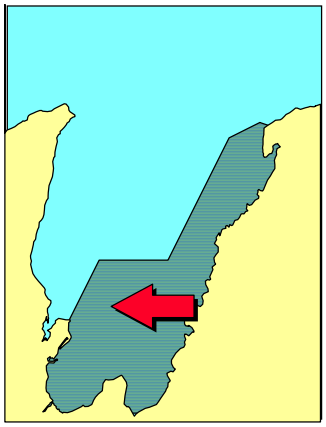


Figure 7.2 Permanent nursery closure areas in Exmouth Gulf prawn fishery

7.1.1 2001 landings

In 2001, the Exmouth Gulf prawn fishery commenced on 10th April. Seven of the thirteen vessels operating in the fishery stopped fishing on 13th November with the remainder ceasing fishing on 17th November 2001. The fishing patterns during the 2001 season have involved flexible fishing arrangements and voluntary industry closures based on an assessment of both king and tiger prawn size and abundance in areas via fishery independent surveys.

The preliminary annual unloading figures for 2001 indicate a total prawn catch of 666 tonnes, comprising 330 tonnes of king prawns, 205 tonnes of brown tiger prawns and 130 tonnes of endeavour prawns. Only one tonne of banana prawns was landed.

Tiger prawn landings improved in 2001 compared to the very low catch level of 82 tonnes recorded in 2000. However, tiger prawns were still below the ten year range of catches recorded from 1990 to 1999 of 250 –550 tonnes. The tiger prawn stocks seem to still be recovering following the negative impacts of Cyclone Vance on the seagrass habitat which impacted the recruitment in 2000. If environmental conditions continue to be favourable and seagrass continues to increase in biomass and spatial extent, the tiger prawn stocks are expected to continue to recover in 2002. King prawn catches in 2001 were slightly below the ten year acceptable catch range of 350 – 500 tonnes. Endeavour prawn catches were in the lower end of the acceptable catch range (120 – 300 t) for this species.

7.2 Fishery independent surveys

As the main spawning and recruitment areas were closed to fishing in the early 1980's, fishery independent surveys were introduced, within the tiger prawn spawning and recruitment grounds to provide the information previously gathered on spawning stock and recruitment catch rates via commercial logbook data.

7.2.1 Recruitment surveys and inshore sampling

Since 1985, three standard recruitment surveys have been conducted in Exmouth Gulf in early and late March and early April (Tables 7.1 and 7.2, Fig. 7.3). In 2000 and 2001, additional inshore sampling (inshore of normal recruitment sampling sites) using try-gear (5m) was also undertaken on 1st and 30th March to look at the abundance of smaller size classes of prawns and their potential predators.

In most years the recruitment surveys are dominated by small prawns with a modal carapace length of about 25 mm – this pattern of size distribution was seen in 2001, when the modal size for male tiger prawns was 24 to 26 mm CL (Figure 7.4). This distribution contrasts with that in 2000 when the prawns were much larger with a modal size of 30 to 32 mm CL indicating that the population consisted mainly of residual prawns from 1999. Therefore, although the recruitment indices were generally higher in 2000 than 2001 (Table 7.1, 7.2), recruitment levels in 2001 are higher than those in 2000 because most of the catch was recruits and not residual prawns. Inshore sampling also indicated slightly higher numbers of juvenile prawns in 2001 than 2000 (Table 7.3). However, recruitment in 2001 is still at reasonably low compared to historical indices (Tables 7.1, 7.2).

Table 7. 1 Indices of recruitment (Area Q3) and spawning (Areas Q1 and Q2) and total annual landings of brown tiger prawns in Exmouth Gulf. For location of areas, see Figure 7.3.

Year	Recruitment Index			Spawning index		Total
	Early March	Late March	Early April	Q 1	Q 2	Landings
1984				7.5		167
1985	14.4	20.7	25.4	6.8		226
1986	24.7	33.8	31.7	7.3		372
1987	57.0	52.2	51.0	12.2		529
1988	26. 8	35.2	42.5	6.3		445
1989	29.6	34.9	41.0	4.9	11.0	231
1990	41.3	43.2	46.4	15.4	17.9	564
1991	17.4	34.1	45.3	15.2	19.4	340
1992	21.0	23.0	31.5	15.3	17.7	339
1993	24.1	20.5	24.9	15.1	14.6	355
1994	43.4	57.8	60.7	15.2	30.9	682
1995	33.4	33.4	31.8	6.9	19.0	306
1996	31.4	34.3	31.6	8.0	14.7	205
1997	33.4	44.9	36.0	8.6	16.4	253
1998	28.3	45.4	62.0	10.4	17.4	377
1999	25.2	-	47.6	25.1	25.7	451
2000	11.1	19.2	13.2	10.3	7.4	82
2001	8.7	14.5	16.5	16.2	18.9	205

Table 7.2 **Indices of recruitment (Area P2) and spawning (Areas Q1 and Q2) and total annual landings of brown tiger prawns in Exmouth Gulf. For location of areas, see Figure 7.3.**

YEAR	Recruitment index			Spawning index		Total
	Early March	Late March	April	Q 1	Q 2	Landings
1984				7.5		167
1985	10.9	15.9	16.2	6.8		226
1986	13.2	29.0	41.2	7.3		372
1987	48.9	40.3	30.0	12.2		529
1988	20.8	23.2	30.7	6.3		445
1989	15.0	18.3	19.5	4.9	11.0	231
1990	37.9	55.9	24.6	15.4	17.9	564
1991	22.0	31.8	28.7	15.2	19.4	340
1992	11.8	18.7	25.0	15.3	17.7	339
1993	18.0	22.5	25.2	15.1	14.6	355
1994	61.9	55.5	45.6	15.2	30.9	682
1995	21.9	12.9	23.2	6.9	19.0	306
1996	15.6	13.8	22.7	8.0	14.7	205
1997	13.6	17.5	16.8	8.6	16.4	253
1998	22.3	17.3	33.4	10.4	17.4	377
1999	12.8	-	28.3	25.1	25.7	451
2000	18.3	33.5	30.1	10.3	7.4	82
2001	8.6	11.0	23.8	16.2	18.9	205

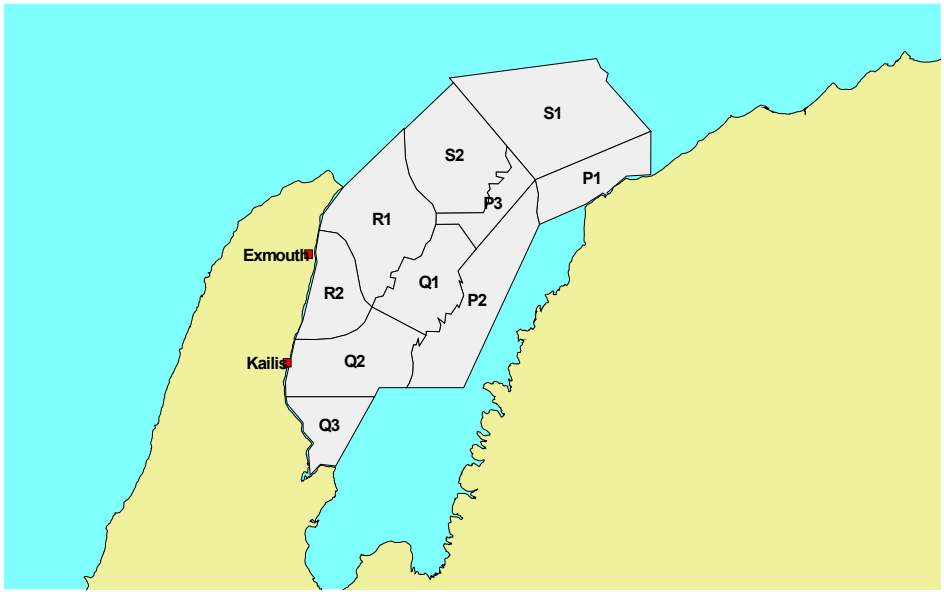


Figure 7.3 **Survey areas in the Exmouth Gulf prawn fishery indicating areas Q3 and P2 (recruitment areas) and Q1 and Q2 (spawning areas).**

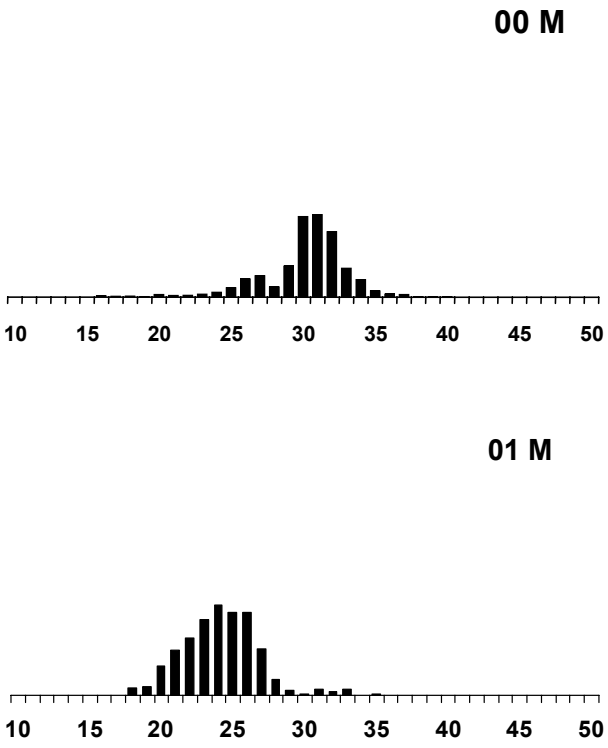


Figure 7.4 Carapace length frequency distributions for male tiger prawns on (a) 27th March 2000 and (b) 31st March 2001

Seagrass surveys in late 2000 indicated increased seagrass/algal abundance at some sites and therefore some improvement in recruitment was anticipated in 2001 (see Chapter 5 - Benthic habitats in Exmouth Gulf). Sampling in December 2001 indicated a further improvement in the biomass and extent of seagrass and therefore further improvement in recruitment levels may occur in surveys conducted in March and April 2002.

Table 7.3 Tiger prawn catches during Eastern shore fishery independent surveys (5 m try-net, 15 minute trawls) in 2000 and 2001.

Site	Latitude	Longitude	Date of sampling		
			30 March '00	01 March '01	30 March ' 01
1	22° 18.7'	114° 16.5'	2	20	32
2	22° 17.3'	114° 19.0'	2	12	7
3	22° 14.9'	114° 19.4'	23	10	17
4	22° 11.5'	114° 19.8'	32	40	84
5	22° 08.5'	114° 22.0'	15	11	10
6	22° 06.7'	114° 24.7'	19	39	110
7	22° 05.4'	114° 26.7'	15	17	32
8	22° 04.3'	114° 20.0'	45	12	6
9	22° 02.0'	114° 29.1'	10	3	0
		Mean	14.8	18.2	33.1

7.2.2 Spawning Stock surveys and SRR relationship

By using historical catch and effort data, it was evident that a strong spawning stock - recruitment relationship existed for the tiger prawn (Figure 7.5, Penn et al. 1995, Caputi et al. 1998).

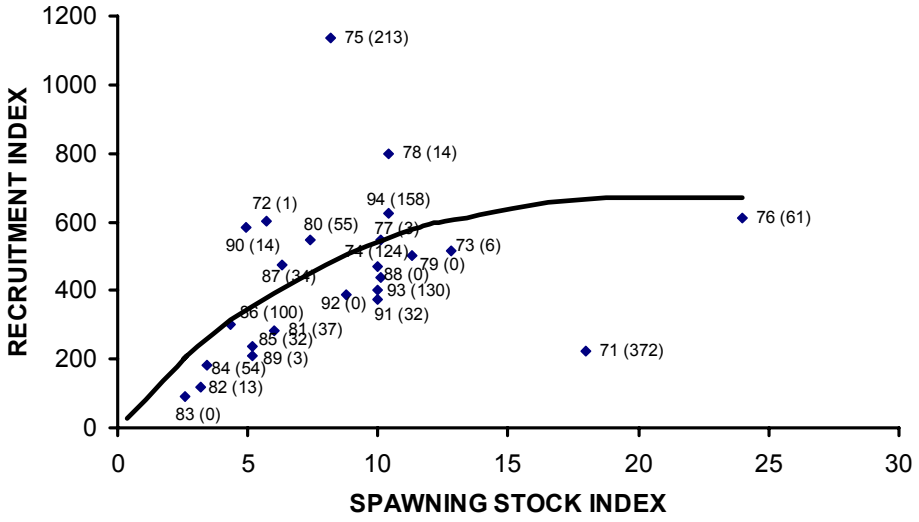


Figure 7.5 **The SRR for the Exmouth Gulf tiger prawn stock using indices from fishery logbook data from 1972 until 1994. Data points show the year of recruitment (*t*) and the January and February rainfall total (mm).** The relationship is given as: $R_t = 85.3\ S_{t-1} \exp(-0.0468\ S_{t-1})$, where *R* is the recruit index and S is the spawning index from the previous year (Caputi et al. 1998).

