# Reducing uncertainty in the assessment of the Australian spanner crab fishery

**I. W. Brown[1], J. Scandol[2], D. Mayer[3], M. Campbell[1], S. Kondylas[1], M. McLennan[1], A. Williams[4], K. Krusic-Golub[5], and T. Treloar[6]**

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Australian Government
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## 1. PROJECT SUMMARY

Spanner crabs (*Ranina ranina*) represent a valuable single-species fishery in Queensland. Although a transparent and effective assessment process was developed some years ago for setting the commercial total allowable catch (TAC), additional information was needed to reduce some of the uncertainty in assessments, and to incorporate fishery-independent information from the DPI&F Long-Term Monitoring surveys into the process. The exploited stock crosses State boundaries and extends into northern NSW waters, but historically quite different approaches to monitoring and assessment have been developed by the two States.

This project set out to clarify conflicting estimates of growth rates, develop an integrated (stock-wide) system for monitoring and assessing the status of the resource, and to examine some environmental variables believed to be responsible for influencing catch rates.

Age-estimation was approached through assaying lipofuscin, a metabolic by-product that accumulates in the neural tissues of a wide range of taxa, using a recently-published chemical extraction method which promised to be more cost-effective than the more traditional histological technique. After considerable experimentation we concluded that the chemical extraction method would not work with this species, so we reverted to the histological method, which estimates the quantity of lipofuscin granules in sections of eyestalk tissue by computer-aided fluorescence microscopy. From this work we established a strong relationship between the size of the crab and the amount of lipofuscin in its optic nerve. However size is a poor substitute for age in crustaceans, and the technique therefore needed to be calibrated against material of known age. Attempts to rear a known-age population of spanner crabs *in aquaria* were hindered by high mortality rates, which resulted in very few of the animals surviving more than about 15 months after being introduced into the aquarium facility as immediate post-settlement megalopa larvae. The lack of older known-age animals precluded the calibration process, so as a final resort an alternative approach was taken, which examined the lipofuscin concentration frequency distribution in a moderately large sample from the wild stock. It was hoped that this might reveal frequency modes that could be interpreted as representing successive age-classes, but (probably because the sample was not large enough) the results were inconclusive.

During the course of the project a different technique for age-determination was investigated. This involves estimating of the length of telomeres, which are non-coding DNA structures on the ends of chromosomes that shorten when the chromosome splits during cell division. On the basis of a small sample of spanner crabs a strong negative relationship was demonstrated between telomere length and body size. Unfortunately this work (outside the project) could not be continued, but it has led to the establishment of a separate project using telomeres as a proxy for age in a number of marine fisheries taxa, including spanner crabs.

In close collaboration with NSW DPI we conducted a year-long 'Crossover Experiment' which compared the effectiveness of the spanner crab monitoring systems used by the two States. This was done by extending the Queensland Long-Term Monitoring survey procedure into NSW waters, and simultaneously conducting a NSW-type survey which extended into Queensland waters. The outputs from this crossover experiment and associated comparisons of sampling gear and bait were tabled and exhaustively reviewed during a Spanner Crab Monitoring Review Workshop, which was attended by fishery managers, researchers, stock assessment personnel, commercial crab fishermen and enforcement/compliance staff from Queensland and NSW. The Workshop developed of a set of recommendations, most of which (including extension of the appropriately-modified Queensland Long-Term Monitoring Surveys into NSW waters to create a unified annual monitoring system) have been implemented already.

Follow-on actions from the Workshop (undertaken primarily by the Queensland Crab Scientific Advisory Group and its technical experts) has led to the formal incorporation of the results of the annual monitoring surveys, along with fishery-dependent data from the compulsory logbook programme, into the stock assessment and TAC-setting process. The use of such fishery-independent information has long been a major recommendation of the Australian Government's Department of Environment, Water, Heritage and the Arts in its response to the State's reporting on the status of the fisheries. It is anticipated that DEWHA will view this as a major step in the sustainable management of the Australian spanner crab fishery.

We examined a number of environmental factors considered by industry and researchers to add uncertainty to our interpretation of estimates of stock abundance. Bottom temperature was shown to have an effect on catch rates, and we established that there is a consistent relationship between temperatures at the sea surface and on the bottom in south Queensland coastal waters. Sea surface temperature can therefore be used as a low-cost proxy for bottom temperature. A study of longer-term climate effects on crab catches revealed not only that catch rates can be predicted from standard climatic indicators, but also that global warming may have a beneficial effect on spanner crab populations along the Australian east coast. We also confirmed the view of many crab fishermen that wave or swell height can affect catch rates, and our preliminary attempts to quantify this relationship show that adjustment of (particularly) fishery-independent stock abundance estimates may sometimes be necessary.

During investigations into the effects of temperature on the abundance, distribution and catchability of spanner crabs, our bottom-water temperature logging programme yielded an unexpected benefit. We found that it was possible to accurately measure soak-time using the temperature loggers, and this led to a closer examination of factors that might be contributing to uncertainty in our understanding of the dynamics of the fishery. As a result of analyses of the effects of increasing soak-time on CPUE indices from the commercial sector, substantial resources were invested by DPI&F into a survey of the spanner crab fleet to quantify changes in vessel fishing power. The output from this project was then used in a management strategy evaluation process (undertaken by DPI&F quantitative assessment staff from the Southern Fisheries Centre) as part of the revision of the stock assessment process and associate decision rules.

## 2. ACKNOWLEDGEMENTS

Specific acknowledgements are included at the end of the report chapters, so are not repeated here. However sincere thanks are due to members of Queensland's Crab Fishery Management Advisory Committee and its Scientific Advisory Group, particularly chairman Dave Mitchell, industry representatives Richard Freeman and Russell Doyle, and CSIRO stock assessment scientist Dr Cathy Dichmont, for their continued interest and support in many ways. The project was supported in large part by the Australian Government's Fisheries Research and Development Corporation

## 3. BACKGROUND

The Australian spanner crab fishery, valued at around $8 million, operates in southern Queensland and northern NSW coastal waters. Queensland accounts for 85%, and NSW 15%, of the annual catch of approx. 2000 t, most of which is exported live to Asia. Recent evidence (Ovenden *et al*., unpubl. data) indicates that the fisheries in both States are exploiting the same genetic stock, but procedures for monitoring, assessing and managing the fisheries differ markedly. This makes it difficult to evaluate the performance of the stock as a whole. Queensland's component of the fishery has been subjected to a detailed Ecological Assessment according to Environment Australia requirements (Brown *et al*. 2001), and is the first of the State's fisheries to have received EA approval. However in his letter of approval, the Minister for the Environment and Heritage (the Hon. Dr David Kemp MP) made certain recommendations to be addressed before the next Commonwealth review. These included (i) "making arrangements with NSW to establish joint monitoring and assessment of the shared stock of spanner crabs with a view to developing future collaborative management arrangements" and (ii) "continuing work to develop a stock assessment model based on sound biological data, and an analysis of the impact of effort creep on the current total allowable commercial catch setting process and management responses".

Conventional biomass-dynamic assessment models applied to the Qld catch-effort data have not been informative, mainly because of lack of contrast in fishing effort and uncertainty about the values of important population dynamics information (Dichmont *et al*., 1998; Brown *et al*., 1999). For this reason the Queensland Spanner Crab Stock Assessment Group (SAG) has developed an assessment and TACC-setting process based on the analysis of a time-series of commercial CPUE data. This yields a suite of performance indicators which are translated into a TACC for the forthcoming year via a set of objective Decision Rules (Brown *et al*., 2001). The process was developed with a high level of inbuilt precaution, but it has been criticised as being too conservative, resulting in unnecessary financial hardship to the industry. Currently there is concern amongst crab fishers that the decision rules will result in continued reductions in annual TACs over the next few years, even during periods when annual CPUEs are increasing. This issue highlights some of the problems associated with using decision rules based on trends in commercial CPUE, and is being addressed as part of a review of the decision rules required by the Management Plan. An annual industry-funded fishery-independent survey of the spanner crab stock is carried out as part of Queensland's fishery Long Term Monitoring Programme (LTMP), but as yet the time-series of survey data is very short, and the information is not yet being used formally as part of the assessment process. Part of the reason for this is the high degree of spatial and temporal variability in biological indicators such as catch rates and sex ratios. While the Qld part of the stock appears stable, LTMP survey data indicates a degree of variability that challenges the reliability of the assessment process.

A FRDC study in NSW (Kennelly and Scandol 1999) developed a model of the NSW component of the fishery, conditioned on catch and fitted to the results of two 2-year surveys carried out 9 years apart. The authors recommended the continued use of this survey design for future assessments of the NSW fishery, and suggested that it could also be applied in Queensland. However Kennelly and Scandol (2002) were not confident in setting a theoretical TACC for this fishery, cautioning that it will continue to operate with uncertainty about sustainable harvest rates, and concluding that any TACC

between 100 and 350 t could be justified. At the time there were indications of a decline in stock size, prompting a call for a third periodic independent survey.

Estimates of spanner crab growth parameters from previous studies are inconsistent, and (despite Chen and Kennelly 1999) absolute age-length relationships and age at recruitment are still unknown. Lack of knowledge about these critical population parameters is limiting our understanding of fundamental stock processes. It is still uncertain as to whether the spanner crab resource is characterised by high biomass and low productivity, or low biomass and high productivity.

The independent monitoring programme will not be subject to biases relating to potential hyperstability of the commercial catch-rate statistics, but because it still uses commercial gear it is subject to the same variable catchability problems. These are related to behavioural cycles of the crabs, habitat patchiness, and the effects of environmental factors such as bottom water temperature. The impact of these factors on catchability, and thus on interpretation of survey and commercial CPUE, needs to be investigated to optimise the survey design. Until this work is done, the Queensland spanner crab fishery will continue to be managed in the context of great uncertainty.

During the first half of 2001 there were concerns that Queensland's spanner crab stock appeared to be in a state of collapse. Commercial catch rates dropped to half of their early-season values in three of the five assessment regions. In the other two Assessment Regions (geographically the smallest) catch rates were already depressed in the early part of the season. This situation caused considerable concern amongst fishers and processors, suggesting that the stock was under significant threat, and was a major stimulus to the development of this proposal. Fortunately, however, catch rates improved over the next few months, but we are unable to explain why this change happened. The size composition of the catch suggests that it was not simply the result of a new batch of recruits entering the fishery. Such fluctuations in apparent abundance, in the absence of an identifiable cause, present major obstacles to the management of the fishery. It seems likely that they may be caused by environmental influences – a number of crabbers believe that inflows of cold water close to the bottom are responsible for reducing the animals' feeding activity. However at present we have no understanding of the hydrography of the spanner crab fishing grounds, so there is no effective way of testing whether changes in catch rate are related to environmental effects.

This application is the direct result of attempts by the Spanner Crab Stock Assessment Group (SAG) to improve the robustness, reliability and scientific basis of the existing arrangement for monitoring and assessing the status of spanner crabs in Queensland, and setting the fishery's TAC. This represents a logical step in the quest for a better understanding of the population dynamics of this important crab resource.

## 4. NEED

In his assessment of the Queensland spanner crab fishery for exemption from the export controls of the EPBC Act, the Federal Environment and Heritage Minister recommended that arrangements for joint monitoring and assessment of the shared stock of spanner crabs be made, with a view to eventual co-management. This process needed to be addressed before the next Commonwealth review of the fishery in five years' time. While the development of complementary management arrangements is ultimately a core-business function of the two State governments, the evaluation of existing monitoring and assessment paradigms and the synthesis of a common reference point-setting process clearly requires significant collaboration between scientists, modellers and statisticians.

A workshop involving scientists and fishery managers from Queensland and NSW was held on 27 September 2002 to examine collaborative options with regard to research and management in the spanner crab fisheries. The meeting agreed that there is a need to conduct simultaneous field trials of the two States' monitoring surveys, to determine their relative cost-effectiveness as fishery-independent measures of stock abundance. The broad principles of such an exercise were agreed to, and details of the experimental design were fleshed-out at another meeting of research collaborators in

NSW in late November.

Previous work aimed at estimating growth rates in spanner crabs has yielded highly divergent results, and none has provided a reliable estimate of age at recruitment. It is essential that this knowledge-gap is bridged because an estimate of age at recruitment is crucial to the successful development of age-based assessment models. Ideally such a model, tuned with the LTMP fishery-independent survey data, would replace the simplistic CPUE regression-based model.

While the fishery-independent spanner crab monitoring programme is expected to overcome hyperstability problems inherent in the commercial statistics, it still requires the use of commercial gear, and is therefore subject to the same problems of variable catchability. These are presumably related to behavioural cycles of the crabs, habitat patchiness, and the effects of environmental factors such as water temperature. The impact of these factors on catchability needs to be investigated if survey and commercial CPUE data are to be interpreted correctly and the assessment process significantly improved

## 5. OBJECTIVES

- Determine the age at which spanner crabs recruit to the fishery.
- Develop a common methodology for monitoring and assessing the Australian spanner crab stock.
- Investigate sources of variability in apparent population density.

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# CHAPTER 1. AGE DETERMINATION

I.W. Brown, K. Krusic-Golub, M. Campbell, S. Kondylas, M. McLennan and T. Treloar.

## 1.1 INTRODUCTION

Attempts to determine the age of spanner crabs have yielded greatly varying estimates, due in part to biases inherent in the different methods used. Estimates from different sources of age at the minimum legal size (MLS) have ranged from 1.75 to 8.83 yr for females and 1.08 to 3.58 yr for males (Kirkwood *et al*., 2005). The methods used to derive these estimates included length-frequency analysis of the recruited sector of the population (Brown 1986, de Moussac 1988, and Boullé 1995), tag release-recapture (Chen and Kennelly 1999), and aquarium studies (Okamoto *et al*. 1997). In an investigation of growth and reproduction in *R. ranina*, Krajangdara and Watanabe (2005) attribute differences in maximum size between the sexes to differential growth rate, but do not provide estimates of absolute growth for either males or females. Kirkwood *et al*. (2005) studied apparent modal progression in small post-settlement juvenile spanner crabs, as part of a previous FRDC project (95/022; Brown *et al*. 1999), and concluded that male crabs reach MLS in approximately 4 years, while the slower-growing females take between 5 and 6 yr to attain MLS. These results place spanner crabs at the slower end of the spectrum of growth rate scenarios, suggesting that the crab stock may take longer than initially thought to recover, should it become overexploited. While the work of Kirkwood *et al*. (2005) was the first published attempt to include data from the juvenile component of the population in growth estimation, the results were based on a small sample size and were derived by forward extrapolation of length-at-age from just two initial year-class modes. There was a need to test the slow-growth hypothesis further and reduce the continuing uncertainty surrounding this aspect of the spanner crab's population dynamics. However in the opinions of Kirkwood *et al*. (2005) it would be wise (i) not to use highly selective commercial tangle-nets to obtain samples, (ii) not to use tag release-recapture methods (which had been shown to adversely affect the growth of spanner crabs in aquaria), and (iii) not to rely on length-frequency analysis (because of great variability in growth between individuals). This left few options for investigations of growth, longevity and size at recruitment.

However there was a possible alternative, which involves the ageing pigment lipofuscin. This substance is a metabolic by-product that accumulates over time in certain tissues, particularly neural tissue of the eyestalk (optic nerve) and brain. The substance is relatively insoluble and in granular form fluoresces, making it detectable by histofluorimetric techniques, and it had been detected in spanner crab tissue by Sheehy (1990). Considerable advances in lipofuscin histofluorimetry have been made in recent years (e.g. Robertson *et al*. 1998; Sheehy *et al*. 1998; Sheehy and Bannister 2002) but the technique is time-consuming and expensive. An assay procedure using chemical rather than histological determinations has been developed recently by Prof R Harvey and Dr Se-Jong Ju (Uni of Maryland), which can be carried out in a basic chemistry laboratory relatively cheaply, and is reported to overcome some of the problems of the histological method. The technique, initially developed with blue crabs (*Callinectes*) (see Ju *et al*. 1999), was also being used to age Antarctic krill as part of the international SO-GLOBEC program in the Southern Ocean. Advice from Prof Harvey indicated that the technique should be equally applicable to spanner crabs.

Any chemical assay requires calibration, and to obtain meaningful results from a lipofuscin determination (regardless of the method used) a reference set of known-age material is essential. This presented a challenge with regard to spanner crabs, as no captive population of cultured crabs of known age was available, and with the exception of work by Minagawa *et al*. (1993) on laboratory-reared zoeal stages, no published information on size-at-age. The only crabs in the wild whose ages

are known (even approximately) are those that have recently settled, following their ~6 week planktonic larval phase. We therefore needed to establish a laboratory population of crabs at the Southern Fisheries Centre (SFC), collected at early post-settlement stage (close to age 0) from the wild, and rear them for as long as possible. During a previous FRDC project we had achieved some success at sampling early post-settlement (megalopa and C1 stage) crabs with a channel dredge. We decided to re-design this device and use it to obtain crabs for the experiment. As the new DPI&F research vessel (RV *Tom Marshall*) was expected to have far greater capacity to tow and handle this sort of sampling gear than the previous vessel, we anticipated greater success in capturing sub-legal crabs of a range of sizes. While we did not anticipate that the dredge would be available for routine use until after the strongest pulse of early post settlement juveniles resulting from the 2003-4 spawning period had settled, we intended to evaluate the device by the other organisms and material it collects (including older crabs), as well as by underwater video footage of the apparatus in action.

An aquarium system would need to be established *de novo* at SFC to maintain a population of known-age crabs, which could be sampled periodically for lipofuscin assay by collaborating staff at the DPI&F Food Research Laboratory (Hamilton, Brisbane). While it was originally anticipated that the Harvey-Ju chemical extraction method would be the primary source of information on lipofuscin concentration, additional samples were to be sent to the Central Aging Facility (CAF) located at the Department of Primary Industries, Queenscliff, Victoria, for a limited number of comparative assays using conventional histofluorometric techniques.

With an estimated natural attrition (mortality) rate of 40% and no sampling of intermediate ages, simple modelling indicated that 400 animals of age 0 would be needed to yield a sample of 85 at age 3. To allow for adequate samples of the younger (age 1 and 2) animals, the supply of juveniles in the known-age populations would need to be replenished each year from wild stock in the post-spawning period. This would also provide some degree of inter-annual replication, further strengthening the experimental design. The success of the experiment would clearly depend on being able to maintain at least a small number of individuals through to age 2.5 or 3 yr. If sufficient numbers were still available at the end of the project, they could be maintained, and some additional funding sought to continue the lipofuscin analysis as long as possible.

On the basis of the calculations above, we anticipated stocking the facility initially with 400 juvenile crabs (20 trays of 20 individuals), which would be fed and inspected for moulting activity every 2-3 days (depending on their size). Moult shells and dead crabs would be removed from the trays and measured. It was intended that a target n = 430 individuals would be analysed over the period of the experiment – the approximate distribution among ages being 200, 100, 80 and 50 for ages 0 and 0+,1 and 1+, 2 and 2+, and 3 yr respectively. Approx. 80% of these were to have been assayed chemically at CFT and 20% histologically at CAF. Throughout the project period an additional 200 crabs would be taken from the wild, including the complete spectrum of available sizes, and assayed chemically at CFT.

While the post-settlement crab sampling apparatus was being developed to the point where it could be used to catch enough animals to stock the aquarium system, tissue extraction and assay trials would be undertaken at CFT using commercially-caught crabs of a range of sizes. This would allow the analytical process to be refined without having to use the known-age animals, and would indicate the extent to which lipofuscin concentration is related to body size. A general correspondence between the two variables would be anticipated, and the establishment of such a relationship was seen as an important milestone in the development of this technique. At six-monthly intervals the known-age crabs would be sampled for neural tissue (eye-stalk and/or forebrain). Tissue dissection would take place at SFC using standard methods, and the samples taken to the Centre for Food Technology (CFT; Hamilton Qld) for analysis. Additional samples taken annually would be preserved and freighted to the CAF for analysis. Chemical determinations would be done at the CFT's chemical laboratories under the supervision of research chemist Mr WA Treloar, and comparative data obtained from histological preparations carried out by Mr K Krusic-Golub (CAF) using procedures and protocols transferred to the CAF (Robertson *et al*., 1998).

## 1.2 MATERIALS AND METHODS

### 1.2.1 Sampling operations

*Dredging*

Various devices, including an hydraulic dredge, have been constructed and tested in the past for sampling spanner crabs smaller than the size-range normally caught in baited tangle nets. The final design for a dredge to collect post-settlement spanner crab larvae was strongly influenced by advice from a retired licensed specimen shell-collector. SFC workshop staff constructed a dredge similar to that which the fisherman recommended – a 1 m wide box dredge with an adjustable-depth V-shaped cutter bar and a top-mounted towing bar. The latter was designed to allow the device to be towed relatively close to the boat with a minimal length of towing wire. Following initial trials in December 2003, certain design modifications had to be effected to make deployment and retrieval simpler and safer, and to ensure that the device tracked along the sea-bed without lifting. After more trials, which included the use of an underwater video camera attached to the mouth of the dredge (Figure 1-1), we were able to optimise the combination of cutter-bar depth, warp length and towing speed so that a uniform operation resulted.

**Figure 1-1.** Benthic box-dredge used to capture spanner crab post-settlement larvae and juveniles, aboard the RV *Tom Marshall*. Note the underwater video camera housing facing in towards the dredge mouth, and three orange floats to prevent the dredge flipping over.

*Light-trapping trials*

We had anticipated being able to re-commence the larval sampling to replenish the captive known-age population during the latter part of the 2004 spawning season (December), but owing to the non-availability of the vessel's skipper, this had to be deferred to early January 2005. While dredging was still the primary collection method to be used, we also wished to test the effectiveness of a light-trap designed by Dr P Doherty (AIMS) for collecting larval fishes (Doherty, 1987). These sampling devices frequently capture the larval stages of various crustaceans including crabs, although little

attempt has been made so far to identify this crustacean bycatch. Two light traps were loaned to us by the Centre for Marine Studies, University of Queensland, to determine whether the highly mobile raninid megalopae can be captured this way. If light-trapping proved effective, we considered that it may represent a far more efficient and for the larvae less physically stressful way of obtaining the numbers we required for the experiment.

*Megalopa swarm sampling*

By early 2005 it was becoming apparent that we would need to do much more dredging to collect the requisite number of small crabs, but a fortuitous observation by the skipper of our research vessel RV *Tom Marshall* revealed another possibility. While at anchor at night off the northern face of Heron Island reef in late March 2005, the deck lights of the vessel attracted a swarm of orange-coloured nektonic organisms near the water surface (Figure 1-2). They were sufficiently large to be retained in a knotless fish landing net (Figure 1-3), and were confidently identified by the boat crew as being spanner crab megalopae.

**Figure 1-2.** Swarm of *R. ranina* megalopae near the water surface, attracted by the ship's deck lights.

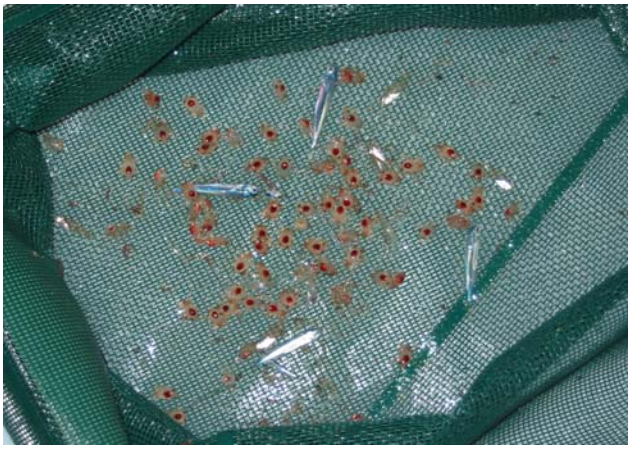

**Figure 1-3.** *R. ranina* megalopae collected at night in a knotless landing net (Heron Is., March 2005).

Several scoops of the landing net yielded around 100 individuals, and over two nights more than 600 megalopae were collected and placed in fish boxes filled with seawater and a layer of coralline sand substrate. The megalopae, which had been captured while swimming at the sea surface, appeared equally at home buried in the substrate, so they were clearly on the point of changing from a planktonic to a benthic lifestyle.

We hoped to progress this by re-allocating ship time from dredging to 'night-lighting' operations during and after the 2005–06 spawning season. Where possible, this work would be piggy-backed on to other project work in the southern GBR and/or Hervey Bay areas. This observation opened up a possible new way of collecting large numbers of effectively known-age spanner crabs (i.e. megalopae) for the remainder of the age-determination work. If the swarming behaviour is widespread, this method of capturing larval crabs could well supersede the laborious, costly and relatively inefficient dredging programme.

It was our intention to re-visit the Heron Island reef where spanner crab megalopa swarms were encountered in 2005 in the hope of seeing the swarm again and collecting large samples of megalopae, during new moon at the end of February and/or at the end of March. Unfortunately these trips had to be cancelled because of adverse weather conditions, including the influence of Tropical Cyclones Larry and Wati, along the central and southern Queensland coast.

Other avenues of identifying and locating megalopa swarms were also investigated. These included a request to the Field Operations Manager at the Heron Island Marine Research Station to keep a lookout for signs of the larvae when research vessels are working at night, either within or outside the lagoon. The AIMS research vessel *Lady Basten* was at the time working in the Capricorn-Bunker Group, and the vessel's skipper was also asked to maintain a watch for the aggregations at night. Tuna vessel skippers have from time to time reported seeing surface-swarming crustacean larvae in quite deep water off the coast, but it was not clear whether they were spanner crabs or some other species. The principal of a fishing company operating tuna vessels out of Mooloolaba was asked to alert his skippers to our interest in the phenomenon and to let us know if and when they next make a sighting. Unfortunately, however, none of these leads produced anything positive, and it became apparent that further opportunities for collecting large numbers of megalopae for the rearing work may have passed.

*Sampling extended size-range with modified dillies (flat tangle nets)*

Towards the end of 2006 it became clear that mortality rates amongst the captive population of known-age crabs were so high that we would not finish up with enough advanced-stage crabs (even under 2 years old) to undertake the original experiment effectively, so alternative arrangements were examined.

We decided to adopt the approach taken by Sheehy *et al.* (1998) on WA rock lobsters, which involved examining a large sample of crabs from the wild to see if there were modes in the lipofuscin-concentration frequency distribution that could be interpreted as age-classes. As the spawning period of *R. ranina* in Queensland is relatively short and discrete (Brown, 1986), we believed that (assuming lipofuscin does accumulate over time) there was a reasonable chance that, regardless of their size, crabs less than a few years old may be able to be identified as distinct modes in the lipofuscin-frequency spectrum, in much the same way as some fish can be aged on the basis of their size frequency.

For maximum effect, this sort of analysis requires large samples and equal representation of all size-classes. Our ability to provide for the former was limited only by the limited resources available for processing the samples and doing the histofluorimetry (approximately 200 samples). However the equality of sample density across sizes was a more difficult problem. Previous experimentation with

nets of different mesh size, denier rating and layer configuration showed that a multi-layered slack-hung small-mesh net caught a significantly greater proportion of small crabs (in the 50–70 mm range) than did the conventional single-layer 1.25" 12-ply nets of the type used in the commercial fishery (IWB, unpublished data). We used a fleet of 4-layer nets, hung slack, and made from light-weight 4-ply multifilament 13 mm mesh. These nets tangled the crabs more effectively than the conventional dillies, but (not surprisingly) were much more difficult and time-consuming to clear without damaging the crabs.

### 1.2.2 Known-age population

*Rearing system*

A system for rearing post-settlement crabs was established at the Southern Fisheries Centre. The system comprised a number of plastic crates floating in a 3-tonne tank, each with a separate adjustable seawater input (Figure 1-4). The crates were suspended in float cradles made from PVC pipe, and had short mesh-covered outlet pipes to allow water (but not the crabs) to escape into the main tank which acted as a water bath to reduce temperature fluctuations. Each crate was lined internally with a close-fitting rigid plastic mesh tray about 50 mm high. A layer of sand (to about 35 mm depth) was placed in the crate to allow the small crabs to bury as they would in their natural habitat.

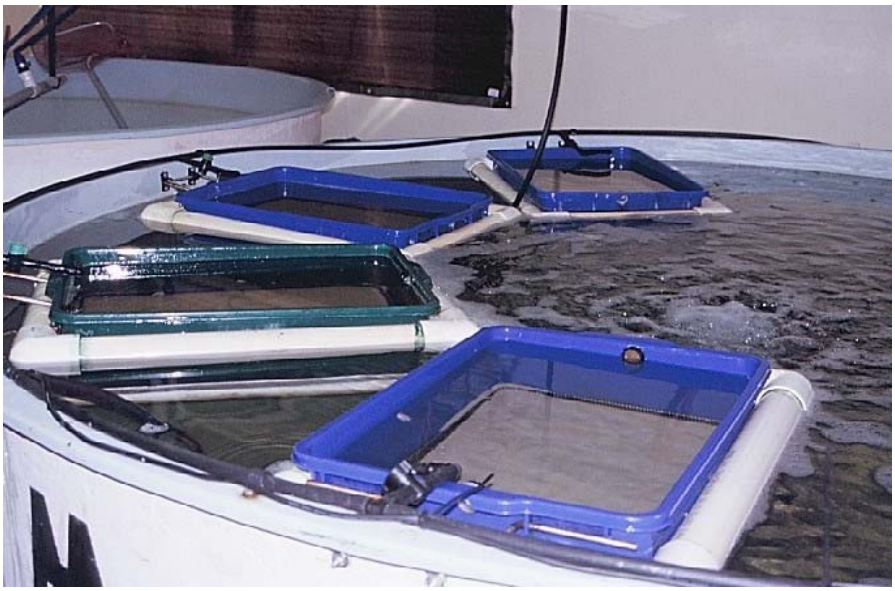

**Figure 1-4**. Rearing tubs containing sand-covered mesh trays at the Southern Fisheries Centre. The main 3000 l tank contained recirculating biofiltered seawater heated in winter but otherwise at ambient temperature.

When the crabs were periodically checked, measured and/or sampled, the mesh tray was carefully lifted up, allowing the sand to fall through the mesh but retaining the crabs (Figure 1-5). This system was designed to provide as natural an environment for the crabs as possible, while at the same time allowing their removal for periodic inspection with the least amount of disturbance. While basically a flow-through arrangement, the system incorporated a microbial-action filter which allowed it to function in recirculating mode in the event of flood rainfall reducing the salinity and increasing the turbidity of the inlet water beyond acceptable limits. Temperatures during the cooler part of the year were raised to levels approximating those of bottom shelf waters using submersible coil heaters.

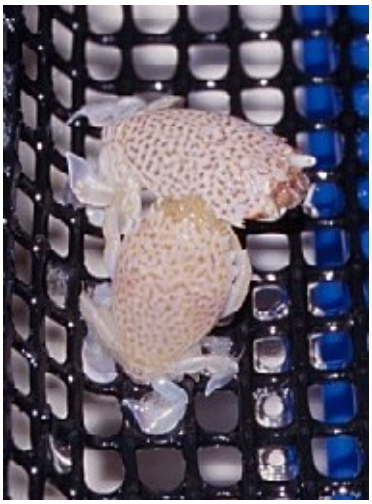

**Figure 1-5.** Juvenile spanner crabs retained by the mesh lining of a rearing tub.

Following the successful collection and transport of a large sample of live megalopae from the Heron Is. swarm, a rearing system was established which enabled better access to the individual crabs for feeding and observation. The system comprised a bench-top tray approx. 5 x 1 m through which there was a constant flow of seawater from the main tanks referred to above. Suspended within this water bath was a number of clear plastic jars comprising a main chamber separated from a second chamber below by a fine-mesh nylon gauze. A small amount of sand (to a depth of about 15 mm) was placed in the main chamber, together with up to four megalopae. Water was pumped into the lower chamber at a pressure that would create an upward flow through the sand above the mesh without disturbing the substrate physically. This arrangement meant that there was not only a constant flow of clean oxygenated water through the rearing chamber, but also that the upward flow would carry uneaten food fragments and metabolic wastes directly out of the rearing chamber via an overflow pipe.

*Feeding and maintenance*

One issue considered a potential impediment to this part of the project was a lack of knowledge about the diet of megalopae and juvenile crabs. Previous experiments (Brown and Kirkwood 1999, unpublished data) suggested that finding a suitable diet might be a significant problem, and considerable effort was invested initially in monitoring the behaviour of the animals when presented with a variety of foods. Because commercial dillies (tangle nets) rarely catch spanner crabs less than about 50 mm CL, we suspected that there may be a dietary shift between small juveniles and adults. If juvenile crabs were not attracted to the bait typically used in the fishery (i.e. pilchards) this may explain why so few are caught in the dillies, but we had no direct experimental evidence of this one way or the other. Making the simplest assumption - that the diets were similar - we set up a time-lapse video recorder and a long-wavelength (IR) light source which monitored the occupants of one of the rearing tubs from dusk, when the food was introduced, to dawn the following morning. The crabs were presented with a variety of foods, including bivalve molluscs, squid, fish and prawn – all food that had been fed successfully to captive adult crabs on past occasions.

### 1.2.3 Tissue sampling for lipofuscin determination

*Overview of method development and tissue selection*

We had intended to base all the lipofuscin work on optic ganglion tissue from the crabs' eyestalks, one of the principal reasons being their ease of sampling. This tissue had been used previously in investigations of age in giant tiger prawns *Penaeus monodon* (Sheehy *et al*. 1995), European lobsters *Homarus gammarus* (Sheehy *et al*. 1996), blue crab *Callinectes sapidus* (Ju *et al.* 1999) and by A. McGaffin in a PhD study of Antarctic krill at the University of Tasmania and ANARE. More significantly, Sheehy (1990) reported having detected lipofuscin in the eyestalks of spanner crabs along with a number of other crustacean species.

Part-way through the Project the CAF reported that the work they were conducting on lipofuscin in giant crabs indicated that the cerebral neural ganglion is generally a more reliable tissue than optic nerve. Trials by CAF on Antarctic krill eyestalks had been unsuccessful, with no evidence of lipofuscin in this tissue having been obtained, and exploratory work was equally unsuccessful in identifying regions of high lipofuscin concentration in the eyestalks of spanner crabs. As a result, CAF recommended that we consider using spanner crab cerebral ganglion tissue instead of optic nerve tissue, even though eyestalks are clearly much easier to sample and dissect than brains. Adapting the procedure from their experience with giant crabs, CAF staff prepared an instruction manual for locating and removing the brain of adult spanner crabs (Appendix 3), and visited SFC in June 2004 to train Project staff in the relevant procedures.

Early trials with the published chemical extraction methodology at CFT failed to produce consistent and repeatable results, even though a low level of fluorescing lipofuscin-like material was extracted from the eyestalk tissues. There was no correlation between lipofuscin concentration indices in left and right eyestalks, suggesting that the technique was not working adequately, as it would be expected that there would be less variation between eyestalks within crabs than between crabs (of significantly different sizes). Even after numerous attempts to refine and improve the extraction process, using different solvents and buffers, and experimenting with a completely different approach based on the ELISA immuno-assay technique, our confidence in the usefulness of the chemical extraction method was reduced to the point where it was agreed to terminate the work at the Hamilton laboratory (CFT) and re-direct our remaining project resources into expanding the histofluorometric technique at CAF.

While the procedure developed for sampling and dissecting out the brains of adult spanner crabs was time-consuming, it worked well insofar as it produced consistently good tissue preparations. However it seemed that the process would not be able to be used effectively on very small animals the size of megalopae and early crab-stage juveniles. If lipofuscin determinations were to be done on young known-age crabs, we would have to develop a different technique for processing the tissue and locating the cerebral ganglion. One option was to fix the whole animal and section it serially, but first the carapace would have to be softened (by decalcification) to prevent tissue distortion and damage to the microtome blade. As the decalcification solution used by Sheehy (1990) has since been classified as a carcinogen, staff at the Melbourne University Veterinary Laboratory (Werribee) were reluctant to use it. Experiments then had to be carried out with a number of different compounds to determine the most appropriate decalcification process. The best procedure histologically was fixation in formalin solution followed by a period of treatment with a decalcifying solution, but while this allowed whole animals to be sectioned successfully, cerebral ganglia could not be located in the very small crabs.

Although by the end of 2005 the focus was on cerebral ganglion tissue, CAF staff had continued to persevere with sectioning eyestalk tissues, and ultimately located a region of the optic ganglion rich in lipofuscin granules which was assumed to be the *medulla terminalis* (see Results section). This region was able to be located successfully and consistently in adult crabs, with best results obtained when the samples were prepared from whole eyestalks that had been sectioned after decalcification

than from those where the neural tissue had been dissected out of the exoskeletal eyestalk sheath. FRDC agreed with our recommendation to terminate the chemical extraction trials and follow the fallback position of histofluometry (at the CAF laboratory) using remaining available project resources.

In early 2006 there was still some uncertainty as to whether to opt for brain or eyestalk tissue. The former seemed to be appropriate for adult crabs, but not for very small individuals. The latter was also appropriate for adults, and there was some expectation that (from experience with the larger animals) the appropriate part of the optic nerve might be able to be located in post-settlement juveniles as well. If this were the case, it may have been worthwhile re-establishing a captive population of known-age crabs again, assuming that adequate numbers of megalopae could be collected from a swarm of the type observed in the previous year. However no further swarms were observed or located so the known-age population option had to be abandoned in favour of the final alternative of investigating lipofuscin concentration frequency distributions from the catchable component of the wild stock, as per Sheehy *et al*. (1998).

The relationship between lipofuscin estimates from left and right eyestalks was investigated to determine whether there was sufficient similarity to allow a single eyestalk to be taken from each crab, or whether it would be necessary for statistical reasons to sample both eyestalks. This would have a significant impact on the size of the sample of crabs required; if only one eyestalk was required the base sample could be twice as large as would be needed if both eyestalks were needed. As the results of these trials showed a very high correlation between the lipofuscin concentration estimates from left and right eyestalks, it was decided to maximise the base sample size by taking only one eyestalk from each crab.

The fact that *R. ranina* has a relatively short, well-defined spawning season suggested that frequency analysis of lipofuscin determinations from samples of the wild population may show modal separation attributable to separate age-classes. While validation would be difficult, even an approximation of the number of age classes in the recruited component of the population would be a valuable piece of information. There is still much uncertainty about the longevity and growth rate of this species of crab, so some knowledge of the number of years the animals are subject to exploitation would be of particular interest to the stock assessment scientists and fishery managers responsible for setting the total allowable spanner crab catch.

The first large sample was collected in July 2006 using tangle nets modified to maximise retention rate and the size range of crabs in the catch. On advice from DPI&F histologists at the Animal Research Institute (Yeerongpilly, Qld) we used Davidson's Solution to fix these samples. This quick-acting fixative is generally preferred for crustacean tissues, which start to autolyse very soon after cell death. An added perceived advantage was that its acid content may effectively decalcify the exoskeletal sheath, speeding up the pre-sectioning process by removing the need for the decalcification step at the Werribee Lab. Unfortunately a large proportion of this sample could not be assayed for lipofuscin because (although well-fixed) there were problems with the histology of the sections and the decalcification process had not worked as well as initial trials had suggested. We could have re-sampled during a related field operation at the same site in September, but notification of the problem was not received in time. Consequently a third operation was mounted (in October 2006) to obtain a replacement sample, again with the modified tangle-nets. On this occasion, however, the eyestalks were fixed in 10% neutral buffered formalin instead of Davidson's fixative, and decalcified with Kristensen's solution at the Werribee Lab.

A proportion (~15%) of these samples was still unusable for various technical reasons, but about 40 additional eyestalks had been sent with the October consignment to substitute for any that were unusable. Unfortunately it was discovered shortly after that the additional samples had been disposed of during a routine clean-up at the Werribee Lab. While partial replacement was possible with some very small specimens collected by a commercial fisher, the sample was small (n = 7) and did not compensate numerically for those that were unusable.

*Cerebral ganglia*

Samples of crabs for cerebral ganglion or brain tissue were taken as soon as possible after capture. The crabs were narcotised in the research vessel's deck freezer for about 30 min., and once they showed a nil response to claw and/or eyestalk stimulation, the anterior part of the cephalothorax was removed by hacksaw, labelled, and immediately placed in 10% formalin solution. These 'head' samples were fixed for at least 2 weeks in cold 10% buffered formalin-seawater, after which time the cerebral ganglia were dissected out. The procedure for this dissection, as developed by CAF staff, is included as Appendix 3. Once the ganglia were removed, they were washed in distilled water, placed into separate plastic vials with a small plug of water-moistened fibre-free tissue and labelled. The samples were then air-freighted to the Melbourne University Veterinary Research Laboratory at Werribee, Victoria, for embedding. After blocking in Paraplast Plus Tissue Embedding Medium™, the tissues were taken to CAF at Queenscliff, Victoria, for sectioning and reading.

*Optic nerve (eyestalk tissue)*

Eyestalk samples were also collected from cold-euthanased (but not frozen) crabs as soon as possible after capture.

For the first chemical extraction trials an initial small sample of legal sized spanner crabs was obtained from a processor (CrabPAK; Kawana Waters) to enable tissue dissection, chemical extraction and lipofuscin determination procedures to be tried and evaluated. Following the Ju-Harvey technique (used on north-American blue crabs *Callinectes sapidus*), eyestalk tissue was dissected out from the tubular exoskeleton, care being taken not to include any retinal tissue. No attempt was made to separate the optic nerve from surrounding glandular or muscular material. The fresh samples were transported on ice to the Hamilton Laboratory for extraction and assay.

Samples taken for histofluorometry needed to be fixed and preserved, as freezing would destroy the structure of the tissue. Both left and right eyestalks were excised near the distal articulation, and the retinal tips removed to improve ingress of the fixative into the tubular chitinous exoskeleton containing the optic nerve and associated tissue. After further experimentation on various fixatives and decalcification options, the final protocol called for formalin fixation and a 24 hr period of decalcification. Each eyestalk pair was placed in a labeled plastic vial with an adequate quantity of 10% formalin-seawater solution and allowed to fix for at least 10 days at the Southern Fisheries Centre. They were then placed (in pairs) in water-moistened tissues folded into small labelled zip-top plastic bags for air-freighting to the Werribee laboratory for decalcification and blocking.

### 1.2.4 Tissue Processing

*Chemical extraction method*

Initial extraction attempts at the Centre for Food Technology revealed some fluorescence activity, but at a relatively low level, suggesting that either the tissue lipofuscin was present in very low concentration or it was not being extracted effectively. Subsequent efforts were centred on adapting a fluorescence method for measuring lipofuscin dissolved from the eyestalks of the crabs following the method of Ju *et al*. (2001). The fresh eyestalk tissue was added to 4 ml of solvent (dichloromethane:methanol 2:1) and then sonicated. The solvent was then dried down, and the dried material reconstituted in 1 ml methanol. This method was difficult to adapt because of problems with sonicating small volumes of non-aqueous solvents and the lack of suitable equipment for the evaporation of small volumes of organic solvents. The process was therefore modified so that the

eyestalk was homogenised using a Potter-Elvehjem homogeniser in 1 ml of the same solvent, and the fluorescence measured directly on the dissolved material.

*Histofluorometric method*

Eyestalks Unstained longitudinal eyestalk tissue sections were examined for potential areas of neuron clusters within the optic ganglia, but apart from one area of approximately 700μm little structure normally associated with lipofuscin aggregation was found, and no lipofuscin was detected. Subsequent examination of 800 serial transverse (6 μm) sections through an eyestalk, starting from the cornea, revealed a neuron cluster assumed to be the *medulla terminalis* between slides 65 and 75, located approximately 2520 μm from the eyestalk tip, and containing small numbers of lipofuscin-like granules. Further investigation showed that the location of this lipofuscin-rich tissue was consistent from sample to sample, and that a relatively small number of sections was needed to include the area of interest.

Brains: The appropriate plane of sectioning was determined by the analysis of both transversely and longitudinally serial sectioned brains. The olfactory lobe cell mass (OLCM) was more easily detectable in longitudinal section than in transverse section and displayed the classic mushroom shape as observed in the brains of other crustaceans (Sheehy 1989; Wahle 1996). The OLCM was situated in the posterior region of the brain approximately 300 μm to 400 μm in from the outer edge (60–72 sections). Samples for longitudinal sectioning were serially sectioned at 6 μm. Un-coverslipped sections were viewed with a Wild compound microscope at 100–400 x magnification to identify the start of the region of interest. Once the appropriate starting point had been identified, the whole OLCM was serially sectioned. This equated to between 70 and 120 sections per animal and ensured that the area including the posterior olfactory lobe (the 'area of interest', *sensu* M Sheehy) was obtained. The sections were fixed to slides, re-hydrated using standard histological techniques, and mounted with DPX (dissolved perspex in xylene), coverslipped and labeled.