Estimates (indices) of the level of spawning stock were obtained in two ways:

1. Historically this has been the standardised catch per unit effort (CPUE) for the fishery in the main tiger prawn spawning areas (Q1, Figure 7.3) during August to October which is the main spawning period.
2. Since 1982, fishery independent systematic surveys of the breeding grounds (Figure 7.6) have been undertaken because the spawning area has been closed to fishing for periods both before and during the critical spawning period. Surveys take place in August, September and October during the quarter moon phase.

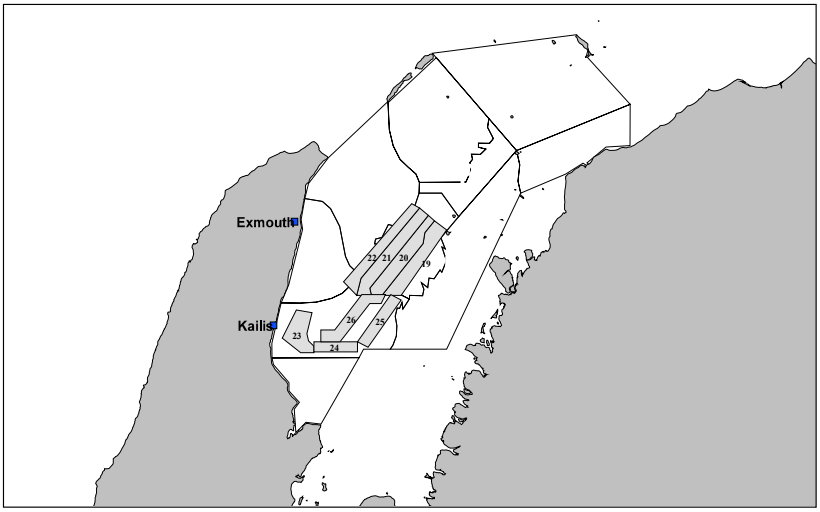


Figure 7.6 Exmouth Gulf spawning stock survey sites

In 2001 additional surveys of spawning stock were also undertaken in November and December to determine the changes in distribution, abundance, size composition and proportion of females spawning during the closure period. The level of spawning stock (Tables 7.1 and 7.2) was higher in 2001 than 2000 and limited fishing on tiger prawn stocks was permitted in the main tiger prawn spawning area in September 2001, until the catch rate fell to the threshold of 19 kg/hr (quad gear). The 2001 level of spawning stock (about 16, Table 7.1) is the fourth highest index of spawning stock since indices were recorded.

7.3 Environmental factors

Low rainfall (about 50 mm) was recorded in 2001, particularly compared with the preceding two years (>300 mm of rain) (Figure 7.7) and no major cyclone activity was reported.

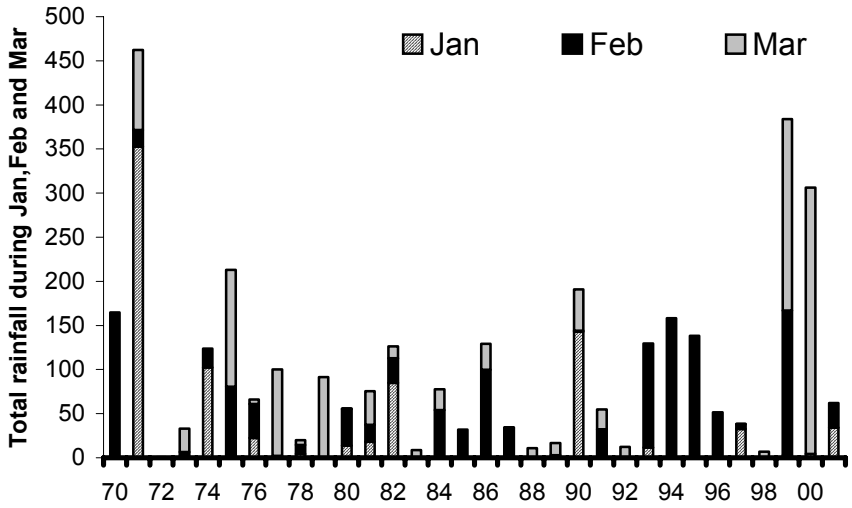


Figure 7.7 Rainfall (mm) in Exmouth Gulf during January to March.

7.4 References

Penn J.W., Caputi N. and Hall N.G. (1995) Stock-recruitment relationships for the tiger prawn *(Penaeus esculentus)* stocks in Western Australia. *ICES mar.Sci.Symp.,* 199: 320-333.

Penn J.W., Watson, R.A., Caputi, N. & Hall, N. (1997). Protecting vulnerable stocks in multi-species prawn fisheries. In: Hancock, D.A., Smith, D.C., Grant, A. & Beumer, J.P., (Eds) *Developing and Sustaining World Fisheries Resources. The State of Science and Management. 2nd World Fisheries Congress* pp. 122-129.

Caputi N., Penn J.W., Joll L.M. and Chubb C.F. (1998). Stock-recruitment-environment relationships for invertebrate species of Western Australia. In: Proceedings of the North Pacific Symposium on Invertebrate Stock Assessment and Management. Jamieson G.S. and Campbell A. (Eds.) *Can.Spec.Publ.Fish.Aquat.Sci.* 125: 247-255.

CHAPTER 8

THE BIO-ECONOMIC MODEL TO EVALUATE THE COSTS AND BENEFITS OF TIGER PRAWN STOCK ENHANCEMENT IN EXMOUTH GULF

Yimin Ye
Neil Loneragan
Bronwyn Harch

Summary

The model from the feasibility study of prawn stock enhancement (FRDC 1998/222) was revised to take into account new parameter values (particularly those from production) and recommendations from International Reviewers (Harry Campbell, Ray Hilborn and Ken Leber), the stock enhancement Steering Committee (George Kailis, Jim Penn, Ian Poiner and Peter Rothlisberg) and a working group meeting in September 2002. We introduced a term for mortality at harvest, transport and release of the juvenile prawns, and one for density dependent mortality from the time of release to capture in the fishery. Because of a lack of data, we assumed a mean mortality for harvest, transport and release of 7% with a triangular distribution from 4% to 10% (imposed immediately after release). We estimated a value for density dependent mortality of tiger prawns in Exmouth Gulf through the use of the stock recruitment relationship of the Exmouth brown tiger prawn fishery (Caputi et al. 1998). Density dependent mortality was assumed to range from 8 to 23% for the 7 weeks between release and emigration from the nursery ground. Because of the lack of information on likely values for density dependent mortality, a uniform distribution was used in the simulations.

From the revised model, a release of 24 million juvenile prawns (1.0 g) was estimated to increase catches of brown tiger prawns by about 113 metric tonnes, or an estimated 18.8 % of the released prawns are caught by the fishery. If an enhancement of 24 million prawns was carried out in one production run, 179 raceways with a final stocking density of 2,700 prawns$\cdot$m^{-2} would be needed. This scale of production requires 36 million 15-day-old postlarvae (PL15) to stock the raceways (initial stocking density of 4,063 prawns$\cdot$m^{-2}) and the collection of about 1,000 fertilised females, assuming that 500 of these individuals spawn successfully.

The median operating costs for “production” in raceways (includes operating costs for hatchery and production, harvest, transport and release) were about AUD$ 1.40 Million (M) (range = $1.16 M to $1.56 M). About 38% of these costs were for the production of postlarvae, with 17% for salaries, and 12 to 13% for each of transport, feed, and electricity. The median index of “profit” estimated for raceways (i.e. marginal revenue from enhancement – operating costs of enhancement) was a loss of AUD -$170,000 with a range from -$950,000 to $1.53 M. Enhancement from production in raceways had a 48.2% chance of being profitable. Note that these estimates of “profit” provide an index of relative profits, not net profit because the capital costs of investment were not included in the calculations. The capital costs for expanding the existing hatchery were about AUD $3.6 M and $4.48 M to establish the 179 raceways for the production of 24 million 1 g prawns. The capital costs would be halved if 2 production runs of 12 million prawns were completed. However, we do not know how successful two releases would be compared with one major release. The simulations of the bio-economic model for 1 g juveniles were compared with simulations for 0.5 g. About 25% greater numbers of 0.5 g prawns (30 million juveniles and 45 million PL15s) are required to produce an enhanced catch of 100 tonnes, which significantly increases the costs of producing postlarvae and extends the time that density dependent mortality affects the prawns in the nursery areas. However, only about half of the raceways (87) are required to produce the 0.5 g prawns because of the lower total biomass of production, which reduces the capital costs of investment.

The model was an invaluable tool for helping to integrate research and identify parameters that have a major influence on the performance of an enhancement. However, the model only evaluates one release in a year and assumes that all prawns are released at one time. Although the model can be widely distributed because it is written in EXCEL, it is difficult to follow and modify because of the many worksheets in the model. The Steering Committee has identified that further work on the model to make it more user-friendly and allow a wider range of enhancement scenarios to be evaluated (e.g. multiple-releases, releases spread over-time), and potentially developing similar models for finfish and shellfish, would be very worthwhile.

8.1 Introduction

The true measure of the success of any stock enhancement program is its ability to increase fishery revenue by more than the cost incurred in the production, release and monitoring of juveniles produced by aquaculture and released into the wild. The cost of evaluating success through a real experiment is high and bio-economic modelling is often used for this evaluation and should be considered as an essential part of any enhancement project (Blankenship and Leber 1995, Rothlisberg et al. 1999).

We have developed a bio-economic model to evaluate the feasibility of stock enhancement programs. The basic structure of this model was discussed by the project participants at the workshop in Perth in July 1998. The model was implemented in EXCEL to make its output and basic assumptions easily accessible to managers and industry (Loneragan et al. 2001a). Comprehensive analyses, however, should only be run by people familiar with the structure and properties of the model. The model employed sub-models for each component of the enhancement: the hatchery, juvenile production, transport, nursery ground, and fishery. A relatively independent component was added for monitoring released juveniles in the wild environment. The model can be used to run bio-economic analyses to evaluate different experimental designs for stock enhancement or to monitor the economic success of the enhancement.

All the components of stock enhancement are subject to uncertainty from either environmental variations or technical deviations. To mimic the real situation as closely as possible, we used Monte Carlo simulation in this study instead of deterministic modelling. Model parameters were not supposed to take single point values, but to assume a certain distribution, which was derived from experiments or expert judgements. With Monte Carlo simulation, we can quantify the effects of these uncertainties on model-output results and then carry out risk analysis for some management targets or probability evaluation for certain outcomes of interest to the industry. Commercially available software, Crystal Ball (Werckmand et al. 1998), was used to conduct Monte Carlo simulations.

The current model is able to evaluate a single release of prawns at any time within the year and assumes that all prawns are released at a single time. It is not able to assess multiple releases in a year or spreading the release over weeks or months.

8.2 The Model

The bio-economic model has 5 components or sub-models (Figure 8.1) and traces two sub-population dynamics: the enhanced and the wild. The enhanced component covers from the spawning of brood stocks through to the end of fishing, and the wild component covers the time from natural recruitment to the nursery grounds through to capture in the fishery. Weekly time steps were used in the model to capture the seasonal nature of tiger prawn growth.

This model was used to evaluate the enhancement of the prawn fishery in Exmouth Gulf, Western Australia. Two enhancement stages were evaluated: (1) an experimental enhancement and (2) a trial commercial scale enhancement (for details see below). Briefly, the aim of the experimental enhancement was to determine the minimum number of juvenile prawns to be released that could be detected by sampling the commercial fishery. The stage for the trial commercial scale enhancement aimed to evaluate how many prawns would have to be released to enhance the fishery by about 100 t. The parameter values used in the second evaluation are given in Appendix 8A, and each component of the model is described below. With modifications, we believe that this model could be applied to other organisms and other fisheries. The modifications to the model could include converting the model to another programming language or languages to allow great ease of use and the logical connections in the model to be clearer than is possible in EXCEL. The new model should also allow the performance of different enhancement scenarios to be evaluated such as: more than one release of animals in a year and releases to be spread over weeks or months.

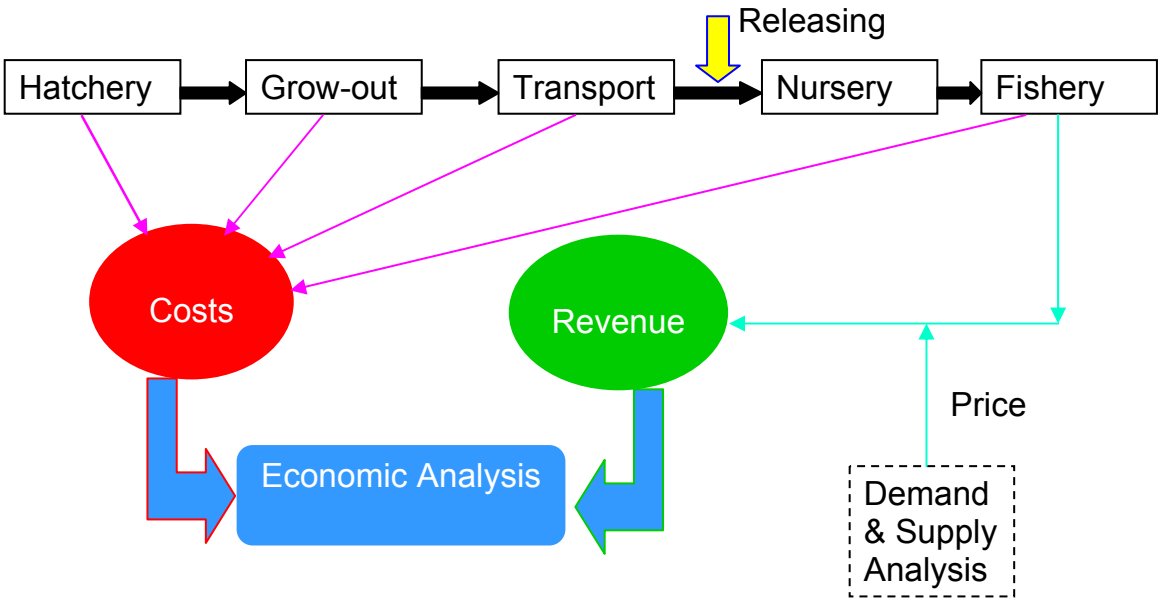


Figure 8.1 Structure of the bio-economic model

Because of a lack of information on the costs of the hatchery and juvenile production in Exmouth Gulf, the feasibility study and initial years of this project, modelling approaches were used to estimate estimates the costs of producing larvae and juvenile prawns. However, during this project, data on the costs of the hatchery and production were through the successful production of 1 g prawns at Exmouth Gulf. The actual costs are now used in the model but both approaches are described below.

8.2.1 Hatchery

The hatchery component comprises the brood stock and the hatched larvae. A detailed prawn hatchery model, containing the costs for using either wild or domesticated brood stock, has been developed by Preston et al. (1999). In the feasibility study and the first stages of the current project, this model was used to estimate the cost of production (Ω) of each larva (termed zoea in Preston et al. 1999) so that the current hatchery component can be simplified to only contain the output of the hatchery (i.e. the numbers of larvae required and their total cost).

After the development of the prawn production facility at Exmouth Gulf and its successful use to produce prawns, the modelled costs were replaced with a cost of production of postlarvae

15 (PL15) for stocking in the raceways of AUD $0.018 per prawn and an estimated survival rate, based on trials in Brisbane and Exmouth (Chapter 3) of 67.5%. These figures mean that about 1.48 million PL15s, costing about $26,647 would be needed to produce 1 million 1 g juveniles in raceways.

8.2.2 Juvenile Production

This part covers the time from stocking the raceways with PL15 (as defined by the stage produced in the hatchery) to the release of the juvenile prawns in the nursery. In this component, the initial approach used a certain level of production of juveniles N_0 of certain weight W_e as the starting point for the calculations. Given the initial number of larvae required to achieve a certain target of production, the total number of the stock is supposed to follow an exponential decay process. If the mortality rate caused by all natural causes is known M_t, the total number is calculated step by step as follows:

$$N_{t+1} = N_t e^{-M_t}$$

where M_t is the natural mortality. In early life stages, mortality rate changes dramatically over time. But its variation is believed to be related more to body size than time. The model, therefore, assumes natural mortality is a function of size:

$$M_t = \phi\alpha\ e^{-\beta L_t}$$

where L_t is the carapace length at week t, α and β are parameters defining how mortality changes with size in the population and ϕ denotes the difference in mortality between the wild and the artificially raised juveniles. In most circumstances, juvenile grow-out production facilities can establish favourite environments and provide high protein feeds for rapid growth or for cost-benefit efficiency. So, the natural mortality rate is lower than those in the natural nursery areas, and ϕ is less than 1.

Individual growth is modelled as an exponential function of parameter δ,

$$W_{t+1} = W_t e^{\delta}$$

To accommodate for the change in growth rate that occurs during the transition from post-larvae to juveniles, a different value of δ is used for the juveniles, i.e. when prawns reach size W_j. Calculations are stopped when W_t reaches the weight of the first larval stage W_0. This determines the week when prawns are seeded to the wild nursery. Individual weight W_t is calculated from the length weight relationship,

$$W_t = a\, L_t^{\,b}$$

Two production systems were considered for the feasibility study: ponds and raceways. However, it was decided to only model raceways for the current project as the initial modelling showed that the index of profit for raceways was much higher than that for ponds (Loneragan et al. 2001a). The number of raceways required y is a function of the area of each raceway A (m^2), the maximum biomass density at which prawns can be held d ($kg{\cdot}m^{-2}$) and the biomass of juvenile prawns (kg) to be held to meet the target of production increase W,

$$y = \frac{W}{d\,A}$$

The production costs considered were the capital costs R of building each raceway, and the operating costs. No attempt was made to calculate the net present value of the total investment and therefore, the desired internal rates of return and depreciation rates, were not considered. Such calculations are, however, easily done within EXCEL.

Operating costs G_t comprised costs of prawn feed, salary and pumping,

$$G_t = y(a\,\sigma + \psi) + B_t\,\eta + (B_t - B_{t-1})\gamma\,\tau$$

where σ is the weekly cost of pumping per m^2 of pond or raceway, η is the cost of pumping per kg of prawns, ψ is the salary costs of maintaining one pond or raceway, γ is the cost of feed per kg of prawns, and τ is the food conversion ratio (i.e. ratio of food: growth in biomass).

Following the successful production of juvenile prawns in raceways at Exmouth Gulf in 2000 and 2001, the actual costs of production per million 1 g juvenile prawns were used in the model (Table 8.1). The major costs of production are the electricity for pumping and aeration and feed (30%) (Table 8.1).

Table 8.1 **Costs of production for 1 million 1 g juvenile prawns in Exmouth Gulf (from discussions with Roger Barnard, Richard McCulloch and Peter Crocos)**

Item	Cost per 1 million 1 g prawns
Electricity for pumping and aeration	$16,600
Feed	$7,450
Labour	$1,100
Water quality monitoring	$700
Discharge monitoring	$700
Contingency	$500
Total	$27,050

8.2.3 Harvest, transport and release

The harvest and transport from raceways to release sites may cause death of some juveniles. Although a preliminary experiment carried out by MG Kailis Group in West Australia showed no transport mortality, for precautionary purposes, a transport-related mortality was still incorporated in the model with a triangular distribution from 4% to 10% with a likeliest value of 7%.

The cost of transporting the juveniles to the release site is assumed to be a function of number of trips required to transport the juveniles and the number of release sites to be used in each trip. The number of trips is determined by the size of the juveniles, their number, the volume of the tanker/vessel used for transport, and the desired ratio of juvenile biomass to water in the tanker. The number of sites per trip is a function of the biomass of juveniles that can be

released at each site. A daily cost rate is used to include the cost of harvesting, transporting and releasing.

The numbers n carried per tanker/vessel were estimated as,

$$n = \frac{V}{W_t(1+\theta)}$$

where

V is the volume of the tanker/vessel,
W_t is the weight of each prawn at the time of transport and
θ is the ratio of seawater/biomass desired during the transport.

The number of trips of the tanker/vessel are therefore N_t / n, where N_t is the number of juvenile prawns produced in the production and to be released in the nursery area.

The minimum number of sites r required to release prawns is a function of the biomass that can be released per site B_s,

$$r = \frac{N_t W_t}{B_s} + 1$$

and the number of days required to release all prawns D,

$$D = \frac{N_t / n}{T}$$

where T is the number of trips per day that can be made. Transport costs are therefore the product of the number of days and the operating costs of each day T_c.

8.2.4 Nursery ground

There are two sub-components in the nursery ground, one corresponding to the wild stock and one to the enhanced stock. They have the same structure and only differ in the timing and the numbers of prawns entering the nursery.

The enhanced stock sub-component includes juvenile prawns from the time they are released to the time they recruit to the fishery. The size of released prawns can range from the size of a larva, to that of a recruit. The timing and number of prawns settling in the nursery can be varied.

The wild stock sub-component includes prawns from larvae to recruits to the fishery. Survival and growth are size dependent. Growth also varies seasonally. Survival can be made dependent on the total abundance of prawns (wild and enhanced) in the nursery. The timing and number of larvae settling in the nursery can be varied.

The abundance of the enhanced stock N_t and wild stock N_t^* in week t are modelled as,

$$N_{t+1} = (1-\lambda)N_t\, e^{-M_t}$$

and

$$N^*_{t+1} = N^*_t\, e^{-M_t}$$

where M_t is the natural mortality that is calculated as:

$$M_t = \alpha\, e^{-\beta L_t}$$

where L_t is the carapace length at week t, α and β are parameters defining how mortality changes with size; λ denotes the coefficient of density dependent mortality. Density dependent mortality can affect both the wild and enhanced prawns if they have the same competitive abilities for food and habitat space. However, for ease of calculation, we assume that density dependent mortality affects only the enhanced prawns, implicitly suggesting that enhanced prawns are relatively more vulnerable because of the sudden change in living environment. Density dependency after release is the most complex issue in stock enhancement, and the calculation of λ will be described in detail in the section on Density dependent mortality in the nursery below.

Growth in weight is modelled as an exponential function of parameter δ, but modified by a seasonal factor K_t that is equal to 1 at the peak of the growing season, and is smaller than 1 in the rest of the season

$$W_{t+1} = W_t\left(1+\kappa_t\left(e^{\delta}-1\right)\right)$$

The biomass of the enhanced B_t and wild stock B_t^* are therefore calculated as,

$$B^*_t = N^*_t W^*_t$$

and

$$B_t = N_t W_t$$

The average individual weight of enhanced juveniles W_t and wild juveniles W_t^* in the nursery area at any particular time t will not necessarily be the same. These weights depend on the week when the wild stock recruits to the nursery area, on the individual weight of enhanced juveniles released in such areas and on the week when these enhanced juveniles are released. Wild juveniles start life in the nursery area during week j_o at weight W_0, the same weight that larvae have when they start in the production. The number of wild larvae starting life in the nursery area N_0^* is a parameter of the model and is the main determining factor for the size of the wild recruitment to the fishery.

The timing for sub adult prawns to migrate out of the nursery is mainly related to size. Migration occurs at the same fixed size L_m for both the enhanced and the wild prawns. Individual weight of migrating prawns is calculated by substituting L_m in the length-weight relationship. The size at migration was selected as 18.7 mm CL and 0.005g based on

discussions during project meetings and results from studies on juvenile *P. esculentus* in Moreton Bay (O'Brien 1994).

8.2.4.1 Density Dependent Mortality in the nursery

Density dependency is a complex issue and depends on the current stock level relative to the environmental carrying capacity and the interaction between the enhanced and wild stocks. To quantify the density dependent effect after release, some extensive experiments would be necessary. However, such experiments are very costly. Due to the limited data on density dependence, we investigated the density dependence exhibited in the stock recruit relationship of the wild stock to provide an estimate of the possible values for density-dependent mortality.