Photomicroscopy: Although the Central Ageing Facility had purchased a Leica DC300F low light fluorescent digital camera in 2003 to alleviate the need for time-consuming and expensive traditional photographic methods, complications with the camera and associated software at the time of analysis meant that traditional photographic methods had to be used during most of the project period.

Slide-mounted sections were viewed at 250x magnification under incident light to determine the area that contained the posterior olfactory lobe cell mass or optic nerve medulla (depending on which tissue was being examined). Magnification was increased to 400x and switched to epifluorescence using an epifluorescence microscope fitted with a 50 watt mercury lamp and an I3 (450-490 nm) excitation filter. All areas of the brain and connecting ganglia were examined for lipofuscin granules.

The whole tissue preparation containing visible lipofuscin granules was imaged using the MPS 60 Photoautomat. Sample information, slide number, position of the section on the slide and the number of frames from each of the olfactory lobe were printed automatically on the bottom of each photomicrograph for identification during image processing. Between 2 and 7 photomicrographs or images were taken from each specimen, depending on the size of the area of interest and the quality of the histological preparation (amount of tissue teasing from the microtome). An example of one area in the OLCM at 400 x magnification is shown in Figure 1-6. Seven images were adequate to cover the whole area of the region of interest. The images overlapped, but (using the image analysis software) a frame was drawn on the image so that no part of the cell mass was measured twice. This approach was considered preferable to taking a number of random potentially overlapping images because the lipofuscin is not distributed homogeneously throughout the region of interest.

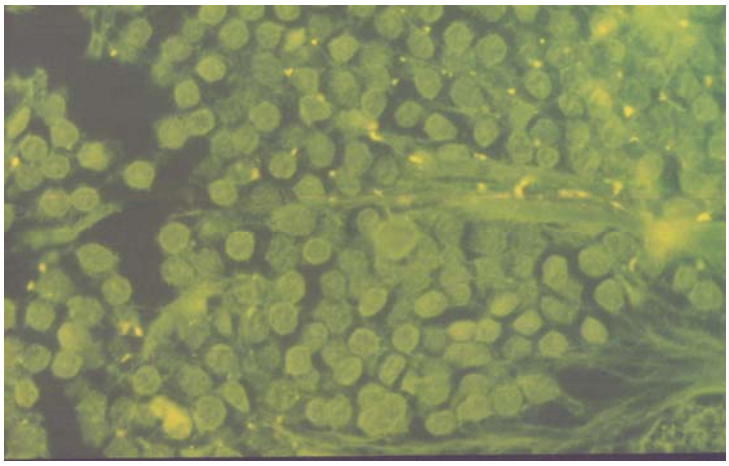

**Figure 1-6.** Photomicrograph of part of the neural ganglion of an adult spanner crab at 400x magnification showing fluorescing lipofuscin granules (yellow) amongst the neuronal tissue (green).

Image processing: The images were loaded to Optimas™ and converted to 8 bit grey-scale (256 levels of grey) for image processing, and a customised macro used to determine the lipofuscin volume ratio in the images (Appendix 4). This involved automated measurement of the area of each fluorescing lipofuscin granule visible within the frame, the total area covered by the frame, and the area of 'negative space' (the gaps between cell masses in the section which resulted from teasing or separation of the tissue during processing or sectioning). The ratio of the total area of fluorescing granules across all images from each specimen to the difference between the total area of interest and the total area of negative space was calculated, as the lipofuscin volume ratio. This quantity expressed as a percentage became the index of lipofuscin concentration in the tissue. The number of fluorescing granules (in each frame) whose areas were estimated with the image processing algorithm was highly variable, ranging from zero to 811. During examination of the slides an assessment was made of their histological quality and usefulness (Table 1-1), information used later to determine which data should be included in the analyses.

**Table 1-1.** Scoring process used to identify the histological quality of tissue preparations used for lipofuscin assay.

| Score | Comment |
|---|---|
| 1 | Very good example and preparation |
| 2 | All area present; slight teasing of tissue (larger negative space) or angled section. |
| 3 | Major teasing of the tissue but most of the region of interest present. |
| 4 | Difficult to be certain whether tissue is compressed, or not all area present |
| 5 | Failed preparation (unusable). |

Statistical analysis.

The relationship between lipofuscin concentrations in left and right eyestalks was tested using simple product-moment correlation coefficient. It was considered if the correlation was statistically significant at $P < 0.01$, there would be sufficient justification for only taking one tissue sample from each crab, which would effectively double the sample size that could be processed with the available project resources.

A fundamental first assumption in using lipofuscin as an indicator of age in spanner crabs was that there should be a demonstrable positive relationship between lipofuscin concentration and body size. It must be assumed that size is at least loosely correlated with age, so for the lipofuscin technique to be considered a viable indicator of age, a correlation with size must first be able to be demonstrated. Inspection of the data distributions indicated the need for logarithmic transformation of both length and lipofuscin volume ratio prior to analysis. The relationship between the two variates was then tested using a general linear regression model in GenStat (2007), and (after initial testing showed a poorer model fit when all data were included) only preparations with a quality score between 1 and 3 were used. The response variate was Ln(Lipofuscin) and the fitted model terms were Ln(Size) + Sex + Ln(Size)·Sex. The interaction term was included to test the null hypothesis that there was no difference in the size-lipofuscin relationship between the two sexes.

*Telomere trials*

During the early stages of the project, discussions with SFC Molecular Biologist Dr Jennifer Ovenden suggested the possibility of using an alternative approach to age estimation in spanner crabs. This genetic technique involves the estimation of the lengths of telomeres – non-coding base-pair sequences at the ends of chromosomes, one function of which is to protect the chromosome from damage when they undergo replication. Each time the chromosome unravels during mitosis there is a probability that the telomeres may be damaged from having base pairs knocked off, thus making them shorter. The greater the number of replications (a function of age), the shorter the telomeres would be expected to be. Genetic techniques have been developed to estimate the length of the telomeres (in terms of the number of their constituent base-pairs), which provides the possibility of using this as a surrogate for age. A considerable literature of gerontological work has demonstrated the inverse relationship between age and telomere length in humans, and the technique is now starting to be applied to other species, especially those whose ages are difficult to determine using conventional methods.

A collaborative study focusing on spanner crabs was developed with Southern Cross University (NSW) to examine the feasibility of such an approach, and it yielded some encouraging initial results. While not formally part of this project (FRDC 2003/046) we report it briefly here because it was a spin-off from our project and the PhD student involved transferred to a different project before completing the work.

At SCU genomic DNA was extracted from spanner crab muscle tissue provided by project staff, and the non-telomeric DNA removed by restriction enzyme digestion. DNA containing telomeres was run into a gel and hybridised following the Southern blotting method to a probe that was specific for invertebrate telomere DNA. The mean length of the telomeres was measured in base pairs after gel calibration and densitometric scanning. DNA from crabs with carapace lengths 40 to 100 mm was analysed on the reasonable assumption that 100 mm crabs were older than 40 mm crabs. The method was modified over a series of experiments from a published procedure developed for vertebrates. For some specimens, data were also obtained for DNA extracted from gill tissue. Telomere lengths from different tissues from the same crab differed by 4–8%. Details of the samples collected, most of which are still in ultra-freezer storage, can be found in Appendix 5.

Consideration was given to expanding the present FRDC project to incorporate a genetic component, but after discussion with FRDC staff it was agreed to progress the work as a separate project. As a result, a joint application for Queensland Smart State & Innovation and FRDC funding to develop an international collaborative project was prepared, but was unsuccessful in attracting support. However a successful follow-up application was developed with a broader focus, including a range of taxa which are difficult to age using traditional methods, and including spanner crabs. This project commenced in late 2007 and has begun telomeric analyses early in 2008.

### 1.2.5 Detection of length modes in wild-caught pre-recruits

Later in the term of the Project it became apparent that, even if we were fortunate enough to locate another swarm of megalopae, we would not be able to rely on having enough known-age animals of sufficient age to undertake the analyses effectively. Previous large-sample length frequency analyses of the catches from standard commercial-style dillies did not yield convincing evidence of different length-frequency modes that could be attributed to different age-classes (Brown *et al*. 1999, Kirkwood *et al*. 2005). As we had decided to use modified nets known to retain larger proportions of small crabs for the final sampling of wild-population crabs for lipofuscin analyses, it was of interest to see whether the extended size-range of crabs in the catch might reveal more about possible age cohorts in the population. Standard graphical methods were used to examine the length-frequency distributions of crabs sampled for lipofuscin determination during the sampling trips off the southern end of Bribie Island in 2005.

### 1.2.6 Condition index (commercial catch sampling)

Crustacean growth can only occur as a result of moulting, and it is sometimes possible to determine objectively when the animal is in the immediate pre- or post-moult condition. It would be valuable and informative to know when spanner crabs of various sizes undergo moulting, particularly if it happens to be reasonably synchronised. This would help identify steps between successive instars, and shed some light on the growth process. Post-moult crabs have soft shells, and this can often be used as an indicator of moult status. Unfortunately soft-shelled spanner crabs are found very infrequently, presumably because the animals stop feeding until the carapace has become hard enough for it to afford some reasonable protection from predators, so that avenue did not appear to be an option. An alternative approach takes advantage of the fact that at after moulting the crabs are light in weight (compared to their size), because although the new carapace has been expanded by the uptake of water into the tissues, the musculature has not had a chance to fill out into the new shell. This is a form of condition index often used as a surrogate measure of the physical health of fish. Commercial crab fishers often maintain that there are certain times of the year when the crabs are light (i.e. in poorer condition) and therefore attract a lower price.

To determine whether there is a seasonal pattern in condition that might correlate with the moulting cycle we instituted a commercial catch-sampling programme to gather data on length and weight of crabs throughout the year. The sampling was carried out at the CrabPak premises in Kawana Waters through the generosity of the company's principal Mr Les Apps and his staff.

Every month (where possible) a visit to the factory was arranged to coincide with the evening unloading of spanner crabs, before they were sorted and allocated to the processor's live-holding tanks. Generally the contents of two (randomly-selected) baskets from each of two or three crab fishers were measured. Carapace lengths were measured with stainless steel vernier calipers, and the corresponding weights measured with a portable electronic Mettler balance reading to 5 kg. Because of the need to process the catches quickly to reduce the exposure time, the data were recorded on a small tape-recorder and transcribed directly onto computer file the following day.

The significance of a seasonal signal in the weight-length relationship (i.e. condition index) was examined using General Linear Modelling in GenStat (2007), after removal of spurious outliers. As the relationship between length and weight is cubic, log transformation of both variables was appropriate prior to analysis. The response variate was Ln(W) and the model terms were Ln(L) + Month, where W was the total live weight in grams and L the carapace length in mm. Sex was not included in the model because few female crabs were represented in the commercial catch samples, and only data for male crabs were analysed.

## 1.3 RESULTS

### 1.3.1 Collection of material

*Dredging*

Because of considerable delay in the final hand-over of the research vessel RV Tom Marshall, we were unable to start these dredging trials until February of 2004, which was well past the peak spawning season for *R. ranina* in Queensland waters. However we expected to capture some megalopae and crab 1 stage animals, because although spawning had finished, there would most likely still be some late-spawned megalopae and first-stage crabs on the ground.

A number of dredging trips, mainly in the area to the north of and just outside Moreton Bay, were conducted over the period Feb–May (Table 1-2), which resulted in the capture of 43 megalopae and 36 juvenile crabs. Capture rates (when adjusted for the length of cutter bar) were similar to those achieved with a smaller device during our previous dredging work in 1997–98. The large (~9 mm CL) megalopae were robust, and survived the standard dredge haul of 15–20 min surprisingly well. The juveniles were more susceptible to damage and consequent mortality, although most survived capture and transportation back to the Southern Fisheries Centre, where there they were placed in the rearing apparatus shown in Figure 1-4.

**Table 1-2.** Numbers of juvenile spanner crabs (megalopae and crab stages 1-3) caught in the first series of dredging operations between February and May 2004.

| Date | Megalopae | Crab 1 | Crab 2 | Crab 3 | Total |
|---|---|---|---|---|---|
| 2/02/2004 | 3 | 0 | 0 | 0 | 3 |
| 19/02/2004 | 18 | 10 | 0 | 0 | 28 |
| 20/02/2004 | 22 | 2 | 0 | 0 | 24 |
| 1/04/2004 | 0 | 5 | 1 | 0 | 6 |
| 2/04/2004 | 0 | 15 | 1 | 0 | 16 |
| 4/05/2004 | 0 | 0 | 1 | 1 | 2 |
| 19/05/2004 | 0 | 0 | 0 | 0 | 0 |
| Totals | 43 | 32 | 3 | 1 | 79 |

At the beginning of 2005 we undertook a number of dredging operations in nearshore coastal waters outside Moreton and Stradbroke Islands, as we had done the previous year. Some of the work was done as part of the 13th International Marine Biological Workshop, run by the South Queensland Branch of the Australian Marine Sciences Association (AMSA) and based at the University of Queensland's Marine Studies Centre at Dunwich, North Stradbroke Is. Five sampling days during January 2005 yielded no megalopae or juvenile spanner crabs, while over six days in February we obtained 13 megalopae and 28 stage-1 crabs. This compared with 38 megalopae and 12 C1 animals during the same month in the previous year.

*Light traps*

We investigated the use of light-traps as a possible alternative to active dredging as a means of catching pre-settlement crabs. Prior to and during the AMSA workshop (Jan-Feb 2005) we deployed a light-trap (designed by Dr Peter Doherty for sampling fish larvae at night), in the hope that this method would be appropriate for crab larvae. This apparatus was considered potentially a less damaging way to collect the crablets than dredging. A few crab megalopae were caught in the light-trap, but none were spanner crabs.

*Megalopa swarm*

The megalopae captured from the surface swarm in March 2005 survived surprisingly well over the several days they were held on the vessel, then transported from Gladstone to Brisbane by car in sealed polystyrene foam containers. In all, around 500 megalopae were distributed among the 12 available rearing tubs on 21 March.

### 1.3.2 Maintenance, survival and growth of captive population.

*Feeding*

Behavioural observations using time-lapse video recording of overnight feeding trials were used to establish an appropriate diet for the post-settlement stage juvenile crabs. Evaluation of the video tapes each morning suggested some movement of the crabs, but there was little evidence that the crabs were coming out of the sand for significant lengths of time to feed. We later realised that the frame frequency was too slow to detect the rapid, almost darting, movement of the crabs while they were moving around the tank. As with the adults, when they were not moving they were buried in the substrate and completely invisible to the camera. Once this behaviour was identified, we were able to adjust the tape speed so that definite evidence of feeding activity was obtained. Subsequent frequent observations of crabs (both megalopae and juvenile stages) approaching and grasping the food, and sometimes attempting to drag it under the sand, confirmed that the early life stages of spanner crabs are attracted to the same range of foods as is eaten by the adults.

Subsequently we fed the crabs (usually daily) with pieces of thawed frozen squid, pipi, prawn, or pilchard. Uneaten food was cleared from each of the rearing tubs within 12 hr of introduction. Despite this, some food was dragged beneath the sand by the juvenile crabs, and could not effectively be located and removed. This resulted in some blackening of the substrate, and to prevent the buildup of anoxic material we need to clear the crabs approximately weekly. This involved carefully lifting the plastic mesh tray so that the sand dropped through, leaving the crabs, moult shells and any pieces of uneaten food. At the same time we conducted a census, to determine the numbers and stages that had moulted and the mortalities (Figure 1-7).

*Survival*

Throughout the first year there was a continuous low level of mortality occurring amongst the animals we had collected by dredging as megalopae or stage-1 crabs following the 2003-04 spawning season. Around mid-May 2004 there was a spate of unexplained mortalities which could not be attributed to poor water quality or excessively low tank temperatures. This resulted in the loss of a significant number of crabs of all stages – but principally during or just after a moult – leaving only 21 live animals from the fist batch at the end of June 2004. Most of the crabs had reached the fourth juvenile instar, at a carapace length of ~20 mm, after a period of 4.5 months. Because of the small numbers that survived, we were unable to carry out the sampling as was originally planned, as it was important to conserve the live animals for as long as possible to provide the best calibration time-series. We assumed we would be able to collect more megalopae and C-1s again the following season.

There was some circumstantial evidence that the animals caught at the crab stage may have survived better in the rearing facility than those caught as megalopae. There was no immediate explanation for this, but it may have been related to the fact that the animals caught first (mostly megalopae) were fed food that had been frozen. Subsequent advice from BIARC crustacean aquaculturist (S. Mikami) prompted us to switch to a diet of fresh squid, fish and shellfish because of the possibility that

freezing may have reduced the amount of certain biochemical compounds critical to successful moulting. Only five crabs survived to the end of the first year, even without any sampling for lipofuscin analysis. This made it very important that we replenish the supply of post-settlement stages to maximise the possibility of obtaining animals of at least two years of age before the conclusion of the project.

The collection of megalopae from the surface swarm in March 2005 enabled us to re-stock the rearing system with about 500 individuals, providing some optimism that we may be able to obtain some known-age material before the Project concluded. Apart from an initial mortality of 10% during the following week, all the megalopae moulted to C1 stage. There was another mortality event in the second half of April, when about 200 of the crabs died, often during the process of moulting. Tank salinity was variable but had not changed appreciably (Figure 1-7), and standard aquarium-water chemical tests did not reveal any significant change in water quality that could be linked to the mortality. The only possible cause that could be identified was a slight (2 C°) drop in water temperature during April, the time when the greatest mortality occurred (Figure 1-7). After consultation with staff of the Bribie Is Aquaculture Centre we began supplementing the diet with enriched pellets. We have no direct evidence that the pellets were effective or even ingested by the crabs, but the mortality rate dropped to about 5% per week thereafter, despite a continuing reduction in ambient water temperature with the onset of winter. Growth rates in 2005 seemed somewhat slower than the previous year, perhaps because of differences in ambient water temperature. Alternatively, the high early mortality rates and reduced rate of moulting may have been due to physiological stresses resulting from the less-than-optimal conditions to which the megalopae were subjected immediately after capture.

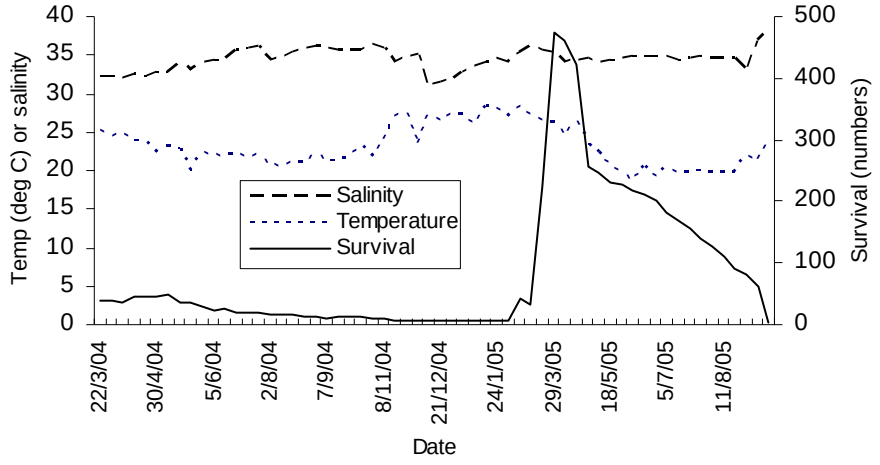


**Figure 1-7.** Survival curve for megalopal and post-settlement juvenile crabs, showing ambient salinity and temperature trajectories in the rearing system at the Southern Fisheries Centre. The survival curve (solid line) includes introductions, the major event being in March ’05 with nearly 500 megalopae.

### 1.3.3 Lipofuscin assay: chemical extraction method

A pilot experiment was carried out to determine whether lipofuscin could be extracted chemically from spanner crab eyestalks, whether the amount extracted is a function of the size of the animal, and whether extracts from left and right eyestalks were consistent. The pilot study involved assaying lipofuscin from each eyestalk of six mature spanner crabs close to or above the minimum legal size (five male and one female). There was some consistency in the lipofuscin indices between left and right eyestalk preparations, and (amongst the males) an indication that higher lipofuscin

concentrations were associated with larger body sizes (Table 1-3). This provided some initial optimism that the method might be appropriate and potentially useful as an indicator of age in spanner crabs.

A follow-up analysis, again using eyestalk tissue, was carried out on nine adult crabs of a range of sizes from 109 to 145 mm RCL (rostral carapace length) in three notional size classes (around 110 mm, 120 mm and >135 mm). The sizes were chosen on the basis that although there may not be a strong relationship between size and age in this crustacean, a male spanner crab of 145 mm CL (at the upper end of the population size rage) would almost certainly be somewhat older than one that is only slightly larger than the minimum legal size.

**Table 1-3.** Results of pilot experiment comparing spanner crab carapace length and lipofuscin (LF) index

| Sample No. | CL (mm) | Sex | LF index[1] |
|---|---|---|---|
| 6 | 89[#] | F | 731, 853 |
| 5 | 96 | M | 242, nd |
| 1 | 109 | M | 338, 315 |
| 4 | 116 | M | 522, 856 |
| 2 | 119 | M | 340, 286 |
| 3 | 121 | M | 377, 376 |

[1] Arbitrary fluorescence units/$A_{280}$

Only small amounts of fluorescence were detected in the spanner crab eyestalk chloroform:methanol extracts in either the pilot or follow-up trials. In the latter there was little agreement between the amount of LF in the left and right eyestalks (Table 1-4), and no significant correlation between the carapace length and the LF index as measured (Figure 1-8).

**Table 1-4.** Results of experiment #2 comparing spanner crab carapace length and lipofuscin (LF) index derived from optic nerve (eyestalk) extracts.

| Sample No. | CL (mm) | Fluorescence Intensity[1] | $A_{280}$ | LF index[2] |
|---|---|---|---|---|
| 2 | 109 | 31.0, 60.0 | 3.73, 4.67 | 0.077, 0.115 |
| 1 | 110 | 59.4, 26.1 | 2.48, 1.95 | 0.215, 0.125 |
| 3 | 110 | 21.3, 61.9 | 2.90, 5.33 | 0.070, 0.104 |
| 9 | 117 | 68.7, nd | 5.21, nd | 0.118, nd |
| 8 | 119 | 59.8, 49.6 | 4.68, 3.66 | 0.115, 0.122 |
| 5 | 121 | 71.1, 26.0 | 3.36, 3.87 | 0.189, 0.063 |
| 6 | 136 | 61.7, 38.9 | 5.22, 4.37 | 0.106, 0.081 |
| 4 | 139 | 94.8, 86.3 | 3.41, 6.20 | 0.246, 0.124 |
| 7 | 145 | 27.1, 75.9 | 6.59, 6.17 | 0.038, 0.110 |

[1] Fluorescence intensity is in arbitrary units; [2] LF index is in the units of µg quinine sulphate fluorescence equivalents per unit $A_{280}$ from each of the eyestalks.

There was only a poor correlation between carapace length and LF index reported by Ju *et al*., which was insignificant in the same region of carapace length used in our experiments. While there is no reason to suppose that the accumulation of lipofuscin in both eyestalks should be identical, the amount of extracted protein varied widely between eyestalks and between the different samples,

which suggests that the extraction was poor, as the amount of protein in the samples would be expected to be relatively constant. Inspection of the extract showed that the chloroform:methanol was dehydrating the tissue and toughening it to mechanical homogenisation. The choice of chloroform:methanol as a solvent for lipofuscin therefore seemed inappropriate, as lipofuscin is a protein aggregate which would not be expected to dissolve readily in solvents such as chloroform:methanol. For this reason, it was decided to experiment with an aqueous buffer as the extracting solvent.

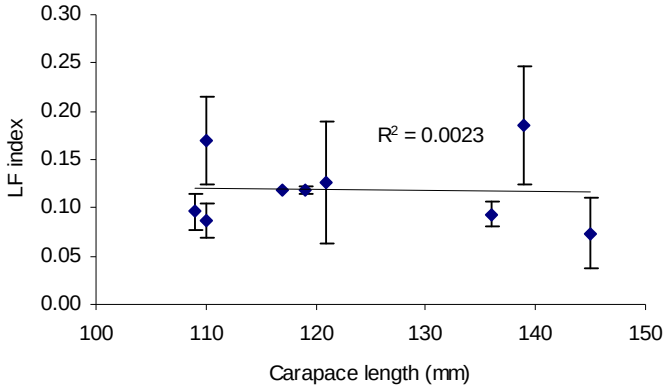


**Figure 1-8**. Relationship between eyestalk lipofuscin index (LF) and size in spanner crabs. Symbols depict means and the range bars represent the difference between left and right eyestalk measurements.

*Alternative extraction method*

A protein extraction buffer, based on a commercially available product, was developed. This buffer contained 50 mM Tris-HCl pH 7.5, 0.15 M NaCl and was trialled in the presence of two detergents, 0.1% (w/v) SDS and 1% (w/v) Nonidet P-40. The Nonidet P-40 was found to be inappropriate because it fluoresced in the same wavelength spectra as those used for the assay itself. Extracts of brain tissue were produced using the buffer in the presence and absence of SDS. No difference in the fluorescence or protein (as measured by $A_{280}$) was observed in the buffer + SDS compared with the buffer alone, indicating that SDS was superfluous. Sonication of the extract was also shown to have no effect. The early experiments used samples which were centrifuged prior to analysis to remove aggregated material. However fluorescence measurement is possible using turbid samples containing fine particulate material, and trials were carried on extracts that had not been centrifuged. The fluorescence of the non-centrifuged samples was higher by between half and one order of magnitude than that of the centrifuged samples, suggesting that most of the lipofuscin aggregates are removed by centrifugation.

*ELISA assay for the measurement of MDA-modified protein*

As a major factor in the production of lipofuscin is the formation of malondialdehyde (MDA)-modified protein, it was thought that the measurement of MDA-modified protein may be also provide an age-estimation method perhaps more suited to large numbers of samples and more accurate than the fluorescence assay. ELISA assays for MDA-modified protein have been used in other laboratories for the measurement of lipofuscin-like substances due to their importance in various mammalian degenerative disorders.

A monoclonal antibody reactive with MDA-modified keyhole limpet haemocyanin (KLH) was obtained from Prof. Koji Uchida, Graduate School of Bioagricultural Sciences, Nagoya University,

Japan. This antibody had been used previously in their laboratory in ELISA assays of oxidatively-modified low density lipoprotein (Yamada *et al*., 2001). A similar inhibition ELISA was devised and control experiments were performed using the anti-MDA-KLH antibody. The control assays showed that anti-MDA-KLH antibody reacted with MDA-modified BSA, and suggested that the inhibition ELISA would function. However, the amount of antibody required to render the assay sufficiently sensitive for the measurement of MDA-modified protein was prohibitive compared to the amount available, and thus the efforts were concentrated on the fluorescence assay. Similar caveats apply with the measurement of MDA-modified protein as a marker as apply with the fluorescence assay.

### 1.3.4 Lipofuscin assay: histological method.

*Identification of appropriate tissues (eyestalk and brain).*

Initial eyestalk trials were unsuccessful in locating a lipofuscin-rich region of the optic nerve, so the focus of this work shifted to brain (cerebral ganglion) tissues early in the project. The mean lipofuscin volume ratios were calculated from the brains of four *R. ranina* ranging in size from 77 to 110 mm CL (Table 1-5).

**Table 1-5.** Mean lipofuscin volume/ratio in spanner crab brain tissue.

| Sample No. | Area of ROI | Area of lipofuscin | Area of negative space | Lipofuscin volume fraction (%) | Carapace length (mm) |
|---|---|---|---|---|---|
| 12 | 438.62 | 1.47 | 152.49 | 0.51 | 77 |
| 2 | 593.113 | 5.37 | 184.43 | 1.31 | 110 |
| 10 | 563.12 | 2.88 | 125.113 | 0.66 | 91 |
| 8 | 426.82 | 2.25 | 176.84 | 0.90 | 109 |

A plot of lipofuscin concentration against carapace length of the sampled crabs (Figure 1-9) shows a positive relationship, with large crabs yielding higher lipofuscin values than small crabs. With an $R^2$ value of 0.74 and a sample size of four, this was considered only indicative, but the fact that there were differences in lf index and that they changed in the expected direction against body size gave some cause for optimism about the methodology.

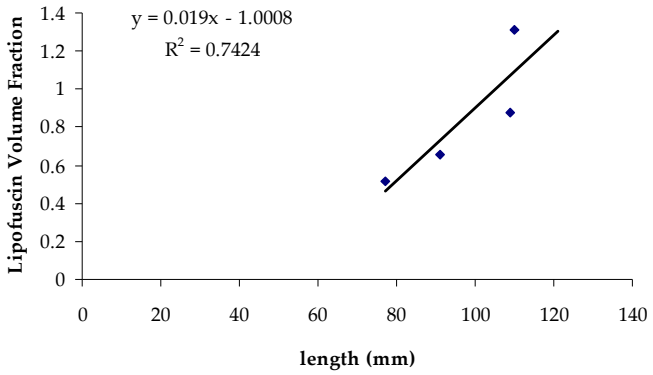


**Figure 1-9.** Relationship between spanner crab brain tissue lipofuscin concentration and body size.

During June 2005, when the replacement/repaired camera equipment became available, considerable effort was put in to investigating the suitability of eyestalks. This yielded mixed results. Initially

three samples of eyestalk neural tissue were prepared by taking serial transverse sections through approx. 6 mm of the eyestalk from the distal tip. Of the three samples, two showed areas of lipofuscin accumulation. In the first sample the area of interest (assumed to be the *medulla terminalis*) appeared similar to that reported in Sheehy's papers on his work with the European lobster. In the other sample, however, the area of neurons appeared slightly different from the first one, possibly because of slight differences in dissection and preparation methods. However the eyestalks were similar in size, and the position of the neuropiles was approx. 2500 μm (400 sections) from the tip.

Subsequently 10 more samples from crabs of different sizes were examined. Rather than serially sectioning the structure right from the tip, the approximate location of the area of interest was estimated (based on the initial sectioning) and sectioning commenced after the equivalent of 300 section-thicknesses from the retina. Approx. 320 sections were taken from each sample. Two of these showed evidence of lipofuscin and neuron aggregation, but the tissue was teased apart and the region of interest broken up, as a result either of the dissection or the sectioning technique, so interpretation and analysis was difficult.

In the eyestalks in which lipofuscin was detected, there were several separate areas within the ganglia of cell clusters with lipofuscin accumulation which appeared to have different accumulation rates. If eyestalks are to be used successfully, the same cell clusters would clearly have to be used each time for consistency. One eyestalk (sample 21A) also showed considerable background autofluorescence, which made image analysis difficult and may have biased the estimate.

Three more brains were prepared for comparison with eyestalk tissue from the same animals. The results of lipofuscin analysis of these additional tissues were consistent with those of earlier brain analyses. However no lipofuscin was detectable in the corresponding eyestalk samples, so the comparison was limited to four samples.

*Whole eyestalk specimen decalcification*

There were indications that eyestalk neural tissues would provide an estimate of the lipofuscin volume ratio, but it was unclear as to the exact position sections should be taken, as the published literature suggested that the location of the *medulla terminalis* can vary substantially between species (Blaustein 1988). Dissection of the eyestalk carapace from the neural tissue is difficult, and can cause the tissue to become teased apart so the structure is spread out after sectioning which may have compromised some of the lipofuscin volume ratio estimates referred to above.

An alternative approach was investigated, involving decalcification of the eyestalk and embedding the entire structure for sectioning. A sample of small (fixed and preserved) crabs from the known-age population was sent to CAF for tests to see whether decalcification is an effective way to access the lipofuscin-bearing tissues without dissection. This was seen as particularly important with respect to the analysis of megalopae and post-settlement juveniles, which are quite small (10–20 mm CL), and it was unlikely that that it would be possible to dissect out the intact neural ganglia of these individuals successfully.

After trials on a small number of post-settlement crabs and several different sized eyestalks, Kristensen's decalcification solution was preferred to Goody and Stuart's solution as it provided for better cell structures after tissue sectioning and mounting. Kristensen's solution consisted of 8 N formic acid (368.24 ml/100ml of water) and 1N sodium formate (68.01 gm/100 ml distilled water) at a ratio of 1:1.

Eyestalks were immersed in solution for a minimum period of 5 days. This was sufficient for decalcification of small eyestalks, but medium and larger eystalks required an additional 2-4 days

depending on size. After 5 days of immersion, large and medium eyestalks were checked each day for sufficient softness and the presence of a colour change (whitening) in the exoskeleton of the eyestalk. Decalcified eyestalks were then processed using the methods described earlier for dissected brain tissue.

The main problem with the eyestalks had been the variability in the size of the structure and the fact that they were examined in transverse section. To be able to process these structures successfully it appeared that most of their length would have to be sectioned to ensure that the fluorescing area is bracketed within the sections. This would require taking and examining 800–1000 sections, clearly a time-consuming process. However after identifying a suitable decalcifying agent, the ability to embed whole eyestalks alleviated the need for time-consuming dissection and greatly improved tissue section quality. Transverse serial sectioning determined the position of the largest area of neuron and lipofuscin accumulation relative to morphological features of the eyestalk. An area of interest was found that corresponded with the proximal end of the retina (Figure 1-10). Several more eyestalks from a range of different sized specimens were processed, with sectioning commencing at a position just below the base of the retina. A total of 300 sections were taken from each sample, and an area of neurons and lipofuscin accumulation was detected between sections 100-200 in all samples examined. The position and shape of the area of interest was consistent between samples, and assumed to be the same cell cluster. Selected slides containing the region of interest were de-waxed and mounted with glass coverslips. Between 4 and 7 images (at 400 x) were taken to ensure that the whole cell cluster was captured.

*Post-settlement stages: whole sectioning for brain tissue.*

Accessing the appropriate tissues for lipofuscin determination in post-larval crabs presented a challenge, as the cerebral ganglion even in adult crabs is quite small. The possibility of being able to dissect them from 10-40 mm CL animals was considered remote. The solution to this was to serially section the whole animals and (given our knowledge of the location of the brains of adults) look for the tissue in the appropriate area. Decalcification of the crabs' exoskeletons by soaking for at least 5 days in Kristensen's solution allowed unlimited 6 µm sections to be taken through the carapace after embedding. Some post-settlement crabs (~12 mm CL) were embedded and serially sectioned, but CAF staff were unable to locate the cerebral ganglia using accepted protocols.

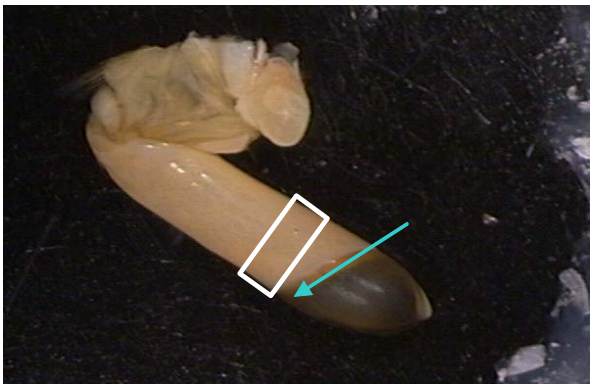

**Figure 1-10.** Eyestalk from *R. ranina* indicating starting point for transverse sectioning (blue arrow) and approximate position of the region of interest (white box).

Three specimens were prepared for longitudinal sectioning and 2 for transverse sectioning. Serial sections (6 µm) were taken throughout the entire specimen, de-waxed and mounted on glass coverslips. Examination of slides from both the longitudinal sections and the transverse sections

failed to detect the brain mass or any associated neuron and lipofuscin accumulation. Due to the fact that the brain mass was not detected and potentially the number of sections required to be taken would be very large (even if this method was successful) it was thought that other approaches would be preferable.

As a result, several more juvenile samples were selected for careful dissection under magnification to determine the size constraints of the dissection method. The samples selected ranged from 9 mm CL to approximately 50 mm CL. Dissections were performed with a dissecting microscope against a black background, under reflected light. The magnification ranged between 6 x and 16 x. Despite earlier concerns to the contrary, the brain mass and associated ganglia were successfully dissected from samples as small as 23 mm CL (Figure 1-11). However the brains from samples less than 20 mm CL could not be dissected from the surrounding tissue successfully. Due to the limitations of size, the dissection process resulted in damage to the brain mass to the extent that they would be unusable for further analysis. An alternative method was to remove only enough tissue from the area containing the brain to allow the brain (and some protective tissue) to be isolated from the carapace (Figure 1-12). This would reduce the risk of damage to the brain and still allow for histological sectioning. While considerable care was required for the dissection of the brain of the juvenile crabs, the time required for dissection was not significantly longer than for adult specimens.

Another determination used brain tissue from a very small (13 mm CL) specimen, presumably a C1 stage crab around 2–3 months post-hatch. As might be expected, the quantity of lipofuscin detected in this animal was much less than in adult crabs. Less successful results were obtained from tissues from a 20 mm and a 40 mm crab, where no lipofuscin or neuronal bundles could be located within the cerebral ganglion preparations. This may, however, be attributable to the fact that these (captive) specimens had died in the rearing system and had very likely undergone some tissue degradation prior to fixation.

*Relationship between brain lipofuscin concentration and body size*

The relationship between body size and lipofuscin in the cerebral ganglion or brain (Figure 1-13) is strong, with carapace length explaining nearly 60% of the variation in brain lipofuscin. A logarithmic function provided the best model fit to the data. The point of major interest relates to the 13 mm individual, at the lower left of the plot area, in which the lipofuscin concentration was less than 0.1%.

This indicates that larger animals have accumulated measurably more lipofuscin than smaller animals. While body size is not considered to be a reliable predictor of age in crustaceans (because of large differences in individual growth rates) it would be reasonable to suggest that large crabs would be older, on average, than small crabs collected from the same area at the same time and sharing the same thermal history. These results suggest that a substantial proportion of the variation in age-pigment is explainable by body size, the remainder probably being due to differences in individual growth rate or metabolic activity.

**Figure 1-11.** Brain mass and ganglia dissected from a juvenile spanner crab 23 mm CL.

**Figure 1-12.** Brain mass and surrounding tissue of juvenile spanner crabs (A) 13 mm CL and (B) 11 mm CL. Arrows indicate hemispheres of the brain.

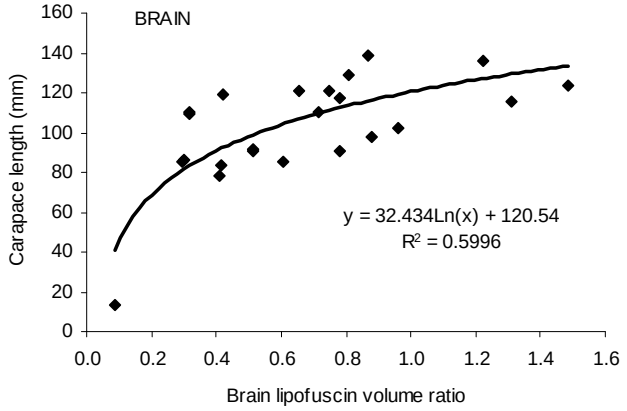


**Figure 1-13.** Relationship between lipofuscin volume ratio (concentration) and size (carapace length) in spanner crab brain (neural ganglion) preparations.

*Variability in lipofuscin concentration between left and right eyestalks.*

This element of the ageing work needed to be completed prior to taking the final sample of crabs, to determine an appropriate sample size. As we wanted to obtain as large a sample of eyestalks as possible from the wild population for the lipofuscin-frequency analysis, it was important to know beforehand whether it would be appropriate to use just one eyestalk from each animal, or whether (because of within-animal variability) both eyestalks would be required. As the available financial resources in the project budget only allowed for a finite number of determinations, this decision could effectively double (or halve) the sample size. A pilot study involving 21 eyestalk pairs revealed a very close relationship between the left and right members of the pairs in terms of their estimated lipofuscin concentration (Figure 1-14).

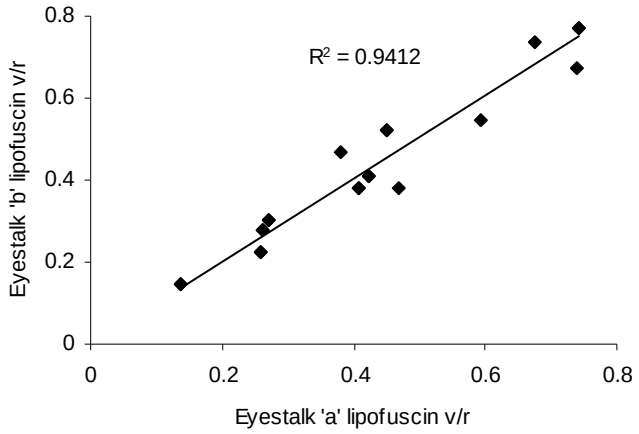


**Figure 1-14.** Relationship between lipofuscin volume ratio (concentration) estimates from the optic nerves of eyestalk pairs. As it is not possible to determine the 'handedness' of the eyestalks, they are referred to as 'a' and 'b'.

The overall sample mean lipofuscin concentrations were 0.417% and 0.423% for the two pair members, a difference of just 0.06. A paired t-test showed that there was no significant difference between the means ($t = -0.59$; $P = 0.560$ on 20 d.f). In this analysis it was not possible to identify the left and right member of the pair, which precluded any assessment of possible left-right bias, but analysis of a further four samples where the left and right members of each pair were identifiable failed to detect any discernable bias. These results provided justification for sampling only one eyestalk of the pair (left or right), thus providing for twice the sample size that would otherwise be the case.

*Sampling with modified tangle-nets for lipofuscin concentration frequency distribution analysis*

We concluded from the above that there was benefit in continuing to progress the histological lipofuscin determination work in an attempt to establish whether there are discernible frequency modes in large samples from the wild stock that can be attributed to age classes. Obtaining samples across as wide a size range as possible would clearly be an advantage for such an analysis. This is particularly so for smaller sizes, as this is the area where the clearest modal separation is likely to occur. The commercial gear is quite selective, rarely taking crabs less than about 65 mm CL. This is a useful attribute for minimising the take of undersized crabs, but it does impact on our ability to sample the smaller size-classes effectively. We attempted to maximise the catch of small animals by using modified dillies with four layers of slack-hung, small fine-denier mesh.

In mid-July 2006 a fleet of 10 multi-layer, light denier 4-ply tangle nets was constructed by Crab 'n' Gear, Clontarf. This design had been field-tested previously and found to catch considerably more crabs than conventional gear (because of lower escapement rates), and a larger proportion of undersize crabs. As we were interested in sampling the greatest possible size-range of animals for the lipofuscin concentration frequency analysis, this apparatus was the best available, although clearance times were very much greater than with conventional tangle nets. The nets were set on three occasions off the sandbanks opposite the southern end of Bribie Island, at about 26° 56'S, 153° 21'E, where we had found significant numbers of undersized crabs on previous occasions.

On 24 July we undertook what was to have been the definitive sampling for lipofuscin. Eyestalks were dissected from 200 crabs caught in 30-40 m depth off the Spitfire Banks between Cape Moreton and Caloundra and immediately placed in labelled vials containing Davidson's fixative. The usual carapace length and sex information was recorded for each individual sample. The bulk of the sample (183 individuals) represented the entire catch from the ten multi-layer nets, and was supplemented by 17 of the largest and smallest crabs taken on the 10 standard nets. In case of any future need for processing the crabs' brains, the heads were also removed, labelled and preserved in formalin. After an appropriate fixation and decalcification period the eyestalk samples were removed from the fixative, wrapped in water-moistened tissue paper, sealed in small plastic bags and mailed to the Werribee lab by overnight express on 30 August.

Another field operation was undertaken on 20 September, using the full fleet of 20 multi-layer tangle nets, to collect length-frequency information and (if necessary) additional eyestalk samples. Unfortunately we were not notified until 21 Sept that there had been a problem with the initial sample. The fixation/decalcification process (using Davidson's fixative) did not work as well as predicted, causing desiccation and shrinkage of the optic nerve tissue and inadequate calcium removal from the exoskeleton. Not only did the entire July sample have to be discarded, but we also missed out on the opportunity of collecting another sample on the September trip.

The final sampling trip was undertaken on 13 October 2006, this time using the fleet of 20 multiple-layer nets on two strings of 10, without any conventional single-layer nets. We tested a new overhead video camera arrangement, which secured the underwater housing about 1.8 m above a crab dilly and enabled video footage to be taken while the gear was being fished in typical commercial

configuration (Figure 1-15). Video footage obtained on two sets above one of the multi-layer nets demonstrated convincingly that escapement rates are much reduced with this gear. Although very small crabs in the 60-75 mm CL range were not as plentiful as in the previous two trips, there appeared to have been more very large crabs. Eyestalk samples were fixed in 10% formalin, and were re-packaged and shipped to Werribee during the first week of November.

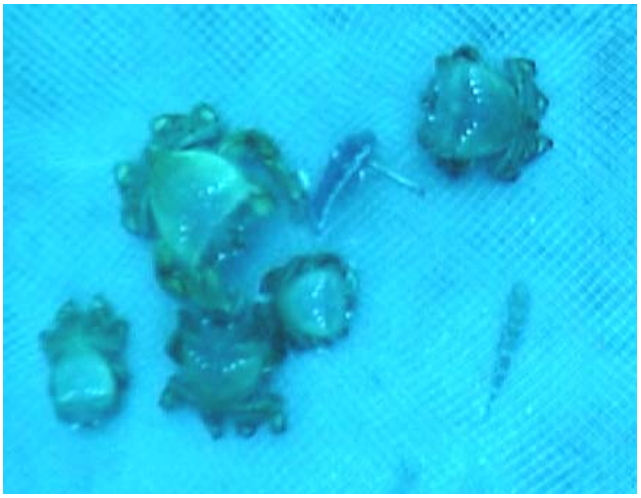

**Figure 1-15.** Spanner crabs surrounding the bait bag on a multi-layer (research) tangle net at a depth of ~30 m.

The relationship between lipofuscin concentration and body size was investigated using general linear modelling. Initial trials indicated that the exclusion of data for preparations where the 'reliability' index was low led to a significant improvement in the model fit, from explaining 21% to over 28% of the variation in observed lipofuscin levels, despite the obvious reduction in sample size. Thus only data where the quality index was between 1 and 3 were used in the analysis. Other preliminary modelling confirmed that the best fit was achieved when both variates (carapace length and lipofuscin volume ratio) were natural log-transformed. The regression analysis (Table 1-6) revealed a significant interaction ($P < 0.001$) between sex and size in the relationship between size and lipofuscin, indicating a difference in the slope of the size-lipofuscin regressions between male and female crabs.

**Table 1-6.** Cumulative analysis of variance of the effects of size (CL, mm) and sex on lipofuscin determinations.

| Source | d.f. | S.S. | M.S. | V.R. | F prob. |
|---|---|---|---|---|---|
| Size | 1 | 6.369 | 6.369 | 38.86 | <0.001 |
| Sex | 1 | 1.4053 | 1.4053 | 8.57 | 0.004 |
| Size.Sex | 1 | 2.5824 | 2.5824 | 15.76 | <0.001 |
| Residual | 151 | 24.7483 | 0.1639 | | |
| Total | 154 | 35.105 | 0.228 | | |

The adjusted mean log-transformed lipofuscin concentrations, together with associated standard errors, showed a strong positive linear relationship with carapace length (Figure 1-16 a). When back-transformed the linear log-relationships resulted in exponential curves, the trajectory for female crabs showing a much more pronounced upward trend than that of the males. While the standard errors suggest a very tight relationship between the two variables, it must be remembered that these are standard errors *of the means*, and the raw data show a great deal of scatter around the fitted

regression line. The two curves in Figure 1-16 b suggest that above a carapace length of about 80 mm male and female spanner crabs of the same size have increasingly divergent levels of accumulated lipofuscin in their neural tissues.

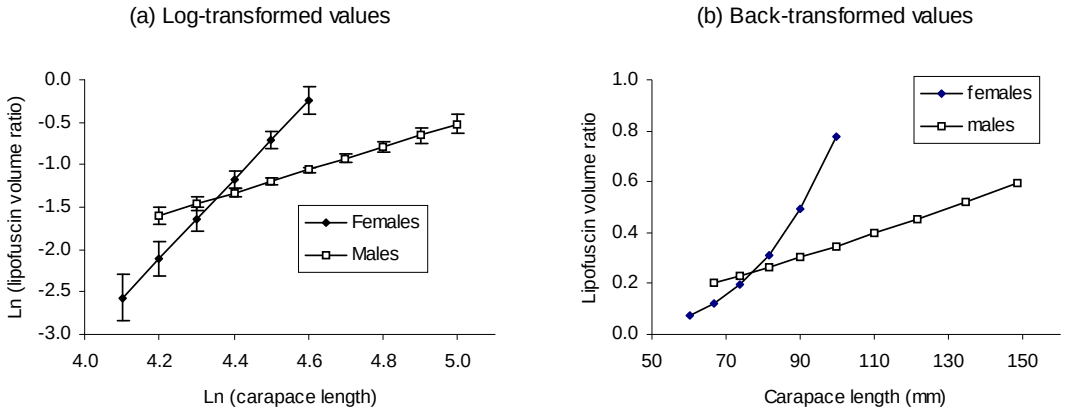


**Figure 1-16.** Relationship between adjusted mean lipofuscin concentration and body size (CL) in male and female spanner crabs on the log-log scale (a) and back-transformed (b).

Because of the relatively small size of the total sample (n = 20 and 135 for females and males respectively) after removal of records where the lipofuscin determinations were judged of doubtful reliability, some aggregation of lipofuscin concentration classes was needed to smooth the frequency distribution. The size of the sample of female crabs was clearly inadequate to obtain any modal resolution in the frequency distribution, with the maximum frequency in any class being only 3 (Figure 1-17). While the male sample showed some possible multimodality, there could be substantially different interpretations of where the modes are situated. On the one hand there could be modes at 0.25 and 0.35%, but on the other it could equally be argued that there is a single modal group spanning values from 0.15% to 0.6% and that one class (0.30%) was slightly under-represented for reasons of chance. The absence of convincing visual evidence of distinct lipofuscin concentration classes indicates that it would be unwise to try to fit a multimodal model using mathematical mode-separation algorithms.

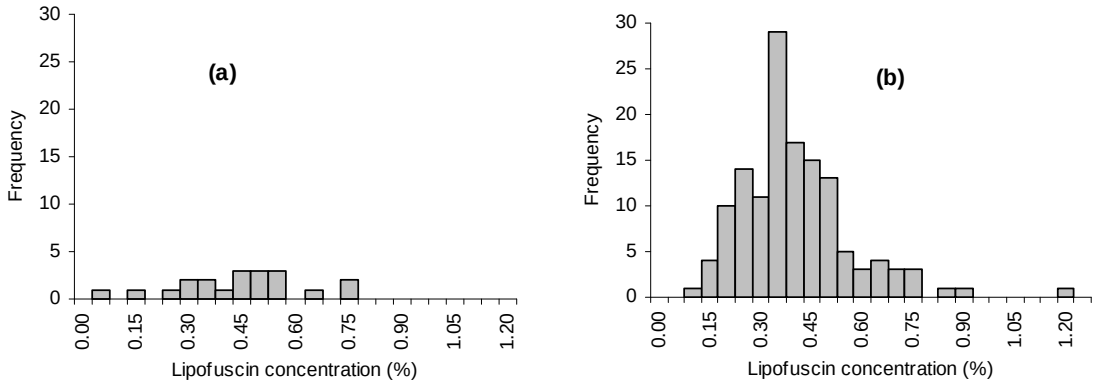


**Figure 1-17.** Lipofuscin concentration frequency distributions for female (a) and male (b) spanner crabs. Class width is 0.05%.

### 1.3.5 Growth indications from modal shift in pre-recruits

The methodology adopted for the larval rearing did not allow for the identification of individual crabs, so it was not possible to accurately follow the moult history of individuals. When censuses were conducted, the instar number of each survivor was estimated from the size of the remaining exuvium and the prior history of the group of crabs within each rearing tub. Accurate measurements of preserved specimens of each of the (supposed) instars were made after the rearing trials were concluded. The length-frequency distributions of each instar are shown in Figure 1-18.

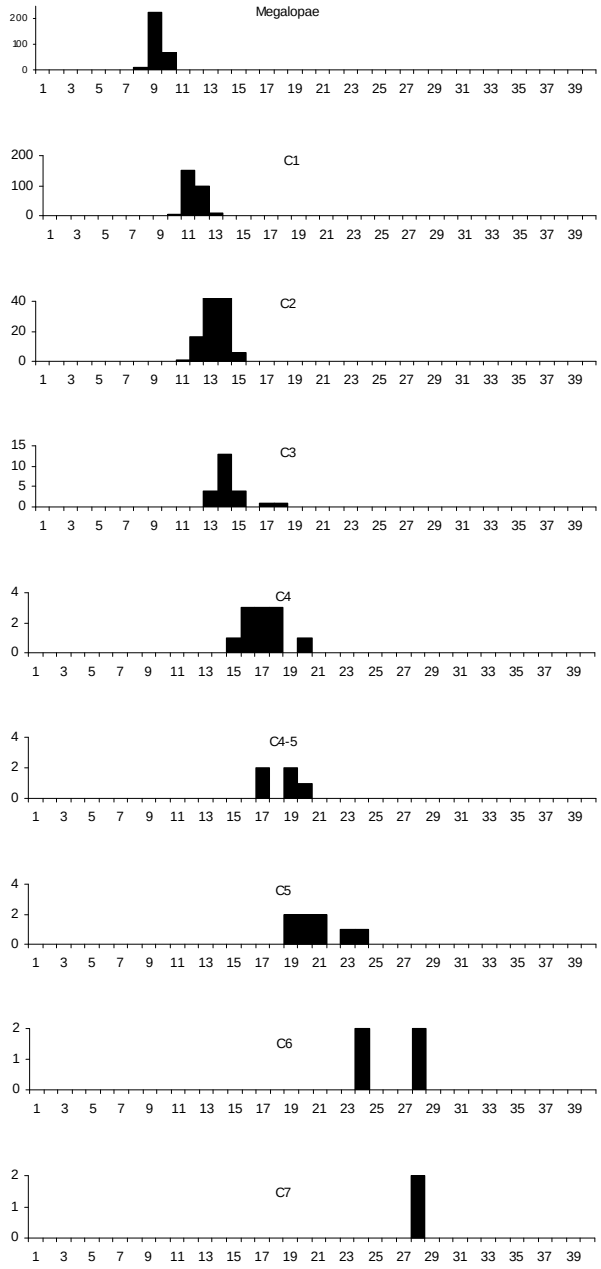


**Figure 1-18.** Length-frequency distributions of megalopae and juveniles from the rearing experiment.

It is likely that some errors were made in assigning instar numbers to some of the juvenile crabs, particularly those in the later stages of development when a greater level of size variation could be expected. This may explain some of the bimodality in the frequency distributions in Figure 1-18, although as the numbers were quite small some departure from normality is quite possible.

To derive some estimate of growth rate, counts of the various stages recorded at each of 21 selected censuses approximately one month apart were tabulated (Table 1-7). The data show the growth patterns of two age-classes, from the 2003-04 and 2004-05 spawning seasons, and indicate a much lower survival rate among the more densely stocked latter cohort.

**Table 1-7.** Numbers of megalopae and post-settlement spanner crabs recorded in the rearing system at approximately monthly intervals between February 2004 and October 2005.

| Date | Census | Megs | C1 | C2 | C3 | C4 | C5 | C6 | C7 | C8 | C9 | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2-20/2/04 | | 43 | 12 | | | | | | | | | 55 |
| 23/02/2004 | 1 | | 49 | | | | | | | | | 49 |
| 22/03/2004 | 2 | | 25 | 18 | | | | | | | | 43 |
| 21/04/2004 | 3 | | 4 | 42 | 1 | | | | | | | 47 |
| 24/05/2004 | 4 | | | 12 | 17 | 6 | | | | | | 35 |
| 22/06/2004 | 5 | | | 6 | 3 | 11 | | | | | | 20 |
| 19/07/2004 | 6 | | | 4 | 7 | 4 | 3 | | | | | 18 |
| 25/08/2004 | 7 | | | 2 | 3 | 3 | 6 | | | | | 14 |
| 25/09/2004 | 8 | | | 1 | 1 | 2 | 8 | | | | | 12 |
| 22/10/2004 | 9 | | | | 2 | | 6 | 1 | | | | 9 |
| 22/11/2004 | 10 | | | | 1 | 1 | 3 | 1 | 1 | | | 7 |
| 21/12/2004 | 11 | | | | 1 | 1 | 3 | 1 | 1 | | | 7 |
| 24/01/2005 | 12 | | | | | 1 | 2 | 2 | 1 | 1 | | 7 |
| 15/02/2005 | | 15 | 28 | | | | | | | | | 43 |
| 28/02/2005 | 13 | 7 | 26 | 2 | 1 | | 2 | 1 | 1 | 1 | | 41 |
| 21/03/2005 | | 500 | | | | | | | | | | 500 |
| 29/03/2005 | 14 | | 453 | 13 | 4 | | | 2 | 1 | 1 | | 474 |
| 28-29/4/05 | 15 | | 84 | 176 | 1 | 1 | | 1 | 1 | 1 | 1 | 266 |
| 25/05/2005 | 16 | | 16 | 209 | 1 | 1 | | 1 | 2 | 1 | 1 | 232 |
| 24-27/6/05 | 17 | | 9 | 191 | 1 | 1 | | | 1 | 1 | 1 | 205 |
| 21-22/7/05 | 18 | | 5 | 152 | | 2 | | | 1 | 1 | 1 | 162 |
| 25/08/2005 | 19 | | 5 | 73 | | 2 | | | 1 | 1 | 1 | 83 |
| 26/09/2005 | 20 | | 1 | 24 | | | | | 1 | 1 | 1 | 28 |
| 18/10/2005 | 21 | | | | | | | | | | 1 | 1 |

Three main ‘introductions’ of new recruits into the system are identified as mid-February 2004, ~15 February 2005, and 21 March 2005. The last of these three comprised the megalopae collected from the surface swarm at Heron Island. The first appearance of a subsequent instar was identified from Table 1-7, and the time from the previous instar estimated, as the number of intervening months. This information was combined with the instar-size data from Figure 1-18, then plotted to show the approximate growth trajectory (Figure 1-19). The growth curve was best described by a quadratic function ($R^2 = 0.992$) with slight upward curvature as shown on the figure. The mean daily growth rate was calculated as 0.063 $mm.d^{-1}$, from the first crab stage (at 11 mm) to the ninth (at 38 mm CL).

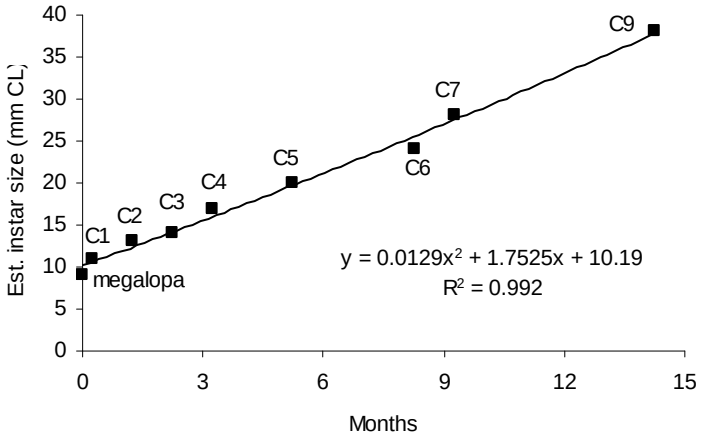


**Figure 1-19.** Approximate growth trajectory for the first nine spanner crab instars, with parameter estimates for the fitted quadratic curve.

### 1.3.6 Genetic (telomere) method

While not formally a component of this project, the telomere work was done collaboratively as a pilot study by Martin Elphinstone through Southern Cross University and the SFC Fisheries Molecular Biology Laboratory under the supervision of Dr Jennifer Ovenden. The pilot project showed a statistically significant relationship between carapace length and telomere length in five spanner crabs ranging from 40 to 100 mm (Figure 1-20). While length is not assumed to be a particularly good estimator of age in crustaceans, this relationship does provide good *a priori* evidence that telomere length is likely to be age-related in this species. When calibrated against animals of known age, such a relationship could be used as a standard curve to estimate the age of wild caught animals with unknown birth dates.

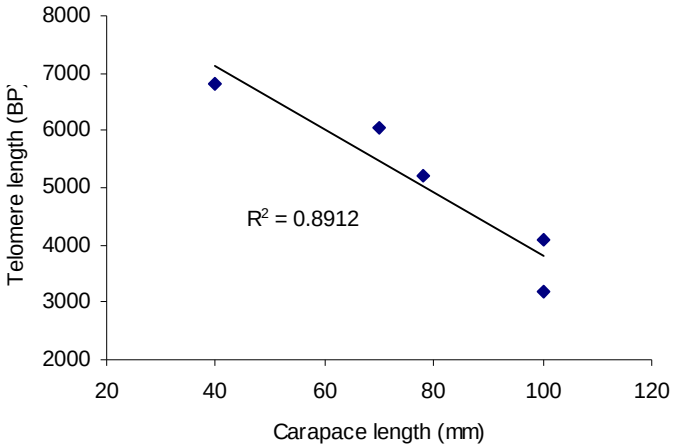


**Figure 1-20.** Indicative relationship between modal telomere length (base pairs) and carapace length in spanner crabs ranging from 40 to 100 mm CL.

Data from studies in vertebrates indicate telomere DNA shortens by 500 base pairs per year. Mark-recapture studies have suggested that spanner crabs grow from 66 to 93 mm over a period of two to four years (Chen and Kennelly 1999). The rate of telomere shortening in our (admittedly limited) data for crabs in this size class are not inconsistent with these figures. Data from larval and early juvenile crabs will be critical in determining the maximum telomere length in this species. We believe that this technique, while still requiring some developmental work to overcome a few DNA extraction difficulties, offers real promise as a new way to estimate the age of animals such as crustaceans.

### 1.3.7 Moult cycle

*Condition analysis*

At a particular time of the year (December through to February) crab fishers report a high proportion of crabs in the catch that are described as being 'weak' or 'light'. Many of these crabs fail to survive being held on deck during the day until the time they're relocated to a refrigerated road transport or unloaded directly at the processor's premises. Crabs in poor condition, whether dead, weak or damaged (missing limbs etc) command a much reduced price compared to live crabs in good condition; the price differential being as much as 50–60%. The purchaser makes a judgment on the state of the crabs and pays the catcher accordingly. To gauge the extent of this issue we approached a commercial crabber who graciously allowed project staff to examine his sales dockets (a record of the quantity landed and price paid for 'live' and 'reject' crabs) for a three-year period. The proportion of rejects (by weight) tended to be highest in January and February, with a mean for the 'sampling' period of about 22% (Figure 1-21). The percentage of rejects then dropped to between about 12 and 15% where it remained until November.

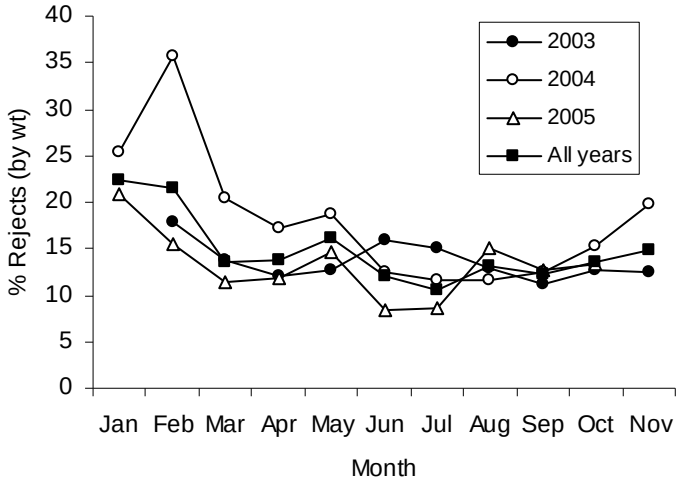


**Figure 1-21**. Monthly variation in the proportion of the catch (by weight) judged to be 'weak' or for other reasons rejected by the processor.

These data show quite clearly that during the first two months of the year the value of the typical crabber's catch is likely to be substantially reduced (by as much as 4-5%) as a result of a higher-than-usual proportion of the catch being classed as rejects.

To investigate a possible cause of the high proportion of weak crabs in January and February we conducted a market sampling programme between June 2004 and July 2006, obtaining (for legal sized crabs) monthly data on size (rostral carapace length) and live weight. After log-transformation it was necessary to remove a number of outliers, which were probably the result of measurement error or more likely mis-recording or mis-transcription from audio-tape. Changes in condition (i.e. monthly mean weight adjusted to the sample mean carapace length) were investigated using general linear modelling. Only data for male crabs were used, as too few females were represented in the samples.

The month effect was highly significant ($P < 0.001$), with a simple seasonal trajectory in condition (Figure 1-22). Maxima occurred in October (back-transformed mean = 505 g), decreasing rapidly to a minimum in January (back-transformed mean = 474 g). This represents a 6.14% decrease in mean weight over three months. From January through to October there was a steady and consistent increase in condition.

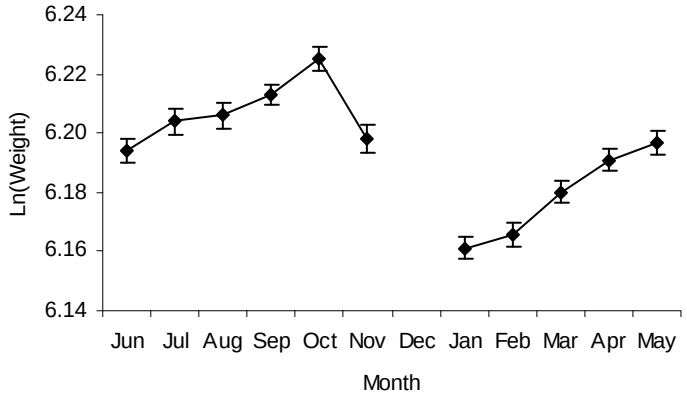


**Figure 1-22.** Monthly changes in adjusted mean (log-transformed) live body weight of male spanner crabs.

There was an approximately inverse relationship between the percentage rejects and adjusted mean body weight (Figure 1-23), suggesting that the rate of rejects in the catch when offloaded at the processing plant is a function of the animals' overall condition at the time.

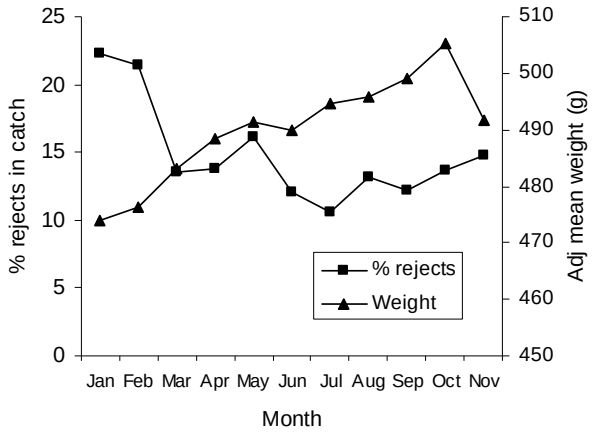


**Figure 1-23.** Synoptic view of month-by-month changes in adjusted mean weight and proportion of catch classed as rejects.

## 1.4 DISCUSSION

The chemical extraction technique for isolating and assaying lipofuscin from the brains and eyestalks of spanner crabs was not successful. The Ju-Harvey extraction process appeared to dry the neuronal tissue and severely limit the solubility of the lipofuscin granules so that fluorescence levels were low and inconsistent. Attempts were made using alternative extraction methods but these also failed to produce consistent results. The lack of recent published literature relating to the successful application of this methodology to age estimation in crustaceans, and the apparent abandonment of an application to Antarctic krill, suggests that it may not be the inexpensive key to crab ageing that was initially promised.

A greater level of success was achieved with the more 'traditional' histological approach, which was included in the project as a fallback position in case the chemical extraction method failed to live up to expectations. We were unable to demonstrate a link between lipofuscin and age, principally because of difficulties in maintaining a known-age population of crabs for use as calibration material. Collection of recently-settled megalopae and early crab-stage instars was possible using our benthic dredge, but the catches were small, and extending tow duration to achieve operational economies may well have resulted in higher mortality rates resulting from turbulence in the dredge. The possibility of an alternative method of collecting megalopae arose with the opportunistic discovery of a pelagic swarm of larvae off a coral reef in the southern GRB. Swarming of the zoeal stage of *R. ranina* has been described by Rice and Ingle (1977), and an unusual stranding of an unidentified raninid megalopa at St Lucia, West Indies, was documented by Chace and Barnish (1976). As *R. ranina* is not known from the Atlantic, the latter observation is unlikely to refer to this species. It would be interesting to determine whether the megalopae behave in the same way in other parts of the distributional range of the adults, or whether there is some entrainment effect which concentrates them in the vicinity of emergent reefs with particular physical characteristics. We were unable to locate any more of these swarms, possibly because they may only occur under certain very specific environmental and hydrological conditions and their location may effectively be impossible to predict. As a result, the supply of early-settled or pelagic larvae (of approximate known age) for rearing was not as abundant as had been hoped, and as a result of significant losses in the rearing tanks the captive population rapidly diminished in number.

Nevertheless, we did establish a positive relationship between eyestalk lipofuscin concentration and body size in spanner crabs. This was a strong indication that lipofuscin does accumulate in the neural tissues of spanner crabs, and that potentially, at least, a reliable method of assaying the substance should be able to be used as an indicator of age in this species. That our work failed to validate the method was largely a reflection of the lack of known-age material, and the fact that it took a considerable time to develop and test the necessary protocols for dissecting, fixing, decalcifying and sectioning the appropriate tissues for histofluorimetry. While Sheehy *et al*. (1998) were able to resolve age-classes in western rock lobsters using a concentration-frequency approach to lipofuscin sampling, this was not the case with spanner crabs. Although there was some suggestion of lipofuscin concentration frequency modes, they were not convincing enough to warrant further investigation with the sample on hand. Even though our (relatively small) sample of spanner crabs was similar in size to that of the rock lobsters analysed by Sheehy *et al.* (1998), it is quite possible that the absence of clear modes may have been due to variability in accumulation rates between the two species in two quite different environments. Perhaps a rather larger sample may have been required to resolve these cohorts in the spanner crab population.

While lipofuscin has been used with some success as an age marker in a number of crustacean species, there is need to be cautious about its use. Lipofuscin is generated by the reaction of various active aldehyde compounds with cellular proteins to form insoluble aggregates which have a weak autofluorescence. Although the production of these reactive aldehydes continues over time and the amount of lipofuscin increases, aging may not always be the predominant factor in the production of

reactive aldehydes. Environmental conditions may also exert an influence on the rate of lipofuscin accumulation, and thus some populations and indeed individuals may accumulate lipofuscin at greater rates than others.

An alternative approach to the estimation of age in spanner crabs was investigated to a limited extent. The length of telomeres – non-coding sections of DNA on the ends of chromosomes – have been the subject of considerable work in human gerontology, and the genetic technique is beginning to see application in the study of the ageing process in animals. Our initial investigations (which were not formally part of the project) showed considerable promise, by demonstrating, albeit on a very small sample, a convincing reduction in telomere length with increasing spanner crab size. As was the case for the lipofuscin determinations, the absence of known-age material across a broad range of sizes meant that we were unable to provide evidence of a direct relationship between telomere length and age. However, as a result of this initial work an independent project has been established to evaluate the application of telomere analysis across a range of exploited marine taxa whose ages have traditionally proven difficult to estimate. This new project is underway, and the required spanner crab samples, obtained from a commercial crabber, are awaiting analysis.

If for some reason the initial inverse relationship between telomere length and body size fails to be sustained in the more detailed investigation, the possibility remains that lipofuscin can still be used as an age proxy, provided the necessary statistical discrimination can be obtained by using much larger samples.

Our attempts to address the issue of growth rates using data gathered as part of the rearing process were limited by the high mortality rates, which severely restricted the age-range of animals that could be used in constructing a growth curve, and also by the fact that the procedure adopted did not allow us to track the moulting history of individual crabs. This was compounded by the need to introduce new 'cohorts' into the system (because of the high mortality rates) which themselves were mixed in with existing stock. Nevertheless, by using indirect methods we were able to gain an approximate idea of the growth rates of post-settlement juveniles from the first to the ninth Crab instar or stage. Over the period of the rearing experiment a small number of crabs survived through a year, by which time they had reached the sixth instar. The pre-settlement megalopa stage of this species is very large (around 9 mm in length) compared to those of most other crabs, and as a result the first crab stage is already 11 mm CL when it emerges from the moult. In contrast to the assumption of Kirkwood *et al*. (2005) these results indicated that growth is at least linear, if not slightly accelerating, during the animals' first year.

Our estimate of a daily growth rate approximating 0.06 $mm.day^{-1}$ is somewhat lower than the 0.09 $mm.day^{-1}$ estimated from length-frequency analysis of wild-caught juveniles by Kirkwood *et al*. (2005). It could be argued that the growth of crabs in captivity, where food is plentiful and there is little need to expend energy on foraging, might be expected to be rather faster than in the natural situation. For example, mud crabs reared under aquaculture conditions can reach marketable size in a very much shorter time than in the wild (B Paterson, pers. comm). However it seems highly likely that the conditions under which we maintained our known-age population were not optimal, as indicated by the significant rate of mortality observed with both year-classes introduced into the system. If conditions are such that survival is severely reduced, it is quite probable that the animals' ability to moult and grow will also be compromised, leading to depressed growth rates. We therefore conclude that the two estimates of daily growth are probably consistent, providing further evidence that spanner crabs grow relatively slowly. Unless contradictory information is gained from the telomeres project, it would be appropriate in any population modelling not to assume that this species grows as quickly as (for example) blue swimmer crabs which to some extent share the same habitat in southern Queensland waters.

## 1.5 ACKNOWLEDGEMENTS

We acknowledge with gratitude the considerable advice and assistance provided by Bribie Island Aquaculture Research Centre staff David Mann and Satoshi Mikami in developing the rearing facility. The generosity of Les Apps (CrabPak) for providing initial samples for lipofuscin analysis is acknowledged with thanks, as is the work of Josie Wilson (Melbourne University Veterinary Laboratory, Werribee) and staff for preliminary tissue processing.

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# CHAPTER 2. DEVELOPMENT OF AN INTEGRATED MONITORING PROCESS FOR THE QUEENSLAND-NSW STOCK.

I.W. Brown, J. Scandol and D. Mayer.

## 2.1 BACKGROUND

The Australian spanner crab fishery, valued at around $5–6 million, operates in southern Queensland and northern NSW coastal waters. Queensland accounts for 85%, and NSW 15%, of the annual commercial catch of approximately 1500 t (DPI&F 2007), most of which is exported live to Asia. Recent evidence (Ovenden *et al*., unpubl. data) suggests that the fisheries in both States are harvesting the same genetic stock, but procedures for monitoring, assessing and managing the fisheries differ markedly between the jurisdictions. This makes it difficult to evaluate the state of the stock and the performance of the NSW and Queensland fisheries as a whole.

Both the Queensland and NSW fisheries have been subjected to detailed ecological assessments according to requirements of the Commonwealth's *Environmental Protection and Biodiversity Conservation Act* (1999) (Brown *et al*. 2001, NSW DPI 2006). The Queensland fishery was the first of the State's fisheries to have been granted approval. Nevertheless the then Minister for Environment and Heritage (the Hon. Dr David Kemp MP) made certain recommendations to be addressed before the next Commonwealth review. These included (i) "making arrangements with NSW to establish joint monitoring and assessment of the shared stock of spanner crabs with a view to developing future collaborative management arrangements".

These sentiments were reiterated in the Commonwealth assessment of the Fishery Management Strategy for the NSW Ocean Trap and Line Fishery, where Recommendation 8 from the Department of Environment and Heritage stated that "NSW DPI to cooperate with other relevant jurisdictions to pursue complementary management and research of shared stocks for all relevant primary and key secondary OTLF species. In particular, DPI will consult with … the Queensland Department of Primary Industries and Fisheries in relation to spanner crab and snapper."

The research presented in this chapter reports on a series of experiments designed to compare the two survey designs that have been developed independently and applied in NSW and Queensland. These experiments occurred at a number of temporal and spatial scales and are reported in two sections: (1) The Crossover Survey, where the LTMP survey was undertaken in NSW waters, and the NSW survey was completed in southern Queensland; (2) Gear and Bait Comparison, where the differences in the sampling gears and baits were compared.

An integral part of the present project was the conduct of a workshop held in December 2005 in the Primary Industries Building, Brisbane. The workshop was attended by researchers, fishery managers, monitoring staff and commercial fishers from both States. The workshop examined and evaluated data from previous monitoring surveys as well as the results of the recently-completed project-based survey work. A common approach to spanner crab monitoring, acceptable to both jurisdictions' was also developed. Outcomes from the workshop are presented in the Discussion and Recommendations sections of this chapter.

The spanner crab fisheries in NSW and Queensland have developed quite distinctly, with different managerial policies (e.g. quota management in Queensland, input control in NSW); and slightly different regulations (such as relate to closure periods and minimum legal lengths). Similarly, the original NSW and Queensland surveys were designed with somewhat dissimilar objectives and operational constraints. Thus there were distinct differences between NSW and Queensland in the research and management of spanner crabs. Identifying and understanding these differences was essential for (i) reducing uncertainty and the potential waste of resources in the management of spanner crabs, and (ii) developing and implementing practical strategies to harmonise the various monitoring and management arrangements.

## 2.2 METHODS

### 2.2.1 Crossover survey

*Original Survey Protocols*

Both the Crossover Survey and the Gear and Bait comparison used a slightly modified form of the existing protocols for NSW and Queensland. These original protocols are described first, then any subsequent modifications are explained as required in the relevant sections. The intent was to keep the underlying structure of the existing surveys intact, so that any time-series that had previously been developed would be maintained and augmented rather than compromised by this project.

*Queensland LTMP Survey*

Protocols for Long-Term Monitoring Programme (LTMP) spanner crab survey were developed in 1999 by a task-group of DPI&F and CSIRO researchers and statisticians. Routine surveys commenced in 2000, using the Department's research vessel RV *Warrego* and four chartered commercial vessels. The protocols called for a survey design involving randomisation of sites within five fixed sub-grids within five fixed regions (Figure 2-1). This remained unchanged from 2000–2003, but in 2005 one sub-grid in region 2 and three sub-grids in Region 3 (shown in red) had to be re-located (new locations shown in orange) after becoming 'green' zones under the GBRMPA Representative Areas Program. To date, this has been the only significant alteration to the original sampling protocol.

The summarised survey protocol is:

- Sample five sub-grids (CFISH 6 × 6 minute grids) within each of the five assessment regions in Managed Area A of the Queensland Spanner crab fishery (5 × 5 = 25 subgrids).
- Sample in May of each year using commercial and research vessels.
- Set 15 individual strings, of 10 dillies per string, in each sub-grid (25 × 15 × 10 = 3750 net lifts). Dillies are set at 50 m intervals on the string.
- Record sex and rostral carapace length (RCL) for each captured spanner crab.
- Record bottom temperature, depth, wind, current and sea conditions.
- Return all spanner crabs to the water.
- Bycatch species are retained for detailed identification and enumeration in two out of every five years.

Detailed explanations of the sampling methodologies are contained in DPI&F (2005). Since their commencement, the annual surveys have been financed primarily by a $40,000 levy across the current quota-holders, with remaining costs topped up from departmental sources.

*NSW Survey*

The survey design for spanner crabs in NSW was based upon the earlier work of Kennelly (1989; 1992) and Kennelly and Craig (1989). This research resulted in development of a standardised survey design for sampling populations of spanner crab off NSW (also see Kennelly and Scandol 2002).

The original survey, in calendar years 1988 and 1989, was repeated 9 years later in 1997 and 1998. An additional (but restricted) repeat survey was undertaken within this project during the fiscal year 2004/05. The following descriptions apply to the original design, and any revisions that made to achieve the objectives of the current project are described in Section 2.2.2. NSW surveys are based upon a fully-balanced and fixed-factor design. Factors are summarised in Table 2-1.

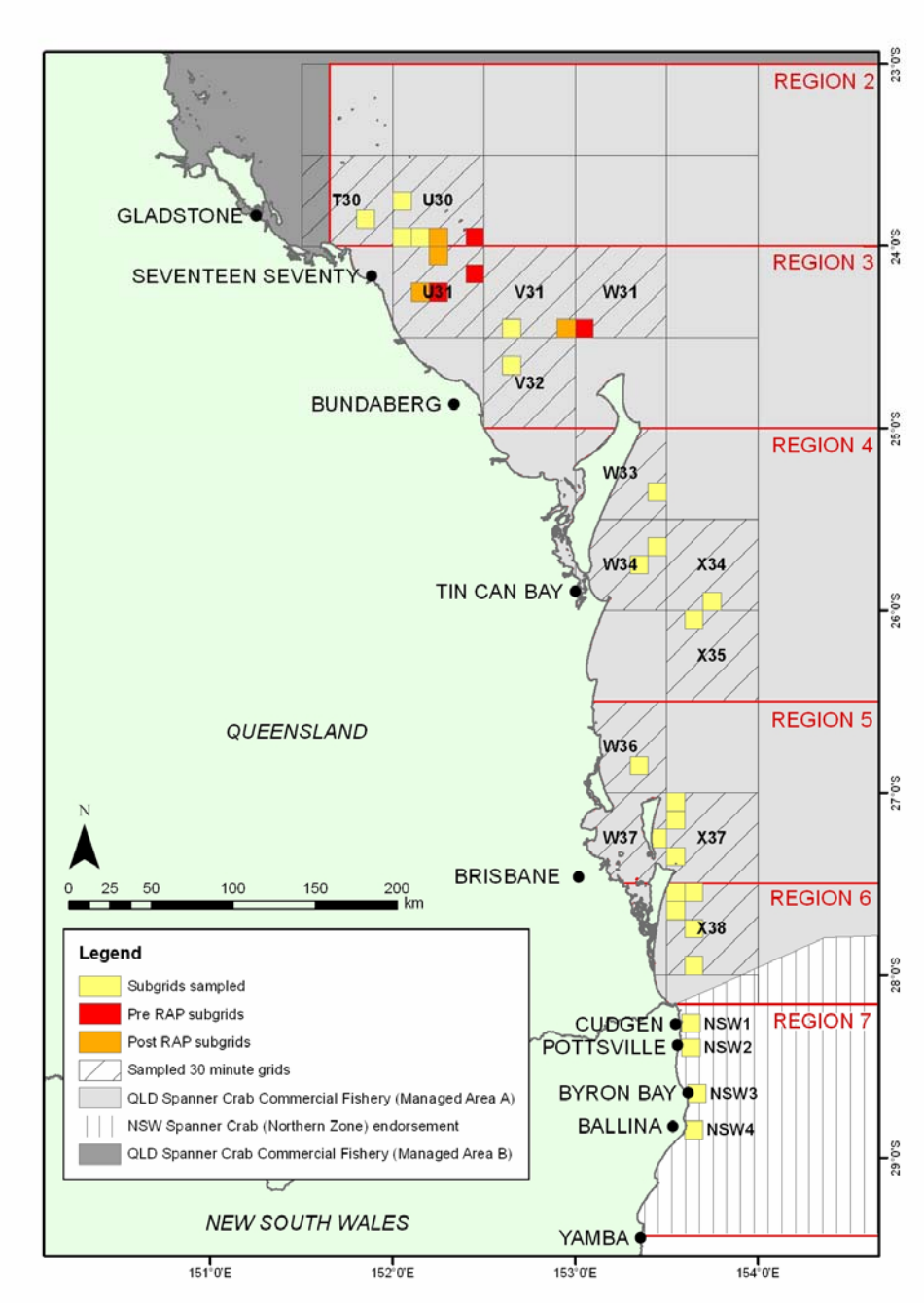


**Figure 2-1.** Chart of the Queensland-NSW spanner crab fishery, showing the location of fixed 30' grids within Regions and fixed 6' subgrids within grids for the extended monitoring survey. Fifteen out of a possible 100 sampling sites within each subgrid were selected randomly prior to the survey.

**Table 2-1.** Summary of the factors in the original NSW survey design.

| Factor | Comments |
|---|---|
| Repeat | Survey identifier |
| Calendar Year | |
| Period | Even numbered month, (i.e. 6 periods per year) |
| Location | Kingscliff, Pottsville, Tallows Beach, Flat Rock (Sand Point), Riordans' Shoal (see Figure 2-2) |
| Depth | Three depths (22 m, 40 m and 58 m) |
| Set | Three replicate sets of traps per depth, location and period. |
| Trap | Five replicate traps per set, which were 60 m apart with a planned soak-time of 1 hr. |

Traps were square frames (1.2 × 1.2 m) of mild steel covered with 85 mm, 4-ply double hung mesh with a 230 mm fall or drop. Fish-frames (mullet) were used as bait and were attached to the middle of the traps within a plastic mesh 'gutter-guard' container. Traps were soaked for a minimum period of 1 hour. Captured crabs were disentangled, counted, sexed, measured (from the posterior edge of the eye-orbit to the posterior edge of the carapace, or orbital carapace length (OCL)) and returned to the sea.

The objective was to complete all the sets for all locations and depths within the prescribed month. Replicate traps were set 60 m apart along trotlines that were placed cross-current. Kennelly and Craig (1989) indicated that traps 60 m apart were statistically independent. Transects were perpendicular to the depth contours (Figure 2-2).

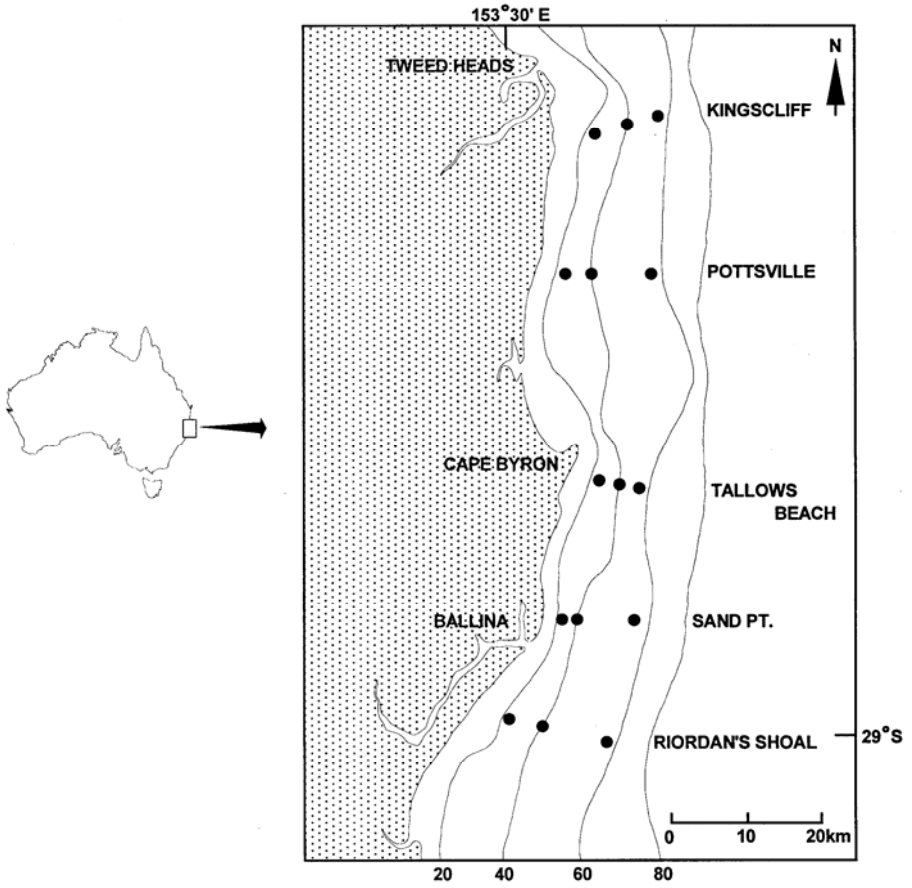


**Figure 2-2.** Northern NSW sampling sites occupied during the 1988/89, 1997/98 and 2004/05 surveys.

Data from the previous surveys were entered, checked and archived in an Access database[1] The information was normalised into several tables and queries used to extract relevant parts of the database. Previous surveys were analysed with a fixed factor ANOVA, but generalised linear models were used in the current project.