The relationship between spawning stock (S) and recruitment (R) can be described in the following general form:

$$R = \alpha S f(S) \quad (1)$$

where α is the slope of the S-R curve at the origin, and f(S) is assumed to be monotonically decreasing and to represent density-dependent process. Two functions are commonly used to depict this density dependence in fisheries, and accordingly the above equation becomes two classic models:

- the Ricker model (Ricker 1954) and
- the Beverton-Holt model (Beverton and Holt 1957).

The Ricker model shows over-compensation, i.e. at high spawner abundances recruitment declines. However, for the Beverton-Holt model, recruitment does not decline at higher spawning stock levels. The density dependent mechanisms in both models are very similar before spawning stock reaches its peak.

In this study, we therefore used the Ricker model to estimate the density-dependent mortality caused by the increased prawn population from enhancement.

Ricker (1954) used an exponential function for the density dependent process

$$f(S)=e^{-\beta S} \quad (2)$$

where β is a density dependent term, and the stock recruitment relationship then takes the following form:

$$R = \alpha S e^{-\beta S} \quad (3)$$

Indices on the recruitment and spawning stock of tiger prawns in Exmouth Gulf were calculated from logbook data from 1972 until 1994 by Caputi et al. (1998) (Fig. 8.2) and used to estimate the parameters in Equation 3 – estimates of α=85.3 and β=0.0468 were obtained.

The above equation can be reorganized as follows

$$R / S = \alpha \quad e^{-\beta S} \quad (4)$$

If there is no density dependence i.e. $\beta = 0$, the recruitment per spawning stock should be constant ($R/S = \alpha$), i.e. the number of recruits from one spawner remains the same regardless of the spawning stock size. However, as the density of recruits increases, competition is likely to take place between individuals for food and habitat. The most important impact of

increased competition is an increase in natural mortality, but increased competition may have other impacts e.g. a decrease in growth rate (see Loneragan et al. 2001b).

With the above stock-recruitment relationships, only natural mortality is accounted for in the density dependent process. The increase in mortality rate reduces the survival rate of juveniles before recruitment, and consequently the recruits per unit spawning stock decreases. This density dependent effect becomes more and more severe with the increasing number of recruits. The maximum R/S is α in Equation 4 when S approaches zero. So, the density dependent effect can be estimated by comparing the value of R per S at its corresponding level of S with the value of α.

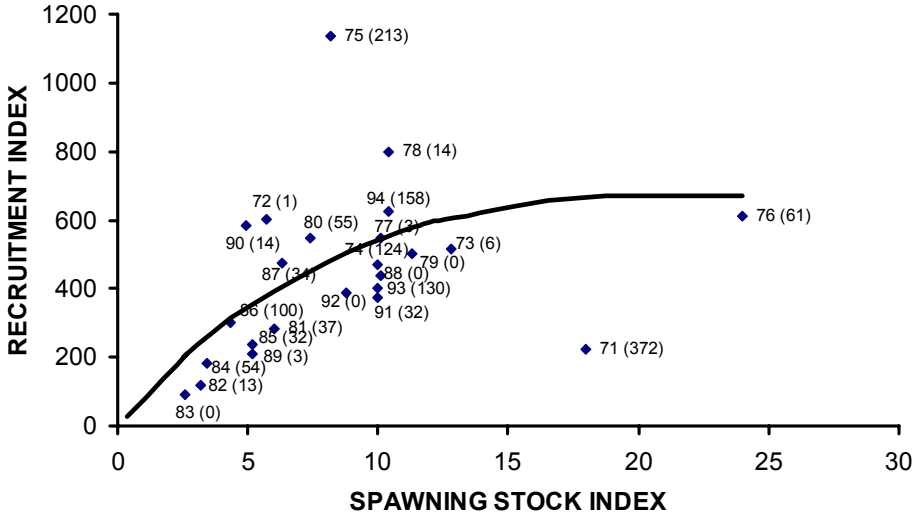


Figure 8.2 **The stock recruitment relationship of the Exmouth Gulf tiger prawn stock. In brackets are the January and February rainfall total (mm), not used in the SR modelling (from Caputi et al. 1998).**

Given the stock recruitment parameters in Equation 3, the relative decrease, p, in recruits per unit spawning stock (R/S) caused by a certain proportion of increase, γ, in spawning stock due to enhancement can be calculated as follows:

$$p = \frac{R_0 / S_0 - R_1 / S_1}{R_0 / S_0} = 1 - e^{-\beta\gamma S_0} \qquad (5)$$

where the subscript 0 represents the original levels of recruitment and corresponding spawning stock, and the subscript 1 represents the increased levels of recruitment after enhancement and their corresponding spawning stocks.

When we try to enhance a wild stock, competition occurs between enhanced and wild stocks. If the released prawns have the same size as the wild counterparts and we assume that they have the same competitive ability, the density dependent process will apply equally to both the enhanced and wild stocks. i.e. we can therefore treat the enhanced recruits as though they are a result of increasing the number of wild spawners. When the current recruitment level and the number of enhanced prawns to be released are known, the decrease in recruit per unit stock caused by the enhancement can be calculated on the basis of Equation 5. Using the stock recruitment parameters to define density dependence of the enhanced stock, we assume

that density dependent processes operate between the size at which we release enhanced prawns and the size at which they recruit.

The tiger prawn landings from the Exmouth Gulf ranged from 250 to 682 metric tonnes between 1990 and 1999 (Fisheries WA, unpublished data). Suppose that the fishery remains at its average level and have annual landings of about 400 tonnes when enhancement is carried out. The target for successful commercial enhancement is to increase the long-term average catch by 100 tonnes, i.e. on average about 25% of the wild stock.

From Equation 5, the percent mortality induced by a 25% increase in recruitment can be calculated at various levels of spawning stock (Fig. 8.3). The density dependent mortality rate increases exponentially with the level of wild recruitment (particularly above a recruitment index of 300). When the index for natural recruitment approaches its maximum average level of 600 (Fig. 8.2), any release of artificially hatched prawns will not be able to survive the density dependence process as explained by the Ricker S-R model. Our calculation, therefore, is limited to recruitment levels that are less than or equal to the maximum after adding recruitment from enhancement.

We next need to decide the spawning stock corresponding to the 400 tonnes of catch. Figure 8.2 shows that a spawning stock of 20 units produces the maximum recruitment. After excluding the extremes of 1971 and 1975, recruitments close to the maximum were seen in 1972 (669 tonnes), 1974 (661 tonnes), 1976 (745 tonnes), 1978 (639 tonnes), 1980 (688 tonnes), 1990 (564 tonnes) and 1994 (682 tonnes). The average catch from these 7 years of 664 tonnes was taken as the maximum catch the stock can produce. The Exmouth tiger prawn fishery is a recruitment fishery, meaning that all its annual catch comes from a single cohort. Thus, catch is directly dependent on recruitment. Under the assumption that this relationship is linearly proportional, the corresponding recruitment level for 400 tonnes of catch was calculated as about 350 units. The density dependent mortality at this level of recruitment is estimated at about 8.18% (Fig. 8.3).

The current level of recruitment has a major impact on the magnitude of density dependent mortality. When recruitment is close to the carrying capacity, the density dependent effect will be greater than at lower levels of recruitment. If recruitment is at the carrying capacity level, enhancement will not have any positive impact of the productivity of the stock because either all the released prawns die if they are not as competitive as the wild one, or the released prawns replace the wild one if they have equivalent competitive abilities for food and habitat space. The workshop held in September 2002 in Perth formed a consensus that the present recruitment level is difficult to determine and should not be taken as a single figure. It is possible that the mean recruitment level of the last ten years, which had an average catch of about 400 t, reaches around 500, and the corresponding mortality would be 23% (Fig. 8.3). Since we do not have any further information, a uniform distribution raning from 8.2% to 23% was used for density-dependent mortality.

As $\gamma=(S_1-S_0)/S_0$, substituting Equation 3 into Equation 5, we have $p=f(R_1, R_0)$, meaning that density dependent mortality is determined by the wild recruitment, R_0, and the enhanced, R_1-R_0. This is exactly the density dependent mortality, $\lambda(N_t,N_t^*)$ in the mortality equation used in the model for the feasibility study (Loneragan et al. 2001a).

It is assumed that released juveniles emigrate from the nursery areas after a few weeks; therefore the instantaneous mortality λ can be estimated by solving the following equation:

$$p = 1 - e^{-TM_\lambda}$$

where T is the number of weeks from the release to the migration out of the nursery ground. When the release size of juvenile prawn varies, the duration of their stay in the nursery will also change. If both *p* and T are known, M_λ can be easily calculated from the above equation. Following out the previous assumption that density dependency occurs only to the enhanced population, M_λ is introduced only to the calculation of the enhanced component. The validation of this assumption depends on the prawn size and the time at release. This simplification should not introduce large bias into the model, but should be revised when more information becomes available.

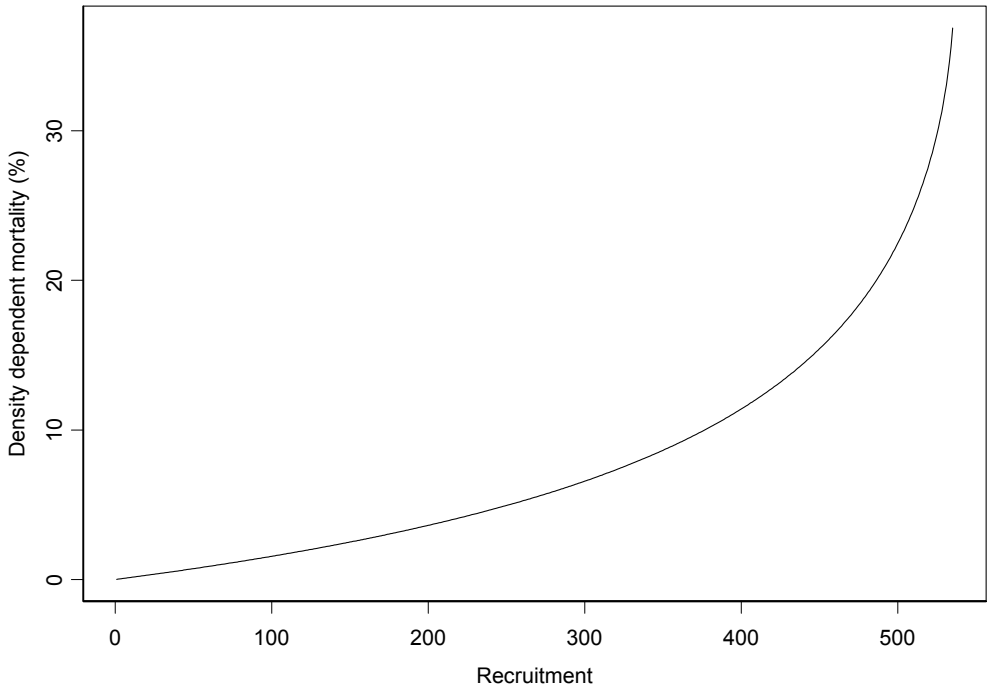


Figure 8.3 The density dependent mortality (%) caused by releasing 25% of the wild recruits at different levels of recruitment. Refer to Equation 5.

The bio-economic model used in this study consists of many parameters. Some parameters can be estimated quietly reliably through experiments e.g. the parameters for weight-length relationship, but others can never be represented well by point estimates e.g. natural mortality rate. For these latter parameters, we specified a distribution to describe the most likely parameter values based on the best possible available information for the parameter. Any change in any parameter's value or the distribution of the parameter, will change the outcome of the model. The influence that a parameter has on the results depends on the combination of all model parameters. Because of the large number of parameters and the complexity of the model, an analytical solution is impossible. We therefore used Monte Carlo simulation (10,000 simulations) to incorporate the uncertainties of model parameters. In each simulation, values of each parameter were randomly varied according to the specified probability distribution, based on the knowledge available. For some parameters (e.g. density-dependent

mortality), an uninformative prior of uniform distribution was used (for details of the parameters and their distributions see Appendix 8A).

8.2.5 Fishery

The fishery consists of two components, one corresponding to the wild stock, and one to the enhanced stock. The model structure of these two components is exactly the same – they have been split to follow changes in numbers of prawns from the wild and enhanced stocks separately. Each component has separate male and female populations. It includes prawns from recruits to adults (up to one year old). Growth is size, sex, and season dependent. Natural mortality is size dependent. Fishing selectivity is size dependent and fishing mortality of a certain size of prawn changes as a function of fishing effort. Egg production is seasonal and also a function of female size.