Prior to this project, full surveys had only been completed twice (FRDC projects 86/63 and 96/135). Each repeat was funded by cash contributions from the FRDC with significant in-kind support from NSW DPI (or its predecessor, NSW Fisheries). Each survey required NSW DPI to fabricate the gear, hire/redirect and train staff and organise charter operations. The surveys were one-off projects rather than part of an ongoing monitoring program. The estimated costs of the NSW spanner crab surveys are around $50-$60 000 each year.

### 2.2.2 NSW vs Qld gear comparisons

To compare and contrast the two survey types (Queensland annual LTMP and NSW periodic), we developed an experimental design that extended the NSW survey into Queensland, and the Queensland survey into NSW. The two surveys were undertaken so that there was a temporal as well as a spatial crossover or overlap. This experiment is referred to as the 'crossover survey'.

*Modifications to the Queensland LTMP Survey design*

The extended Queensland survey was run in May 2005. The design, in line with standard LTMP procedures, involved sampling five blocks (6' × 6' logbook sub-grids) in each of the five Assessment Regions (Regions 2 - 6 inclusive) between the NSW border and Yeppoon. The survey was extended into northern NSW waters (Region 7) with the placement of one 6' × 6' survey block over each of the four northernmost NSW survey transects shown in Figure 2-1).

Within each block 15 randomly-selected sites (from a regular grid of a possible 100 sites) were sampled, each with a single string of ten Queensland commercial-design dillies and a minimum soak of 40 minutes. The dillies were attached to the ground-line with shark clips at (marked) intervals of 50 m.

While the 6' × 6' blocks are normally fixed (as a result of an initial selection process described elsewhere), we were forced to abandon one block in Region 2 and three in Region 3 because of the location of the recently-established closures for the GBRMPA Representative Areas Programme. Alternatives were selected on the basis of closest proximity to the original blocks and the need to be situated outside the GBRMPA closed areas (Figure 2-1).

After retrieval, the crabs were removed from the nets and stored temporarily in baskets, but no attempt was made to keep the catches from the individual nets separate. Thus in this survey the 10-dilly string becomes the sampling unit and the 15 random sites the survey replicates within each (fixed) block. The minimum distance between the starting points of any two sets or strings was 0.6 n.mi.

The survey work was carried out in Queensland waters by four chartered commercial vessels and the DPI&F RV *Tom Marshall*, and in northern NSW waters by two local chartered vessels. As is normally the case, a member of the LTMP staff was aboard each of the Queensland boats, while the *Tom Marshall* was crewed by Project staff. The NSW vessels were accompanied by both Queensland and NSW technical personnel. Note that the May 2005 LTMP Survey was straddled by two of the sampling months (April and June 2005) of the NSW Survey.

[1] Microsoft Access 2003: SpannerCrabSurvey.mdb (NSW DPI G:\RAAM\Spanner Crabs\Data)

*Modifications to the NSW Survey design*

Three modifications to the NSW survey design were required to undertake the Crossover Survey. The first was to undertake only four of the five previously surveyed offshore transects in northern NSW (Kingscliff, Pottsville, Tallows Beach and Sand Point/Flat Rock) and add a transect in southern Queensland. The Queensland extension comprised a transect that included the same depth zones as in NSW, and was sited offshore from the coastline between Caloundra and Mooloolaba within subgrid 19W36 (Figure 2-1). This transect (referred to as 'Mooloolaba') was chosen on the basis of available depths rather than any prior knowledge of the abundance or otherwise of spanner crabs in the area. This was consistent with the procedure used originally to establish the NSW sites.

The second modification was that the NSW survey was only completed for one fiscal year, commencing in August 2004 and finishing in June 2005, with sampling occurring every two months (as per the original protocol). Resources were unavailable to complete the NSW survey for the two full calendar years as had been done previously. This enabled a balanced, but not replicated, comparison of years with the two prior surveys.

The third modification, described in more detail in Section 2.2.3 (modifications to the NSW survey design required for the Gear and Bait Comparison), was to augment the NSW Survey Design with an identical number of Queensland-style traps. These traps were identical to the traps used in the LTMP survey, enabling a direct comparison of the catch rates between the two surveys.

Two commercial vessels were chartered to undertake the surveys in NSW, and the DPI&F research vessel RV *Tom Marshall* (crewed by project staff) used to sample the Mooloolaba transect every 2 months.

*Data analysis*

Owing to the very different survey designs adopted by the two States, it was not possible to include both data sets from the Crossover experiment in one statistical model. Instead, generalised linear models were fitted to the two data-sets separately, and the resultant adjusted means compared graphically. Potential reasons for differences were then explored with further analysis of the effects of bait type and gear design.

The LTMP counts data for all years since the programme was established were analysed using a generalised linear regression model with a Poisson distribution and log-link function (GenStat 2007). The model structure was as follows:

```
Catch = Constant + lnSoakTime + Nets + Grid + Year + DepthZone + Grid·Year +
  Grid·DepthZone + Year·DepthZone + Grid·Year·DepthZone
```

where the response variate (catch) was the total number of crabs of both sexes and all sizes caught on a 10-net string. Adjusted means were referenced to a standard set of 10 nets and a standard soak-time of one hour because of occasional net losses and variability in soak time. They were then scaled to counts per net (rather than counts per string) for graphical presentation and ease of comparison with the NSW-survey data, which are reported at the 'per net' level. The standard errors were similarly re-scaled for an approximate comparison.

For the NSW-type survey analyses, Queensland sub-grids (blocks) were mapped to NSW transects as:

19W36 ↔ Mooloolaba
1NSW ↔ Kingscliff
2NSW ↔ Pottsville
3NSW ↔ Tallows
4NSW ↔ Flat Rock (Sand Point).

### 2.2.3 Gear and Bait Comparison

*Modifications to the Queensland LTMP Survey*

The Gear and Bait Comparison did not require any modifications to the Queensland LTMP Survey except that the resources normally allocated for the LTMP Survey in 2004 were re-allocated to experiments undertaken in this project.

*Modifications to the NSW Survey Design*

The Gear and Bait Comparison required two additional modifications. First, three additional sets of Queensland-style traps were interspersed with the three sets of NSW-style traps. Second, pilchards were used as bait rather than mullet (the sampling in August 2004 used both types of bait to enable a comparison of bait type to be made).

Sampling was expanded to incorporate the use of Queensland-style dillies so that the relative efficiencies of the two different gear types could be evaluated and the differences quantified. This would allow for retrospective adjustments to be made to the prior data sets should there be a change to the characteristics of the sampling gears.

In the first of the bi-monthly series of samples (August 2004) we also incorporated a comparison of the effectiveness of two baits (mullet frames, as used in the NSW survey, and pilchards, as used in Queensland). Previous work by Kennelly and Craig (1989) had compared various baits, but these had not included West-Australian pilchards, which are the industry standard in Queensland, and therefore the preferred bait for the LTMP surveys.

Along each transect, sampling was carried out at each of three depths or 'sites' (12, 22 and 32 fathoms [22, 40 and 58 m]). At each depth six strings of five dillies were set for a minimum soak of one hour. Three strings carried NSW (survey-type) dillies and the other three carried Queensland (commercial type) dillies. The first net-type to be set at each site was determined randomly, and subsequent strings were set alternately. All nets were attached to the ground-line with bridles clipped onto 2 m branch-lines which had been permanently spliced into the ground-line at 60 m intervals. There were thus five transects (4 in NSW and 1 in Queensland) × 3 depths × 6 sets or strings × 5 dillies = 450 net-lifts per bi-monthly survey, or 450 × 6 = 2700 net-lifts in total.

In the bait trials mullet and pilchards were used alternately along the string of dillies, the 'starting' bait having been determined randomly. The sequence was continued through the six strings of nets to ensure that equal numbers of each bait type were used.

After the nets were retrieved, the crabs were removed and those from each of the five nets on the string were kept separate in labeled baskets. As soon as practicable, the crabs were measured (orbital carapace length in NSW and rostral carapace length in Queensland) and appropriate records made, along with setting and retrieval times, depth and other location data. In cases where a crab had been damaged to the extent that it could not be accurately measured, a capture was recorded against an appropriate code.

Because of the design differences associated with the third survey, a modified version of the database was developed to contain the additional information on bait, Queensland-style traps and the Mooloolaba transect.

*RCL and OCL Conversions*

NSW and Queensland scientists had adopted differing protocols with respect to the measurement of spanner crabs. Queensland measured rostral carapace length (RCL), whilst NSW measured orbital carapace length (OCL). Regression equations from Brown (1986) were used to effect conversions between these measurements where required (Table 2-2). Note that the legal size limit is the same in both jurisdictions i.e. 93 mm OCL or 100 mm RCL.

**Table 2-2.** Formulae for converting spanner crab rostral carapace lengths (Queensland protocol) to orbital carapace length (NSW protocol), and *vice versa*.

| Sex | RCL to OCL (mm) | OCL to RCL (mm) |
|---|---|---|
| Males | OCL = 0.946 × RCL – 1.499 | RCL = (OCL + 1.499)/0.946 |
| Females | OCL = 0.939 × RCL – 0.906 | RCL = (OCL + 0.906)/0.939 |
| Unknown | OCL = 0.9425 × RCL – 1.2025 | RCL = (OCL + 1.2025)/0.9425 |

### 2.2.4 Spanner Crab Monitoring Review Workshop

A workshop was held in Brisbane on 7-8 December 2005 to evaluate the results of the project experiments, review procedures for collecting commercial and survey data from the spanner crab fishery in Queensland and NSW, and develop a strategy for implementing a cross-border 'whole-of-stock' monitoring arrangement. Participants included researchers, fishery managers, enforcement/compliance staff, commercial fishers and representatives of GBRMPA and FRDC. The workshop was highly successful and resulted in a number of key recommendations which are outlined in Section 2.7.

### 2.2.5 LTMP data update

The LTMP spanner crab survey data covering the entire period (2000-2006) were analysed by generalised linear modelling (GLM) in GenStat to determine the significance of the effects of a variety of factors – notably year, region, and depth – on catches.

The regions included in the analysis were those used in the spanner crab assessment and TAC-setting process: from 2 in the north to the NSW border, and (in 2005 and 2006) including the northern NSW region (7). Except for 2004, when the industry contribution to the LTMP survey was redirected to the experimental component of this project, all years from 2000 to 2006 are represented in the analysis. The factor depth comprised three levels: shallow (level 1, < 32 m), mid-depth (level 2; 32–49 m) and deep (level 3, ≥ 50 m).

Catches were defined as the total number of crabs of various categories subdivided by sex (M and F) and size (small or sub-legal, and large or legal, where the minimum legal size is 100 mm RCL [rostral carapace length]). Even though the survey protocols specified standard gear and consistent deployment methods, it was necessary to account for slight variations in soak time and the number of 'effective' nets on the string or trot-line, neither of which was able to be kept constant at all times. While the number of nets set on a string was always 10, there were occasions when nets were lost, due usually to snagging on hard bottom or hook-ups. The 'sample' of crabs used in the analysis was therefore the number caught adjusted in the GLM for the numbers of nets retrieved and the soak time (the difference between time of setting and time of retrieval). Rather than calculating a catch-per-unit-effort statistic this approach was taken so that the effects of net numbers and soak time could be accounted for individually, and their separate effects on catch tested in the model.

A small number of crabs (est. < 0.5%) were damaged to varying extents by predators (probably sharks, turtles and rays). If they were not able to be measured, (e.g. when the whole body of the crab was eaten, leaving only the legs entangled in the net), they were ignored in the analysis.

The generalised linear model specified a Poisson distribution with logarithmic link function for the following response variates: large, small and total males (ML, MS and M); large, small and total females (FL, FS and F), large crabs (L), small crabs (S), and total crabs (All Crabs). The fitted model terms were as follows:

```
Constant + lnSoakTime + PotsLifted + Region + Year + DepthZone + Region·Year +
   Region·DepthZone + Year·DepthZone + Region·Year·DepthZone.
```

### 2.2.6 NSW Survey Update

Data collected during fiscal year 2004/05 using the NSW survey protocols were analysed together with a subset of the previous survey results. In particular, data from Riordan's Shoal was excluded, as was data collected outside of the fiscal years 1988/89 and 1997/98. This resulted in a fully balanced dataset of three repeats (or fiscal years), six months, four locations (Kingscliff, Pottsville, Tallows and Flat Rock, three depths (shallow, medium and deep) and three sets of five traps (i.e. a total of 3420 records). Bait was a confounding aspect of this analysis but was not corrected for here as there are no reliable records of the bait species used in previous surveys. Furthermore this factor would have unbalanced the design. Soaktime was not included as a covariate in the analysis as there were too many incomplete records from the previous surveys (particularly the data from the 1988/89 fiscal year).

## 2.3 RESULTS

### 2.3.1 Crossover survey

The crossover survey was used to compare the results of the NSW Survey Design in April and June 2005 with the results of the LTMP Survey conducted in May 2005. Comparisons were made at 5 locations and at 3 depths as well as all depths pooled. Only crabs sampled during the NSW Surveys using Queensland-style traps and baited with pilchards were included. Given the very different designs of these surveys it was not expected that the results would be in complete agreement, but a general pattern of consistency would be expected.

Factors of statistical significance in determining catch rate from the LTMP survey were soak time, location (grid), year, depth, and the interaction terms grid·year and grid·depth (Table 2-3). For the NSW survey, many factors (and their interactions) were significant due to the treatment of trap-lifts rather than strings as the unit of replication. Table 2-3 presents the accumulated analysis of deviance for the fitted model, from which the adjusted means were generated for subsequent comparison.

**Table 2-3.** Accumulated analysis of deviance table showing significance of various factors on the catch (count) of spanner crabs in the LTMP data set, including data for the extended survey into Region 7 (NSW).

| Source | d.f. | Deviance | Mean deviance | Deviance ratio | Approx. F prob. |
|---|---|---|---|---|---|
| lnSoakTime | 1 | 1779.9 | 1779.9 | 171.2 | <0.001 |
| Nets | 1 | 35.8 | 35.8 | 3.4 | 0.064 |
| Grid | 32 | 14820.4 | 463.1 | 44.6 | <0.001 |
| Year | 4 | 1201.7 | 300.4 | 28.9 | <0.001 |
| Depth | 2 | 1170.2 | 585.1 | 56.3 | <0.001 |
| Grid.Year | 92 | 5515.5 | 60.0 | 5.8 | <0.001 |
| Grid.Depth | 26 | 1009.6 | 38.8 | 3.7 | <0.001 |
| Year.Depth | 8 | 98.1 | 12.3 | 1.2 | 0.308 |
| Grid.Year.Depth | 45 | 661.6 | 14.7 | 1.4 | 0.037 |
| Residual | 1723 | 17913.2 | 10.4 | | |
| Total | 1934 | 44205.9 | 22.9 | | |

**Table 2-4.** Accumulated analysis of deviance table showing significance of various factors on the catch (count) of spanner crabs in the NSW Survey data set, including data for the extended survey into Queensland (Mooloolaba).

| Source | d.f. | Deviance | Mean deviance | Deviance ratio | Approx. F prob. |
|---|---|---|---|---|---|
| Location | 4 | 788.6 | 197.1 | 358.2 | <0.001 |
| Month | 5 | 143.7 | 28.7 | 52.2 | <0.001 |
| Depth | 2 | 596.0 | 298.0 | 541.5 | <0.001 |
| Location·Month | 20 | 375.8 | 18.8 | 34.1 | <0.001 |
| Location·Depth | 8 | 841.0 | 105.1 | 191.0 | <0.001 |
| Month·Depth | 10 | 88.2 | 8.8 | 16.0 | <0.001 |
| Location·Month·Depth | 40 | 216.2 | 5.4 | 9.8 | <0.001 |
| Gear | 1 | 188.9 | 188.9 | 343.2 | <0.001 |
| Bait | 1 | 5.7 | 5.7 | 10.4 | 0.001 |
| Residual | 2607 | 1434.8 | 0.6 | | |
| Total | 2698 | 4678.8 | 1.7 | | |

Figure 2-3 indicates that neither survey sampled many crabs in shallow water (~22 m). In contrast, deeper waters yielded higher catches for both surveys at Flat Rock. Both surveys gave generally consistent results in medium and deep water with the exception of Tallows (where LTMP catch rates were very low) and Mooloolaba (where no crabs were caught on the deep water transect of the NSW Survey). The absence of a result for certain combinations of depth and location (e.g. May LTMP, Mooloolaba, shallow; Figure 2-3) is due to the fact that none of the 15 randomly-selected sampling sites within the particular 6 × 6 n.mi. subgrid fell within the relevant depth-range. In other cases (e.g. Kingscliff and Pottsville, shallow sites) there were effectively zero catches despite standard levels of sampling effort.

With few exceptions, the results from the LTMP survey indicated lower catch rates from the same sampling gear, possibly reflecting the lesser selection of spanner crab 'habitat' that would have resulted from a random selection of sites within the sub-grid (Figure 2-3).

At none of the three depths on the Tallows transect (3NSW) was the LTMP procedure particularly successful (Figure 2-3). There is no reason to expect such low catch rates in this area given that the NSW Survey had consistently founds crabs in this area (Fig 4 *in* Kennelly and Scandol 2002).

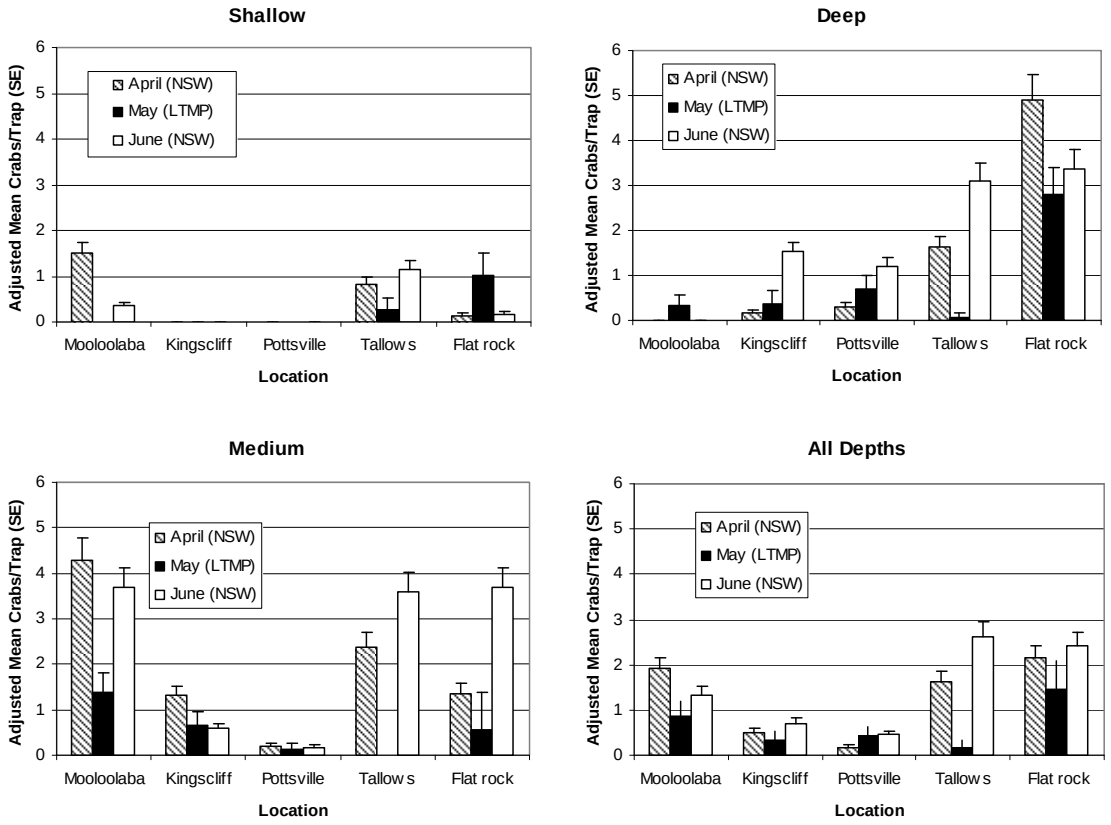


**Figure 2-3**. Comparison of the results from the NSW Survey and LTMP Survey in April, May and June 2005 for the five locations and three depths sampled in this study. The adjusted mean (and s.e.) crabs/trap-lift are estimated from the general linear model fitted.

**Table 2-5.** Summary of the adjusted mean (± s.e.) number of crabs/trap-lift for the Crossover Survey by location, month and depth class.

| Location | Month (Survey) | Depth | | | |
|---|---|---|---|---|---|
| | | Shallow (~20 m) | Medium (~40 m) | Deep (60 m) | All |
| Mooloolaba | April (NSW) | 1.50 (0.22) | 4.27 (0.51) | 0.00 (0.00) | 1.92 (0.24) |
| | May (LTMP) | | 1.39 (0.41) | 0.33 (0.25) | 0.86 (0.33) |
| | June (NSW) | 0.35 (0.09) | 3.68 (0.45) | 0.00 (0.00) | 1.34 (0.18) |
| Kingscliff | April (NSW) | 0.00 (0.00) | 1.32 (0.20) | 0.17 (0.06) | 0.50 (0.09) |
| | May (LTMP) | 0.00 (0.01) | 0.64 (0.32) | 0.36 (0.31) | 0.33 (0.21) |
| | June (NSW) | 0.00 (0.00) | 0.59 (0.12) | 1.52 (0.23) | 0.70 (0.11) |
| Pottsville | April (NSW) | 0.00 (0.00) | 0.21 (0.06) | 0.31 (0.08) | 0.17 (0.05) |
| | May (LTMP) | | 0.13 (0.15) | 0.71 (0.29) | 0.42 (0.22) |
| | June (NSW) | 0.00 (0.00) | 0.16 (0.06) | 1.21 (0.19) | 0.46 (0.08) |
| Tallows | April (NSW) | 0.83 (0.15) | 2.37 (0.31) | 1.63 (0.24) | 1.61 (0.23) |
| | May (LTMP) | 0.26 (0.25) | | 0.08 (0.09) | 0.17 (0.17) |
| | June (NSW) | 1.16 (0.19) | 3.59 (0.44) | 3.10 (0.39) | 2.62 (0.34) |
| Flat Rock | April (NSW) | 0.15 (0.05) | 1.37 (0.21) | 4.91 (0.57) | 2.14 (0.28) |
| | May (LTMP) | 1.02 (0.48) | 0.56 (0.81) | 2.81 (0.60) | 1.46 (0.63) |
| | June (NSW) | 0.17 (0.06) | 3.68 (0.45) | 3.37 (0.42) | 2.41 (0.31) |

### 2.3.2 NSW vs Qld gear and bait comparisons

Observations from the August 2004 surveys were extracted to compare the relative effectiveness of the two bait types used: mullet frames and Western Australian pilchards. This resulted in data from 5 locations, 3 depths, 2 gears, 3 sets and 5 traps per set, or 450 observations in total. Baits were distributed on the traps as described in Section 2.2.3. Counts (crabs per trap-lift) were made of all crabs, male crabs, female crabs, legal sized crabs (≥ 93 mm OCL) and sub-legal (< 93 mm OCL) size crabs.

A GLM with a Poisson distribution and a log link function was fitted to the observed catch of traps per trap-lift (GenStat 9.1, VSN International 2006). This approach enabled the skewed distribution of the catch data to be modeled without the need to transform the data. Models of increasingly complexity were fitted iteratively to the data until no further terms could be justified on the basis of statistical significance (an appropriate method for the 450 observations of the bait trial). The final fitted model was:

```
CrabsPerLift = Constant + Soaktime + Gear + Bait + Depth + Location + Depth·Location
```

The dispersion coefficient was estimated to be 1.49, thus the observations were over-dispersed for a Poisson distribution. The same model was fitted to the count of male, female, legal and sub-legal crabs, and the resulting models had similar characteristics (such as the significance of various factors and the distribution of residuals). Figure 2-4 indicates the adjusted means of these four response variable for the two types of bait used. Note that the bait effect indicates a (non-significant) reduction in the mean catch rate of ~15% on sub-legal crabs and a significant 35% reduction on legal crabs from mullet-frame to pilchards.

A similar analysis was used to measure the relative catchability of the NSW and Queensland-style gears. In this case, observations were repeated every two months (during 2004/05) but the design became unbalanced because the bait comparison was only completed in Aug 2004 (for the other five months, WA pilchards were used). This resulted in 2700 observations of the five response variables described above.

The same statistical method was used on this larger dataset with the exception that the deviance ratio was used for model selection. The final fitted model was:

```
CrabsPerLift = Constant + Bait + Depth + Gear + Location + Depth·Location +
    Location·Month + Depth·Month
```

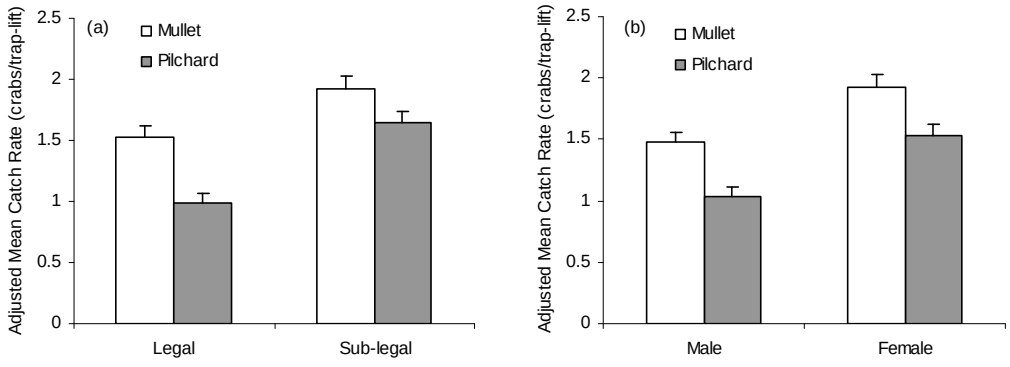


**Figure 2-4.** Differences in **a**djusted mean catch rate (± s.e.) between mullet frames and WA pilchards from the August 2004 bait trial: comparison between (a) legal and sub-legal crabs; (b) male and female crabs.

The dispersion coefficient for model was 1.6 (indicating that the data were over-dispersed for a Poisson distribution). Inclusion of covariate soak-time could not be justified in this model on the basis of statistical significance. The same model was fitted to the other response variables, and all five models had very similar characteristics. Figure 2-5 illustrates the adjusted catch rates from the analysis. There was a significant difference in the catch rate of both the legal and sub-legal crabs between the NSW- and Queensland-style traps, where the Queensland traps were only 57% and 40% as efficient as the NSW traps for legal and sub-legal crabs respectively. Note that these GL models were used to summarise the results from this study and report the adjusted means. The statistical significance of various differences between contrasts can be determined by examining the overlap (or otherwise) of the confidence intervals of the adjusted means.

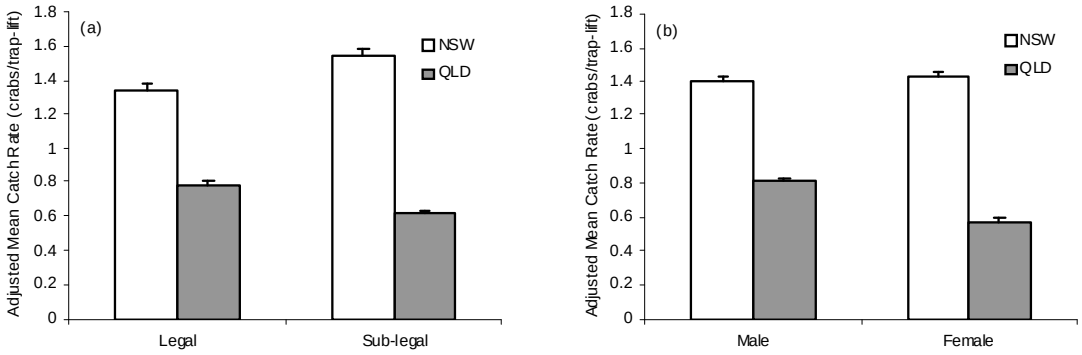


**Figure 2-5.** Adjusted mean catch rate (± s.e.) between NSW-style traps and Queensland-style traps from the 2004/05 gear comparison trial: comparison between (a) legal and sub-legal (a) legal and sub-legal crabs; (b) male and female crabs.

The greater efficiency of the NSW-style traps was expected given the larger surface area, finer thread diameter, and slacker hanging. To more fully investigate this size-selectivity effect, frequency histograms of the size distribution of trapped crabs are presented (sexes separately) by gear type (Figure 2-6).

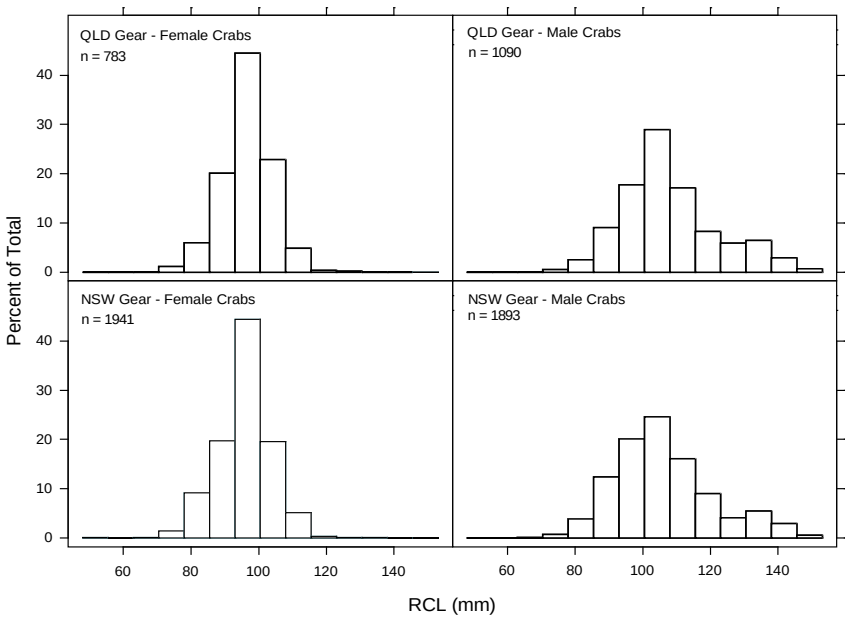


**Figure 2-6.** Length frequency distributions of male and female spanner crabs captured by NSW and Queensland gears during the 2004/05 gear comparison trial. Lengths are measured as rostral carapace length (RCL).

Two-sample Kolmogorov-Smirnov tests indicated there was a significant difference in the length distributions of crabs trapped with NSW and Queensland gears for male crabs (P = 0.001) but not for female crabs (P = 0.095). This result was somewhat surprising given it was assumed that the NSW gear was less selective. The most likely cause of this result is that there were few small crabs sampled during 2004/05.

To double-check this result, five additional sets of five NSW-style traps were randomly interspersed with the Queensland traps at the four NSW locations during the 2006 LTMP surveys. The results of this study were similar (Figure 2-7) with two-sample Kolmogorov-Smirnov tests indicating there was a significant difference in the length distributions of crabs trapped by NSW and Queensland gears for male crabs (P = 0.0001) but not for female crabs (P = 0.104).

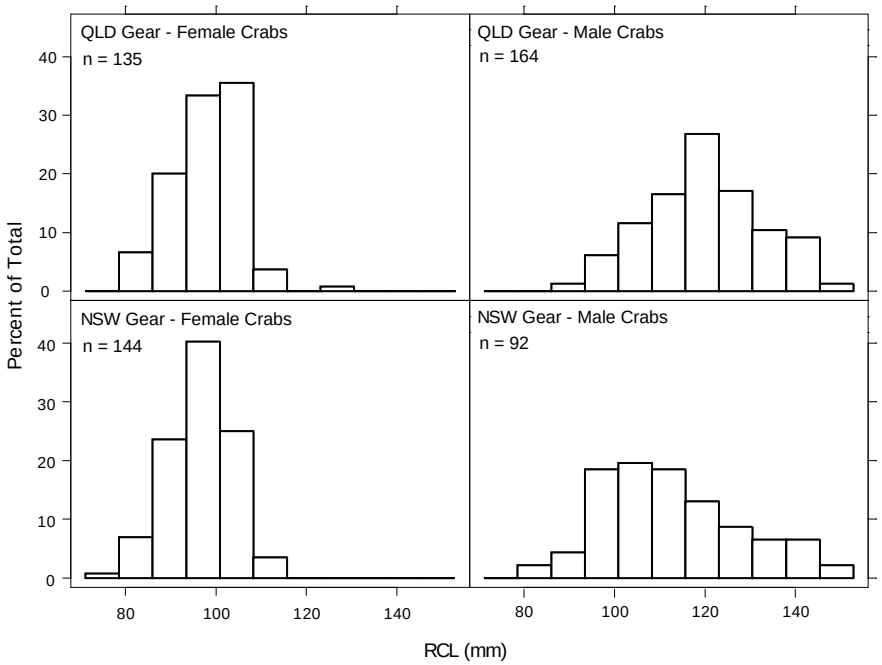


**Figure 2-7.** Length-frequency distributions of male and female spanner crabs captured by NSW and Queensland gears during additional tests carried out in 2006. Lengths are measured as rostral carapace length (RCL).

### 2.3.3 LTMP survey update

An update of the Queensland Long-term Monitoring Programme's annual spanner crab survey results was carried out in late 2006 to include data from the May 2006 survey. As a result of the Workshop recommendation to extend the survey into northern NSW waters and make it a whole-of-stock monitoring process, this update includes the set of NSW survey sites, identified collectively as Region 7.

The GLM analyses for all response variables were highly significant (P < 0.001). In all cases the accumulated analyses of deviance indicated that the three main effects (soak-time, number of pots or dillies lifted, region and year), as well as their second- and third-order interaction effects, were significant. The results of a typical analysis, where the response variable is the total number of crabs, is shown in Table 2-6. This confirms the significance of the soak-time effect, which was until recently assumed not to be influential after about an hour.

The mean catch-rate of legal-sized, sublegal and total crabs adjusted for all other factors varied considerably over the LTMP survey period. Note that the catch rate is the count of crabs per set, standardised to 10 nets and 1 hour soak-time, i.e. 10 net-hours. Region 4 (Fraser Is to Noosa) was the most productive area, with adjusted means more than twice those of surrounding regions (Figure 2-8). The lowest means were from Region 7 in northern NSW. Interestingly, in all regions except Region 5 the modeled proportion of large

crabs in the catch was substantially greater than that of the sub-legal sized crabs. However in Region 5 the situation was the reverse, with very low numbers of large crabs being caught relative to those below the minimum legal size.

**Table 2-6.** Analysis of deviance table from GLM showing significance of main and interaction effects on the total catch of crabs (M+F, all sizes).

| Source | d.f. | Deviance | Mean deviance | Deviance ratio | approx. F probability |
|---|---|---|---|---|---|
| lnSoakTime | 1 | 1457.66 | 1457.7 | 84.1 | <0.001 |
| PotsLifted | 1 | 156.56 | 156.6 | 9.0 | 0.003 |
| Region | 5 | 6895.98 | 1379.2 | 79.6 | <0.001 |
| Year | 5 | 2502.37 | 500.5 | 28.9 | <0.001 |
| DepthZone | 2 | 1504.11 | 752.1 | 43.4 | <0.001 |
| Region·Year | 21 | 2831.77 | 134.9 | 7.8 | <0.001 |
| Region·DepthZone | 8 | 901.06 | 112.6 | 6.5 | <0.001 |
| Year·DepthZone | 10 | 325.53 | 32.6 | 1.9 | 0.044 |
| Region·Year·DepthZone | 30 | 866.53 | 28.9 | 1.7 | 0.013 |
| Residual | 2286 | 39620.93 | 17.3 | | |
| Total | 2369 | 57062.51 | 24.1 | | |

The annual adjusted standardised catch rates declined gradually over the first three years of the survey period (2000-2002), but immediately increased again to previous levels in 2003 (Figure 2-9). In 2005 and 2006 catch rates surpassed all previous estimates, with adjusted means of 20-22 crabs per 10 net-hours, of which 10-15 were above minimum legal length. The ratio of large to small crabs in the sampled population was near unity in 2000, but in all subsequent years there were considerably more large animals than small (Figure 2-9).

The significant effect of depth on catch rates is clearly apparent in Figure 2-10. Not only was total crab abundance (as estimated by survey catch rate) almost identical at the mid-depth and deep sites, but the proportions of legal to undersize crabs (1.6:1) were also almost the same. On the other hand at the shallow (< 32 m) sites catch rates adjusted for all other factors in the model were 30% lower than at deeper sites, and the proportion of large to small crabs was much closer to unity (1.3:1).

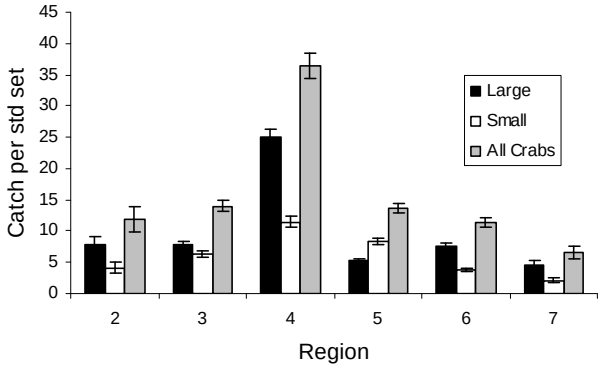


**Figure 2-8.** Effect of region on the adjusted mean catch (counts) of legal-sized and sublegal spanner crabs.

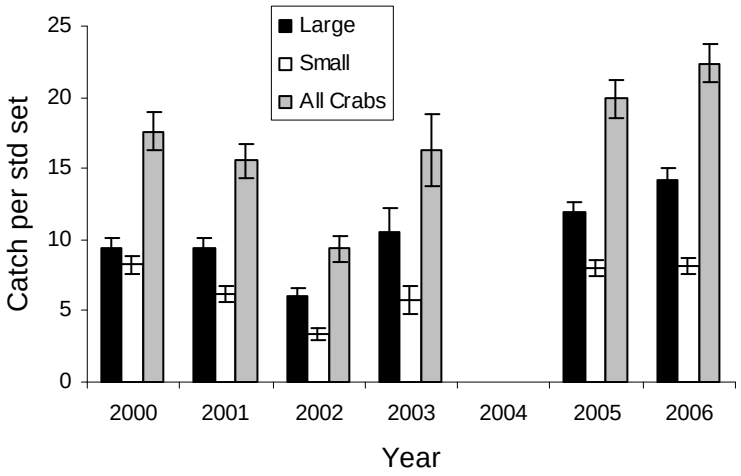


**Figure 2-9.** Effect of year on the adjusted mean catch (counts) of legal-sized and sublegal spanner crabs.

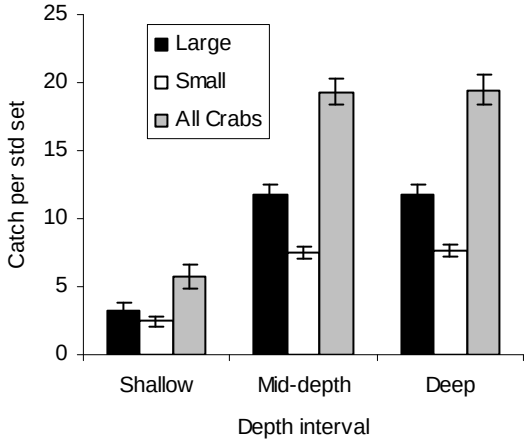


**Figure 2-10.** Effect of depth on the adjusted mean catch (counts, ± s.e.) of large (≥ 100 mm CL) and sublegal (< 100 mm CL) spanner crabs.

Regional differences are in the proportion of males and female crabs in the population have been consistently evident in the LTMP data. Apart from a peak in Region 4 (Noosa to Fraser Is.), the M:F sex ratio shows a steady decline in a north-south direction from Region 2 (Bustard head to Yeppoon) to Region 7 (Northern Rivers, NSW) (Figure 2-11). This appears to be due more to a reduction in the absolute abundance of male crabs rather than an increase in the abundance of females over the geographic range, although the catch rates for females in the north (Regions 2 and 3) were rather lower than those further south.

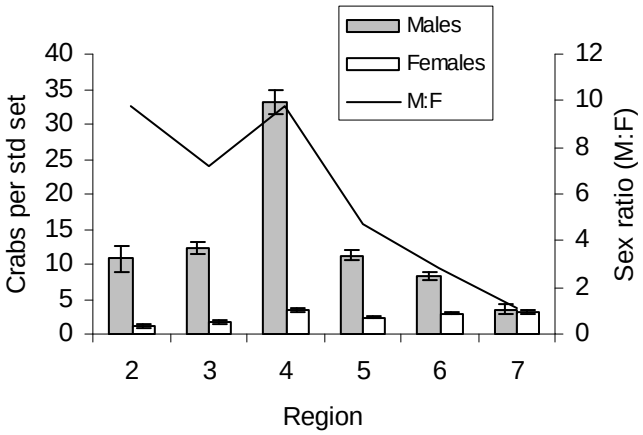


**Figure 2-11.** Effect of region on the adjusted mean catch (counts) of male and female spanner crabs, and the resultant sex ratio.

Over the first four years of the LTMP surveys there was a gradual increase in the M:F sex ratio from about 5.8:1 to 7.4:1 (Figure 2-12). However the trend did not continue, and by 2005 (after the 2004 break in sampling) it had dropped back to 5.3:1. By the following year (2006) the sex ratio had increased slightly again to 6.1:1, about the same as in 2000-01.

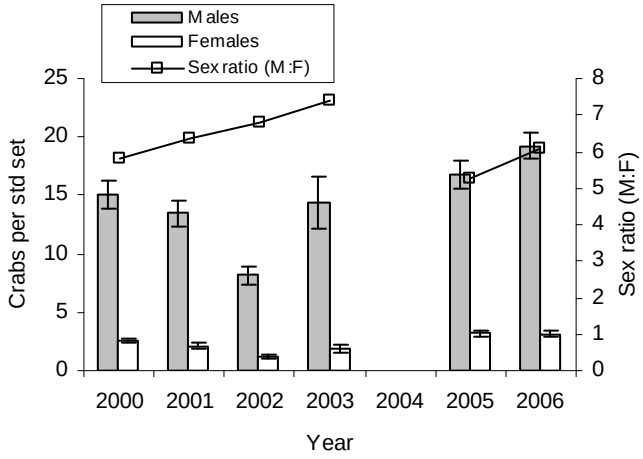


**Figure 2-12.** Effect of year on the adjusted mean catch (counts) of male and female spanner crabs.

The highest M:F sex ratios, as calculated from the mean catch rates of the sexes separately and adjusted for year and region, were observed at sites in the mid-depth ranges (Figure 2-13). At these depths male spanner crabs were seven times as abundant as females. The sex ratio at the deepest sites was somewhat less (5.6:1), and at the shallowest sites it was slightly less again (5.0:1).

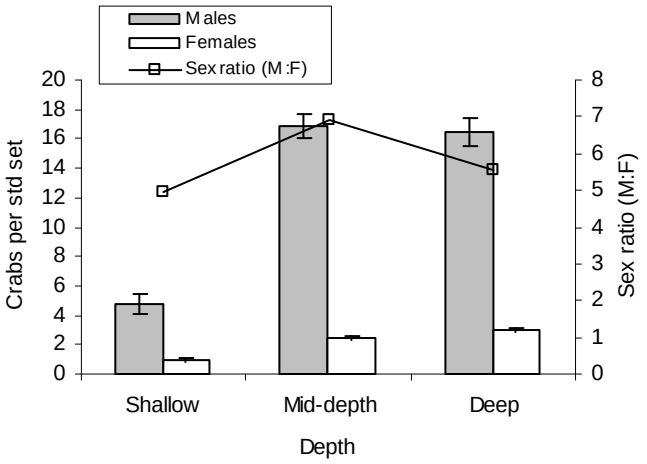


**Figure 2-13.** Effect of depth on the adjusted mean catch (counts) of male and female spanner crabs.

### 2.3.4 NSW Survey Update

A GLM with a negative binomial distribution and a logarithmic link function was fitted to the observed catch of crabs per trap-lift using GenStat. Models of increasingly complexity were fitted iteratively to the data until the reduction in the deviance ratio associated with additional terms reached a threshold of ~20. The final model fitted was:

```
CrabsPerLift = Constant + Location + Depth + Year + Month + Location·Depth + Depth·Year
   + Location·Month + Location·Year
```

Three factor interactions were not included in the model. Examination of the residuals indicated that this model effectively represented the data, which were under-dispersed, with a dispersion coefficient of 0.85.

The adjusted mean number of crabs per trap was calculated and the standard errors estimated from the model. This analysis was repeated for male crabs, female crabs, legal crabs (OCL ≥ 93 mm) and sub-legal (OCL < 93 mm) crabs. Summary results for all five of these analyses are presented in Table 2-7, while Figure 2-14 shows the adjusted mean catch rates for each depth and location for the total numbers of crabs only.

## 2.4 DISCUSSION

### 2.4.1 Crossover Survey

It is clear that the relative abundance estimates obtained by the LTMP survey in May 2005 were generally lower than those obtained by the NSW-type survey in the adjacent months (April and June) of the same year (Figure 2-3). Equally clear was the significance of the effects of depth and location, with medium depths tending to yield higher catches than shallow or deep areas across locations. The trend with location across depths was not consistent, with catches from shallow and intermediate depths at the Kingscliff and Pottsville sites being lower than at the more northern and southern locations, while in the deeper areas catches were substantially higher in the south (Tallows and Flat Rock) than in the north (Mooloolaba, Kingscliff and Pottsville). Spatial patchiness in spanner crab populations, as (presumably) reflected in variable catch rates, is a characteristic of this resource (Kennelly 1992). Passive fishing gear such as the baited tangle nets used in this fishery are certainly not ideal for quantitative estimation: their efficiency will probably vary with the

**Table 2-7.** Adjusted mean catch rates of the total number of: crabs, male crabs, female crabs, legal crabs and sub-legal crabs for the three repeats of the NSW survey. Only data from a balanced design was included in this analysis. Adjusted means and standard errors (in parentheses) were estimated from the GLM.

| Category | Adjusted mean crabs per trap-lift | % of sampled population |
|---|---|---|
| All Crabs | | |
| 1988/89 | 5.50 (0.21) | |
| 1997/98 | 4.51 (0.18) | |
| 2004/05 | 2.98 (0.13) | |
| Male Crabs | | |
| 1988/89 | 2.33 (0.09) | 44.1 |
| 1997/98 | 2.15 (0.09) | 46.6 |
| 2004/05 | 1.07 (0.05) | 36.6 |
| Female Crabs | | |
| 1988/89 | 2.95 (0.13) | 55.9 |
| 1997/98 | 2.42 (0.11) | 53.4 |
| 2004/05 | 1.89 (0.09) | 60.4 |
| Legal Crabs | | |
| 1988/89 | 1.39 (0.05) | 25.7 |
| 1997/98 | 1.19 (0.05) | 26.3 |
| 2004/05 | 1.32 (0.06) | 46.1 |
| Sub-legal Crabs | | |
| 1988/89 | 4.08 (0.16) | 74.3 |
| 1997/98 | 3.39 (0.14) | 73.7 |
| 2004/05 | 1.60 (0.08) | 53.9 |

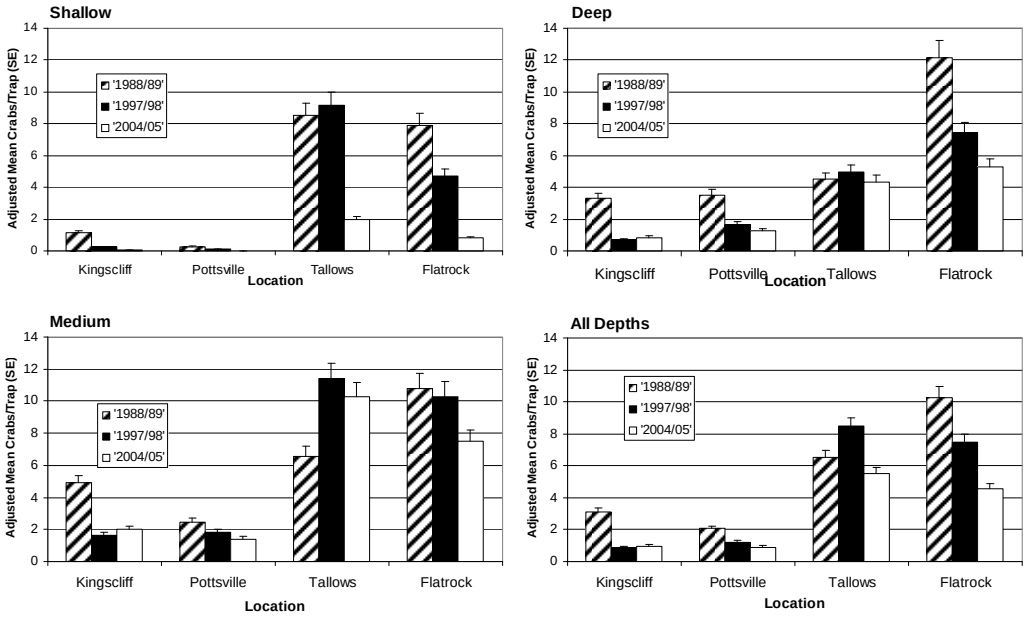


**Figure 2-14.** Comparison of the results from the three repeats of the NSW Survey for the four locations and the three depths (and all depths pooled). The adjusted mean crabs/trap-lift are estimated from the generalised linear model fitted to the data.

current strength and substrate type. Furthermore, the feeding behaviour of the crabs will influence their catchability.

An unexpected result of the analyses has been the finding that soak-time is very influential to the size of the catch in the LTMP surveys. Kennelly (1989) reported significantly larger catches (total numbers of crabs) with a soak-time of 60 min than 15 min., but no significant increase in catch between soak-times of 60 and 120 min. This has led to a tendency to ignore soak-times, provided the nets have been in the water for about an hour. Our results (which are derived from analyses with soak-time as a covariate rather than a fixed or controlled factor), and a much larger sample of observations, show it to be a highly significant determinant of catch size. This realisation coincided with a change in the fishing behaviour of some crab fishermen, who reported that they were finding it more economical to lengthen their soak-times (IW Brown, pers. comm.). Proportionately more crabs could be caught by leaving the gear soaking for longer periods (sometimes up to 3 hours), with corresponding savings in steaming time and fuel costs. At a time when the commercial catch data were indicating progressively increasing catch rates, this prompted the Crab Scientific Advisory Group to establish a second spanner crab fleet survey, the aims of which were to (a) identify the prevalence and influence of this behavioural change across the whole fishery, and (b) investigate other potential factors that might be implicated in a possible undocumented increase in effective fishing effort or fishing power. The results of the fleet survey have already been applied to the improvement of Queensland's spanner crab assessment and TAC-setting procedures.

The reason why the LTMP survey (a randomised block design) was so much less effective in terms of catch rate than the fixed-site, depth-stratified NSW-type survey was investigated as part of the experimental design of the Crossover Survey. Differences in dilly construction and bait type between the two States' sampling designs prompted us to assess the influence of these factors on catches from the two types of survey.

The comparisons yielded interesting results for both bait and gear issues. Kennelly and Craig (1989) compared three types of bait, sea mullet (*Mugil cephalus*), luderick (*Girella tricuspidata*) and grey morwong (*Nemadactylus douglasii*) and found there was no significant effect from bait on catch rates. In contrast, the bait comparison trial undertaken in August 2004 indicated a significant reduction in the catch rate of legal crabs of 35% when using Australian sardines (*Sardinops neopilchardus*) compared to mullet. Sardines are the industry standard bait in Queensland and also used in NSW, but it seems unusual that fishers should deliberately use a less efficient bait. It may be that the convenience and availability of pilchards outweighs their lower catch rates, or that the colder conditions in August accentuated the effect of bait, though this is doubtful as the experiment in Kennelly and Craig (1989) was undertaken in July. Note that the effect of bait was analysed in this study with generalised linear models, whilst Kennelly and Craig (1989) used ANOVA.

When undertaking a detailed experiment of the effect of sampling gears, Kennelly and Craig (1989) identified a significant effect of mesh-size, mesh-ply and hanging style (single or double) on the catch of the total number of crabs. The gear comparison trial undertaken in this study indicated very significant differences on the catch rate of crabs (including subsets of this aggregate, such as male, female, legal and sub-legal crabs). NSW traps clearly have higher retention rate than the Queensland-style traps. It was, however, also assumed that the NSW-style traps were less selective than the Queensland traps. While there is some evidence for this assumption from this project and the follow-up trials undertaken in May 2006 (Figure 2-7), the evidence is not particularly strong. Kennelly and Craig (1989) did not complete a full analysis of selectivity but did present experimental results on various size-classes and sexes of spanner crabs.

### 2.4.2 Survey Updates

The analysis of the NSW data indicated a significant reduction in the catch of all types of crabs over the three comparable surveys. The adjusted mean fell from 5.50 ± 0.21 (s.e.) crabs per trap in 1988/89, to 4.51 (± 0.18) in 1997/98 and then to 2.98 (± 0.13) in 2004/05. Further inspection of the results indicated that this last reduction was essentially due to the lower catch rates of smaller crabs in the 2004/05 NSW survey. Indeed the adjusted mean catch rate of legal crabs did not change significantly between 1997/98 and 2004/05, whilst the adjusted mean of sub-legal crabs fell from 3.39 to 1.60 crabs/trap-lift (i.e. a 52% reduction).

This reduction in the catch of sub-legal crabs occurred at all locations and depths, but was more pronounced in shallow waters, which saw an 85% reduction in sub-legal crabs. The most parsimonious explanation for this result is that there were fewer sub-legal crabs than when previously surveyed. Given the patchy nature of crab distribution this is a plausible hypothesis. At one stage there was concern that the selectivity of the gear in 2004/05 was not the same as the gear used previously. However, discussions with skippers of the charter vessels (who were the same for all surveys) and technicians involved with the earlier surveys indicated that this was unlikely. Furthermore, the mesh used in the sampling gears for the 2004/05 surveys was exactly the same as that used in 1997/98. The bait effect was probably responsible for a reduction in catch rates (in the order of 15% for sub-legal crabs), but certainly not the large reductions that were observed in the final repeat of the NSW survey.

In contrast, the updated LTMP data (i.e. from 2000-2006 inclusive except for 2004) revealed a somewhat different trend. During that period – which did not include the first two NSW surveys – there was no indication of a stock decline, although survey catches in 2002 (~ 0.9 crabs/net-lift) were significantly lower than in any of the other years (~1.6 crabs/net-lift). The 2002 minimum in the time-series of total catches of crabs was reflected equally in the catch rates of both large and small animals, suggesting that the drop was not the result of a recent poor recruitment, in which case the numbers of undersize crabs would have been expected to lag those of the larger adults. Interestingly, 2002 was not a particularly poor year in the commercial fishery, as the logbook data indicated that catch rates were consistent over the three years from 2001 to 2003. Despite this anomaly, the trends in the two data sets since that time have been remarkably consistent.

The LTMP surveys showed that, after statistical removal of spatial (region) and temporal (year) effects, there was a significant effect of depth on adjusted mean catch rates, with intermediate and deep waters yielding 2-3 times the catch rate as shallow areas. It is unlikely that depth *per se* is the determinant here; rather we suggest it is more likely to be a function of reduced populations in the more accessible areas close to the coast and major ports resulting from historical spatial distribution of fishing effort. The Crossover Survey indicated that in one area (off Mooloolaba) the deepest zone yielded the lowest catch rate, but this was almost certainly the result of variation in habitat type along the survey transect rather than the result of population depletion in deep water.

A curious phenomenon apparent from the LTMP data is the general north-to-south decrease in the ratio of male to female crabs in the catch. With the exception of Region 4, where male crabs were particularly abundant, there was a tendency both for the catch rate of males to decrease and for females to increase between the northern-most and the southern-most regions. The reduction in males could be explained in terms of historical changes in fishing intensity, with greater fishing effort having been applied in relatively smaller geographical areas in the south, particularly Regions 6 (Pt Lookout to the NSW border) and 7 (northern NSW). However it is difficult to explain why the absolute abundance of female crabs should be less in the north than in the south.

The LTMP and NSW survey comparisons (updated to include data obtained since the Monitoring Workshop) were valuable in strengthening the available time-series. The inconsistencies between the trends observed in the long-term 'three-snapshot' surveys in NSW and in the shorter-term annual surveys in Queensland serve to highlight the value in frequent sampling, which at present is annual. Ideally there should be more frequent stock abundance surveys to take account of shifts in seasonal patterns that may result from climate variability, but this is unlikely to be feasible with existing resources and may not be justifiable in terms of cost-benefit.

### 2.4.3 Uptake of Monitoring Workshop recommendations

Two LTMP surveys (2006 and 2007) with the NSW extension into Regions 7 have been successfully carried out, and planning for the 2008 survey is underway at the time of writing. There has been excellent cooperation between NSW and Queensland staff in regards to this programme, and the procedures are now well established, so there is no reason why the process cannot be carried forward into the future. Monitoring in 2008 is also likely to include an assessment of bycatch from the LTMP survey within NSW waters.

As yet it has not been possible to carry out the second part of the first recommendation – i.e. conducting simulation-model evaluations of different survey designs to see whether it can be modified to achieve a greater degree of cost-efficiency. The simulation model to be used in this task has only just been completed (early December 2007), and is currently being utilised in a high-priority evaluation of the spanner crab TAC-setting decision rules. The need to modify the decision rules only became apparent after the Workshop, and this work needed to be done in time to enable DPI&F staff to set the TAC for 2008-09. The work was done by the PI in collaboration with Mr Michael O'Neill and Mr Alexander Campbell, using a MatLab-based simulation model comprising a process sub-model interfaced with a management sub-model. The model will later be applied in the context of investigating possible survey changes, which is an important consideration at the moment, as increasing costs are requiring the LTMP to consider various cost-cutting options across the board, including the annual spanner crab survey. Preliminary work has also been undertaken to investigate how the Queensland decision rules could be applied within the NSW fishery. Such a development would be a significant advance for stock-based (rather than State-based) management of the shared spanner crab resource.

Recommendation 2 from the Workshop has recently been completed. In November 2007 a Working Group of Queensland's Crab Management Advisory Committee agreed upon a procedure for incorporating the results of the fishery independent (LTMP) surveys with the compulsory daily logbook data from the commercial fishery, with equal weighting, into the data-based stock assessment and TAC-setting arrangement. The Working Group comprises the PI, the Project's consultant biometrician Dr David Mayer, CSIRO Stock Assessment Modeller Dr Cathy Dichmont, Fishery Manager Phil Gaffney, Commercial spanner crab fisher and MAC member Richard Freeman, and DPI&F stock assessment scientists Michael O'Neill and Alexander Campbell. In full consultation with industry, a data-based stock assessment and TAC-setting process was developed and tested, and early in 2008 the procedure was used set the 2008-10 annual Total Allowable Catch.

The third Workshop recommendation has been implemented, with the collaborative extension of the Queensland survey into NSW waters, a process that has been carried out seamlessly and to great effect.

Recommendation 4 has also been adopted without question by the Decision-Rules Working group, in that all estimates of CPUE or catch rate will be derived from generalised linear modelling (GLM) analyses which will account for as many influential variables as possible. This is expected to provide a more accurate estimate of standardised catch than can be derived from the logbook catch rate data alone.

## 2.5 ACKNOWLEDGEMENTS

We are grateful to the Fisheries Business Group of the Department of Primary Industries and Fisheries, and to the Crab Fishery Management Advisory Committee (CrabMAC) for allowing the Project to make use of the industry funds to run the Crossover Survey. These funds would otherwise have been used to finance the LTMP fishery-independent survey in 2004. The contribution of all participants (from both Queensland and NSW) in the Monitoring Review Workshop is gratefully acknowledged, as are all the DPI&F and NSW DPI staff, including Jason McGilvray and Eddie Jebreen in particular, who contributed in one way or another to the smooth running of the cross-border surveys. The support of the Fisheries Research and Development Corporation, and particularly that of Matt Barwick is recognised with gratitude. The authors would also like to thank crab fishers Jack Lavis, John Spedding and John Joblin (NSW) and Richard Freeman (Queensland) to their contributions to this research over the years. Dr Steve Kennelly made valuable suggestions about the necessary modifications to the NSW survey design that enabled the crossover analysis.

We appreciated the assistance from the following support staff from NSW DPI: Alan Robertson, Douglas Rotherham, James McLeod and Jim Craig, and from Queensland DPI&F: Matt Campbell, Mark McLennan, and research vessel skippers Brett Davidson, Stelios Kondylas, and Sean Maberley.

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## 2.7 APPENDIX: WORKSHOP OUTCOMES

The workshop participants agreed that:

- A single process for monitoring the east coast stock is extremely desirable for the consistent management of the resource in the two States. Differences in design between the existing methods would make it quite difficult, if not impossible, to synthesise the results so as to allow their application to a single stock assessment.
- There is a clear need to continue some form of fishery-independent monitoring of the east coast spanner crab stock. Data from sources independent of the fishery must be available to complement existing commercial fisheries statistics, which are currently the sole basis of Queensland's assessment and quota-setting arrangements.

- The purpose of monitoring the spanner crab stock is to provide the assessment and Qld TAC-setting process with a reliable index of stock abundance that is not prejudiced by changes in fisher or fleet behaviour, or undocumented changes in effective fishing effort. While commercial catch and effort data are comprehensive, the experience with many fisheries throughout the world is that reliance upon such data is risky and can result in undetected overfishing and significant socio-economic costs.

And, with respect to comparisons between the two States' survey methodology

- NSW survey nets outfish the Queensland nets either because of their larger size, lighter ply rating, double layer of mesh or some combination of the above.
- Mullet frames are slightly superior to pilchards in attracting crabs to the net.
- There was no evidence of a substantial difference in the size-selectivity of the Queensland and NSW nets as used in the respective surveys.
- There was no clear evidence that either survey design is superior to the other with respect to providing useful data for routine stock assessment.
- The results of the Queensland and NSW survey data concur with the current interpretation of recent changes in the stock using the fishery-dependent datasets.

and recommended that:

- The current LTMP design should be run for the next 2 years (2006 and 2007). In the mean time, analyses should be carried out within the context of the current FRDC project to determine whether any cost-savings can be made by improving the efficiency of the survey. These analyses would take the form of simulations involving the random exclusion of data to estimate the degree of signal degradation resulting from a reduction in sampling intensity.
- Arrangements should be made to incorporate the fishery independent abundance indices into the Review rules used to set the TAC by the time of the review of the Queensland Spanner Crab Fishery Management Plan schedule for 2009.
- The survey be run using existing LTMP protocols, with a relatively low-cost extension into NSW waters to be funded by NSW DPI.
- That modelled estimates rather than the raw data estimates should be used as the index (or one of the indices) of abundance.

**Review of main features of the two survey approaches**

Participants agreed that this issue had been addressed adequately on day 1.

**Development of a coordinated monitoring survey system**

Participants discussed and provided comment on the following issues:

- *Transects (depth-stratified) or grid blocks* (previously addressed).
- *Fixed or randomised sites*- The capacity to access and assess the subgrid blocks which had to be discontinued because of the GBRMPA Representative Area Process appears remote. The cost of justifying their re-establishment may not be worth it, although it means a loss of continuity in the data series. Group agreed that the new replacement subgrid blocks should continue to be used instead. All other subgrids should be retained for sampling, as it was considered that a randomised pattern within fixed blocks is an appropriate survey design for the fishery.

- *Seasonal or 'one-off' survey* – costs and logistic difficulties of undertaking six bi-monthly surveys would be prohibitive in Qld. Agreed that a survey at one time of the year is appropriate. Results from the NSW surveys do not indicate that May is an unrepresentative month to complete the survey.
- *Large-mesh, double layer ('research') or small-mesh, single layer ('commercial') dillies* – Towards the conclusion of the Workshop an analysis was carried out (see Fig. 6) to compare the size-frequency distributions of the catches from the two apparatus types. This indicated that, contrary to current belief, there was little difference in the selectivity of the two apparatus types. Consequently there is little justification (on the grounds of increased sub-legal catch) to adopt the 'research' net design. Although catches overall are higher (a useful attribute for a sampling device) the NSW nets are more fragile and prone to damage and require more frequent maintenance. In addition, clearing times are significantly greater than for the Qld dillies. Participants agreed that the existing Qld dilly design is suitable for the survey.
- *Bait*- Agreed that Western Australian pilchards should continue to be used, even though they are slightly less effective as bait than mullet frames. Pilchards are much easier to store and use, generally more convenient, and are more readily available than mullet.
- *Use of bait containers* – In regions where predators are prevalent there seems little option but to use bait cylinders rather than bait bags. In NSW wire bait bags tend to be used in the commercial fishery rather than plastic mesh because of the problem with leatherjackets. It was agreed that existing practices within LTMP should be maintained.
- *Method of measurement* – Each of the two methods of measurement has its advantages and disadvantages. To avoid confusion, it was agreed that rostral carapace length (RCL) should be used throughout the survey area (including NSW). There is no critical reason why either of the commercial fisheries should be required to change from one method of measurement to the other, as the relevant legislated minimum legal sizes are equivalent regardless of the method of measurement.
- *Dilly spacing*- David Mayer referred to his analyses that showed little significant difference in the independence of dillies as sampling units at a spacing of 50 m (as in the Qld protocol) compared to 60 m (as in the NSW protocol). It is a 'grey area' statistically, but there was no clear signal suggesting that at 50 m the Qld nets could not be considered to be functioning independently. There was, however, no clear statistical advantage of a single trap being the sampling unit (unless there was need to identify high-order interactions in the design), and there are significant logistical advantages of using the 10-dilly string as the sampling unit (not having to keep the catch from 10 dillies separate from each other prior to measurement and release). The group, therefore, agreed that the 10-dilly string is suitable for the broad-area surveys.
- Trotline arrangement (rope diameter, lanyards vs. bridles, fixed branchlines or marked groundline*)* – No change to existing LTMP protocols recommended.
- *Survey timing* – previously addressed (no change necessary).
- *Sampling intensity*- previously addressed, to retain existing arrangement. However it was recommended that statistical modelling should be undertaken to see whether any improvements in sampling efficiency and cost-effectiveness could be achieved.
- *Protocol for dealing with damaged crabs where measuring/sexing is impossible* – Need to continue with some standard procedure for recording part-crabs following attack by predators etc.
- Discussion extended to the need for recording data in some fields on the LTMP data sheets, as it appears unlikely that some of the abiotic measurements will be used, and others can be derived from alternate sources that may be quantitatively more reliable. The question was raised as to whether the 'nets set' field is necessary, given that there is a 'nets lifted' field and the standard number of nets set is 10. Ian Brown agreed to investigate these issues and recommend which fields (if any) might be dropped from the database.

- *Accounting for protocol changes in interpretation of time-series data* – this will only apply in the case of standardising the existing NSW survey time-series to the LTMP format, which can be done with adjustment factors derived from the crossover experiments.
- *Operational division of labour between States* – This will be addressed at a higher level, although it was suggested that it would be advisable for NSW to co-ordinate the vessel charters for that part of the survey occurring in northern NSW waters.
- *Financing options* – Again, this will have to be taken up at a senior Departmental level, although it is anticipated that NSW DPI should cover the cost of any component of the monitoring process done by Qld LTMP in NSW waters.

**Incorporation of survey results into current assessment and TAC-setting process**

The Chair advised participants of the process involved to change the review rules. The Chief Executive makes review rules on the basis of recommendations from CrabMAC. As the review rules relate to the 'scientific method', the MAC would seek technical advice from the SAG. Any amendment to the rules requires that they be sent out to licence-holders for a period for comment. Given that (i) DPI&F has a new Minister and will shortly have a new DDG, (ii) the requirement for 14-21 days for industry to comment on any proposed changes to the Rules, and (iii) the next SAG meeting scheduled for mid-January, any recommendation from here would not affect the TAC for the next two years. We should work towards incorporating an LTMP-based Review rule in time for the 2008 assessment.

Discussion: We should register an intention for change – to go through the SAG. We are not in a position at this stage to alter the way the pooled index or individual indices are calculated – this would need a six year time-series of survey data. There is no urgency to add a review rule or amend an existing rule until the next round of TAC-setting in 2008, as the survey data are not at odds with current interpretation of the commercial CPUE statistics. A way of incorporating the fishery independent data into the decision-rule process should be done in the context of simulation modelling, using (if possible) the existing DPI&F/CSIRO size-structured model. In addition, it is strongly recommended that the results of the crossover experimentation be written up as a peer-reviewed paper.

**Development of a coordinated management system**

*Opportunities for harmonising management legislation* – Sonya Errington advised that NSW and Qld Crab Fishery Managers have already been communicating on possible ways of coordinating management arrangements in the east coast spanner crab fishery. One possible area could be an extension of the spawning closure in Qld along the lines of that in NSW, where there is a ban on taking of female spanner crabs between 20 October and 20 January. There would need to be close co-operation because of TAC implications. The issue should be put on CrabMAC agenda, preferably with a request from NSW. The difference between NSW and Qld in the method of measurement is not seen to be an issue.

*Feasibility of incorporating the NSW fishery into the Queensland quota-management arrangement.* Indications from the NSW Fishery Manager are that the very small size and low value of the NSW component of the east coast spanner crab fishery could not sustain the high cost of a quota management system, at least not for some considerable time, or until lower-cost approaches for TAC-based management were investigated. In the meanwhile, NSW will continue to use input controls in the management of this fishery.

# CHAPTER 3. LOCAL-SCALE ECOLOGICAL EFFECTS: TEMPERATURE, HABITAT TYPE AND WAVE ACTION.

I.W. Brown

## 3.1 INTRODUCTION

Many spanner crab fishers believe that changes in bottom-water temperature play an important role in determining catchability, but there are few useful data available for coastal waters in areas where the spanner crab fishery operates. At a local scale and on the upper part of the continental shelf there can be short-term changes in bottom water temperature regimes resulting from tidally-induced currents and the southward progress of gyres and meanders relating to the East Australian Current. While these systems are predominately well offshore in oceanic waters, there is a strong likelihood that their effect extends to shelf waters. Crabbers frequently report periods of the day when dramatic changes in catch rates appear to coincide with an altered pattern of surface-water movement (usually referred to as a 'change in tide'). Most often it is reported that catch rates are low when the tide is 'slack' and increase to varying extents when the tide 'picks up'. This is consistent with the findings of a small study by Craig and Kennelly (1991) who compared a series of current measurements (using a simple flow metering device) with spanner crab catch rates and found a positive relationship between the two. There are two possible explanations for such a finding – that catchability is a function of water movement rate (through dispersal of the bait odour plume in the bottom water layers), or that it is a function of changes in water temperature associated with current patterns (through changes in activity levels resulting from altered metabolic rates).

There is very little available information on water circulation or movement patterns in the shelf waters of southern Queensland. This is in contrast to many other areas of the Australian coastline (e.g. Great Barrier Reef, central NSW, Bass Strait, and Western Australia) whose oceanographic characteristics are comparatively well-understood and documented. While it is possible to obtain data on a number of hydrological parameters at a range of spatial scales and at any given depth, these are essentially predictions from ocean-climate models, and their accuracy at small temporal and spatial scales is insufficient for time-specific comparisons of catch rates in restricted geographical areas. Relatively few reliable sets of observational oceanographic data exist for the area between the southern end of the GBR and the mid-north coast of NSW.

As a demersal species which spends a large part of the time (when not foraging) almost completely buried in the substrate, it would not be surprising to find that bottom type plays a significant part in determining the distribution and abundance of the spanner crab stock. Previous work (e.g. Brown 1986) reported highest catch rates from areas of fine, well-sorted sand in clean oceanic environments. Spanner crabs have not been reported from the mud and silt substrates of central and western Moreton Bay, but they are found on and around the sandbanks on the eastern side of the Bay, particularly in the north where there is a major exchange of oceanic water. Given their natural burying behaviour, spanner crabs would not be expected to occur in significant numbers in areas where the sea-floor is predominately rock or gravel, although Boullé (1995) reported a coral and sand substrate to be the preferred habitat of *R. ranina* in the Seychelles. The propensity for the standard fishing gear to become 'hooked up', and consequently damaged or lost, on hard bottoms means that little fishing is done in those areas (which can be readily identified by a competent observer using typical echo-sounding equipment), and consequently there is a paucity of actual observational data from which comparative tests can be done.

The continental shelf off southern Queensland is by no means uniform in composition or physical characteristics, with large areas of rocky reef and hard bottom occurring within a broader sandy substrate (Marshall, 1977). Most of the significant reef areas within the spanner crab fishing grounds have been

charted, and there is no doubt that most spanner crab fishers have a good idea of the location of hard or otherwise non-fishable grounds in the particular parts of the fishery they frequent. However, apart from the annotations on Admiralty charts (which may date back many decades) there is no overall description available of the distribution of bottom types throughout the area of interest. Such a chart would be of considerable value to the fishery, particularly if it could clearly identify the seabed characteristics of greatest significance in determining the distribution and abundance of the target species.

Another factor believed to be of importance in determining catch rates is the wave or swell height. During periods of heavy seas there is likely to be a significant wind, both of which reduce the ability of the fleet (particularly the smaller vessels) to operate safely. The 'weather' effect is a major constraint in this fishery to the TAC being achieved (the annual catch is typically about 10% less than the TAC). For example, in the three months from mid December 2007 to mid-March 2008, one crabber had been able to fish for only three days. Admittedly this is unusual, but it may be an indication of one of the impacts of climate change that could become apparent in future years, if the trend in wind patterns continues. Apart from the reduction in fishing time due directly to local weather conditions, swell influences are believed by many crabbers to affect catch rates indirectly. For the first few days following a period of heavy swell, even though conditions are appropriate for fishing, catch rates are reported to be lower than usual, and it appears to take several days before they return to levels that are considered normal. If environmental data on wave height were available across the fishery it may be possible to make appropriate adjustments in the modelling process used to derive a standardised catch statistic (index of stock abundance) to use in the TAC-setting process. While of less significance to the fishery-dependent (logbook) information which is collected daily throughout the year across the entire fleet, the ability to account for changes in CPUE resulting from short-term 'weather' phenomena may be of great value in deriving equivalent statistics from the fishery-independent annual surveys which have attracted some criticism from industry because they are conducted over such a brief (1-3 week) time-period each year.

This study explores the hypotheses that catch rates are influenced by changes in water temperature; that the distribution of the spanner crab stock is dependent upon the simple indicators of sea-bed characteristics; and the extent to which catch rates are reduced immediately following periods of heavy ocean swell.

## 3.2 MATERIALS AND METHODS

### 3.2.1 Water temperature

*Data collection*

Miniature TidBit™ submersible temperature data-loggers with download software and hardware were distributed to each of ten fishers selected to provide the greatest possible geographic coverage of the fishing grounds (including both Queensland and northern NSW). The fishers were encouraged to deploy the loggers at least once per week, then download the data to disk or e-mail it directly to the laboratory, along with location data. This provided bottom water temperature readings at a number of sites throughout the year that could be correlated with catch rates reported through the logbook system on the relevant days.

In an initial publicity release and subsequent radio interviews we highlighted the way commercial crab fishers could contribute valuable environmental information to the Project via the temperature data-logging programme. A subsequent article in *The Queensland Fisherman* focussed on this part of the project, seeking expressions of interest from cooperative fishers. The response rate was poor, so during the Spanner Crab Alternative Gear Workshop in 2003 we promoted the data-logging programme to the nine commercial crabbers in attendance. This resulted in five (all from Queensland) registering their interest. Collaborating NSW scientist Dr James Scandol also undertook to seek interest from commercial crabbers in northern NSW while arranging for charters for the NSW periodic spanner crab abundance survey.

To determine whether sea surface temperatures could be used as an index of bottom temperatures in the area of the southern Queensland spanner crab fishery we used historical data collected during various CSIRO oceanographic cruises undertaken during the 1950s–1970s by the *Kalinda*, *Diamantina*, *Sprightly*, *Gascoyne*, *Kanimbla* and other vessels when operating in south Queensland waters. The hydrography of the southern section of the Queensland continental shelf (i.e. beyond the southern end of the GBR) is extremely poorly known, and although it is possible to obtain estimates of many oceanographic parameters in the area of interest, these are based on few empirical data and involve much interpolation. The historical CSIRO data sets, comprising observations derived from water sampling rosette casts, had been provided to the PI previously, and were used to directly compare surface and bottom temperatures at various depths.

*Data analysis*

Prior to analysis the data from the TidBit™ output files (which were received by e-mail) had to be interpreted. This involved using the Boxcar™ utility to manually retrieve the start and finish times of all sets on a particular day. Then the mean temperatures for each set were calculated or estimated (or simply read if there was no variation). These data were then recorded, along with the contributor's boat record number, date, fishing depth and geographical coordinates, and any comments provided by the fisher in his accompanying e-mail, and ultimately entered into a Microsoft Access relational database. It should be noted that the participating crabbers were not asked to provide coordinates for each set, but rather an approximate fix for where most of the day's fishing was carried out. However they were asked to note the depth of each set on which the logger was deployed. Some 1100 records, relating to 245 fishing days, were collected in total. These included a number of observations from Project operations involving the *RV Tom Marshall*.

Corresponding indices of crab abundance (i.e. CPUE as kg.net $lift^{-1}$) were calculated from the DPI&F CFISH logbook database by matching the boat record numbers and dates from the two datasets. Thus a catch rate was appended to each daily observation of bottom temperature, which was calculated as the mean of the (normally 5-7) daily set temperatures. The catch rate and temperature data were specific to the individual boat.

Historical CSIRO oceanographic data were tabulated so that (ideally) for each rosette cast there were pairs of depth and temperature observations at the surface, at every 20 m depth interval to a maximum of 100 m, and at the sea floor. The recorded depths were categorised into those on the continental shelf (the shelf break was arbitrarily set at 100 m) and those in deeper oceanic water on the continental slope and beyond. A general linear regression model in Genstat (2007) was used to determine whether there was a consistent relationship between depth and temperature, and whether this relationship is dependent on the gross geographic location of the water mass. It was assumed that there would be a seasonal effect on temperature, so month (from the sample date) was also included as a term for adjustment.

### 3.2.2 Habitat type

Indices of the roughness and hardness of the sea-floor were obtained from a Sea Scan™ 100 system installed on the DPI&F research vessel *Tom Marshall*. The system comprised a console-mounted unit housing the processing hardware and software, which was interfaced with the vessel's echo-sounder (Furuno FCV 1100, 50 kHz; transducer: 1 kW) and computer/plotter running the navigation software MaxSea™. The first and second echo return signals from the sounder were post-processed by the Sea Scan unit, yielding a pair of voltage values (converted to fixed-length ASCII strings each ranging from 0.000 to 9.999) representing the hardness and roughness of the sea floor. The scale is such that a sandy seabed has a nominal roughness of 1.000 and a nominal hardness of 1.000. These R and H signals were fed to the ship's navigation computer together with other data as an NMEA sentence, which was converted to a colour-graded 2-dimensional visualisation on the plotter. A 3-D display was optional, with bottom depth as the third dimension.

The navigation software was not easily able to output the R & H voltages simultaneously with GPS coordinates and time-date stamp, which were needed in order to analyse the data. With the invaluable help of technical staff from the DPI&F Tor St Centre (Toowoomba) we developed a system of hardware and software to enable the required information to be logged onto a single laptop file in real time. The core of the system was a 4-port USB multiplexing unit, which received signals from the ship's GPS unit (providing latitude, longitude, date and time) and from the Sea Scan unit (providing depth, hardness and roughness). An S+ computer program that coordinated I/O, effected some necessary data filtering and post-processing, and wrote the record to computer file at intervals of 1 sec, was developed by Mr Les Zeller (DPI&F, Toowoomba).

Initial trials identified the need to operate the Sea Scan system in unreferenced mode (i.e. using factory defaults) to ensure that data sets from different regions and times were numerically comparable. Some considerable time was also spent on determining what (if any) effect changes in echo-sounder gain and range settings and vessel speed would have on the results. Eventually we concluded that (depending on sea conditions) vessel speeds up to about 12 kt would yield consistent results. It was found that range setting did have an effect, so we opted to make all recordings at the deepest range setting likely to be required throughout the project. We did not develop a statistical relationship between range setting and R/H voltage, but it is expected that if a deeper range setting were required at some time in the future it would certainly be possible to develop an algorithm to adjust the existing data for comparability.

Two sets of seabed roughness and hardness data were obtained from the Sea Scan system. The first was from subgrid 19W36 (Spanner Crab Managed Area A, Assessment Region 5) off Mooloolaba (see Chapter 2, Figure 2-1) on 11-12 January 2007. The reason for this choice was that we had collected a time-series of catch and effort data from a number of accurately identified fixed sites in three depth ranges during the Qld-NSW Crossover Survey experiment in 2004-05 (see Chapter 2, Section 2.3.2). It became apparent early in this experiment that for some reason spanner crabs were consistently absent from the deepest (60 m) sites. This observation persisted throughout the entire experiment, and we deduced that the crabs' absence was most likely due to sea-bed characteristics, since at many other locations crabs were quite abundant at 60 m depth. We believed that this set of observations could provide a good initial test for using seabed roughness and hardness characteristics as a predictor of spanner crab abundance.

The second data set was obtained from subgrids 10W34 and 14W34 (Spanner Crab Managed Area A, Assessment Region 4) off the southern end of Fraser Is. (see Chapter 2, Figure 2-1) on 30-31 August 2007. The reason for this choice was that these subgrids are also sampled annually by the DPI&F Long Term Monitoring Programme, and the LTMP database had shown some sites within these subgrids to be consistently high-yielding and others low-yielding in terms of spanner crab catch rates, suggesting a certain variability in habitat type. The area surveyed by the LTMP surveys (approx. $2 \times 6^2 = 72$ sq. n.mi.) was considerably greater than the area surveyed off Mooloolaba during the Crossover experiment, but much less clear-cut in terms of crab abundance.

*Data screening and analysis*

A considerable amount of screening and filtering was required to remove anomalous and erroneous records from the data set. There were three main sources of erroneous records: data drop-out, straying beyond system depth limits, and (presumably) transducer interference. The first of these was dealt with by running a Visual Basic filter program in Excel to remove records where any of the critical fields were truncated or missing. The second source of error was due to the fact that the SeaScan 100 is designed to operate in depths greater than ~10 m; in shallower depths the data are quite unreliable. Unfortunately at depths < 10 m the depth field itself can also be unreliable, so careful manual editing of the data was required to ensure that all these records were culled out. Finally, there were instances where (for no apparent reason) the R and H fields suddenly dropped by an order of magnitude to 'suspiciously' low levels (e.g. < 0.01), sometimes accompanied by a large change in depth which was not consistent with the console echo-sounder readout. These periods of anomalous data were infrequent, but sometimes lasted for several minutes, potentially

resulting in the loss of data over distances of up to 300–400 m. We found that resumption of 'normal' data values could often be achieved by rapidly reducing then increasing the vessel's speed, suggesting that the problem may have been related to transducer interference, either from cavitation or fouling by driftweed or other floating material. It is also possible, however, that it could have been the result of random electrical interference from other ship-board instrumentation. Whatever the cause, it was necessary to remove the corrupted data from the dataset, and this was achieved by filtering out records where either R or H was less than an arbitrary 0.01.

Scatter-plots of the Mooloolaba data were examined initially to visualise the relationship between depth and each of the two seabed indices. Follow-up analyses investigated spatial differences in seabed structure across the ~5 x 30 = 150 $km^2$ swath-transected survey area. The data were divided into three depth strata (shallow < 31 m; intermediate 31–50 m, and deep > 50 m), and single-factor analyses of variance (GenStat 2007) were performed on roughness and hardness indices separately. Next a binary generalised linear model (GenStat 2007) was used to test the effect of seabed roughness and hardness on the presence of spanner crabs; catches having been made at the shallow and intermediate-depth sites but not at the deep sites. For this analysis only the seabed data in close proximity to the three spanner crab sampling locations were used, to avoid spatial extrapolation into areas where no catch rate data were available.

The Wide Bay data were structured somewhat differently from the Mooloolaba data, in that reliable catch rate estimates from the LTMP surveys were available for most of the 100 sites within each of the two grids (10W34 and 14W34). Not all sites had been occupied over the time-series of LTMP operations because only 15 of the 100 possible sites were selected randomly each year for sampling. Swath transects using the Sea Scan system were designed to cover all 100 sites in both grids, although not all sites were ultimately quantified because of data dropout and errors described above. Spatial seabed data were aggregated to site using the MS Access FIX function at the 0.005° level, and the site means and variances (a measure of within-site structural variability or heterogeneity) were calculated. Depth-related trends in bottom type were investigated visually using scatter plots of depth against the means and variability of R and H indices. The site-by-site seabed indices were then linked to the corresponding LTMP survey catch rate estimates (total crabs caught per net-hour) and the relationship between seabed characteristics and apparent spanner crab abundance tested using general linear modelling (GenStat 2007). The catch rate data were derived by dividing the total catch of spanner crabs (all sizes and both sexes) taken at each site over the six annual LTMP surveys by the corresponding total amount of sampling effort spent at each site, measured as the sum of net-hours.

Because there were many sites where no crabs were caught (particularly in Grid 14W34) the catch rate (CPUE) data were grouped into four classes as follows: (1) CPUE = 0; (2) 0 < CPUE < 1.0; (3) 1.0 ≤ CPUE < 5.00; and (4) 5.0 ≤ CPUE prior to analysis. With catch rate class as the response variate, a main-effects generalised linear model with Poisson distribution, log-link and estimated dispersion parameter (Genstat 2007), was fitted to the data. The fitted terms were:

```
Constant + Grid + DepthAve + DepthVar + RoughAve + RoughVar + HardAve + HardVar.
```

After re-ranking the significant terms, 2-degree polynomial (quadratic) sub-models were incorporated to test for linearity among the main effects. Where significant curvature was identified by the parameter estimates, the quadratic term was retained in the final reduced model, from which adjusted means and associate standard errors were then estimated.

### 3.2.3 Wave height effects on catchability (Waverider Buoy data)

The effect of wave height on spanner crab catch rate was investigated using a subset of Waverider Buoy data provided by the Environmental Protection Agency (Queensland) and concurrent catch rates (CPUEs) calculated from catch and effort statistics in the DPI&F daily logbook data.

The Mooloolaba Waverider Buoy, jointly operated by the Environmental Protection Agency and Queensland Transport, was installed in May 2005 some 10 km north of Pt Cartwright at 26° 33.95'S, 153° 10.89'E. The Datawell 0.9 m GPS Waverider Buoy is anchored in 32 m depth, and records a number of surface hydrological and oceanographic variables, including wave direction, maximum zero up-crossing wave height (m), significant wave height (m), SST (°C), and wave length (m). We used significant wave height (Hsig) as the measure of wave height as this is the value used by the Bureau of Meteorology (BOM) in their wave height forecasts. Hsig is defined as the average of the highest one-third of the zero up-crossing wave heights in a 26.6 min wave record, and approximates the value a person would observe by eye (www.epa.qld.gov.au/environmental_management/coast_and_ocean/waves).

From the 2006 data the daily mean Hsigs were calculated, and then the days on which the mean Hsig equalled or exceeded 2.0 m were identified. Frequently there was a 'run' of 2 or more days when mean Hsig ≥ 2.0 m, but sometimes the large waves were recorded on just one day. The days following the last identified 2 m Hsig were then numbered, from 1 to 6 or to the next 2 m wave-height day if one occurred within 6 days of the previous record. Subsequent days (from 7 onward) were ignored, as it was assumed that the effect of the heavy swell would have dissipated by that time. Thus the wave data set included a series of 'days following a large Hsig event', the dates being identified by a number between 1 and 6. These data were then matched with the daily mean CPUE data for all of Region 5 (26° 30'S to 27° 30'S; see Figure 2-1), assumed to be subject to the same wave conditions as measured by the Mooloolaba Waverider Buoy. To improve data accuracy only CPUE records where total daily effort (pot-lifts) ≥ 400 were used. The reduced data were analysed by general linear regression in GenStat (2008), with CPUE as the response variate and the five-level factor days elapsed (since Hsig ≥ 2.0) as the predictor.