The price of prawns is considered to be dependent on size, but independent of season. For more reliable modelling in the future, price should be predicted on the basis of econometrics analysis. Only the variable costs of fishing effort have been incorporated in the model. The profit obtained from the harvest of wild stock is the difference between the revenue and the variable cost associated with the wild stock harvest. For the enhanced catch, the marginal revenue is calculated to reflect the fact that the cost of harvesting the "extra" (=enhanced) stock is lower than that of harvesting the wild stock i.e. the costs for the enhanced stock included the processing costs but not the costs of fishing. The profit from the enhanced stock is then the difference between the marginal revenue from the enhanced catch and the cost of the enhancement program.

Abundance of the enhanced stock N_t and wild stock N_t^* in week t for sex j are modelled as,

$$N_{j,t+1} = N_{j,t}\, e^{-M_{j,t}-F_{j,t}} \qquad N^*_{j,t+1} = N^*_{j,t}\, e^{-M_{j,t}-F_{j,t}}$$

where F_t is the fishing mortality rate in week t, calculated as,

$$F_{j,t} = q f_t S_{j,t}$$

where q is the catchability coefficient, f_t is the weekly fishing effort and $S_{j,t}$ is the selectivity factor for prawns of sex j ($j=1$ for females and $j=2$ for males), and size L_t calculated as,

$$\begin{cases} L_{j,t} < L_0 \cdots\cdots\cdots\cdots\cdots\cdots & 0 \\ L_0 < L_{j,t} < L_{100} \cdots & \dfrac{L_{j,t-} L_0}{L_{100-} L_0} \\ L_{j,t} > L_{100} \cdots\cdots\cdots\cdots\cdots\cdots & 1 \end{cases}$$

where L_o is the largest length where the probability of retention is zero and L_{100} is the smallest length where probability of retention is one.

Natural mortality after leaving nursery grounds is supposed to be independent of abundance but calculated as a function of length,

$$M_{j,t} = \alpha e^{-\beta L_{j,t}}$$

where length is modelled with the Von Bertalanffy equation,

$$L_{j,t} = L_{\infty j}\left(1-e^{-k_j t}\right)$$

Note that, the natural and fishing mortality rates upon the enhanced and wild stocks in the fishery are assumed to be the same if they have the same carapace length. Biomass is then calculated as the product of stock size and individual prawn weight,

$$B_{j,t} = N_{j,t} W_{j,t} \qquad B^*_{j,t} = N^*_{j,t} W^*_{j,t}$$

where $W_{j,t}$ is calculated from the weight-length relationship,

$$W_{j,t} = a_j L_{j,t}^{b_j}$$

Weekly prawn catch of the enhanced stock $C_{j,t}$ and wild stock $C^*_{j,t}$ in week t are estimated from the normal fishing equation:

$$C_{j,t} = \left(N_{j,t} - N_{j,t+1}\right)\frac{F_{j,t}}{F_{j,t}+M_{j,t}} \qquad C^*_{j,t} = \left(N^*_{j,t} - N^*_{j,t+1}\right)\frac{F_{j,t}}{F_{j,t}+M_{j,t}}$$

and the yields (catch in weight) are estimated as the product of the numbers caught and prawn weight,

$$Y_{j,t} = C_{j,t} W_{j,t} \qquad Y^*_{j,t} = C^*_{j,t} W^*_{j,t}$$

Egg production E_t is calculated as the product of the female number in the stock, the proportion of mature females ν_t, and the number of eggs produced by each female χ_t,

$$E_t = N_{1,t}\, \nu_t\, \chi_t$$

ν_t is a function of size and week and is calculated as

$$\begin{cases} L_{1,t} < E_0 \cdots\cdots\cdots\cdots\cdots\cdots\cdots 0 \\ E_0 < L_{1,t} < E_{100} \cdots \quad \rho_t \dfrac{L_{1,t} - E_0}{E_{100} - E_0} \\ L_{1,t} > E_{100} \cdots\cdots\cdots\cdots\cdots\cdots\cdots \rho_t \end{cases}$$

where

E_o is the largest length where the probability of being mature is zero,
E_{100} is the smallest length where probability of being mature is one and
ρ_t is the proportion of mature females in week t.

The numbers of eggs produced by each female (fecundity) is also a function of size,

$$\chi_t = \xi + \varepsilon L_{1,t}$$

We have assumed that the revenue derived from harvesting enhanced prawns is best calculated from estimates of the marginal revenue of enhanced prawns caught. This simplifies calculations and does not require estimates of the total cost of fishing. The value of the enhanced catch V_t is a function of the marginal revenue per kg for female and male prawns $\omega_{j,t}$ at week t and the weight of the catch,

$$V_t = Y_{1,t}\,\omega_{1,t} + Y_{2,t}\,\omega_{2,t}$$

where marginal revenue per kg $\omega_{j,t}$ is a function of the individual prawn weight and is defined by the price of commercial size categories $p_{j,t}$,

$$\omega_{j,t} = \frac{p_{j,t}}{p_{\max}}\omega_{\max}$$

and where p_{max} is the price per kg for the largest prawns and ω_{max} is the marginal revenue per kg for the largest prawns.

8.2.6 Monitoring

To assess the real impact of stock enhancement on stock abundance and catch, a monitoring plan has been designed and incorporated in the model. With this sub-model, we can estimate the sample requirements to effectively calculate the proportion of released prawns in the total population with a certain statistical power. It estimates the minimum sample sizes required to achieve a minimum number of recaptures of enhanced prawns. The probability *p(n)* of obtaining at least *n* enhanced prawns in a sample of *m* prawns randomly sampled from the commercial catch is,

$$p(n) = 1 - F(n)$$

where *F(n)* is a binomial distribution function with parameters, *m* and θ, the proportion of enhanced prawns found in the catch,

$$\theta = \frac{\sum_j \sum_t N_{j,t}}{\sum_j \sum_t N_{j,t} + \sum_j \sum_t N^*_{j,t}}$$

The only costs of monitoring considered are the cost of obtaining adult prawns at sea ι for genetic analyses and the cost of genetic analysis for each prawn π. Note that costs were included to catch prawns for monitoring because it may be necessary to sample prawns independently of the fishery. Total monitoring costs are therefore calculated as ι plus the product of π and the minimum number of prawns required to be genetically screened g_s.

Note that currently, the real costs of genetic analyses are not known because the technology for screening large numbers has not been developed and the feasibility of using micro-satellites for identifying enhanced prawns has not been full developed (see Chapter 4). The

model uses a cost based on the assumption that the screening will be feasible. It should be noted that the monitoring component assumes that the enhanced prawns mix randomly with the wild prawns (i.e. enhanced and wild prawns are homogenous).

8.2.7 Model parameters

All the parameters in different components of the model were put in a special worksheet called 'Parameters'. When available, parameter estimates from the Exmouth fishery were used. If no existing estimates were available, alternative values from nearby Australian fisheries or similar species were used. In the feasibility study and the first stages of this project, estimated values from prawn aquaculture studies in Queensland were used for parameters of the hatchery and production of juvenile prawns. All parameter values used in the model of the Exmouth fishery are given in Appendix 8.

Generally, few estimates of parameter uncertainty were available. Some parameters can be precisely estimated (e.g. length weight relationship, gear selectivity), and some others have limited impact on the uncertainty of the predictions (e.g. salary costs of production, size at emigration from the nursery), although their estimates do contain uncertainty. These two kinds of parameters were excluded from the uncertainty analysis. For other parameters, we examined the minimum and maximum values of the parameter reported from different sources for different prawns (e.g. growth parameters) and/or the level of natural variability in the parameters (e.g. survival rates in the nursery, prawn prices). For all other parameters, we guessed the minimum and maximum likely values of the parameters. Three types of probability distributions were used, normal, uniform and triangular. Correlation was incorporated for those parameter pairs that were known to be highly correlated (e.g. growth parameters, prawn price and marginal revenue).

8.2.8 Output indicators

The main indicators of the performance of enhancement were calculated in weekly and annual steps. Each indicator is stored in a separate worksheet. The indicators considered were:

- Cost of juvenile production
- Juvenile biomass of enhanced and wild stocks in the nursery
- Biomass of enhanced and wild stocks in the fishery
- Catch in weight of enhanced and wild stocks in the fishery
- Egg production from enhanced and wild stocks in the fishery
- Revenue from wild stock, marginal revenue from enhanced stock and cost of fishing.

8.2.9 Uncertainty and sensitivity analysis

A commercial add-on to EXCEL called Crystal Ball (Werckman et al. 1998) was used to incorporate parameter uncertainty in the analysis. This add-on uses Monte Carlo simulation to estimate the uncertainty associated with the predictions of the model. It can also estimate the sensitivity of predictions to uncertainty in the parameters. We used Crystal Ball to estimate the uncertainties involved in the output indicators of the model.

The Optquest function within Crystal Ball was used for various parameter optimisation procedures such as:

- to refine parameter estimates so that predictions were consistent with observations from the wild fishery (e.g. to estimate the magnitude of natural recruitment)

- to estimate the production requirements for a particular enhancement objective (e.g. obtain 100 t of enhanced stock)
- to estimate the statistical precision of parameter estimates obtained during monitoring (e.g. precision of the estimate of the ratio enhanced/wild prawns in the catch).

8.3 Results and Discussion

During the feasibility study, two scenarios were considered for the production of juvenile prawns: raceways and ponds (Loneragan et al. 2001a). As the industrial partner of the project, MG Kailis Group, is using only raceways for grow-out, many parameters, cost and growth information in the grow-out in particular, are from raceways. There were no reliable data available for ponds. Therefore, only raceways were modeled with the bio-economic model. To consider the possibility of releasing different sizes of juveniles into the nursery, we considered two sizes: 1 g and 0.5 g.

8.3.1 1g release size

The simulations showed that the wild catch ranged from 116 t to 968 metric tonnes (t) and was normally distributed with a median of 417 t and a standard deviation of 123 t (Fig. 8.4). These estimates match the range of catches recorded for the last 10 to 15 years of the Exmouth fishery. As expected, the enhanced catch varied considerably less, between 52 t and 171 t, and its distribution was close to normal (Skewness = -0.09), with a median of 113 t and a standard deviation of 17 t (Fig. 8.5). If we assume an average weight at capture of 25 g, about 4.52 million enhanced prawns are caught, a recovery rate of about 18.8%. This compares with reported recapture rates of kuruma prawns *Penaeus japonicus* in the Seto inland sea of 22 and 27.6% in two consecutive years (Tanida et al. 2002). These recapture rates are higher than those reported for *Penaeus chinensis* (= *Fenneropenaeus chinensis,* 5 to 12.5%) and *P. japonicus* (= *Marsupenaeus japonicus*) (6.8% to 10.2%) in China (Wang et al. 2002).

To produce 113 t of prawns, we need to release 24.0 million of 1 g juveniles that requires 179 raceways. The total cost of production in raceways ranged from AUD $1.16 million (M) to $1.56 M with a median of $1.40 M (Fig. 8.6).

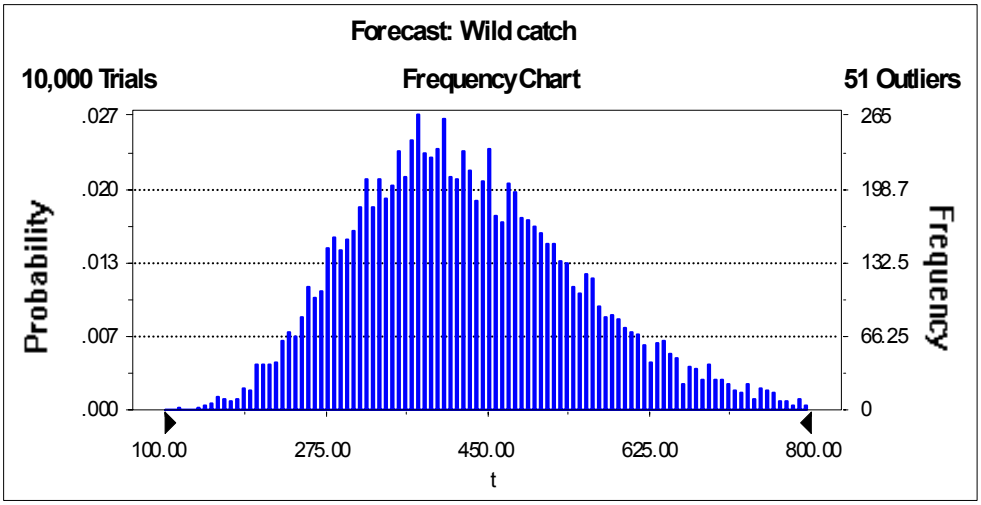


Figure 8.4 Catch predictions for wild stock during a trial commercial-scale (113 t) enhancement.