## 3.3 RESULTS

### 3.3.1 Water temperature

Six crab fishers agreed to participate in the temperature data-logging programme. Their areas of operation ranged from the Tweed/Gold Coast region north to Noosa, providing a good spread over the southern half of the fishing grounds. It was expected that one of the participants, operating a dual-licensed boat out of the Tweed, would be shifting operations more into northern NSW waters, which would extend the southerly range of our information base. However this did not eventuate, so we enlisted the aid of a cooperative NSW crabber working out of Ballina. We were unable, however, to identify a skipper working north of Fraser Is who was prepared to join the temperature logging programme. This meant that information from the northern part of the fishery (Regions 2-4) were not represented in the study. The PI personally visited all these crabbers, providing them with a logger and associated equipment, and instructed them in its use. Typically the crabber would 'launch' the logger the night before anticipated deployment, and attach it to a dilly where it accumulated data at 1-sec intervals throughout the day. On return home they would download the day's data onto a PC, then send the data files to the PI as an attachment to an e-mail containing other information about the day's fishing activities (e.g. depth and location).

The data files typically contained a temperature profile with a series of plateaus (of minimally-varying temperature) relating to the time the dilly was on the sea-floor during each set, separated by spikes of varying temperature relating to the setting, lifting or inter-set period (Figure 3-1). The data in this figure were collected in late May, when air temperatures were cooling but bottom-water temperatures were still fairly high. Later in the year the situation reversed, with air temperatures higher than bottom temperatures. During the intermediate periods when water and air temperatures were similar, there was less contrast between the two media and interpretation of the set commencement and completion times was not so clear. However in these cases we were guided principally by the periods of uniform temperature, which indicated when the logger was on the bottom.

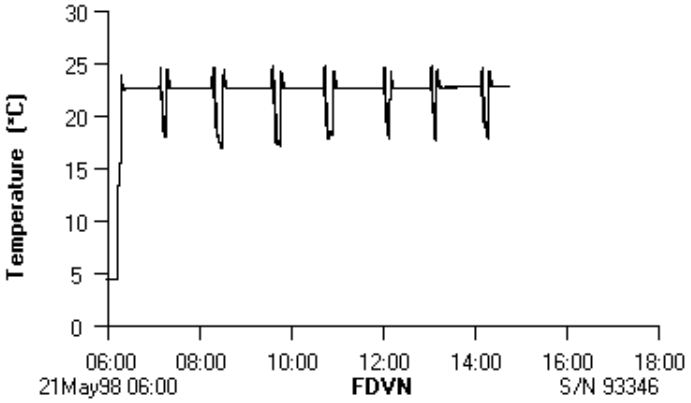


**Figure 3-1.** Typical daily temperature log provided by a commercial spanner crab fisher. Plateaus represent periods (and temperatures) while the logger was on the sea-floor.

Analysis of variance in catch rates revealed that both month and bottom water temperature (as measured by the industry-deployed TidBit data loggers) were significant factors in determining spanner crab catchability (Table 3-1). After adjusting for the very strong ($P < 0.001$) seasonal signal and the residual effect of depth, the modelled mean log-transformed catch rates showed a clear positive relationship with temperature (Figure 3-2). Back-transformation of the log values to natural scale indicate that, over the temperature range reported, catch rates increased by a factor of two, from 0.6 kg.net$^{-1}$ at 18° C to 1.2 kg.net$^{-1}$ at 26° C (Figure 3-2).

**Table 3-1.** Accumulated ANOVA table showing the significance of month, depth and temperature on the catchability of spanner crabs.

| Change | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Month | 11 | 9.8085 | 0.8917 | 5.02 | <0.001 |
| Depth | 1 | 0.4043 | 0.4043 | 2.28 | 0.134 |
| Temperature | 1 | 1.8686 | 1.8686 | 10.53 | 0.002 |
| Depth.Temperature | 1 | 0.6386 | 0.6386 | 3.6 | 0.06 |
| Residual | 117 | 20.7644 | 0.1775 | | |
| Total | 131 | 33.4844 | 0.2556 | | |

It was not possible to confirm that possible temperature changes resulting from changes in tides or current patterns within a very short time-scale could have a marked effect on catch rates. This was because (i) the catch rate data were obtained only at the scale of the fishing day, and (ii) there were relatively few instances where logged bottom water temperature changed significantly during the course of a set.

Having established the influential effect of bottom water temperatures on spanner crab catch rates, we then explored the possibility of using sea surface temperature as an index of bottom temperature in the area of the fishery. As SST is clearly a much more readily available statistic than information from data loggers, it would be useful to know whether there is a consistent relationship between surface and bottom temperatures, and how this is influenced by depth. If SST is a good indicator of bottom temperature, broad-scale satellite-derived data could be used to adjust for temperature effects on spanner crab catchability in stock assessments (such broad-scale effects are examined in detail in Chapter 4).

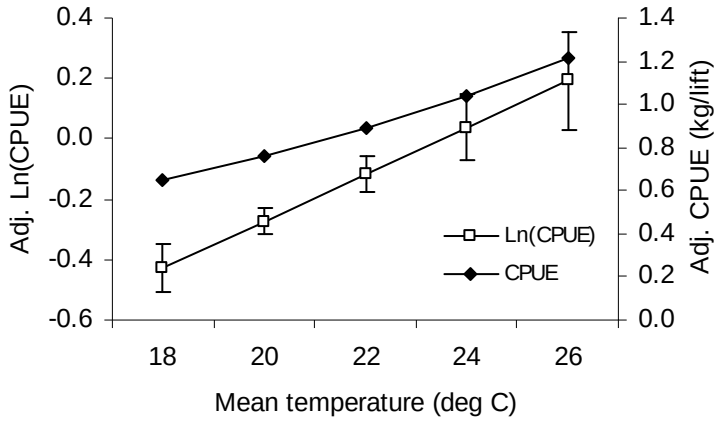


**Figure 3-2.** Response of modelled mean catch rates to changes in bottom temperature (open symbols: log scale, solid symbols: back-transformed values).

Potentially influential factors included in the general linear model were depth zone (i.e. whether on-shelf or off-shelf, with the shelf break arbitrarily set at 100 m), month and depth. Initial tests showed that fitting a 2-degree polynomial to depth normalised the otherwise skewed distribution of residuals, so the polynomial form was retained in the model. All terms in the model were highly significant, including the depth·depthzone interaction (Table 3-2), which indicates that the relationship between temperature and depth differs between shallower shelf waters and deeper offshore waters.

**Table 3-2.** Accumulated analysis of variance table showing the effects of depth (as a 2-degree polynomial), depth zone (shelf or slope) and month on water temperatures on the southern Queensland spanner crabbing grounds.

| Source | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Dzone | 1 | 200.96 | 200.96 | 174.68 | <.001 |
| Month | 9 | 462.38 | 51.38 | 44.66 | <.001 |
| POL(Depth; 2) | 2 | 3638.19 | 1819.10 | 1581.16 | <.001 |
| POL(Depth; 2).Dzone | 2 | 21.94 | 10.97 | 9.54 | <.001 |
| Residual | 320 | 368.15 | 1.15 | | |
| Total | 334 | 4691.62 | 14.05 | | |

The mean on-shelf temperatures at depth, adjusted for seasonality, ranged from 22.7° C at the surface to 18.6° C at 100 m (Figure 3-3). There was a surprising uniformity in these results, as demonstrated by the tight error-bars, indicating a predictable relationship between surface and bottom water temperature in the area of interest.

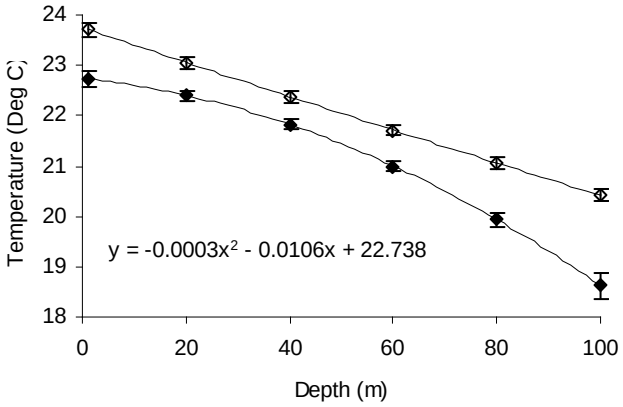


**Figure 3-3.** Seasonally-adjusted mean water temperatures at depth (± s.e.) on the continental shelf (depths ≤ 100 m; solid symbols) and beyond the shelf break (depths > 100 m; open symbols). Note that the equation refers to the shelf curve.

### 3.3.2 Habitat type

*Mooloolaba Crossover Experiment area*

The surveyed area off Mooloolaba is shown in Figure 3-4. , together with the three Crossover Experiment depth-stratified zones where spanner crab sampling had been carried out bi-monthly for 12 months. Coordinates of the starting points for each of the six replicate spanner crab sampling sets in each depth zone are shown in Table 3-3. Depths recorded by the Sea Scan system (Figure 3-4. ) closely approximated those from the national bathymetric grid.

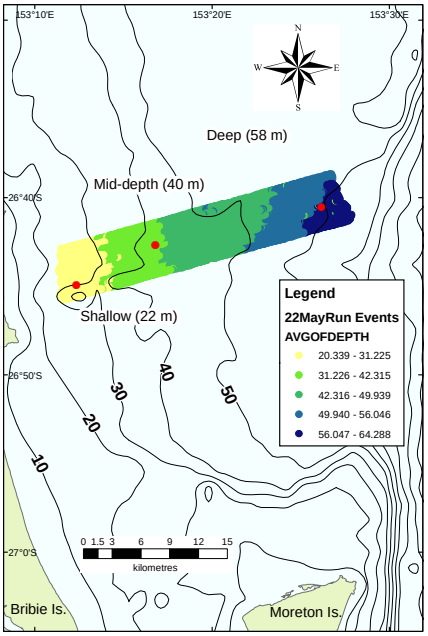


**Figure 3-4.** Area off Mooloolaba surveyed by Sea Scan (transect lines) and the locations of the spanner crab sampling sites (in approx. 22, 40 and 58 m; red symbols) used during the Crossover Experiment. Depth contours are in metres.

Initial visualisations of the seabed characteristics (roughness and hardness) revealed considerable variation in both parameters within the survey area (Figure 3-5). Interestingly, the sea floor was rougher at the shallow and deep ends of the transects than in the intermediate depths, but harder in the deeper parts than in either shallow or intermediate depths.

**Table 3-3.** Geographical coordinates of the Crossover Experiment sampling sites shown in Figure 3-4. .

| Replicate | Shallow | | Mid-depth | | Deep | |
|---|---|---|---|---|---|---|
| | Latitude | Longitude | Latitude | Longitude | Latitude | Longitude |
| 1 | 26.7602 | 153.2004 | 26.7235 | 153.2736 | 26.6848 | 153.4356 |
| 2 | 26.7552 | 153.2002 | 26.7186 | 153.2721 | 26.6802 | 153.4361 |
| 3 | 26.7489 | 153.2057 | 26.7114 | 153.2800 | 26.6756 | 153.4361 |
| 4 | 26.7445 | 153.2051 | 26.7057 | 153.2806 | 26.6708 | 153.4390 |
| 5 | 26.7395 | 153.2028 | 26.6999 | 153.2809 | 26.6661 | 153.4419 |
| 6 | 26.7350 | 153.2004 | 26.6953 | 153.2788 | 26.6617 | 153.4454 |

These trends were clearly evident in scatter plots of roughness and hardness against depth which also indicates the spatial variability in both parameters. In particular, hardness indices were not only greater, but were very much more variable in deeper areas (50–60 m) than in shallower areas, signifying a more heterogeneous sea-floor.

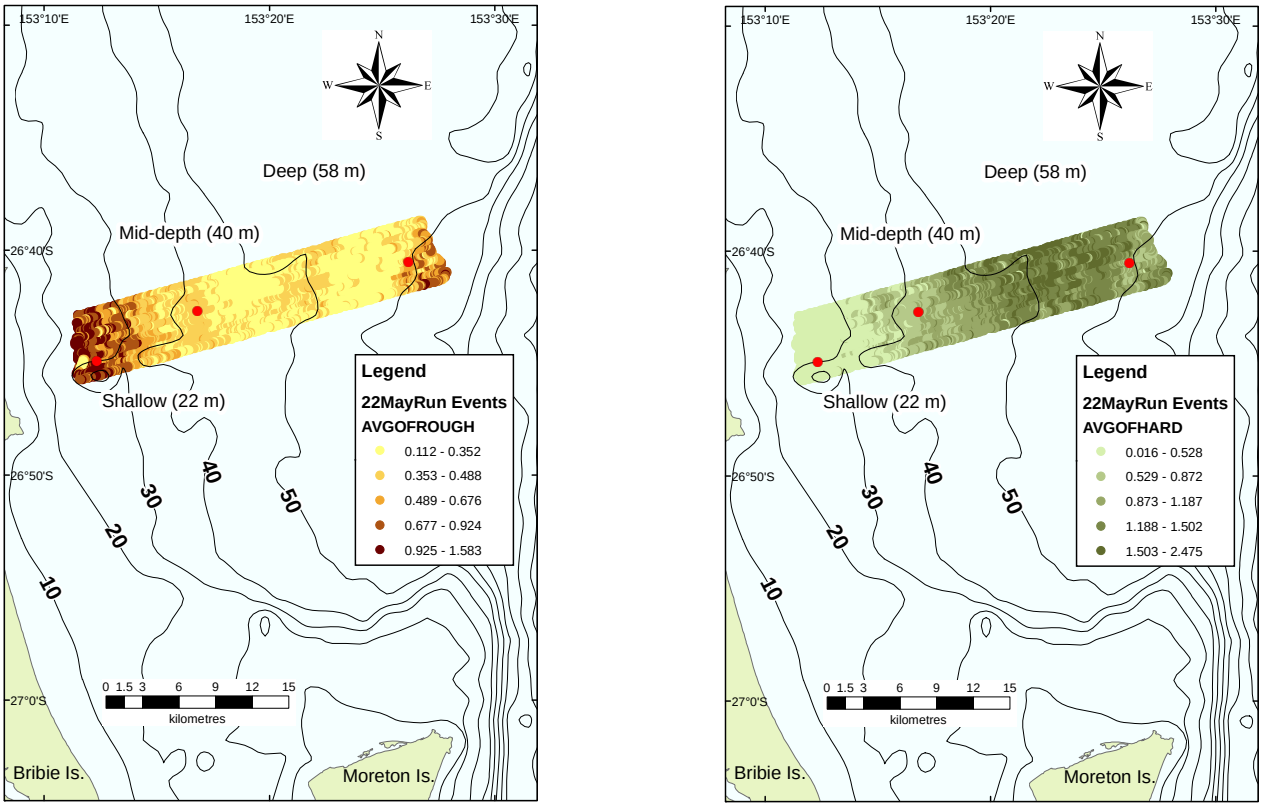


**Figure 3-5.** Seabed characteristics at the Mooloolaba survey area: mean indices of roughness (left ) and hardness (right). Depth contours are in metres.

Analyses of variance confirmed that differences in sea floor roughness and hardness were highly significant ($P < 0.001$) between three depth zones (shallow < 31 m; moderate 31-50 m; deep > 50 m). Depth zone means and their standard errors from the ANOVA are shown in Table 3-4.

**Table 3-4.** Estimated mean roughness and hardness indices (± s.e.) in three depth zones across the Mooloolaba survey area.

| Depth zone | Roughness | | Hardness | | n |
|---|---|---|---|---|---|
| | Mean | s.e | Mean | s.e. | |
| Shallow | 0.8416 | 0.0025 | 0.3285 | 0.0057 | 3999 |
| Moderate | 0.3670 | 0.0014 | 1.0401 | 0.0033 | 12115 |
| Deep | 0.3849 | 0.0020 | 1.3035 | 0.0045 | 6376 |

These results suggest that, in this area, bottom roughness *per se* is not a strong determinant of spanner crab distribution, as very similar mean roughness indices were recorded from moderate depths, at which crabs were caught, and deep areas, where the crabs were absent (0.37 and 0.38 respectively). There was a greater distinction in hardness indices between the depth zones, with relatively soft sediments occurring in the shallow zone, moderately hard sediments in intermediate depths, and (in the context of the range of data recorded at the Mooloolaba survey area) quite hard substrates in the deepest strata.

A generalised linear model was used to determine the effects of roughness and hardness on the presence or otherwise of spanner crabs. Both roughness and hardness, as well as their interaction, were highly significant terms in the model ($P < 0.001$; Table 3-5), which explained about 55% of the deviance in the probability of encountering spanner crabs.

**Table 3-5.** Effect of seabed characteristics on the likelihood of capturing spanner crabs in the Mooloolaba survey region.

| Source | d.f. | Deviance | Mean deviance | Deviance ratio | Approx. F-prob. |
|---|---|---|---|---|---|
| Hardness | 1 | 226.541 | 226.541 | 723.050 | <.001 |
| Roughness | 1 | 8.356 | 8.356 | 26.670 | <.001 |
| Hard.Rough | 1 | 3.730 | 3.730 | 11.910 | <.001 |
| Residual | 616 | 193.000 | 0.313 | | |
| Total | 619 | 431.626 | 0.697 | | |

The significance of the interaction term indicates that the main effects are not independent, which is further demonstrated by Figure 3-6. While there was a downward trend in the modelled adjusted mean probabilities of capture on soft sediments with increasing roughness, the reverse was the case when on hard sediments. In other words, when the sea floor is smooth, the probability of finding spanner crabs is high if the substrate is soft, but low if the substrate is hard. However with increasing roughness (i.e. with more vertical structure on the sea floor) the difference in capture probability between soft and hard sediments decreases. The standard errors of the adjusted means indicate that at maximum roughness (group 4) capture probabilities are similar across all four hardness categories.

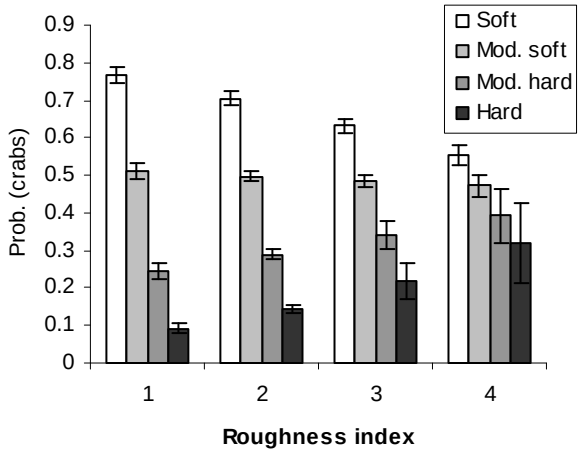


**Figure 3-6.** Effects of seabed hardness and roughness on the probability of capturing spanner crabs in the Mooloolaba survey region.

*Wide Bay Long-Term Monitoring sites*

The Wide Bay transect data, collected on 31 August 2007, were analysed using generalised linear regression initially to screen the likely main effects of grid, depth, roughness and hardness on the catch rate of spanner crabs as recorded by the Department's annual long-term fishery independent crab surveys. Initial data scans provided an overview of the relationship between depth and the two measures of bottom structure (roughness and hardness) at the two 6 x 6-minute grids. At 10W34 there was a tendency for roughness to decrease and hardness increase with increasing depth (Figure 3-7), but at 14W34 roughness appeared non-linear while hardness showed little change with depth (Figure 3-8). In other words there was not a consistent relationship between either roughness or hardness and depth at these adjacent locations. This effect is also apparent from Figure 3-9 (b and c), which shows that the sea-bed is relatively non-uniform with respect to both parameters.

Grid was included of necessity because of the clear differential in overall catch rates between the two grid blocks (Figure 3-9). The response variate was catch rate (CPUE) class, derived by grouping the overall effort-standardised catches of crabs into four categories. In addition to the average roughness and hardness indices, the effects of bottom-type variability (as variances) were also specified in the model.

There were 129 observations across the two grids (out of a possible 200) because not all sites were occupied by the LTMP survey. This was a result of the process of randomly selecting 15 of the 100 sites per grid block for each annual survey. Also, a small number of sites lacked Sea Scan data because of data drop-out problems. The number of sea-bed 'observations' at each sampling site ranged from 11 to 160, with a mean of 125.2, and it is from these samples that the various site means and variances were derived.

The first-pass full model (Poisson distribution; log-link) identified the following factors and covariates as having a significant effect on categorical catch rate: grid ($P < 0.001$), average roughness ($P < 0.001$), and depth variability ($P = 0.015$), with hardness variability having a weak effect ($P = 0.076$). To test whether any of the sea-bed parameter responses showed evidence of curvature, the next iteration of the model included polynomial sub-models. The quadratic forms of depth and hardness showed no improvement over their linear forms and were therefore dropped, but there was evidence of significant non-linearity in the roughness response, so this polynomial term was retained.

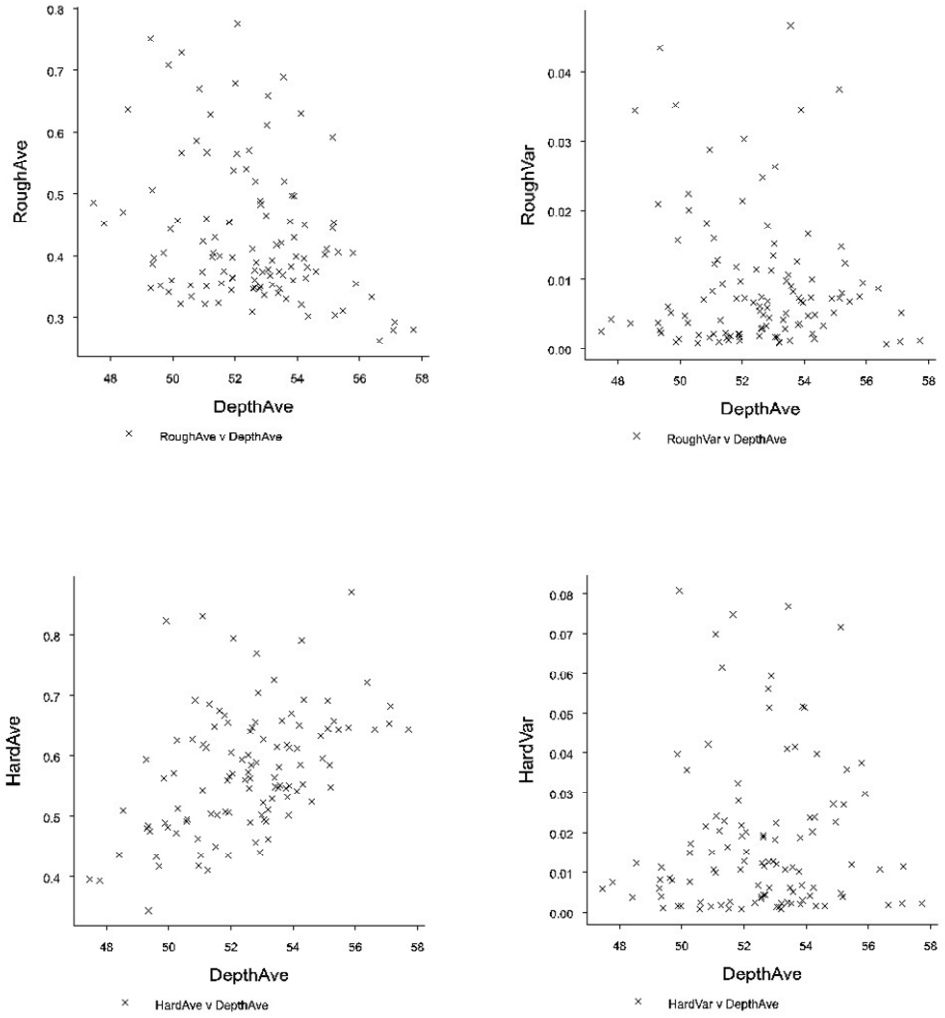


**Figure 3-7.** Relationship between means (left) and variances (right) of roughness (top) and hardness (bottom) and depth at Sea Scan sites in Grid 10W34.

After re-ordering the significant model terms and dropping those that were non-significant, the final reduced model was of the form

```
Grid + POL(RoughAve; 2) + DepthVar + HardVar
```

whereupon both DepthVar and HardVar became non-significant (P = 0.22 and 0.45 respectively). This left just grid (P < 0.001) and the quadratic mean roughness (P < 0.001) as the only significant terms.

Adjusted mean catch rates were then estimated for each grid separately, and at five nominal levels of average roughness within the range of the data (0.25, 0.35, 0.45, 0.55 and 0.65). It should be noted that in the following presentation of results, adjusted mean 'catch rates' refer to the four catch-rate classes (1-4) rather than to actual CPUE values.

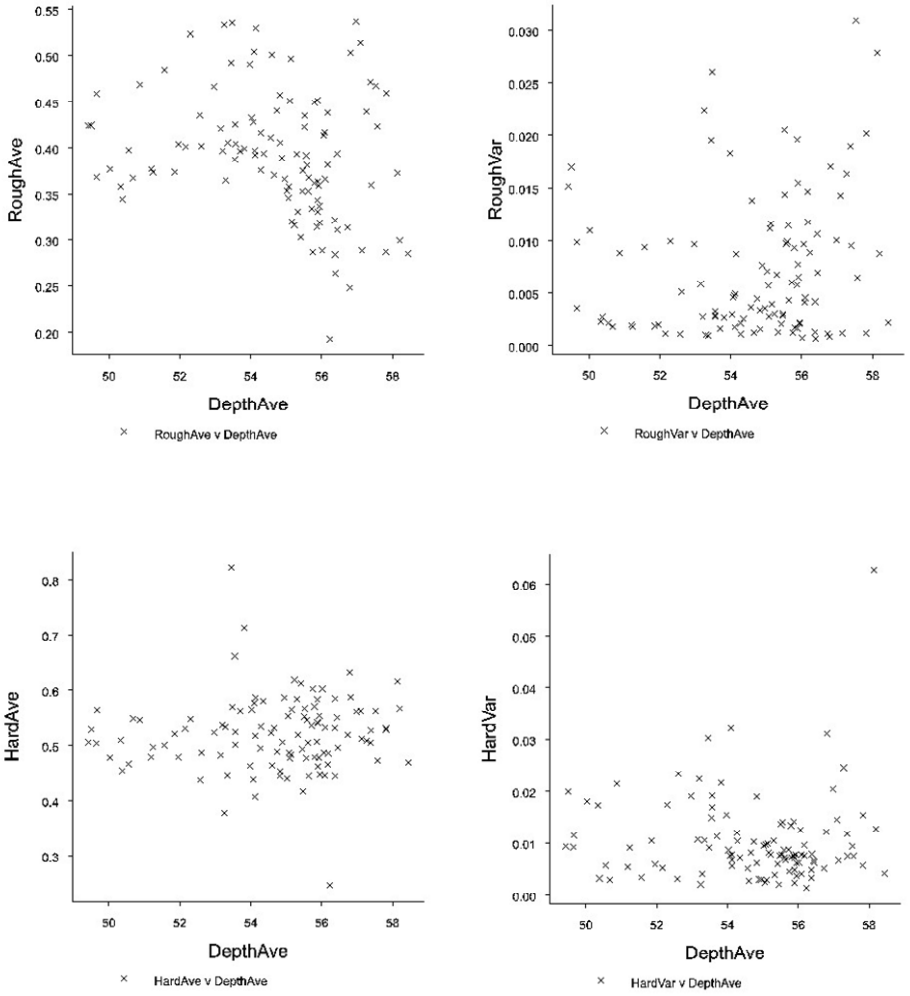


**Figure 3-8.** Relationship between means (left) and variances (right) of roughness (top) and hardness (bottom) and depth at Sea Scan sites in Grid 14W34.

The modelled adjusted means () reflect the significant difference in apparent abundance of crabs between the two transected areas. In both cases the catch rate was higher at intermediate levels of sea-floor roughness than at 'smooth' or 'very rough' locations. At grid 10W34 the difference was (in real terms) from about 1 crab.net-hour$^{-1}$ on a smooth sea-floor (R = 0.25) to 4 on a moderately rough sea-floor (R = 0.45) then down to 0.2 on a comparatively rough (R = 0.65) substrate. A similar trend, but with significantly lower values, was evident at grid 14W34. Although this suggests a two-fold difference in apparent abundance, the difference is actually a great deal more than that, because (for example) the average CPUE for catch rate class 3 (2.9 crabs.net-hour$^{-1}$) is seven time that for class 2 (0.4 crabs.net-hour$^{-1}$).

This set of results contrasts with those from the Mooloolaba survey area, where the abundance of spanner crabs was affected both by sediment roughness and hardness, and much higher roughness index values were recorded (particularly at the shallower site) than in the Wide Bay grids.

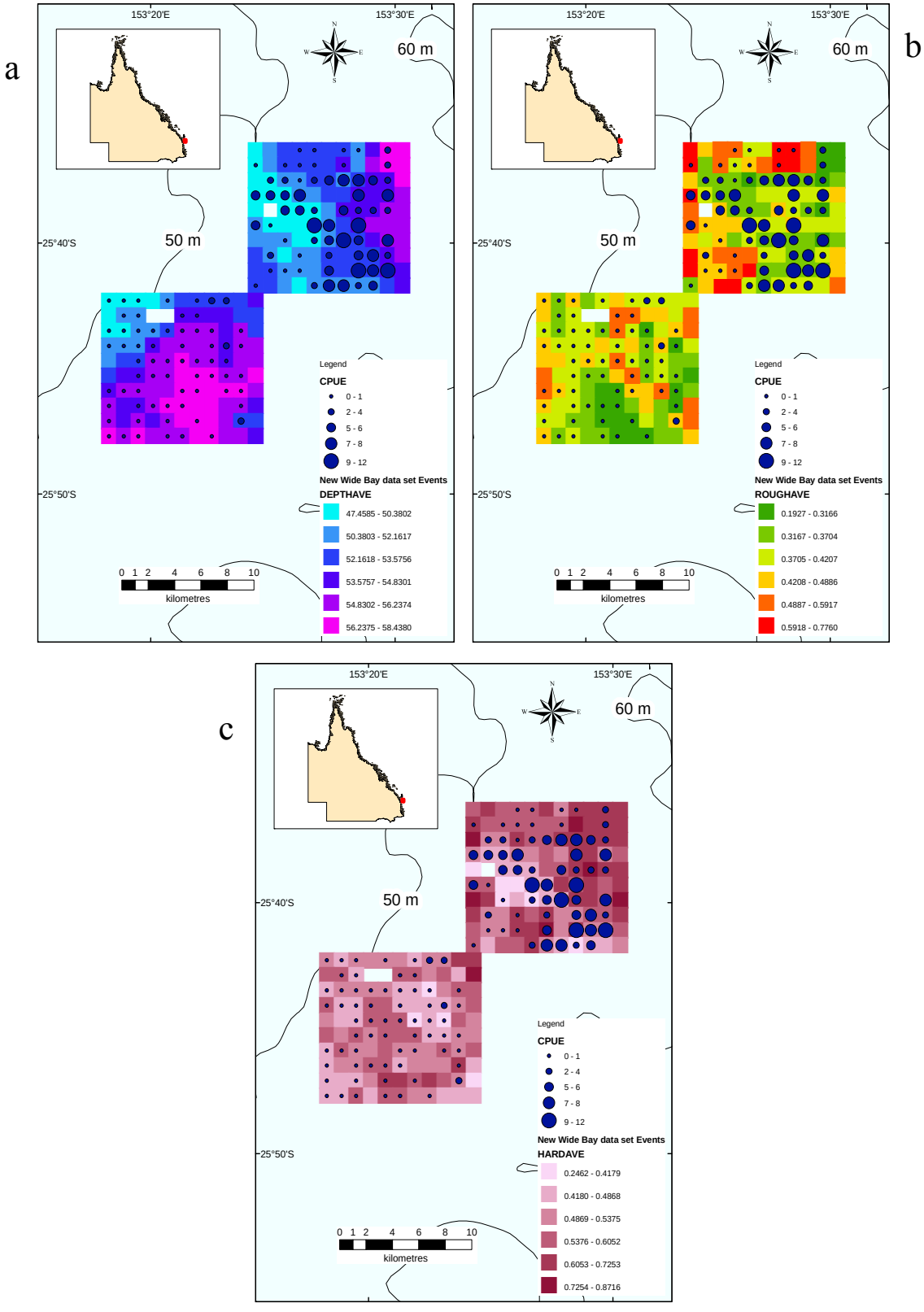


**Figure 3-9.** Mean depth (a), roughness (b), and hardness (c) indices across grids 10W34 (upper) and 14W34 (lower), with overall catch rates (crabs per net-hour) superimposed. Blank sites indicate Sea Scan data dropout, and sites with no CPUE symbol had not been sampled for crabs during the LTMP period.

An alternative approach was then taken to analysing the Wide Bay data because of the structural differences in the data from the two grids. In contrast to grid 10W34 which identified only four sites where no crabs had been caught, over 55% of the 65 sampled sites in 14W34 yielded a nil catch. The two data sets were analysed separately, 14W34 by a binary-response (present/absent) model with logit link, and 10W34 by general linear regression modelling with log-transformed CPUE (i.e. ln(CPUE+0.1)) as the response variate. Logarithmic transformation normalised the data satisfactorily.

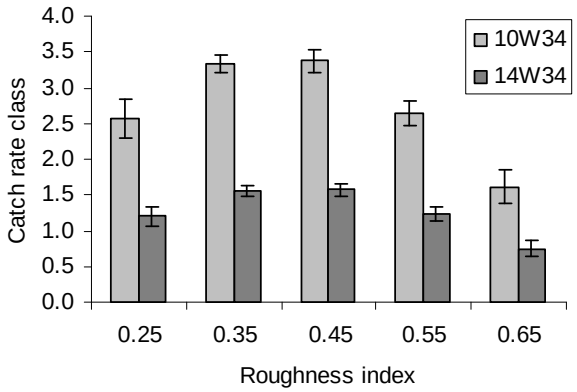


**Figure 3-10.** Effect of seabed roughness on the adjusted mean catch rate of spanner crabs at two 6' x 6' grid blocks off the south of Fraser Island. Note that the catch rates relate to four classes rather than actual CPUEs.

The binary model on presence or absence of spanner crabs at grid 14W34 fitted the data very poorly, explaining only 10% of the observed deviance. Average depth was a significant determinant (P = 0.044) but neither hardness nor roughness, nor any of the three variance measures, approached statistical significance.

On the other hand, the data from 10W34 showed a clear trend with respect to the roughness statistic (Figure 3-11). The general linear model on log-transformed CPUE (crabs.net-hr$^{-1}$) identified the quadratic form of average roughness as being highly significant (P < 0.001), while all other terms, including the roughness-hardness interaction, were non-significant. While only 49% of the variance was accounted for by the model, this was a substantial improvement on the binomial model fit for grid 14W34.

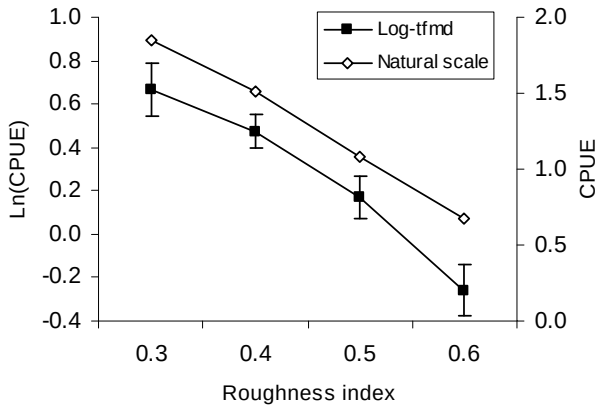


**Figure 3-11.** Effect of sea-floor roughness on the catch rate of spanner crabs in grid 10W34. Adjusted mean estimates are shown in the log scale (with standard errors) and back-transformed on the natural scale.

The adjusted means across a range of roughness values are shown in Figure 3-11. There is a clear tendency for the catch rate to diminish with increasing sea-bed rugosity, from 1.8 crabs.net-hr$^{-1}$ in areas with low roughness (R = 0.3) to 0.7 crabs.net-hr$^{-1}$ in areas with substantial vertical profile (R = 0.6).

### 3.3.3 Wave height effects on catchability

Wave heights in the Mooloolaba area during 2006 were typically between 1.0 and 1.5 m, but periods of heavy swell (with Hsig ≥ 2.0 m) occurred on nine occasions throughout the year (Figure 3-12). It is the period of up to 6 days following each such event for which the catch rate of spanner crabs was modelled.

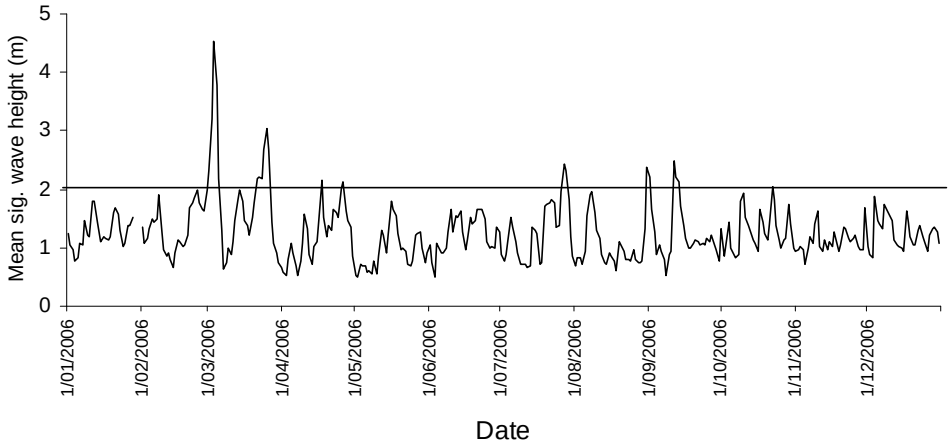


**Figure 3-12.** Mean daily significant wave heights (m) throughout 2006 measured by the Mooloolaba Waverider Buoy. Horizontal line shows periods when Hsig ≥ 2.0 m.

An initial regression analysis ($a$ = 1.1996 and $b$ = -0.1443 ± 0.043 s.e.) showed that there was a significant overall effect of wave height on catch rate (P < 0.001), although there was a great deal of scatter as signified by the low $R^2$ of 0.05 (Figure 3-13). The data indicate that for each unit increase in significant wave height the catch rate is likely to decrease by a little over 14%.

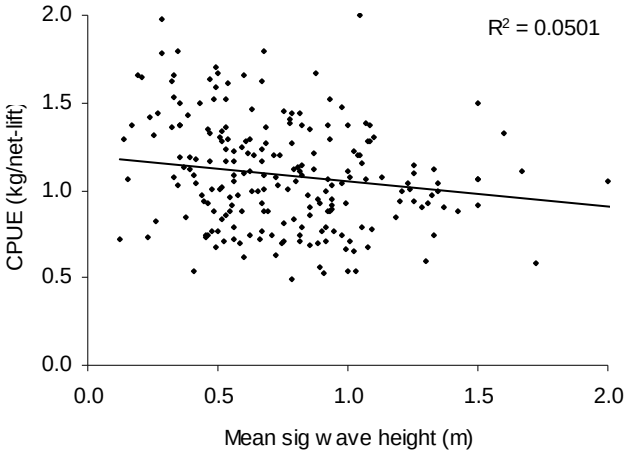


**Figure 3-13.** Effect of wave height on spanner crab catch rate. Each point on the graph represents a day on which some spanner crabbing was carried out in Region 5 and a record of average Hsig was obtained from the Mooloolaba Waverider Buoy.

General linear modelling revealed a tendency for catch rates to be low immediately after a period of heavy swell, and to increase over the next 5-6 days. However the trend was not statistically significant (P = 0.388), presumably because of the influence of other unaccounted factors. The adjusted mean catch rates increased from 0.6 kg.net$^{-1}$ on the first day after a significant wave-height event to about 0.9 kg.net$^{-1}$ on the fifth day (Figure 3-14), an increase of 50%.

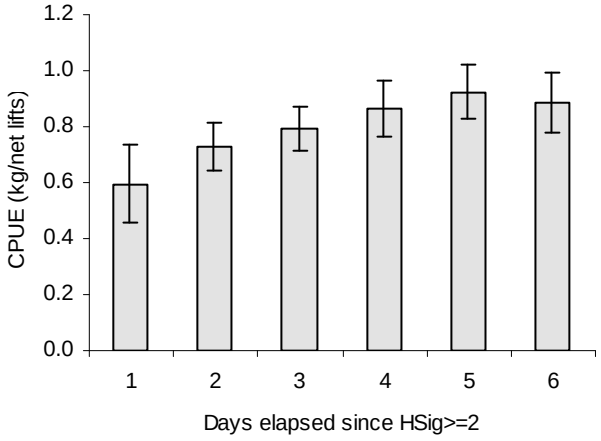


**Figure 3-14.** Adjusted mean catch rates (kg/net-lift ± s.e.) over the 6 days following a period of heavy swell (Hsig ≥ 2.0 m). Data relate to nine heavy swell events in the vicinity of Mooloolaba during 2006.

## 3.4 DISCUSSION

### 3.4.1 Water temperature

Small, relatively inexpensive and robust temperature data-loggers were used very successfully by industry participants to collect data on bottom water temperatures on the spanner crab fishing grounds during their commercial fishing activities. The dedication of a small number of crab fishers resulted in a data set that revealed (after seasonal adjustment) a strong relationship between spanner crab catch rates and bottom water temperature. It is assumed that the effect is due to a temperature-mediated increase in metabolic activity which translates into more active foraging and an increased likelihood of capture. In other words, at this level of analysis it appears to be a function of increased catchability rather than increased population abundance. We were not able to establish whether, at the scale of hours, changes in current flow patterns alter bottom temperatures enough to influence catch rates, as is frequently reported by commercial operators. There is no evidence to suggest that this might not be the case, but it is also possible that changes in water movement patterns alter the direction and flow of the bait plume to the extent that it disrupts the crabs' foraging behaviour and (at least temporarily) reduces their catchability.

Water temperature profile data gathered during CSIRO oceanographic cruises in southern Queensland waters several decades ago showed that (after seasonal adjustment) there was a consistent quadratic relationship between temperature and depth on the continental shelf. This suggests that at larger temporal and spatial scales sea-surface temperatures (SST) derived from remote sensors such as satellites could be used as proxies for bottom temperatures in adjusting spanner crab catch rates (CPUEs) for prevailing temperature. This could be of considerable value in interpreting the results of the Department's fishery-independent spanner crab surveys, which are conducted over a short time-period each year. Knowledge of prevailing SST conditions at the time of the LTMP survey could help account for unexpected differences between the fishery-independent

(survey) estimates of stock abundance and those obtained throughout the year from the fishery-dependent (logbook) records.

An unexpected spin-off from the temperature-logging programme arose from our ability to accurately determine the duration of individual 'sets' of the fishing gear deployed by the cooperating fishers. The period during which the loggers (attached to a dilly line or string) were on the bottom was able to be estimated to the minute from the logger's data file because of the relative constancy of the bottom water temperature over time periods of the order of 1-2 hr. This provided valuable evidence during the Queensland Crab Scientific Advisory Group's review of effort creep in the fishery when it came to light that some fishers had found (contrary to published research evidence) that their catch rates did not decline appreciably if they left their gear to soak for periods considerably longer than an hour. Dilly saturation did not appear to be an issue, so where a productive area had been identified, these fishers were tending to leave their gear for increasingly long set times. The effect of this was (potentially) to inflate CPUE values because the equivalent daily catch was being taken with fewer dilly-lifts. While not a significant issue across the whole fishery, this did focus attention on the need to more closely examine un-documented sources of variation in our estimates of fishing effort in this fishery. This in turn led to a directive from CrabMAC to undertake a survey of the fleet specifically targetted at determining changes in fleet fishing power, the results of which were subsequently incorporated into a revised procedure for assessing the status of the east coast spanner crab stock and the development of a suitably modified set of decision rules used to set the biennial commercial TAC.

### 3.4.2 Habitat type

Difference in format and structure between the Mooloolaba and Wide Bay data sets required different analytical approaches to understanding the influence of sea-bed characteristics on the distribution and abundance of spanner crabs. However, there was a general agreement between the various analyses that bottom roughness or rugosity is a major determinant. It is evident from the Mooloolaba data that depth *per se* does not influence the distribution of spanner crabs, even though all the crabs were caught in shallow and intermediate depths, and none in the deep water. Any superficial depth-related trend can be accounted for by differences in the structure of the sea-floor. The range of depths sampled at the Wide Bay grids (47–58 m) was very much less than at the Mooloolaba area (22–58 m) and was unlikely to have introduced any discernable signal into this data set.