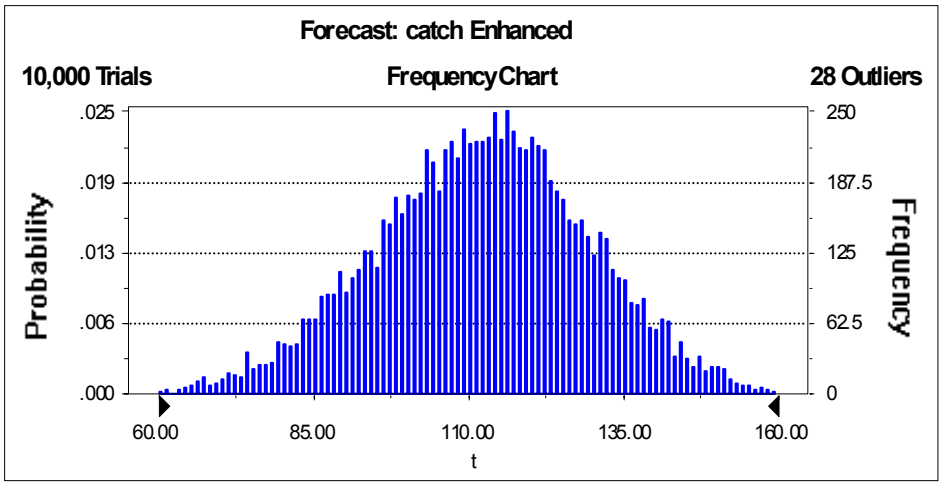


Figure 8.5 **Catch predictions for the enhanced stock during a trial commercial-scale (113 t) enhancement.**

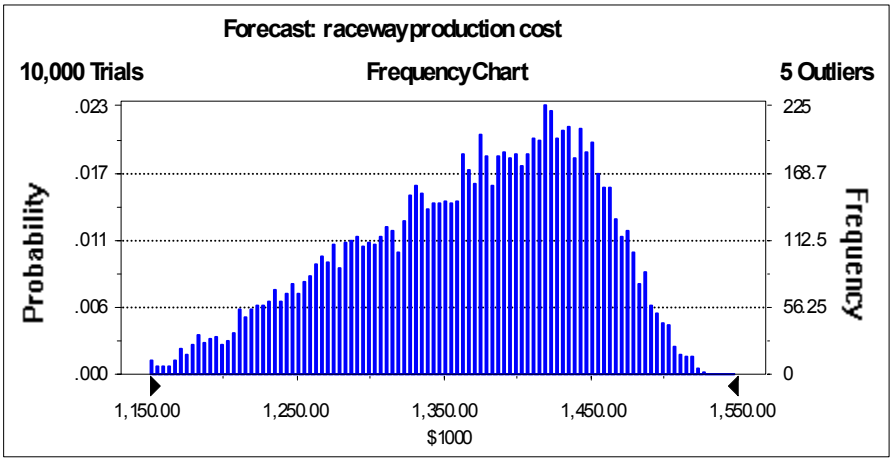


Figure 8.6 **Raceway production costs during a trial commercial-scale (113 t) enhancement.**

The revenue from the wild stock varied by a factor of 9 between AUD $3.10 M and $23.63 M (Fig. 8.7), mainly because of the large variation in the wild catch (Fig. 8.4). The median revenue of $8.42 M for the wild stock is similar to the one reported for the Exmouth fishery.

The marginal revenue for the enhanced stock varied from AUD $0.41 M to $2.93 M (Fig. 8.8), which is consistent with the small range in enhanced catches (Fig. 8.5). The median marginal revenue from the enhanced catch was $1.35 M.

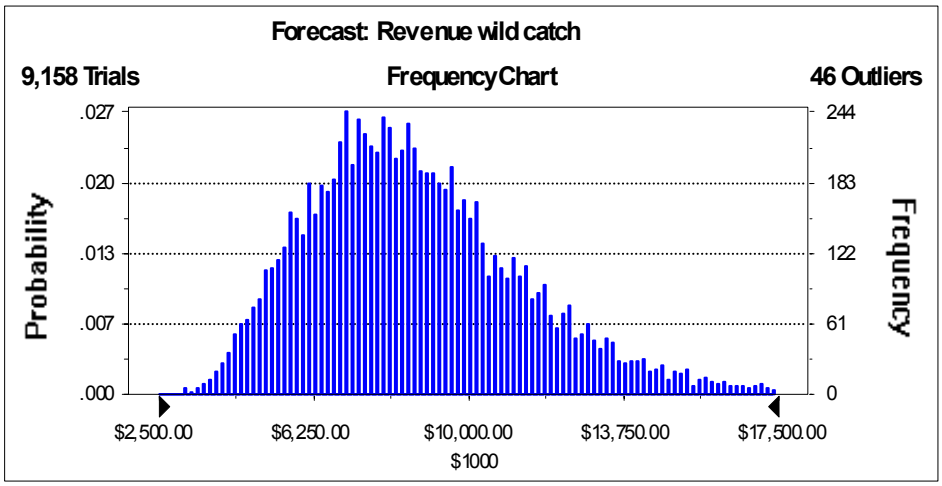


Figure 8.7 **Revenue obtained from the catch of the wild stock during a trial commercial-scale (113 t) enhancement.**

Our results did consider part of the costs of monitoring, specifically the cost of monitoring the contribution of the enhanced stock to the total catch assuming that the identification of enhanced from wild prawns by micro-satellites is feasible and that a screening process can be developed (see Chapter 4 for details). Under these assumptions, the costs of monitoring are not large, around AUD $26,000 for the trial enhancement of 24.0 million juvenile prawns, mainly for obtaining adult prawns at sea and genetic analysis. This cost corresponds to the optimum monitoring level for an "average" fishing season. We defined optimum as the minimum number of samples required to obtain an estimate of the contribution of the enhanced stock to the total catch within 15% of its real value (with an 80% statistical power). In years of exceptionally high catches of wild stock (800 t), the statistical power of our sampling would drop to around 70%. Other costs of monitoring related to for example, disease management have not included in these costs.

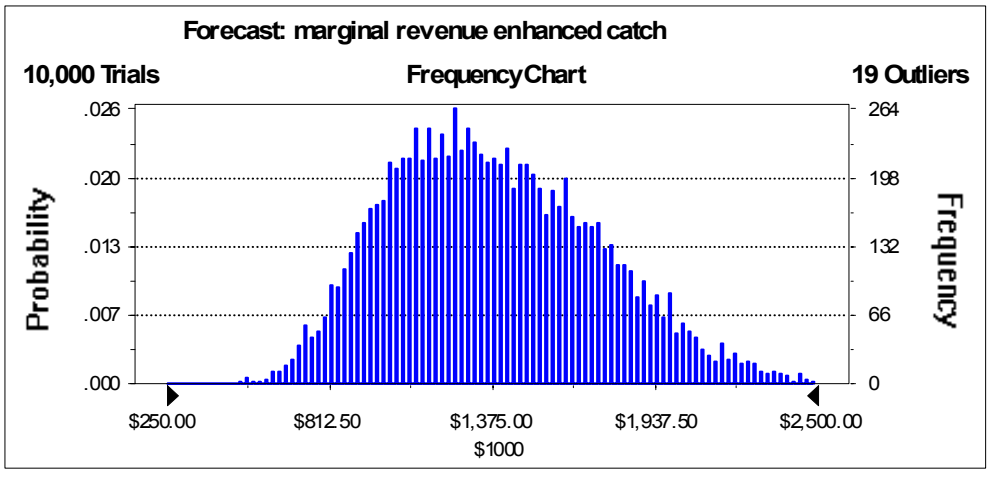


Figure 8.8 **Marginal revenue obtained from the catch of the enhanced stock during a trial commercial-scale (113 t) enhancement.**

For raceways, the difference between costs (hatchery, production, harvest, transport and release) and the marginal revenue from enhancement (an index of "profit") varied between AUD $-0.95 M and $1.53 M, with a median of $-0.17 M (Fig. 8.9), meaning on average the

enhancement would make a loss. There was a 48.2% chance of making a "profit" from enhancement. The calculation of profit in this study did not take into account the cost of capital investment required to build the grow-out raceways because the MG Kailis Group may offset these capital costs against other aquaculture activities. Additional use of the existing raceways for grow-out does not add extra costs to the company, which controls over 90% of the Exmouth Gulf tiger prawn fishery and will also be the major beneficiary of enhancement. In this very special case, the way to calculate profit can be justified, but for other normal enhancement projects, capital investment and depreciation on the capital, must be included in the cost-benefit analysis.

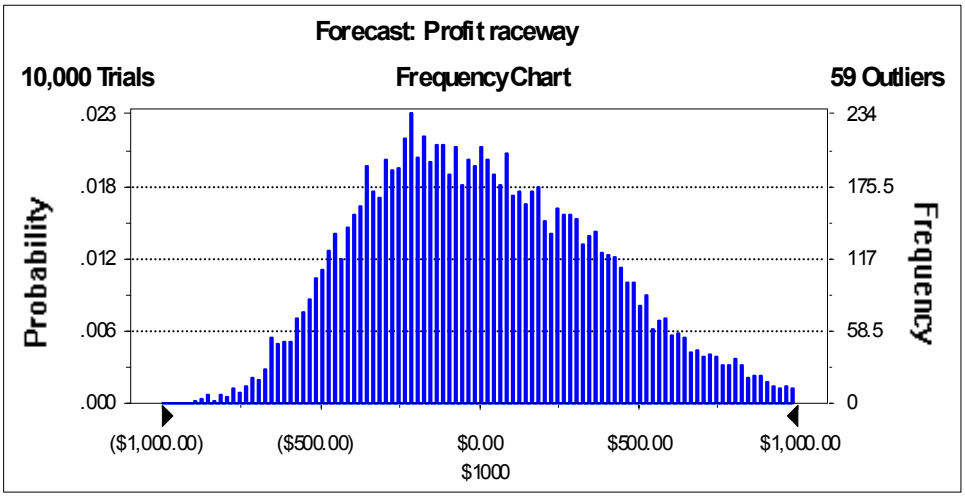


Figure 8.9 **Difference between production costs in raceways and marginal revenue obtained from the catch of the enhanced stock during a trial commercial-scale (113 t) enhancement.**

The model simulated all the mortalities in various stages. For precautionary purpose, an extra function was added to the model to get an idea of the risk associated with the other potential sources of mortality, outside those we have included in the model. We explored the impact of additional "unaccounted mortality". In the case of 1 g release size, as the probability to make profit is already less than 50%, any extra mortality that is to be introduced into the model will definitely lower the probability of making profit further. If the unaccounted mortality is 10%, for example, the chance of making a profit will decline to 32.7%. (Fig. 8.10). The only way to increase the probability of making profit is to reduce the mortalities that were already taken into account in the model. It is worth noting that the density dependent mortality rate for the post-release period was set rather high (8.2% to 22.3%), thus taking a conservative approach.

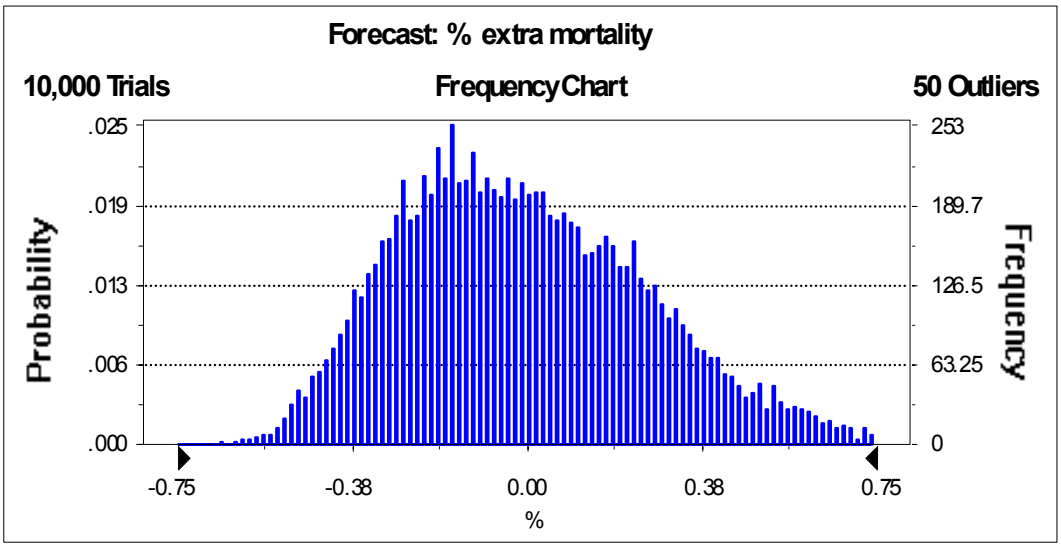


Figure 8.10 **Extra mortality (not accounted for by the model) that would nullify profits from the catch of the enhanced stock during a trial commercial-scale (113 t) enhancement.**

In this study, it was assumed that all prawns were produced in one run, and, therefore, 179 raceways were required. The capital costs associated with building 179 raceways would be substantial – about AUD $4.48 M. For economic efficiency, it is very well justified to produce the juveniles in two or three runs over a certain time period although the biological and ecological feasibility of such release strategies should be carefully studied. The current model is not capable of assessing the performance of multiple releases, but a more generic model in the future should have this capability.

8.3.2 Sensitivity analysis

The bio-economic model consists of many parameters. Each parameter has different importance in providing reliable outputs of the model. A sensitivity analysis was carried out to investigate how different variables affected the forecasts from the model. The sensitivities were calculated as a percentage of the contribution to the total variation in forecast values. For raceway production, the largest contributor to the overall uncertainty of profit is the marginal price of enhanced prawns (35.2%), followed by the price of wild prawn (34.7%), and the female growth parameter k (12.6%). All other parameters contributed less than 10% to the total variation in forecast values and were – male growth parameter k (5.6%), female L_∞ (4.5%), cost per larvae (3.2%), male L_∞ (2.2%), density dependent mortality after release (1.0%), mortality of sub-adults (0.4%), and transport mortality (0.4%), respectively. These results suggest that prawn price following the increased supply from enhancement is the largest factor affecting the profit, and parameters of the growth equations have large impact as well. More efforts should be made to refine estimates of these parameters in the future. It is worth mentioning that the uncertainty of an assumption depends on the type of distribution and the parameters of that distribution. If some assumptions in the model are changed, the uncertainty for a certain parameter might overcome the model sensitivity.

8.3.3 0.5 g release size

When we release prawn juveniles at a smaller size of 0.5 g, the number of juveniles to be released increases to 30 million for about 100 t catch, i.e. a 25% increase in comparison with

the number of juveniles required when the release size was 1 g. The mortality rates in the grow-out were based on the experiments carried out by CSIRO Marine Research in Cleveland and MG Kailis Group in West Australia. The reduced release size means that the juvenile prawns were released to the nursery younger and at a smaller size than the 1 g prawns, and then the mortality rate from 0.5 g to 1 g would be higher than in the optimally controlled grow-out environment. In this simulation, it was calculated by extending the length-dependent mortality for post 1 g juveniles to 0.5 g. These results may be provide an overly optimistic estimate because the smaller the release size, the more unpredictable the mortality rate. The number of raceways required to produce 30 million juveniles declined to only 87 because the density of juveniles in the raceway was assumed to be regulated by biomass per square meter.

The simulations showed that the enhanced catch ranged from 50.0 t to 150.0 t with a median of 104 t (Fig. 8.11). The distribution is close to normal (Skewness = -0.05) and is similar to the distribution for the 1 g simulation of enhanced catch (Fig. 8.5) because the enhancement of about 100 t was the same for both sizes of juvenile prawns. The total cost of producing 30 million juveniles of 0.5 g ranged from $1.20 M to $1.65 M with a median of $1.47 M (Figure 8.12), increasing 5.0% from the cost of $1.40 M for producing 24 million juveniles of 1 g. Although the costs in raceways decreased because of the reduced number of raceways required and shortened duration in raceways while release size is reduced, the total cost still increased. This is because 45.5% of the total cost comes from larval production, and the decreased release size demands the greater numbers of PL15s required for stocking the raceways.

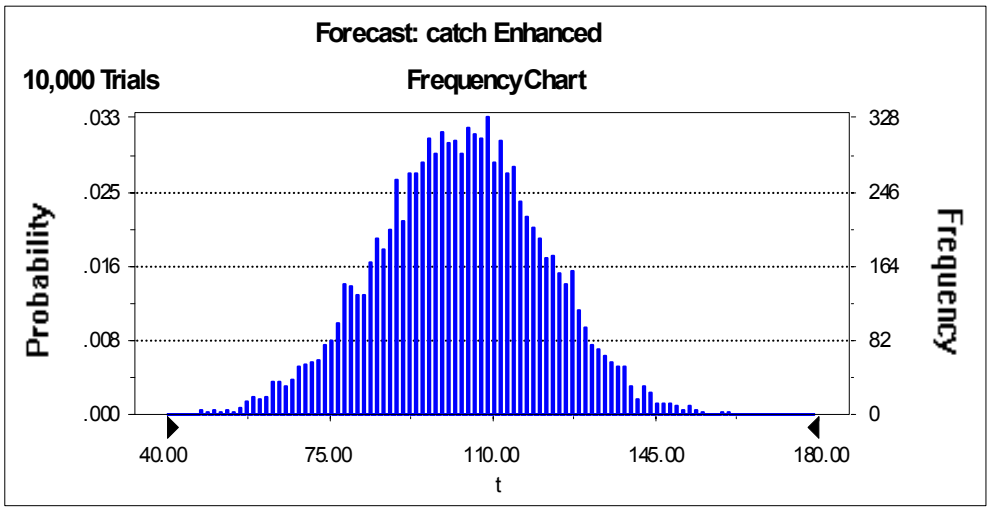


Figure 8.11 **Catch predictions for the enhanced stock during a trial commercial-scale (104 t) enhancement with a release size of 0.5 g.**

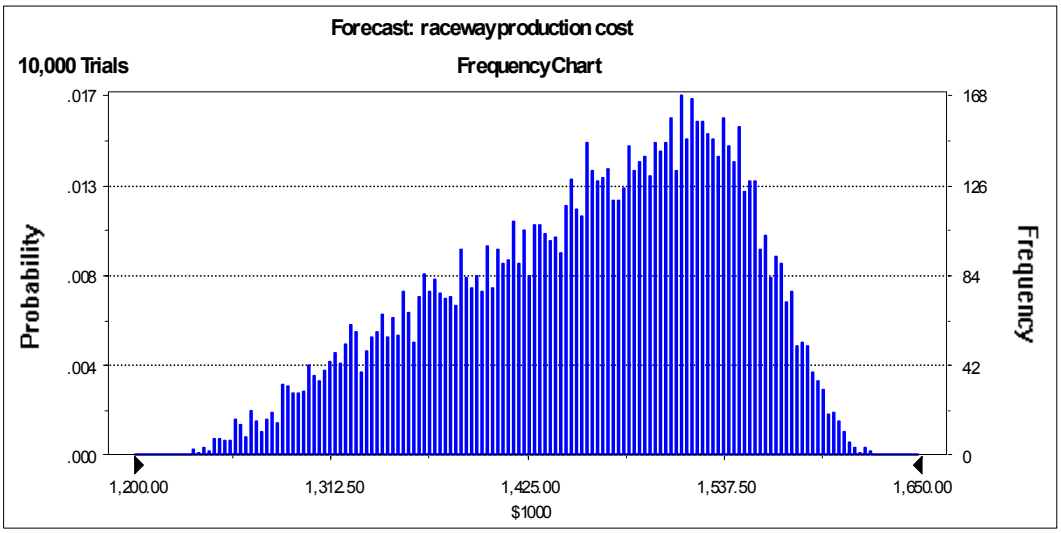


Figure 8.12 **Raceway production costs during a trial commercial-scale (104 t) enhancement with a release size of 0.5 g.**

The revenue from the enhanced stock is distributed pretty close to normal, ranging from $ -1.25 M to $ 0.75 M with a median of $-0.25 M (Fig. 8.13). The probability of making a profit was only 24.9%, significantly lower than that when the release size of juveniles was at 1 g. This may appear straightforward because the production cost of PL15 postlarvae constitutes a major portion of the total cost (46% with a release size of 1 g) and reducing the release size to 0.5g demands an increase in the number of juveniles to be released by 25% to 30 million.

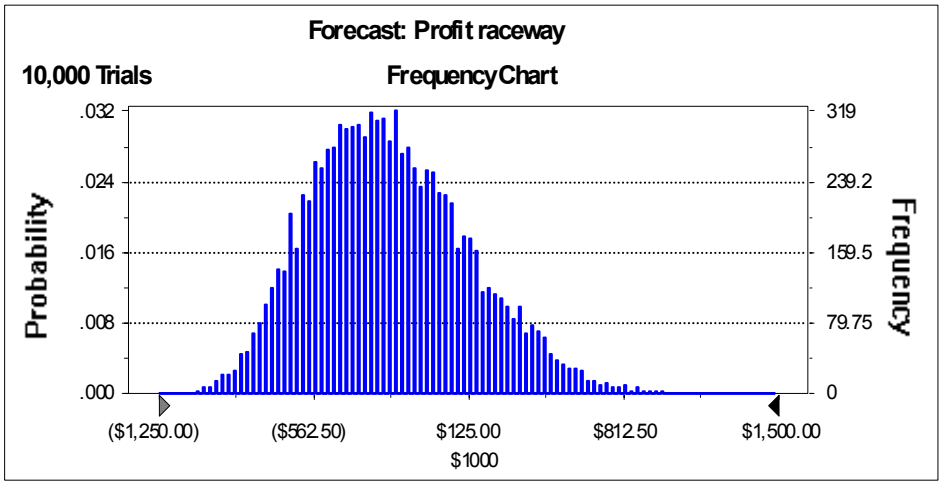


Figure 8.13 **Marginal revenue obtained from the catch of the enhanced stock during a trial commercial-scale (104t) enhancement with a release size of 0.5g.**

We compared the success of the 1 g and 0.5 g release sizes. Under the current cost structure of juvenile prawn production and the assumptions of natural mortality, releasing 1 g juveniles is the preferred option because the probability of making a profit is almost doubled compared with releasing 0.5 g juveniles. This conclusion seems reasonable when no capital costs for building raceways were considered, as was the case in this study. However, this may represent a rather unusual case as the already existing raceways can be used with only

additional operational costs. When capital investment in grow-out facilities is required, the production cost structure of juveniles may change dramatically, and the proportion of cost from larva production will shrink. Therefore, the decline in the number of raceways for grow-out may present a big saving in cost, and then a smaller release size might be preferred.

8.3.4 Further research and development

The development of the bio-economic model has been invaluable for focussing enhancement research and improving the understanding of the project team on the enhancement system. Although the bio-economic model is readily accessible in EXCEL, it is not easy to use, modify or document different enhancement scenarios e.g. different times of release, two releases in one year compared with one. The existing model needs to be translated to another programming language or suite of interacting programs to improve its ease of use. The new software could be constructed so that new enhancement models for different species could be installed in the future.

Future developments of the model should investigate implementing options to compare the potential success of enhancement at different times of the year and evaluate multiple smaller scale enhancements compared with a single large enhancement. These changes to the model would be much more efficiently implemented after moving the model from EXCEL to another modelling platform.

8.4 References

Beverton RJH and Holt SJ 1957. On the dynamics of exploited fish populations. *Fisheries Investment, Cons. Int. Explor.Mer*. 140: 67-83.

Blankenship H.L. and Leber K.M. (1995) A responsible approach to marine stock enhancement. *American Fisheries Society Symposium* **15**: 167-175.

Caputi N, Penn JW, Joll LM, Chubb CF (1998). Stock-recruitment-environment relationships for invertebrate species of Western Australia. In: Proceedings of the North Pacific Symposium on Invertebrate Stock Assessment and Management. Jamieson GS, Campbell A (Eds.) *Canadian Special Publications in Fisheries and Aquatic Sciences* **125**: 247-255.