At the Mooloolaba survey location the probability of encountering crabs decreased with increasing sea-bed rugosity, although the effect was significantly modified by the prevailing hardness of the substrate. Capture of crabs is most likely where the bottom is smooth and soft, and least likely where it is smooth and hard. Intermediate probabilities of capture occur where the sea-floor is rough, with relatively little real difference between soft and hard substrates.

At the (combined) Wide Bay LTMP grids the probability of capture also decreases (in a non-linear fashion) with increasing roughness, but there was also a very significant difference due to grid. The reason for this difference, occurring at only a marginally greater spatial scale than within-grid, is not known, as the ranges of roughness, hardness and depth were similar between the two grids. An additional factor not measured or incorporated into the model must have been responsible for the disparity in catch rates. It is possible that the crab population in grid 14W34 had been reduced by greater fishing pressure to a lower level than in 10W34, as it is slightly closer to the nearest port and therefore somewhat more accessible to the fishery, but this explanation has not been explored. Alternatively, the structural characteristics of the sea-floor which translate into the index of roughness differ qualitatively between the two areas. This may be the case if the vertical structure, which is assumed to be the basis of the roughness index (derived from back-scattering of the echo-sounder signal) is produced in one area by (for example) algal growth, but in the other by current-induced surface rippling of the sea-bed sediment.

Separate analyses of the data from the two Wide Bay grids failed to detect a significant effect of any of the sea-bed characteristics on the presence or absence of spanner crabs in 14W34, but in the higher-yielding

10W34 there was a highly significant roughness effect. Consistent with the analyses described above, increasing sea-floor roughness was associated with decreasing catch rates. While this may seem to be at variance with the Mooloolaba result (where crabs were abundant at the shallow 'rough' site) it could be because the contrast within roughness and hardness indices at Wide Bay was much less than at Mooloolaba, and the interaction effect between the two variables was too weak to achieve significance.

These results, while by no means definitive, provide good evidence that the distribution of spanner crabs is affected – probably in a fairly complex way – by the composition and structure of the sea-floor. It also demonstrates that the simple measures of bottom type obtained from swath transecting with the Sea Scan 100 system can provide a suitable basis for a broad-brush sea-bed classification of value to the fishing industry, although more effort needs to be focussed on ground-truthing by sediment analysis or visual methods to determine the reasons for meso-scale differences in crab abundance such as were observed between the two Wide Bay grid blocks.

### 3.4.3 Wave height

Along with changes in bottom water temperature, we showed that wave action can also affect the catch rate of spanner crabs, at least in the short term. While the observed changes in catchability (a ~30% drop in the first couple of days following a significant wave-height event) were perhaps smaller than anticipated, the study did confirm the contention of commercial fishers that catches are significantly reduced immediately after a period of heavy swell. This effect is evidently the same regardless of whether the swells were generated by winds from a local storm event or by a remote weather system perhaps hundreds of kilometres off the coast. It is postulated that the rotational movement of water beneath a wave (the effect of which diminishes with increasing depth) can be sufficient to interfere with the crabs' ability to determine the direction from which a bait plume is coming, and thus affect their ability to locate the bait. In extreme circumstances, particularly in shallower areas, the seiching effect induced by the increasingly elliptical particle movement close to the sea-floor may actually make locomotion difficult for the crab if it emerges from the stability of the substrate.

The use of such information, derived quite readily for select areas where Waverider buoys and other suitable recording instruments are located, could be used in the same way as SST surrogates for bottom water temperature to assist with interpreting and adjusting the fishery-independent survey results. This may be quite important if (as is argued by some industry representatives) there is some likelihood that the results of these very short-term surveys are compromised by coinciding with periods of heavy swell.

## 3.5 ACKNOWLEDGEMENTS

Thanks are due to Jim Waldron (Environment Protection Agency, QLD) for making a large set of Waverider Buoy data available to the Project. Les Zeller (DPI&F, Tor St Centre, Toowoomba) provided invaluable assistance and advice in setting up the multiplexing system for recording the output from the Sea Scan unit, and wrote the S+ controller programme. The dedication and commitment of all the commercial crabbers who agreed to participate in the seafloor temperature-logging component of the project is acknowledged with extreme gratitude; particular thanks being due to Richard Freeman, Andrew Maclean and Richard Hamilton who between them contributed most of the temperature data. Thanks are also due to skippers Brett Davidson, Stelios Kondylas and Sean Maberley for assistance with field operations aboard the RV *Tom Marshall*, and to Erin Kenna and other SFC staff for helping with GIS data processing and mapping.

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# CHAPTER 4. BROAD-SCALE CLIMATE EFFECTS ON SPANNER CRAB STOCKS

A. Williams and I.W. Brown

## 4.1 ABSTRACT

A first-pass assessment of the sensitivity of spanner crabs to climate variability was carried out in 2001. That study concluded that spanner crab catch-per-unit-effort was correlated with local off-shore climate and also global-scale climate indices. However, while cross-validated, these results were interpreted with caution because the sample covered only a relatively brief (13 year) time-span, from 1988 to 2000.

In 2007 the results were reassessed using updated datasets and found to be consistent with the earlier results, although the statistical significance varies. Specifically, catch rate (CPUE) is influence by the strength of the eastward component of the ocean current, and salinity. These two climate variables and CPUE are also related to the autumn value of ENSO indicators (such as the Nino3.4 SST value). This means that the annual CPUE amount can be predicted by the end of autumn. Given the accumulating evidence that climate change is significantly impacting on marine ecosystems, a more thorough climate impacts analysis is recommended.

## 4.2 INTRODUCTION

Commercial harvest fisheries in southern Queensland Australia are operating in a unique climatic environment. Not only is the interannual rainfall the most variable in Australia (Nicholls *et al.* 1997) but it is also the region with the strongest relationship to the global-scale atmospheric-oceanic oscillation *El Nino* Southern Oscillation, known as ENSO (Nicholls and McBride 1983). Other regional atmospheric features such as temperature and wind are also significantly influenced by ENSO (Stone *et al.* 1998). Given that Queensland's commercial fisheries are the third largest in Australia (annual gross value of production estimated at $350M, Williams 2002), knowledge of the climate forcing of the catch and its predictability could have significant implications to the State's economy.

In other parts of Australia, fisheries managers utilise climate forcing for stock assessment and fishery management (e.g. tailor in Western Australia [Lenanton *et al.* 1996], for prawns via stock-recruitment models in Western Australia, [Vance *et al.*], Northern Prawn Fishery). This demonstrates that variations in catch or catch rates resulting from climatic forcing needs to be considered for stock assessment models which will consequently be of use for predicting possible changes in stock abundance.

Spanner crab (*Ranina ranina*) comprises approximately 18% of Queensland's total seafood harvest by weight, and is the largest commercial fishery in the State in terms of total catch of an individual species (Williams 1997). Little is known about the effect of interannual atmospheric and oceanic variability on *Ranina ranina*, the only studies on the effect of hydrological conditions (e.g. temperature and salinity) having been done in Japan on the development of the larval zoeal series by Minagawa *et al*. (1993).

This study aims to establish whether there is a link between global-scale oscillations and the abundance of spanner crabs. We examine the effect of interannual variability of local climate on the fishery, patterns of coherent variability, and resultant climate models of catch rates; the effect of ENSO on inter-annual catch rates and consequential predictive ability; and present hypotheses on the impact of global warming.

## 4.3 MATERIALS AND METHODS

### 4.3.1 Climate variability

Time-series of observations of catch and environmental conditions were used to investigate environmentally induced variation in the harvest rates of the spanner crab fishery. Catch-per-unit-effort (CPUE) was used as an indicator of stock abundance (e.g. Beentjes and Renwick 2001), which allows the formation of hypotheses on the effect of the abiotic factors on both recruitment and catchability. However, there are a wide range of assumptions and limitations that must be taken into account when using CPUE data in that manner. These are well documented (e.g. Williams 2002, p 15) and hence not discussed here.

Catch and effort data from 1988 to 2001 were obtained from the Queensland Government's Commercial Fisheries Information System (CFISH) database. This system, which began in 1988, relies on the use of compulsory logbooks and is collated in 0.5 degree grids of latitude and longitude. The fishery primarily operates between 22°S and 28°S.

### 4.3.2 Local climate

A total of 18 monthly atmospheric and oceanic variables were selected to provide a representation of the local environmental conditions relevant to the spanner crab fishery. The atmospheric data were obtained from the Australian Bureau of Meteorology (BOM). Monthly mean sea level pressure (MSLP), wind easterly and northerly vector data were obtained for 9 a.m. and 3 p.m. at 3 locations on the Queensland coastline: Mackay, Gladstone, and Cape Moreton. The twice-daily recordings of these variables were averaged. Gridded rainfall data were obtained from BOM.

The ocean data were obtained from the National Centre of Environmental Prediction/National Ocean Atmospheric Administration (NCEP/NOAA) Climate Modelling Branch (CMB). This model-derived data are hindcast results derived from a model based ocean analysis system. Observed surface and subsurface ocean temperatures as well as satellite altimetry sea-level data from TOPEX/POSEIDON are assimilated into a Pacific Basin ocean general circulation model. The model is forced with weekly mean NCEP operational atmospheric analyses of surface winds and heat fluxes (Behringer *et al.* 1998, Ji *et al.* 1995, Ji and Smith 1995). The variables used in this analysis are the zonal and meridional ocean velocity for the surface, 50 m and 100 m depths, sea level anomalies and salinity. Monthly data from Jan 1980 to Dec 2001, and the one-degree grids were averaged over the longitudes 148.5°E to 156°E and latitudes 35.5°S to 21.5°S.

The Reynolds and Smith monthly sea surface temperature (SST) data (Reynolds and Smith 1994) from November 1981 to December 2001 were obtained. The SST fields were blended data from ship, buoy and bias-corrected satellite data. As well as using the individual time series from the one-degree grids, the gridded data were also averaged over longitudes 151.5°E to 154.5°E, and latitudes 23.5°S to 27.5°S to provide a single regionally-averaged SST indicator.

### 4.3.3 ENSO indicators

A selection of ENSO indicators enabled assessment of the response of fisheries to larger scale global variability. A monthly timeseries Southern Oscillation Index (SOI) was obtained from Queensland Department of Primary Industries, as were the Sea Surface Temperature (SST) ENSO indices known as Nino1, Nino2, Nino3, and Nino4 (the well documented regions in the Pacific Ocean linked to the driving source of ENSO) originally sourced from the United Kingdom Meteorological Office's GISST2.3b model (Rayner *et al.* 1996) and NCEP's Centre for Climate Prediction (www.cpc.ncep.noaa.gov) (Figure 4-1).

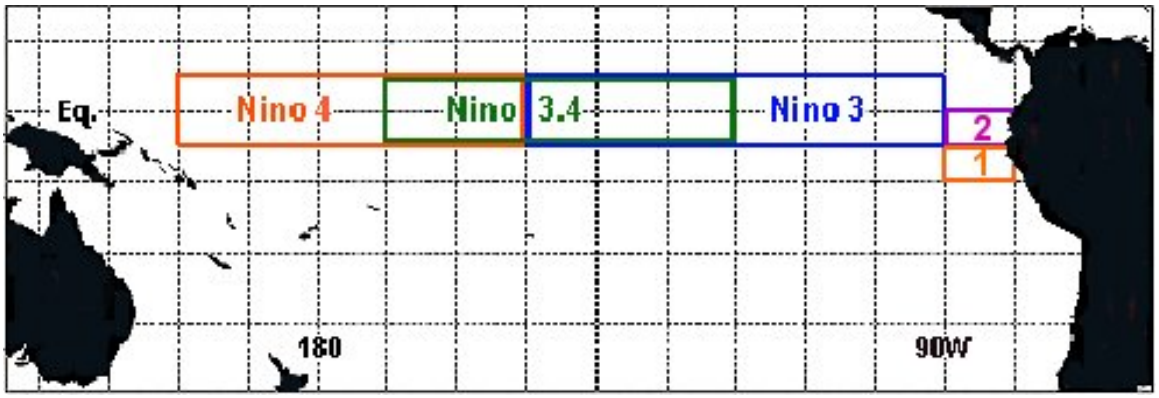


**Figure 4-1.** Location of the SST regions referred to as Nino1, 2, 3, 3.4 and 4.

Although most of the data were available on a monthly time-step, much of the monthly CPUE data is considered to be more reliable (due to methods of data collation by fishers) if smoothed into annual data especially the spanner crab data (Ian Brown pers. comm.). Given that the scale of interest of climate variability effects is interannual, this smoothing is unlikely to compromise any results. Therefore annual means of all variables were compiled by averaging monthly values from January to December. This also has the useful effect of increasing the signal to noise in the data. Three-monthly seasonal averages of ENSO indicators were also used for predictive purposes.

For these analyses all data sets were checked for normality using histograms and the Shapiro Wilkes W test. If the *W* statistic is significant, then the hypothesis that the respective distribution is normal should be rejected. If the data were not normal they were appropriately transformed. All data were subsequently standardized to unit variance.

## 4.4 RESULTS

### 4.4.1 Climate variability

ENSO indices were tested for coherence with the fisheries data. The interannual relationships between climate variables and CPUE were assessed using a range of techniques including Pearson correlation, multiple linear regression (stepwise), spectral, and cross-spectral analyses as described in the following paragraphs. The validity of the relationships was tested using cross validation as well as more traditional measures of skill such as p-levels. This is essential because of the small number of years in the CPUE data set (1988-2001). Unless otherwise specified, levels of significance were set at 0.05 throughout the analyses.

### 4.4.2 Linkages between environmental condition, CPUE, and ENSO

The correlation analysis for the initial climate assessment (1988-2001) showed that spanner crab CPUE had strong, statistically significant ($p < 0.05$) relationships with the zonal component of the surface current, the meridional wind component at Mackay, and salinity. The former two relationships had a positive correlation, and the latter a negative. That is, between 1988-2001 annual spanner crab CPUE was higher in years when the eastward zonal current was stronger, southerly winds at Mackay were weaker, and salinity lower.

When the analysis was extended to include data up to and including 2007, the relationship between CPUE and zonal current was continued (P = 0.1), but the relationship with salinity weakened to -0.25 (P = 0.32). While not directly used for predictive purposes, these simultaneous relationships between CPUE and environment are useful for identifying the type of environment in which high/low CPUEs are found. It is of interest to know if there is a relationship between ENSO and these CPUE environments as it increases confidence in any statistical relationship between ENSO and CPUE itself.

ENSO is used to predict rainfall and temperature in Australia, and it is tested here for predictability of the local climate variables and also CPUE in Section 4.4.3. The effect of ENSO on Australian atmospheric circulation has been well documented in many previous studies (e.g. Allan 1988, Wright 1988). During La Niña events the MSLP over Queensland (and most of Australia) is reduced. In winter, the anticyclonic circulation dominating southern Australia's climate weakens and the southeast trades affecting Queensland decrease allowing the moister easterlies to have influence. The increased westerly zonal flow south of about 20°S brings more frequent and more northerly frontal activity and promotes the occurrence of northwest cloud bands. North of about 25°S meridional flow is anomalously northerly in both winter and summer. However in summer there is weaker easterly zonal flow north of about 25°S, but a stronger easterly component south of this region. The monsoon trough is displaced further south. During El Niño events the subtropical ridge is more intense and the southeast trades increase. Zonal wind is dominated by stronger easterlies between 15 and 30°S. South of 25°S the southerly meridional component increases.

There are significant correlations between the SOI and the other relevant climate variables, *viz.* MSLP, salinity, and zonal current velocity. In La Niña events MSLP is lower, salinity is higher, and zonal current tends to be westward.

### 4.4.3 Prediction of CPUE using local climate and global climate patterns.

Correlations between spanner crab CPUE and ENSO indicators are strong. When the initial research was done in 2002, using data from 1988 to 2001, annual catch rates were significantly correlated (P < 0.05) with the SOI, Nino3, Nino4, and Nino3.4 from February through to August (Figure 4-2). The strongest correlations were with May Nino3.4 values; more importantly using cross-validation this was the month that had the highest $R^2$ values which implies greater model robustness than other months. This means that the January-December total CPUE can be predicted with the May Nino3.4 SST values. The relationship is positive, which means that spanner crab CPUE is higher in when Nino3.4 SSTs are higher (often called El Niño years). The negative correlations between the SOI and effort, while not significant at the 95% level, indicate a tendency toward more effort in El Niño years than in La Niña, with wind strength tending to be the most strongly related variable.

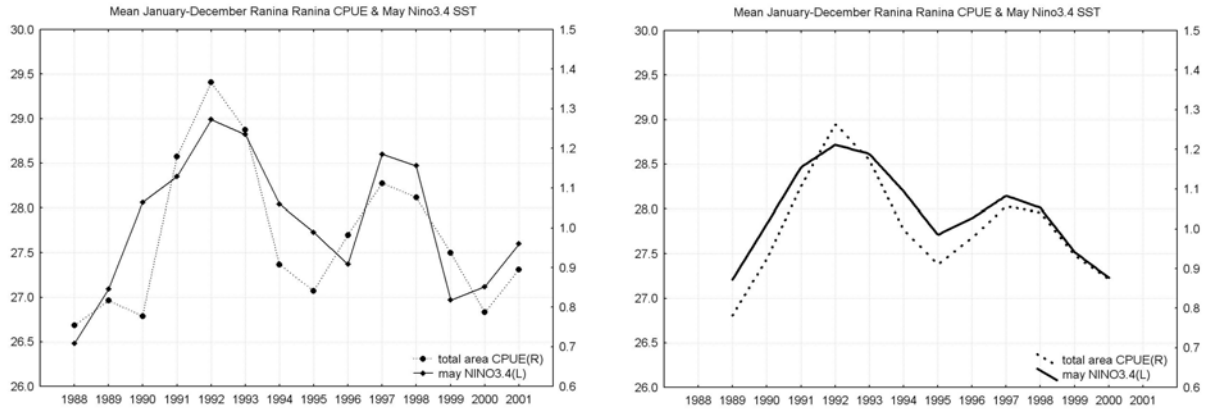


**Figure 4-2.** Mean January-December CPUE (dotted line, right axis) plotted with the May Nino3.4 SST (solid line, left axis) between 1988 and 2001. On the right is the three-year running average of each dataset (centred on the year shown).

The dataset was updated in 2007 with data from 1988 to 2006 and the results are shown in Figure 4-3. The correlation between annual total CPUE and May Nino3.4 was still significant, at 0.78.

Although limits of ENSOs predictability are the subject of considerable debate, many models predict ENSO with lead times of between 6 and 12 months. This could be useful for prediction of CPUEs. For example, if in October 2007 there is confidence in a prediction of the following years ENSO phase (remembering that ENSO phases tend to run from May to the following May), the resultant prediction of CPUE could be consolidated the following May 2008 using the above statistical model.

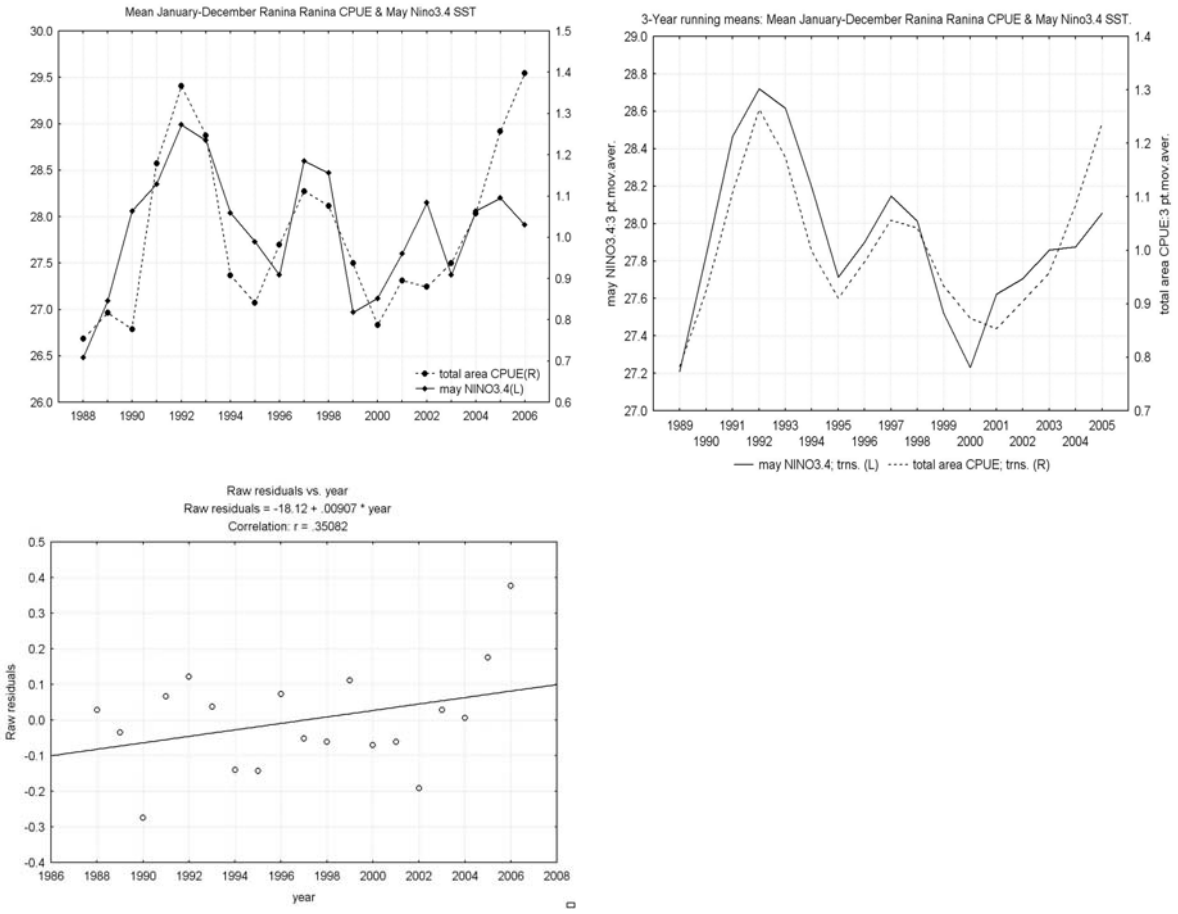


**Figure 4-3.** Updated time-series data: mean January-December CPUE (dotted line, right axis) plotted with the May Nino3.4 SST (solid line, left axis) between 1988 and 2007. The bottom left plot of the residuals of the CPUE ~ May Nino3.4 relationship have a slight but not statistically significant upward trend over time.

### 4.4.4 Nonlinear relationships

Cyclical relationships between the fisheries data and environmental data were also modelled, using the non-linear time series technique of cross-spectral analysis. Cross-spectral analysis using Fast Fourier Transforms (FFT) was employed to firstly deconstruct the non-stationary climate and CPUE time series into their main underlying sinusoidal functions of particular wavelengths and secondly to assess the relationship between the frequencies of the different time series. This is a well-documented technique with details available in Shumway and Stoffer (2000). To enable easier identification of periodicities, the linear trend is removed from the time series and the mean is subtracted.

## 4.5 DISCUSSION

It is well documented that ENSO is quasi-periodic, with a periodicity of between 2 to 7 years (Allan 1988).The individual power spectra for the climate variables of the spanner crab CPUE and the May Nino3.4 SSTs approximately 6 and 5 years respectively. Cross spectral analysis determined the frequencies at which the CPUE and SSTS are most coherent. The statistics of interest are the coherence spectrum which estimates, at given frequencies, the correlation between the sinusoidal cycles of two contemporaneous series; and the phase spectrum which estimates the tendency of cycles in the 2 series at a particular frequency to lead or lag each other. The estimate is most precise when the coherence is high. For the CPUE and Nino3.4 analysis there is a peak in the co-spectral density at 5-7 years which has a squared coherency of approximately 0.8.

There is substantial literature suggesting that increasing levels of carbon dioxide in the atmosphere has the potential to not only significantly affect global and local climates (IPCC 2000), but also has the potential to impact on many other aspects of human life and our environment, including ecosystems and many industries. Decadal scale shifts in climate have already been shown to have dramatic effects on many fisheries in the northern hemisphere.

The climate impacts from anthropogenic global warming have been modelled and are not inconsistent with many observations of climate parameters relevant to this study. On a global scale the oceans are becoming less saline as is consistent with GCM results (Salinger *et al*. 1996). General circulation models are also suggesting that another possible impact of global warming is an enhanced El Niño state. That is, SSTs in the eastern equatorial Pacific will become warmer. There are also suggestions of more frequent El Niños (as has been recorded over the past 30 years or so). The correlation analysis presented here shows that higher CPUE is recorded in El Niño years and when the local salinity is lower. The implications for industry from this extremely simplistic viewpoint is that global warming may well have beneficial impacts on the spanner crab resource. However the ecosystem in which the fishery operates is extremely complex, and the impact of global warming still requires appropriate in-depth analysis.

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# CHAPTER 5. BENEFITS, OUTCOMES AND APPENDICES

From the outset it was intended that the research undertaken during this project would be of benefit principally to the fishery managers and stock assessment personnel involved in evaluating the status of spanner crab stocks on the Australian east coast (both Queensland and NSW). While the spanner crab fishery was the first of Queensland's fisheries to receive accreditation under the EPBC Act, the Australian Government's then Department of Environment and Heritage identified several issues that needed to be addressed prior to the next review of the fishery. These included (i) "making arrangements with NSW to establish joint monitoring and assessment of the shared stock of spanner crabs with a view to developing future collaborative management arrangements" and (ii) "continuing work to develop a stock assessment model based on sound biological data, and an analysis of the impact of effort creep on the current total allowable commercial catch setting process and management responses".

The lack of knowledge about growth rates, age at entry into the fishery, reproductive lifespan, and the longevity of this important crab species was a continuing source of frustration to the Management Advisory Committees in the two States, and particularly to the Stock Assessment Group of Queensland's CrabMAC, responsible for setting the annual Total Allowable Catch. Although the existing data-based assessment process (using commercial CPUEs and a set of decision rules) had worked very effectively and with industry's support for a number of years, it was not seen as a 'proper' stock assessment process as it was unable to estimate parameters such as virgin biomass and fishing mortality rate. The potential for using length-based assessment models was doubtful because of the apparently high variability in individual growth rates (as judged from previous work on large length-frequency samples which showed no modal separation attributable to age classes).

This project was designed to develop an improved, cross-border arrangement for monitoring the entire spanner crab stock, and to provide a better understanding of critical aspects of the dynamics of the spanner crab population and ecological factors influencing their distribution, abundance and catchability. During the course of the project the fishing industry's key stakeholders were kept informed of developments and project findings by the Principal Investigator at every CrabMAC meeting, either with written research reports or verbal presentations. CrabMAC membership included representatives from the commercial and recreational peak bodies, the processing/marketing sector, as well as conservation, management, and compliance agencies. Co-Investigator Dr J Scandol presented similar periodic project updates to the Trap and Line MAC in NSW. However it is in the context of the Stock Assessment Group that the most direct and influential transfer of research findings has taken place, largely because of the PI's direct involvement as a member of the group and close working relationship with the DPI&F Fishery Manager responsible for the State's crab fisheries. The PI and Co-Investigator together planned and ran the successful Spanner Crab Monitoring Workshop which provided an opportunity for key stakeholders to study and evaluate the results of our project work and contribute to the development of the new cross-border stock monitoring arrangements.

These arrangements, which include a coordinated, collaborative stock monitoring process and an improved method of undertaking stock assessments and translating these into total allowable catch recommendations for the commercial fishery, were accepted and adopted by management, even before the project was completed. This is due principally to the close collaboration established between research, industry and the management agencies and maintained throughout the project.

## 5.1 PLANNED OUTCOMES

One of the highly significant outcomes of the project has been the development of a coordinated approach to the annual population monitoring process. The outputs from the gear and bait comparisons and the NSW-Qld crossover experiments were tabled and exhaustively reviewed during the highly successful Spanner Crab Monitoring Review Workshop. This workshop was attended by fishery managers, researchers, stock assessment personnel, commercial crab fishermen and enforcement/compliance staff from Queensland and NSW, and culminated in the development of a set of recommendations, almost all of which have been accepted, if not already acted upon. Extension of the appropriately-modified Queensland Long-Term Monitoring Surveys into NSW waters, with both States contributing to the organisation and resourcing of the annual field operations, as well as collaborating with the data processing and analyses, has been a major benefit. Of equal importance was the follow-on work (undertaken primarily by the Queensland Crab Scientific Advisory Group and its technical experts) which has led to the formal incorporation of the results of the annual monitoring surveys, along with fishery-dependent data from the compulsory logbook programme, into the stock assessment and TAC-setting process. The use of such fishery-independent information has long been a major recommendation of the Australian Government's Department of Environment, Water, Heritage and the Arts in its response to the State's reporting on the status of the fisheries, and it represents the first instance in Queensland where a seamless and transparent process has been developed for the periodic setting of the TAC in a major fishery. It is expected that this development will be embraced by DEWHA as a major step in the sustainable management of the Australian spanner crab fishery.

In the process of investigating the effects of environmental factors on the abundance, distribution and catchability of spanner crabs, our bottom-water temperature logging programme (which relied entirely on the goodwill and cooperation of a number of commercial crab fishermen), had an unexpected spin-off. We found that it was possible to accurately measure soak-time using the temperature loggers, and this led to a closer examination of factors that might be contributing to uncertainty in our understanding of the dynamics of the fishery. As a result of analyses of the effects of increasing soak-time on CPUE indices from the commercial sector, substantial resources were invested by DPI&F into a survey of the spanner crab fleet to quantify changes in vessel fishing power. The output from this project was then used in a management strategy evaluation process (undertaken by DPI&F quantitative assessment staff from the Southern Fisheries Centre) as part of the revision of the stock assessment process and associate decision rules.

Environmental factors claimed or proposed as potentially confounding our interpretation of both fishery-dependent and fishery-dependent estimates of stock abundance were examined, at least at a preliminary level. We found that temperature does have an effect on CPUE, presumably via changes in catchability, at varying temporal scales. Sea surface temperature can be used as a low-cost proxy for bottom temperature (where the crabs live), and a study of longer-term climatic effects on crab catches revealed not only that catch rates can be predicted from standard climatic indicators, but also that global warming may have a beneficial effect on spanner crab populations along the Australian east coast. This will be good news for fishermen, at a time when all the published information seems to indicate that global warming will have universally disastrous consequences for all marine populations. However it should be pointed out that there may possibly be compensatory adverse effects on the population in the longer term, as a result of ocean acidification. In certain situations wave or swell height can affect catch rates, and our preliminary attempts to quantify this relationship show that adjustment of (particularly) fishery-independent stock abundance estimates may sometimes be necessary.

Reliable estimates of the growth rate of spanner crabs continue to be elusive. A new, purportedly quick and relatively inexpensive method for chemically assaying lipofuscin in crustacean neural tissue was not successful with this species. The fallback ('traditional') method using histofluorometry showed clearly that lipofuscin levels in spanner crab eyestalks are a function of body size, but as yet we have been unable to calibrate the process because of the lack of known-age material. This work has led to an alternative (genetic) method that has much promise, and the development of a separate collaborative project examining telomere length as a proxy for age in a number of invertebrate taxa. Indirect analysis of data from the rearing programme appears to support the hypothesis that spanner crabs grow relatively slowly, which is consistent

with the growth-rate scenarios used in the simulation modelling undertaken during the recent review of the fishery decision rules and TAC-setting process.

These investigations have contributed to increasing our understanding of the dynamics of the Australian spanner crab stock and fishery, and have already helped significantly in reducing the uncertainty in assessing the status of our *Ranina ranina* resources.

## 5.2 FURTHER DEVELOPMENT

Further development of the results of the research reported here would be advisable in the following areas:

- Continuation of efforts to obtain a more robust estimate of age. With the establishment of the telomeres project this work is continuing, and an indication of whether the new genetic method can be used to age spanner crabs effectively will become apparent within the next 12-18 months. If it is successful, there will still be a need to calibrate the technique, and the availability of known-age material for this species is very limited. One approach to this might be using genetic markers to identify individual crabs, in an area closed to fishing, which can be sampled periodically (and non-lethally) over a period of years. Another might be to undertake a one-off large-scale sampling of the natural population and look for frequency-distribution modes in telomere length which may be interpretable as different age-classes. If on the other hand the telomere method does not appear successful, the histofluorometric approach could be re-visited now that we have identified the appropriate tissues and developed a set of standard protocols. This would again focus on a large-sample analysis for lipofuscin-frequency concentration modes which was unsuccessful in the current project mainly, we suspect, because of an insufficient sample size.

- Opportunities are available for more detailed investigations of the effects of some of the environmental factors that have been shown to influence stock abundance indicators. Of particular importance is the seabed characterisation work, which should be able to used to better define the limits of the species' distribution. Our seabed mapping work with the Sea Scan system was effective to a large extent, but further work needs to be done to determine the causes of data drop-out. More ground-truthing is essential to determine why (for example) the differences in crab abundance are so marked between areas where the bottom types appear similar. The Sea Scan system represents a very cost-effective way of collecting basic seabed data, but additional work is needed in the areas of calibration and data filtering and processing. The potentially adverse cumulative effects of ocean acidification on crab populations in general has been raised by an anonymous reviewer. This is an area that may benefit from experimental physiological research on crabs under controlled pH conditions, as acidification due to increasing $CO_2$ emissions is posited as a serious threat to the marine environment.

- It is recommended that the Crab Scientific Advisory Group should examine ways in which the results of the climate, temperature and wave-height analyses can be integrated into the stock assessment process in order to adjust (where necessary) stock abundance indicators.

## 5.3 CONCLUSIONS AND RECOMMENDATIONS

The project's three objectives were related to (i) age determination, (ii) developing an integrated monitoring and assessment process for the Australian fishery, and (ii) quantifying the effects of environmental variables on catch rates. The various outcomes and the extent to which we met the objectives are summarised as follows:

- Determine the age at which spanner crabs recruit to the fishery.

  Age and growth rates are still uncertain. The limited information able to be used in an indirect analysis of growth does not support a change to the current hypothesis that spanner crabs grow rather slowly in comparison with other exploited crab species in the same region. Although this final objective was not met, the project did successfully develop a suite of protocols and methods appropriate for eyestalk tissue dissection, preservation, decalcification, histological sectioning, and fluorometric analysis, after considerable experimentation and the investment of significant resources. This groundwork provides the basis for any follow-up work that may be needed if the main alternative method (telomere length analysis) proves less successful than anticipated.

- Develop a common methodology for monitoring and assessing the Australian spanner crab stock.

  This objective was met in its entirety, an extremely successful outcome having been obtained. An integrated, inter-State monitoring programme has been established, and has already commenced. In addition, a procedure for integrating the fishery-independent (survey) data with the fishery-dependent (logbook) data has been developed and accepted by management and the industry. It has been applied already in one biennial assessment and to set the spanner crab commercial TAC for 2008-10.

- Investigate sources of variability in apparent population density.

  The effects of environmental variables on catch rates or apparent population density have been investigated successfully, if perhaps at only a preliminary level. Factors such as SOI/ENSO climate indicators, water temperature, wave height, and sea-floor structure and composition have all been shown as a result of these investigations to have a significant effect on spanner crab catch rates. Some of these effects deserve more rigorous examination – particularly the seabed indices, where unidentified factors also contributed significantly to variation in abundance. While recognising that this work has barely scratched the surface in terms of our understanding of the way environmental drivers influence the abundance and catchability of spanner crabs, we contend that the work presented here will substantially help reduce uncertainty in the interpretation of estimates of key population abundance indicators.

## 5.4 REFERENCES

Relevant references are included at the end of each chapter, so are not repeated here.

## 5.5 APPENDIX 1: INTELLECTUAL PROPERTY

The information generated by this research is in the public domain, and not subject to intellectual property considerations.

## 5.6 APPENDIX 2: STAFF ENGAGED ON THE PROJECT

Dr IW Brown (DPI&F; Principal Investigator)

Dr J Scandol (Principal Stock Assessment Scientist, NSW DPI Fisheries, NSW)

Mr K Krusic-Golub (Central Ageing Facility, Queenscliff, Victoria)

Dr D Mayer (Principal Biometrician, DPI&F)

Dr A Williams (Senior Climatologist, Department of Natural Resources & Water)

Mr M Campbell (Fisheries Technician, DPI&F)

Mr M McLennan (Fisheries Technician, DPI&F)

Mr B Davidson (Skipper RV *Tom Marshall*, DPI&F)

Mr S Kondylas (Fisheries Technician, DPI&F)

Mr S Maberley (Skipper RV *Tom Marshall*, DPI&F)

Mr A Treloar (Chemist, DPI&F)

## Appendix 3

# Dissection techniques for the removal of the brain in the Spanner crab, *Ranina ranina*

Kyne Krusic-Golub

April 2004

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http://www.dpi.vic.gov.au/


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ISBN

# Executive Summary

This manual was created to facilitate the removal of the brain and eyestalks from the spanner crab, *Ranina ranina*. It was prepared primarily as a first step in the completion of the Task 3 of the FRDC project 2003/046. The step by step manual will allow for the appropriate dissection techniques for crustacean brain removal to be transferred to different organisations. This will allow staff at the Queensland Department of Primary Industries to successfully extract and preserve the brain and eyestalks from spanner crab samples collected for this project.

This manual is only a guide to the dissection techniques and the methodology provided may be adapted and refined to facilitate ease of dissection.

**1)** Rinse head under tap. Using the combination of tweezers and the water pressure remove the loose digestive glands. The head and the associated digestive glands (arrows) and the resultant clean head can be seen in Figures 1 and 2. Herein, all cutting should be made with a snipping action to ensure that the connective nerves to the brain are not stretched and ultimately lead to damage to the brain.

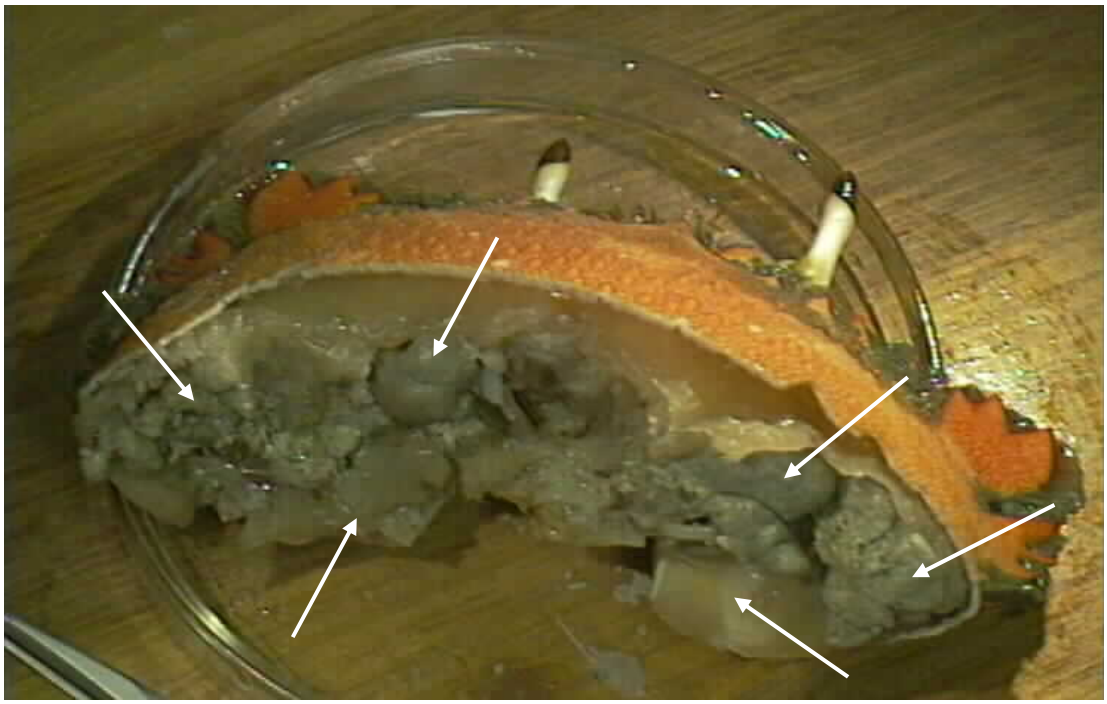

Figure 1. Anterior section of the spanner crab showing digestive glands (arrows).

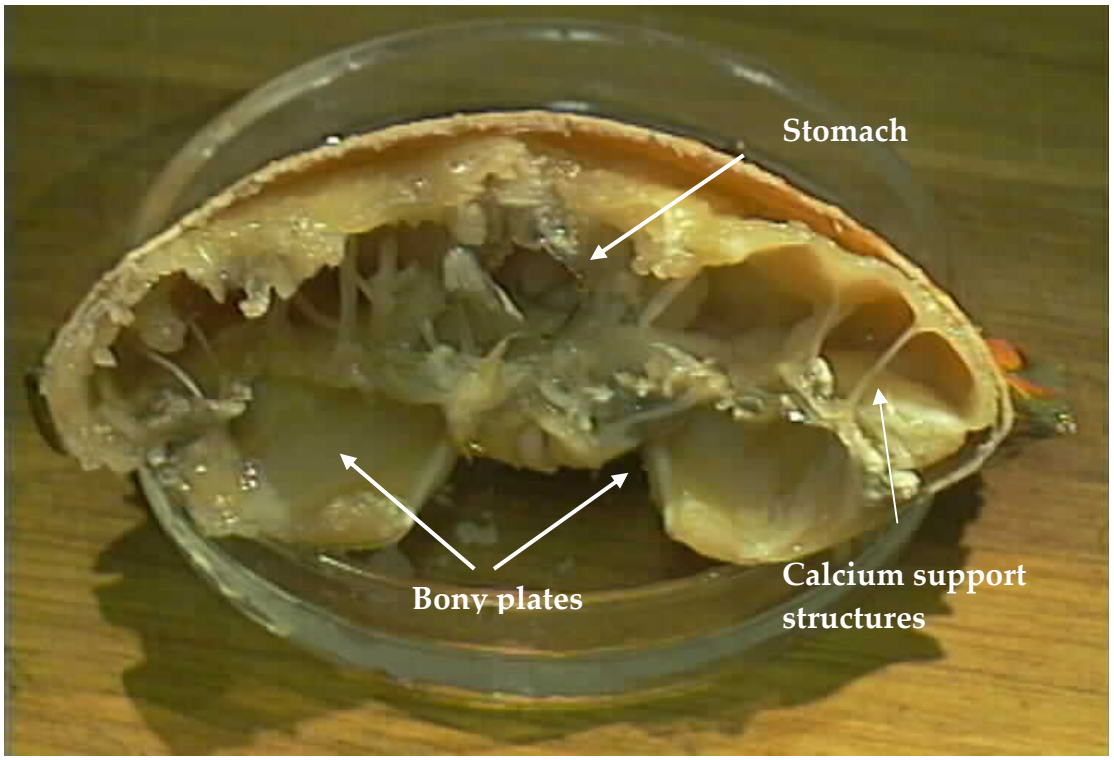


Figure 2. The anterior portion of the spanner crab after the removal of the loose digestive glands and tissue. Arrows indicate the position of the stomach, calcium support structures and hard calcium plates (removed at a later stage).