Loneragan NR, Die DJ, Kailis GM, Watson R, Preston N (2001a). *Developing and assessing techniques for enhancing tropical Australian prawn fisheries and the feasibility of enhancing the brown tiger prawn (Penaeus esculentus) fishery in Exmouth Gulf*. Revised Final Report to FRDC for project 1998/222.

Loneragan NR, Heales DS, Haywood MDE, Kenyon RA, Pendrey RC, Vance DJ (2001b). Estimating the carrying capacity of seagrass for juvenile tiger prawns (*Penaeus semisulcatus*): enclosure experiments in high and low biomass seagrass beds. *Marine Biology* **139**: 343-354.

O'Brien CJ (1994a). Population dynamics of juvenile tiger prawns *Penaeus esculentus* in south Queensland, Australia. *Marine Ecology Progress Series* **104**: 247–256.

Rothlisberg PC, Preston NP, Loneragan NR, Die DJ, Poiner IR (1999). Approaches to reseeding penaeid prawns. **In:** Moksness E, Svasand, Howell B (eds). *Stock Enhancement and Sea Ranching*. pp 365-378.

Preston NP, Brennan DC, Crocos PJ (1999). Comparative costs of postlarval production from wild or domesticated Kuruma shrimp, *Penaeus japonicus* (Bate), broodstock. *Aquaculture Research* **30**: 191-197.

Ricker WE (1954). Stock and recruitment. Journal of the Fisheries Research Board of Canada **11**: 559-623.

Tanida K, Ikewaki Y, Aoyama E, Okuyama Y, Nozaka M, Fujiwarra M (2002). Investigation of stocking effectiveness for kuruma prawn (*Penaeus japonicus*) in the eastern Seto inland sea. Book of abstracts, *Second International Symposium on Stock Enhancement and Sea Ranching*, Kobe, Japan 28th January – 1st February 2002.

Wang Q, Zhuang Z, Deng J (2002). The development and prospects of stock enhancement and sea ranching industry China. Paper for *Second International Symposium on Stock Enhancement and Sea Ranching*, Kobe, Japan 28th January – 1st February 2002.

Werckmand C, Hardy T, Wainwright E (1998). *Crystal Ball User Manual*. Decisioneering, Colorado.

Appendix 8A: The parameter values used in the application of the bio-economic model to the Exmouth fishery in Western Australia.

Parameters where uncertainty is incorporated are in bold and are recognised for having a letter denoting the probability distribution used in the simulations and by having their corresponding parameters in parenthesis: Triangular T (Min, Mode, Max), Uniform U (Min, Max) and Normal N (Mean, Stdev).

Hatchery

cost per nauplius Ω	
$	T(0.01,0.018,0.02)

Grow-out

		raceway
	density (kg/m2)	T(2,3,3.5)
	size (m2)	50
pumping costs	per m2	2.25
	per kg	1.6
conversion	feed/bio	T(1.15,1.3,1.4)
	feed cost	4.75
	Salary	125

Growth

Adults	L_{oo}	K	a	b
Females	N(40,1)	0.052	0.00000373	2.574
Males	N(33,1)	0.06	0.00000207	2.764
	Larvae			
Alpha	T(2.7E-08,3.0E-08,3.3E-08)			
Delta	0.9 (wild)	0.82 (grow-out)		

Mortality

Larvae-Juveniles		Subadults-adults
Alpha	**T(0.9,1,1.1)**	**T(0.095,0.1,0.105)**
Beta	-0.29	-0.03
Culture	**T(0.06,0.07,0.08)**	

Recruitment

	Wild		enhanced
Release weight (g)			0.001
Number of Wild recruits	1.25E+10	Number of Enhanced recruits	24,000,000
Week	5	Week	2
length out of nursery (mm)		18.1	
weight out of nursery (kg)		0.005	

Appendix 8A: Parameter values in the bio-economic model (cont.)

Fishing mortality

selectivity	
Length0	25
Length100	30
Q	0.001

Reproduction

egg a	-5.36E+05
egg b	22573
Matur. 0len	28
Matur. 100len	36

Transport

seed biomass/site	20
tanker volume	1000
water volume/biomass	U(17.5, 25)
trips/day	5
cost per day	750
Transport mortality	U(0.04,1.0)

Seasonal patterns

month	Week	fishing effort	relative growth	maturity		month	week	fishing effort	relative growth	maturity
1	1	0	1	0.7		7	27	105	0.6	0.05
1	2	0	1	0.6		7	28	105	0.6	0.1
1	3	0	1	0.5		7	29	105	0.6	0.1
1	4	0	1	0.5		7	30	105	0.6	0.1
2	5	0	1	0.5		8	31	105	0.6	0.2
2	6	0	1	0.5		8	32	105	0.6	0.2
2	7	0	1	0.4		8	33	75	0.6	0.2
2	8	0	1	0.4		8	34	50	0.6	0.3
2	9	0	0.9	0.4		8	35	25	0.6	0.3
3	10	0	0.9	0.3		9	36	0	0.7	0.3
3	11	0	0.9	0.3		9	37	0	0.7	0.4
3	12	0	0.9	0.3		9	38	0	0.7	0.4
3	13	0	0.9	0.3		9	39	0	0.7	0.5
4	14	105	0.8	0.3		10	40	0	0.8	0.6
4	15	105	0.8	0.2		10	41	0	0.8	0.7
4	16	105	0.8	0.2		10	42	0	0.8	0.8
4	17	105	0.8	0.2		10	43	0	0.8	0.9
5	18	105	0.7	0.2		11	44	0	0.9	0.9
5	19	105	0.7	0.2		11	45	0	0.9	0.9
5	20	105	0.7	0.1		11	46	0	0.9	0.9
5	21	105	0.7	0.1		11	47	0	0.9	0.9
5	22	105	0.7	0.1		11	48	0	0.9	0.9
6	23	105	0.6	0.1		12	49	0	1	0.9
6	24	105	0.6	0.1		12	50	0	1	0.9
6	25	105	0.6	0.05		12	51	0	1	0.9
6	26	105	0.6	0.05		12	52	0	1	0.8

Fishing Cost (per day): $ 1,500

Appendix 8A Parameter values in the bio-economic model (cont.)

Economics

Count/lb	wt(kg)	Price $	Marginal revenue $		Count/lb	wt(kg)	Price $	Marginal revenue $
60	0.00756	5	3.75		30	0.01512	7	5.25
59	0.007688	5	3.75		29	0.015641	10	7.5
58	0.007821	5	3.75		28	0.0162	10	7.5
57	0.007958	5	3.75		27	0.0168	10	7.5
56	0.0081	5	3.75		26	0.017446	10	7.5
55	0.008247	5	3.75		25	0.018144	15	11.25
54	0.0084	5	3.75		24	0.0189	15	11.25
53	0.008558	5	3.75		23	0.019722	15	11.25
52	0.008723	5	3.75		22	0.020618	15	11.25
51	0.008894	5	3.75		21	0.0216	15	11.25
50	0.009072	5	3.75		20	0.02268	17	12.75
49	0.009257	5	3.75		19	0.023874	17	12.75
48	0.00945	5	3.75		18	0.0252	17	12.75
47	0.009651	5	3.75		17	0.026682	17	12.75
46	0.009861	5	3.75		16	0.02835	17	12.75
45	0.01008	5	3.75		15	0.03024	17	12.75
44	0.010309	5	3.75		14	0.0324	17	12.75
43	0.010549	5	3.75		13	0.034892	19	14.25
42	0.0108	5	3.75		12	0.0378	19	14.25
41	0.011063	5	3.75		11	0.041236	19	14.25
40	0.01134	5	3.75		10	0.04536	19	14.25
39	0.011631	5	3.75		9	0.0504	20	15
38	0.011937	5	3.75		8	0.0567	20	15
37	0.012259	5	3.75		7	0.0648	20	15
36	0.0126	5	3.75		6	0.0756	20	15
35	0.01296	5	3.75		5	0.090719	20	15
34	0.013341	7	5.25		4	0.113399	20	15
33	0.013745	7	5.25		3	0.151199	20	15
32	0.014175	7	5.25		2	0.226799	20	15
31	0.014632	7	5.25		1	0.453597	20	15

APPENDIX A

RELATED RESEARCH

FRDC Report Project No. 2002/209 – "Understanding and removing the barriers to *Penaeus monodon* domestication" – Principal Investigator: Nigel Preston.

FRDC Report Project No. 96/302 – "Development of the aquaculture capability of the brown tiger prawn, *Penaeus esculentus*" – Principal Investigator: Peter Crocos.

APPENDIX B

INTELLECTUAL PROPERTY

Some aspects of the project in relation to the development of technology for the production in raceways, harvest, transport of juvenile brown tiger prawns (Area 1) have been identified as Valuable Information and therefore have been kept confidential (see Intellectual Property Schedule for the Project below). The agreed timeframe for the public release of the information is 1 January 2006 unless otherwise agreed between the parties. All other Project Results and outcomes (including parts of Area 1 and all of Areas 2 to 5) have been included in the final report.

Intellectual Property Schedule – 17 February, 2000

Intellectual Property for FRDC Project 1999/222 will be developed in the following five main areas:

1. Development of technology for the production in raceways, harvest, transport and release of juvenile brown tiger prawns (AREA 1);
2. Assessment of the best release strategies for juvenile tiger prawns by surveying potential nursery habitats, and the distribution and abundance of juvenile prawns and their predators;
3. Developing a 'tag' for enhanced prawns using microsatellite DNA markers;
4. Developing a bioeconomic model to assess the success of any stock enhancement of tiger prawns in Exmouth Gulf, and
5. Information on the fishery to monitor changes in commercial catches.

The parties agree that each party retains all rights to any intellectual property or other property contributed by that Party to the project.

Each of the parties grants to the other Party a royalty free, non-exclusive right to use intellectual property brought to the Project to the extent that it is reasonably necessary for the carrying out of the Project.

The Parties agree that the intellectual property rights subsisting in any inventions, discoveries, improvements or reports created during the course of and as a direct result of carrying out the project (the "Project Results") will be owned between the Parties as provided in the FRDC Agreement.

After the completion of the project CSIRO and FRDC agree that MG Kailis will have a royalty free, non-transferable, non-exclusive licence to use the project Intellectual Property.

Management and participation in intellectual property

In the event that a Party or Parties wishes to exploit the Intellectual Property generated from the project, the Parties shall develop a commercialisation strategy for this purpose.

The parties agree that they will at a future date develop a mutually agreed Intellectual Property sharing formula that reflects the proportional investment in the project.

In the event that a Party or Parties wishes to make an additional investment in developing a component of the Intellectual Property that may be generated from the project the Parties further agree that they will consider an appropriate sharing formula that recognises the additional risk and investment relating to that component of the Intellectual Property.

Notwithstanding the above, should a Party not wish to proceed to register or otherwise protect the Intellectual Property then the other Party or Parties at their cost have the right to obtain that protection.

Confidentiality

In relation to Area 1 of the Intellectual Property, the Parties agree that any inventions, discoveries, improvements or reports created during the course of and as a direct result of carrying out the project (the "Project Results") will be considered to be Valuable Information (as per clause 15 of Part 5). The Parties agree that subject to further agreement the project results will become public information two years from the completion of the proposed Stage 3 (Trial enhancement) or 1 January 2006 which ever occurs earlier.

All other results of the project will be published and in the public domain as the project progresses.

APPENDIX C

STAFF

CSIRO Marine Research

Neil Loneragan (Project Leader)
Rob Kenyon
Peter Crocos
Mick Haywood
Fiona Manson
Yimin Ye
Stuart Arnold
Michele Burford
Melony Sellars
Peter Grewe
Bob Ward
Ian Poiner (Steering Committee)
Peter Rothlisberg (Steering Committee)
Steve Blaber (Steering Committee)

CSIRO Mathematical and Information Sciences

Bronwyn Harch
Peter Toscas
Mark Bravington

CSIRO Livestock Industries

Sigrid Lehnert
Jennifer Meadows

Fisheries Western Australia

Nic Caputi
Mervi Kangas
Jim Penn (Steering Committee)

MG Kailis Group

Richard McCulloch
Roger Barnard
George Kailis (Steering Committee)