**2)** Cut away the surrounding shell and tissue leaving the area shown in the Figures 3 and 4. Ensure that the tissue and calcium support structures are cut away with sharp scissors to ensure that the nerves are not stretched. The remaining carapace can be cut with heavy scissors. Remove the hard plates located either side of the mouth with heavy scissors. (Inset Figure 4)

Figure 3. Dorsal-anterior view of the crab indicating area required for further dissection

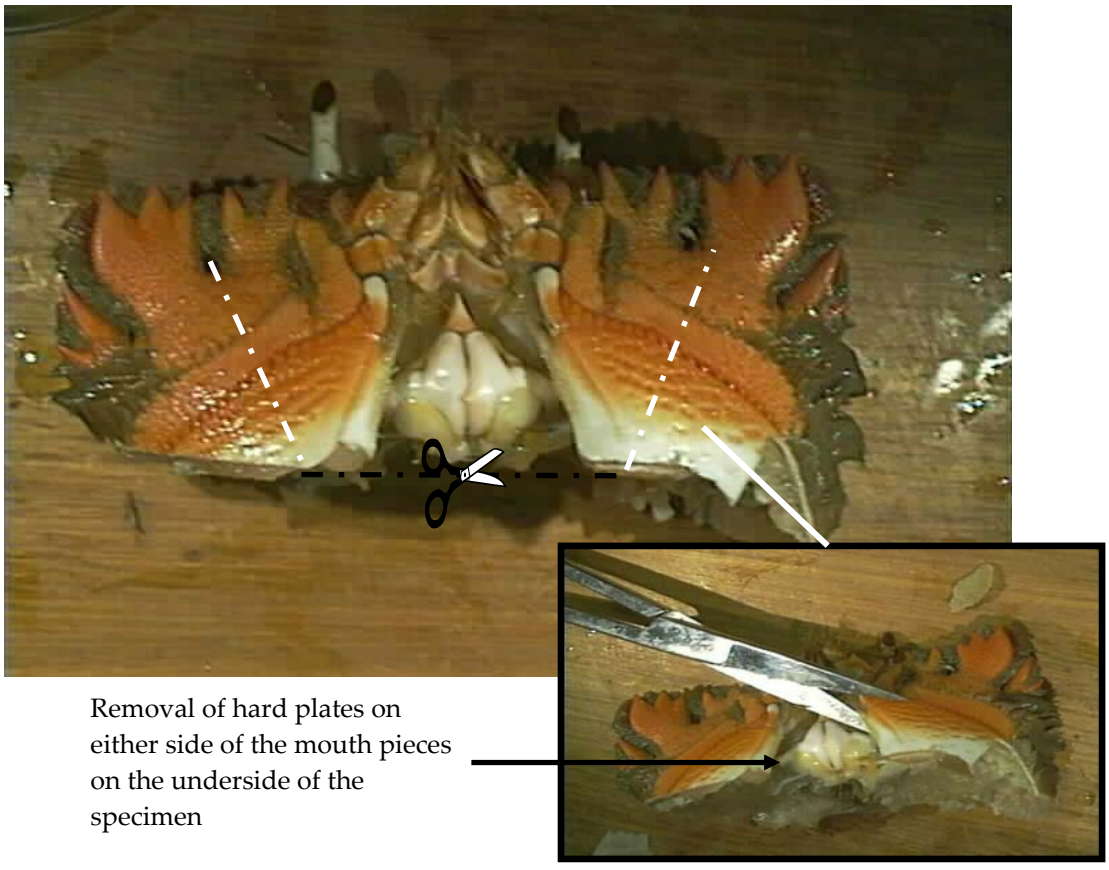


Figure 4. Ventral-anterior view of crab indicating the area required for further dissection.

**3)** Remove the mouthpieces and 1st and 2nd antennae as close to the body as possible (Figure 5.). Cut the eyestalks as close to their base as possible, remove and store in formalin for future dissection. The brain lies directly above/inside the protective area (diamond piece of carapace) indicated in figure 6.

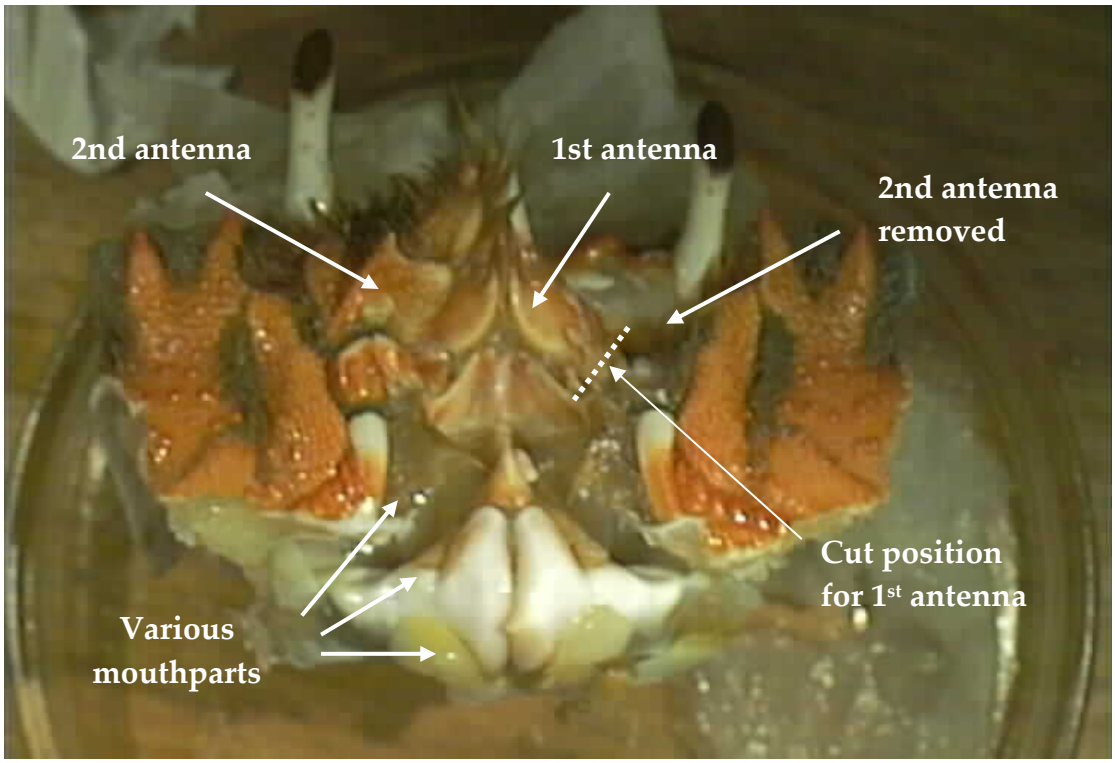


Figure 5. Ventral anterior region indicting the mouthparts and antennae.

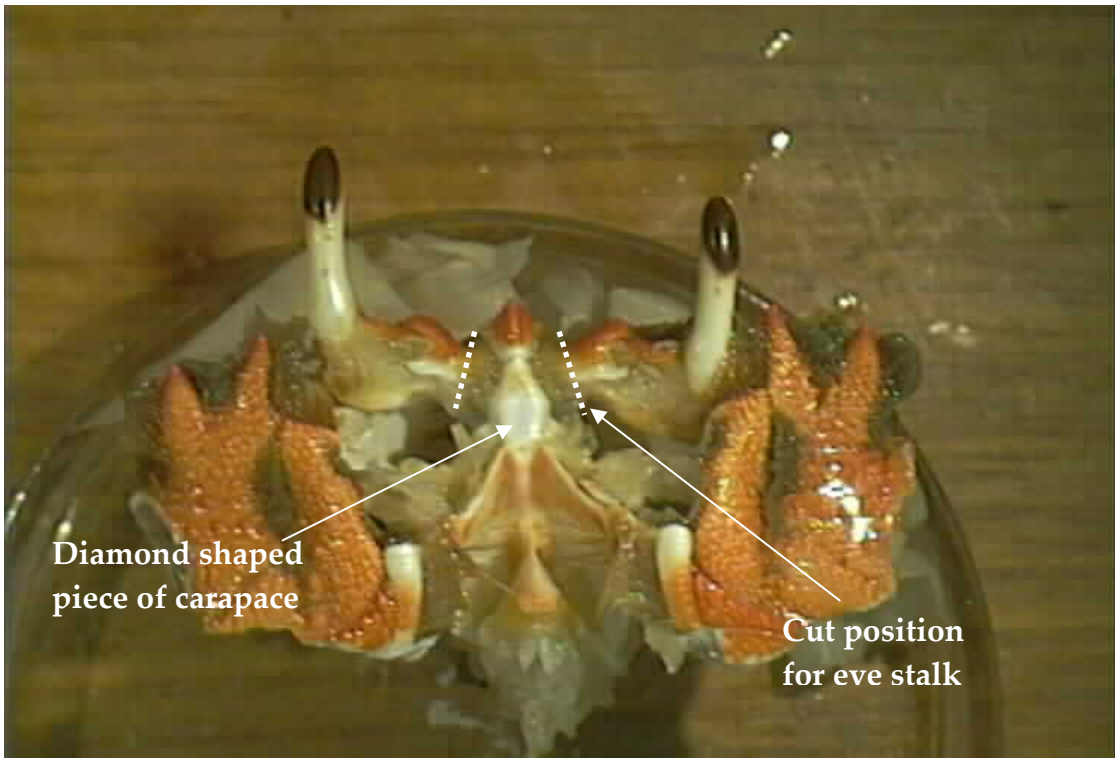


Figure 6. Ventral anterior region of the crab head after the removal of the antenna and mouthpieces showing approximate position of brain (diamond shaped piece of carapace).

**4)** Carefully cut and remove the tissue around the stomach area (Figure 7) and expose the oesophageal commissures. These run vertically from the brain to the posterior of the carapace and can be used to locate the position of the brain within the carapace.
**Note**: Constant washing with water in each step will greatly facilitate the dissection process.

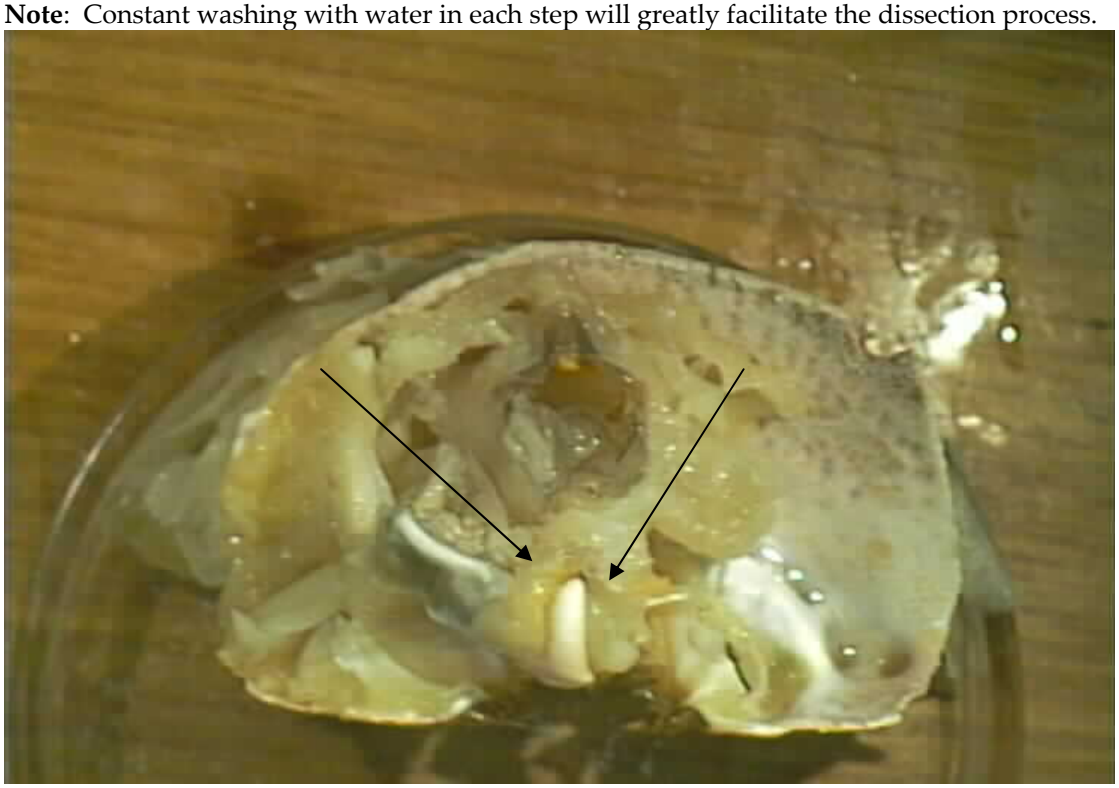

Figure 7. The tissue and remaining intestanal mass on the right side has been dissected away from the cavity. Arrows indicate the appropriate position of the oesophageal connective commisures.

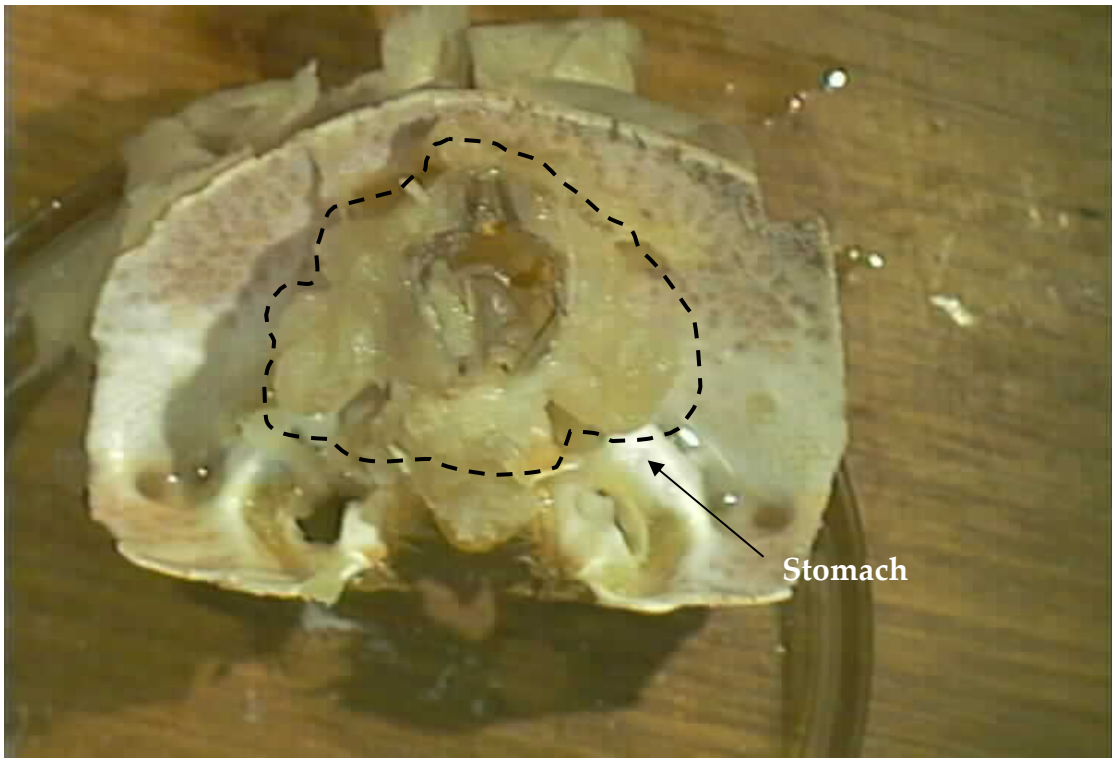


Figure 8. The remaining stomach after the removal of the surrounding tissue and associated intestinal mass.

**5)** Remove the stomach and any remaining calcium support structures carefully with sharp scissors. To make removal of the stomach easier, invert the specimen and then make the appropriate cuts. The stomach will only be attached by the mouth tissue and will hang away from the carapace. Cut through the connective tissue and remove the stomach ensuring that the oesophageal connective commisures are not damaged (Figure 9). Remove the remaining tissue carefully with fine tweezers.

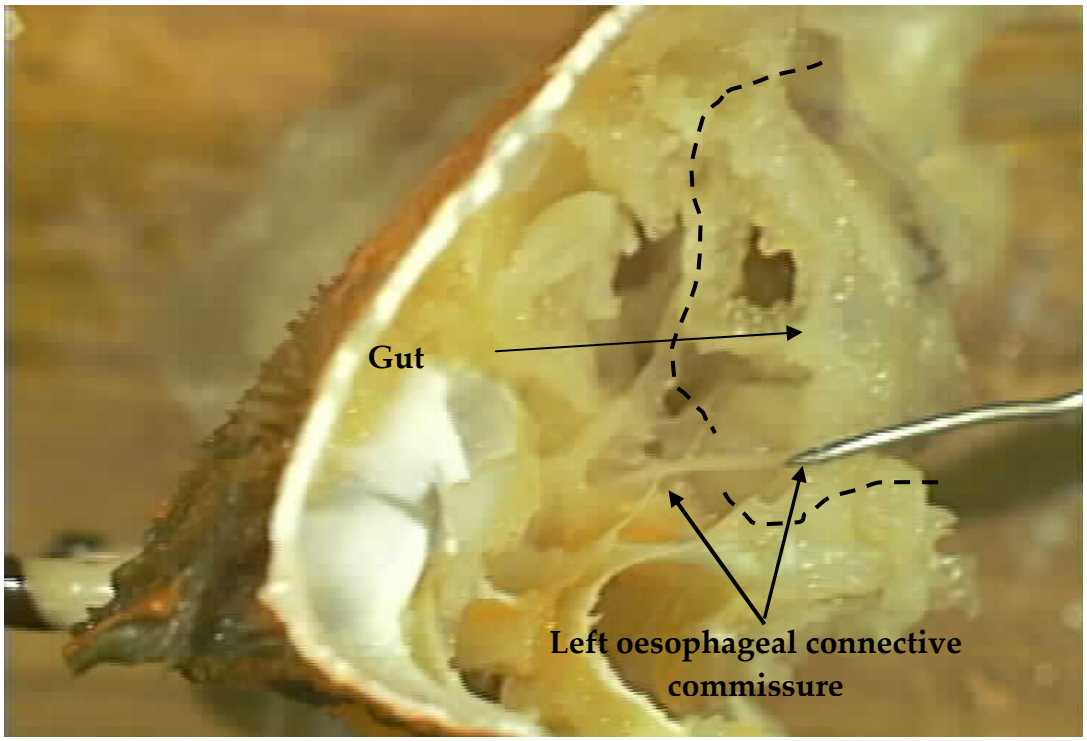


Figure 9. Inner region of the anterior section of the head indicating the stomach prior to removal and the left commisures (nerves).

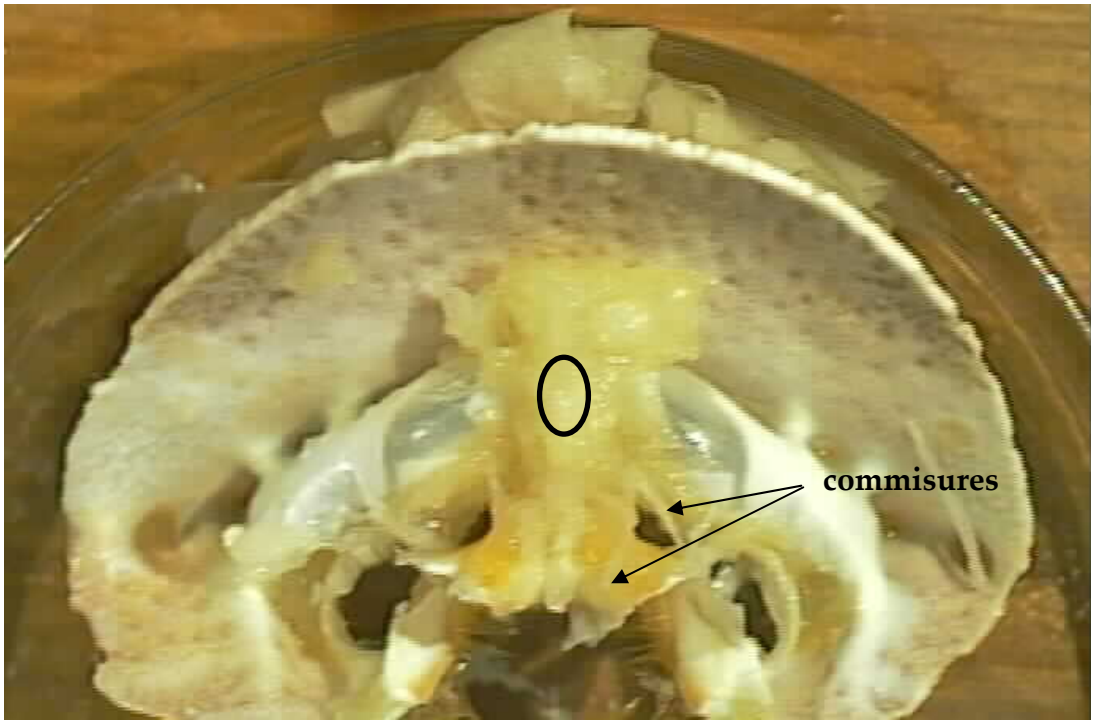

Figure 10. Anterior section after the removal of the stomach. The circle represents the approximate position of the brain.

**6)** After removal of the majority of tissue around the region of interest, make the cuts indicated below on the right side of the crab head (Figure 11). Repeat for the left side.

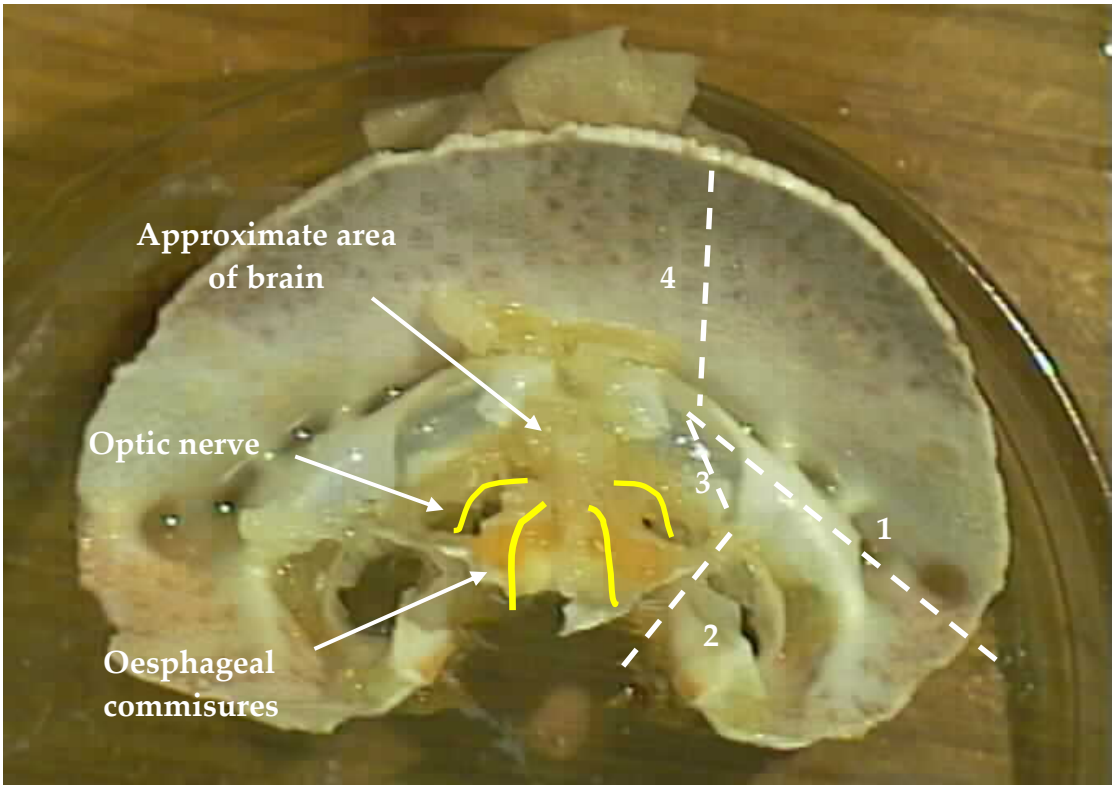


Figure 11. Anterior region of the crab after the tissue and stomach has been removed. The cuts required are shown with dashed lines. Yellow lines highlight the optic nerve and oesophageal connective commisures.

**7)** Remove the dorsal carapace by cutting the attachment between the dorsal carapace and the eye sockets. (Figure 12). Care must be taken not to cut any surrounding tissue as the brain is situated very close to this area. Carefully remove the shell around the eye socket to enable access to the brain mass (Figure 13).

Figure 12. Removal of the remaining dorsal carapace.

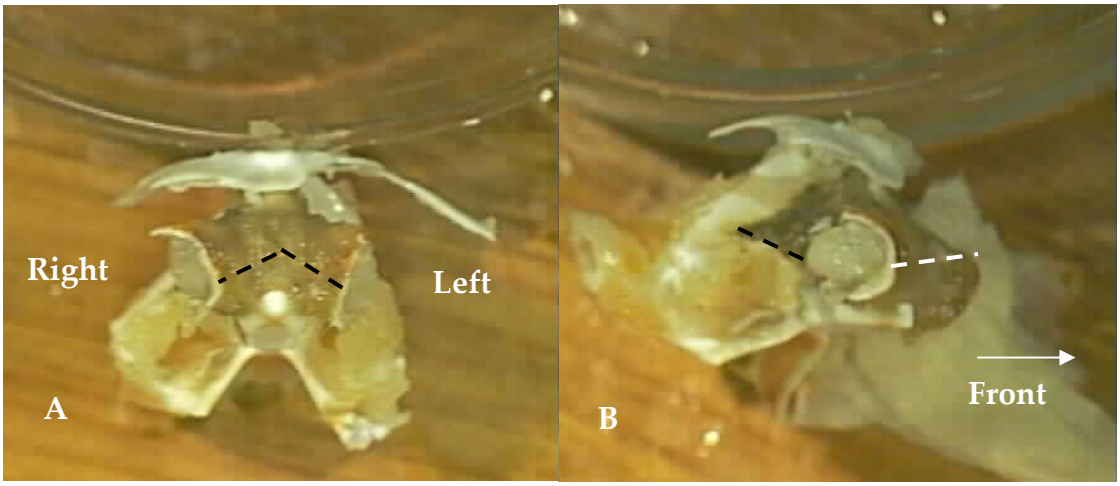


Figure 13. Remaining carapace and brain mass after the dorsal carapace has been removed. Cut positions from the front (A) and the side (B) are indicated with broken lines.

**8)** The brain is located under two relatively thick strands of tissue that protect the brain. These are not connected to the brain and can be removed by making a posterior cut and gently peeling away from the brain mass. Care must be taken to prevent catching the commissures when removing the protective tissue

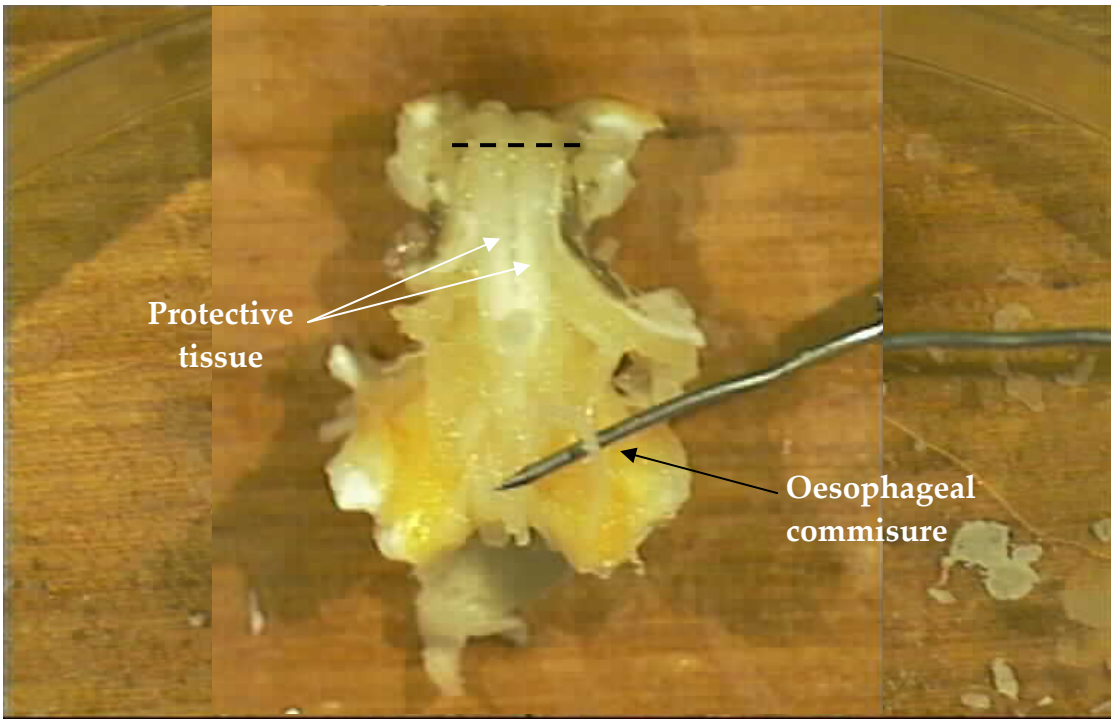


Figure 14. Region of interest showing the protective tissue over the brain.

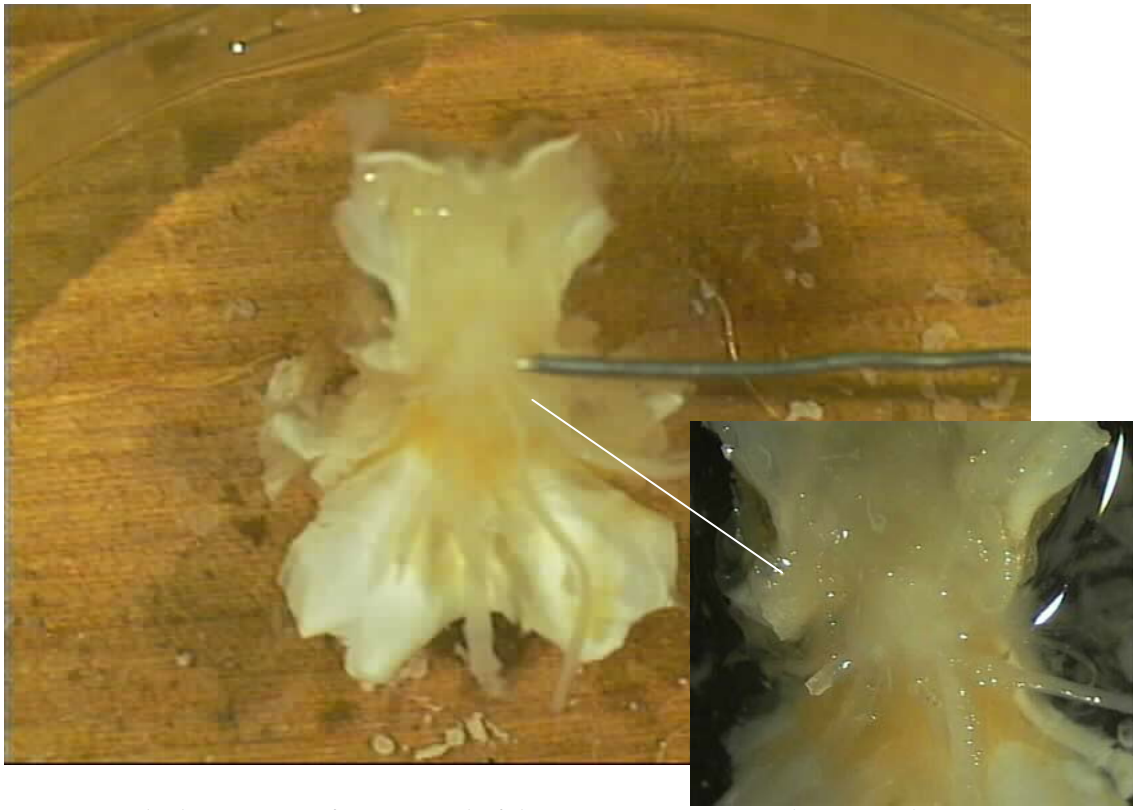

Figure 15. The brain mass after removal of the connective tissue. The insert shows a magnified view of the brain and surrounding tissue.

**9)** Extract the brain mass and surrounding tissue away from the carapace using curved scissors. Carefully cut underneath and around the brain ensuring that the cuts are made against the carapace. Once removed from the carapace the tissue can be gently teased away from the brain with tweezers and a fine probe. The brain is now ready for further fixing.

Figure 16. Brain and surrounding tissue after removal from carapace

Figure 17. The end product after the remaining tissue has been cleaned away from the brain.

## APPENDIX 4. LIPOFUSCIN QUANTIFICATION MACRO

The purpose of this macro is to quantify the lipofuscin granule area in sections of the olfactory lobe of crustacea. The images area taken at 400x and illuminated using epifluorescence with an I3 filter block barrier / excitation filter. The granule sizes in each image, the negative space (where the microtomy has teased apart the tissue) and the area of the olfactory lobe are quantified using these macro's. These data are recorded.

The control and use of the two macros used for the quantification are described in this document. Overviews of these macro's are described below:

The user opens an image of the lipofuscin sample.
1. The image of the sample is zoomed to 50% of the original size.
2. The image is converted from 24-bit color to 8-bit grey.
3. The user then finds the threshold values using the multiple threshold dialog.
4. The user then uses these threshold values to determine class membership.

The second macro Lipofuscin_macro when run then:
5. Traces the area of the olfactory lobe.

The classification is automated and the data is transferred to Excel.
6. The user examine the data in Excel to check transfer and make additional notes.

A more detailed description of operation is given in the following images and text.

These macros are written to run in the Optimas 6.0 environment. Excel must be open to a blank workbook before analysis.

```
Macros

Lipofuscin1 Macro

// Gets the volume of the lipofuscin volume fraction from
// the olfactory lobes of crustacea
//
// Central Ageing Facility November 2002
// Author : Central Ageing Facility
// Keywords : Lipofuscin, volume fraction, automatic classification


Lipofuscin 2 macro

// Use this macro first to determine thresholds

// Open config file lipofuscin
OpenConfiguration ("D:\\Optimas 6.5\\Config\\Lipofuscin.cfg");

// Convert image to 8 bit grey from 24 bit and zoom 50%
       SetColorMode (0 : 1 : 8 : 1 : 1 : 3);
       ZoomFactor = 0;

//Open threshold dialog and the object class dialog
RunMacro ("D:/OPTIMAS 6.5/macsrc/colormac/multhrsh.mac");
ObjectClass ();

/ Gets the volume of the lipofuscin volume fraction from
// the olfactory lobes of crustacea
//
// Central Ageing Facility November 2002
// Author : Simon Robertson
// Keywords : Lipofuscin, volume fraction, automatic classification
```

```
// Use this macro second

// User create area which is then converted to ROI
   SelectFullScreen (optSelectFullViewedArea);
   hAreaID = CreateArea();   /* have the user create a area */
   SetExport(ArPoints,1,TRUE);  /* ArPoints "To DDE" */
   Extract();      /* get ArPoints */
   AreaOfROI=ArArea;
   ImageMask (1, hAreaID, 0);
/*
//  Set array for lookup tables
   Upramp = (0..255);
   RedLUT[0..255] = Upramp;
   RedLUT[100..255] = 0;
   GreenLUT[0..255] = Upramp;
   GreenLUT[175..255] = 255; // highlight gray values from 200-255 in CYAN
// GreenLut[0..15]=00;
   BlueLUT[0..255] = Upramp;
   BlueLUT[75..255] = 255;
// Bluelut[0..45]=0;
   delete (Upramp);


   OutputLUT ("IntensityMap", 0.0);
*/

// Create areas and display
   CreateEmptyClass( "Space", 2, 'A');
   CreateEmptyClass( "Lipofuscin", 2, 'B');
   CreateArea(,,TRUE);
   UpdateAllClassData();
   A=Ar_Lipofuscin_Tally_B;
   B=Ar_Space_Tally_A;
   SetExport(mArArea,1,True);
   MultipleExtract(True);

// get the sum of the lipofuscin and negative space
   TotalCount=Ar_Lipofuscin_Tally_B+Ar_Space_Tally_A;
   SpaceSum=Sum( mArArea[Ar_Space_Select_A]);
   Liposum=Sum(mArArea[Ar_Lipofuscin_Select_B]);

// get a 1-D vector and assign the lipofuscin measurements
// the vector length is used to determine the size of the loop in the export to excel
   LipoGranules=mArArea[Ar_Lipofuscin_Select_B];
   hLen=VectorLength(LipoGranules);

/* Used in checking macro
Macromessage(Liposum, SpaceSum,AreaOfROI);
Macromessage("Lipofuscin Volume fraction is ",liposum /(AreaOfRoi-SpaceSum)*100, " %");
*/

// Now calculate the volume fraction as a percentage
   VolumeFraction=liposum /(AreaOfRoi-SpaceSum)*100;

// Now the export to Excel
// The routine uses two sheets, the first sheet takes the summary info
// while the second sheet takes a maximum of the first

// Set The DHT's if loop object exists, if not establish the row counter for excel
if(!IsObject("loop")) {
   // Establish a loop Counter
   GLOBAL INTEGER loop=2;
}

// Initiate conversation
   hChanSheet1=DDEInitiate("Excel","Sheet1");
   hChanSheet2=DDEInitiate("Excel","Sheet2");
```

```
// Summary info headers for sheet one - poked in each time through
   DDEPoke (hChanSheet1,"R1C1","Image");
   DDEPoke (hChanSheet1,"R1C2","Area of ROI");
   DDEPoke (hChanSheet1,"R1C3","Area of lipofuscin");
   DDEPoke (hChanSheet1,"R1C4","Area of Negative space");
   DDEPoke (hChanSheet1,"R1C5","Lipofusin volume fraction");

// poke the name of the image into excel eachj export
   DDEPoke (hChanSheet1,"R":totext(loop):"C1",Imagefile);

// now the data to sheet one - area of roi, total lipofuscin, total negative space and
lipofuscin volume fraction
   DDEPoke (hChanSheet1,"R":totext(loop):"C2",AreaOfRoi);
   DDEPoke (hChanSheet1,"R":totext(loop):"C3",Liposum);
   DDEPoke (hChanSheet1,"R":totext(loop):"C4",SpaceSum);
   DDEPoke (hChanSheet1,"R":totext(loop):"C5",VolumeFraction);


// loop=2; // used in macro dev.
// sheet 2 - lipofuscin granule sizes

// poke the name of the image into excel each export
   DDEPoke (hChanSheet2,"R1C1","Image");
   DDEPoke (hChanSheet2,"R":totext(loop):"C1",Imagefile);
   DDEPoke (hChanSheet2,"R1C2","Number of granules");
   DDEPoke (hChanSheet2,"R" : totext(loop) :"C2", hLen );

// now the headers for the sheet Max of 250 granules per image
if(loop==2){
   For (i=1; i <= 250; i++){
      DDEPoke (hChanSheet2,"R1" : "C" : totext(i+2),"G_" : totext(i));
      }
   }

// now the lipofuscin granules (if less than 250 granules)
if (hLen <=250){
   For(i=0; i <  hLen; i++){
      DDEPoke (hChanSheet2,"R" :totext(loop) : "C" : totext(i+3), lipogranules[i]);
      }
   }

// now the lipofuscin granules (if more than 250 granules)
if(hLen > 250){

   For(i=0; i <  250; i++){
      DDEPoke (hChanSheet2,"R" :totext(loop) : "C" : totext(i+3), lipogranules[i]);

      }
   }

// Terminate conversation channels with each sheet in reverse of opening
   DDETerminate(hChanSheet2);
   DDETerminate(hChanSheet1);

// use this as a step from cursor to delete the global var loop
// delete(loop);

// use this as a step to decrement the line counter
// loop--;

// increment the line counter
Loop++;

// Clear screen markers and select full screen roi
ClearScreen();
SelectFullScreen(optSelectFullViewedArea);
```

## APPENDIX 5. MATERIAL COLLECTED FOR TELOMERE ANALYSIS.

Capture location of specimens RR5–RR14 incl. was offshore between Caloundra and Mooloolaba. Storage condition codes are (a) frozen at -20° C; (b) chilled to euthanase, then stored in 70% ethanol; (c) 70% ethanol.

| Sample Number | Carapace | | Tissue type* | Capture date | Storage conditions |
|---|---|---|---|---|---|
| | length (mm) | width (mm) | | | |
| RR1 | 110 | | m, g, h, s | unknown | a |
| RR2 | 115 | | m, g, h, s | unknown | a |
| RR3 | 100 | | m, g, h, s | unknown | a |
| RR4 | 100 | | m, g, h, s | unknown | a |
| RR5 | 104 | 87 | m, h, s, g, p, t | 13-Dec-2004 | a |
| RR6 | 102 | 89 | m, h, s, g, p, t | 13-Dec-2004 | a |
| RR7 | 107 | 90 | m, h, s, g, p, t | 13-Dec-2004 | a |
| RR8 | 107 | 91 | m, h, s, g, p, t | 13-Dec-2004 | a |
| RR9 | 76 | 63 | m, h, s, g, p, t | 13-Dec-2004 | b |
| RR10 | 73 | 60 | m, h, s, g, p, t | 13-Dec-2004 | b |
| RR11 | 73 | 62 | m, h, s, g, p, t | 13-Dec-2004 | b |
| RR12 | 115 | | m, h, s, g, p, t, cm | 13-Dec-2004 | b |
| RR13 | 120 | | m, h, s, g, p, t, cm | 13-Dec-2004 | b |
| RR14 | 110 | | m, h, s, g, p, t, cm | 13-Dec-2004 | b |
| RR15 | 10 | | whole megalopa: tail and claw | unknown | c |
| RR16 | 11 | | whole megalopa: tail and claw | unknown | c |
| RR17 | 10 | | whole megalopa: tail and claw | unknown | c |
| RR18 | 11 | | whole megalopa: tail and claw | unknown | c |
| RR19 | 10 | | whole megalopa: tail and claw | unknown | c |
| RR20 | 10 | | whole megalopa: tail and claw | unknown | c |
| RR21 | 9 | | whole megalopa: tail and claw | unknown | c |
| RR22 | 22 | | c6 crablet, may be exuvium only: tail and claw; gill | unknown | c |
| RR23 | 17 | | c4 crablet: tail and claw | unknown | c |
| RR24 | 19 | | c6 crablet: tail and claw; g; s; m | unknown | c |
| RR25 | 16 | | c6 crablet: tail and claw; g | unknown | c |
| RR26 | n.d. due to | 65 | g, m | 1-Feb-2005 | a |
| RR27 | n.d. due to | 58 | g, m | 1-Feb-2005 | a |
| RR28 | 47 | 38 | g, m | 1-Feb-2005 | a |
| RR29 | 40 | 34 | g, m | 1-Feb-2005 | a |
| RR30 | 44 | 36 | g, m | 1-Feb-2005 | a |
| RR31 | 13 | | whole megalopa, 2 weeks post-settlement | 19-Feb-2005 | a |
| RR32 | 14 | | whole megalopa, 2 weeks post-settlement | 19-Feb-2005 | a |
| RR33 | 8 | | whole megalopa | 1-Mar-2005 | a |
| RR34 | 8 | | whole megalopa | 1-Mar-2005 | a |
| RR35 | 8 | | whole megalopa | 1-Mar-2005 | a |
| RR36 | 8 | | whole megalopa | 1-Mar-2005 | a |
| RR37 | 8 | | whole megalopa | 1-Mar-2005 | a |
| RR38 | 8 | | whole megalopa | 1-Mar-2005 | a |
| RR39 | 8 | | whole megalopa | 1-Mar-2005 | a |
| RR40 | 8 | | whole megalopa | 1-Mar-2005 | a |
| RR41 | 8 | | whole megalopa | 1-Mar-2005 | a |
| RR42 | 8 | | whole megalopa | 1-Mar-2005 | a |
| RR43 | 8 | | whole megalopa | 1-Mar-2005 | a |
| RR44 | 8 | | whole megalopa | 1-Mar-2005 | a |
| RR45 | 8 | | whole megalopa | 1-Mar-2005 | a |
| RR46 | 8 | | whole megalopa | 1-Mar-2005 | a |
| RR47 | 8 | | whole megalopa | 1-Mar-2005 | a |
| RR48 | 8 | | whole megalopa | 1/3/05 | a |
| RR49 | 8 | | whole megalopa | 1-Mar-2005 | a |
| RR50 | 8 | | whole megalopa | 1-Mar-2005 | a |
| RR51 | 8 | | whole megalopa | 1-Mar-2005 | a |
| RR52 | 8 | | whole megalopa | 1-Mar-2005 | a |
| RR53 | 8 | | whole megalopa | 1-Mar-2005 | a |
| RR54 | 8 | | whole megalopa | 1-Mar-2005 | a |
| RR55 | 8 | | whole megalopa | 1-Mar-2005 | a |
| RR56 | 8 | | whole megalopa | 1-Mar-2005 | a |
| RR57 | 8 | | whole megalopa | 1-Mar-2005 | a |
| RR58 | 8 | | whole megalopa | 1-Mar-2005 | a |

*m: muscle; g: gill; h: hepatopancreas; s: shell (carapace); t: tail; cm: claw muscle