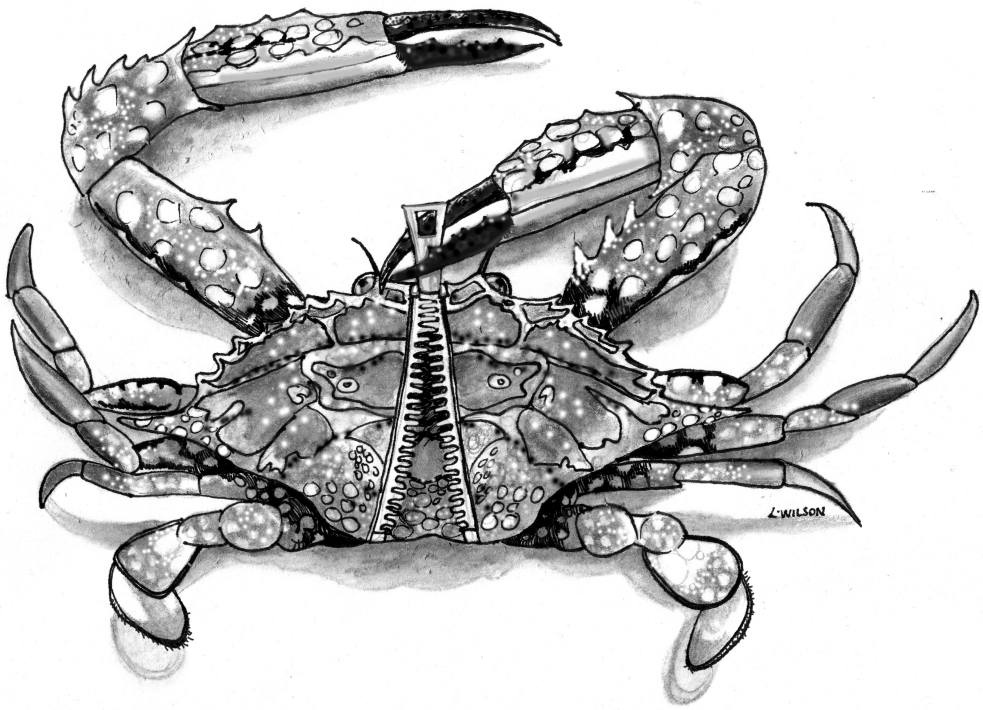
L·WILSON

The Bioaccumulation of Metals and the Induction of Moulting in the Blue Swimmer Crab, *Portunus pelagicus* (Linnaeus 1766)

Ross G. McPherson

This Thesis is all original work and should be cited as this document, unless published in journal format.

Thesis submitted for the degree of Doctor of Philosophy at the University of Technology, Sydney.

University of Technology, Sydney

June 2002

Certificate

I certify that this thesis has not already been submitted for any degree and is not being submitted as part of candidature for any other degree.

I also certify that this thesis has been written by me and that any help that I have received in preparing this thesis, and all sources used, have been acknowledged in this thesis.

Signature of Candidate.

Dedication

This work is dedicated to the
memory of my father, John McPherson

"You care for nothing but shooting, dogs,
and rat catching, and will be a disgrace
to yourself and your family."

Darwin's Father

Acknowledgements

This thesis is dedicated to the memory of my late father, John McPherson, who taught me the value of hard work and instilled in me a work ethic which has enabled me to achieve a great deal in my life to date. He is sorely missed by all his family and friends.

This thesis has been completed on a part time study basis, as have all of my previous studies. The interaction between the research published within these pages and my work colleagues through my career so far has been extensive and I apologise now to anyone who I might have inadvertently omitted.

At the start of my studies I was working at Sydney Technical College, in the School of Biological Sciences, people supporting me during this period included, George Hassapladakis, Barry Cole, Malcolm Bruce, Peter Klein, Trevor Kyriokou, Irene Tchubaski and the late Minni Ralph. All contributing to my education in the field of biochemical instrumentation and supplying helpful hints for the conduct of this work. Also while I was working at Sydney Technical College, the Staff at Sydney Aquarium assisted with the provision of aquarium space during the earlier experiments. Mr. John West, from the Taronga Zoo aquarium, taught me the practical application of aquarium science without which much of this work would not have been possible.

During a secondment from Sydney Technical College to the Australian Institute of Marine Science in Townsville I was fortunate enough to meet and work for Dr Randolph Olson, who is an internationally recognised larval ecologist. While working with Dr Olson on the Crown of Thorns Starfish I learnt a valuable lesson, that it was possible to do science to an international standard and at the same time enjoy yourself immensely. A period of work with Randolph at the University of New Hampshire, in the USA, working on the larval life cycle of the American Lobster, was one of the great highlights of my working life. I now count him as a good friend and credit him with my decision to make a career for myself in marine science.

There was then a period of employment at the University of Technology, Sydney, in the Biological Sciences Department. This was under the direction of my supervisor for this thesis, Associate Professor Ken Brown. I have now known Ken since 1982 and acknowledge him as both a friend and a mentor who has guided my career and my studies in such a way as to inspire my love of science.

Mr. Brett Nudd, Mrs. Joanne Scarsbrick and Mr. Patrick Wong also assisted me extensively during this period with both field and laboratory work. Patrick especially helped with the design and construction of aquaria and the monitoring of the moulting experiments. I have worked with Patrick on a number of occasions since and my respect for him only increases through time.

Thanks also to Mr. Terry Haake who is a professional fisherman on the Hawkesbury River and assisted on a number of occasions with the collection of crabs. I must also thank the staff at Windybanks Bait, Mt Colah, who helped with the supply of food for my charges. Thank you also to Michael Hall from Hallprint Australia for assistance with tag design and supply.

Employment at the New South Wales Environment Protection Authority again taught me a great deal about the conduct of environmental research. I gratefully acknowledge the assistance afforded by Mr. Graham Sherwin, Mr. Rob Smith and Dr Danny Roberts.

Staff at the University of Technology, Sydney have also assisted in a variety of ways and I sincerely thank, Narelle Richardson, Peter Jones, Dr David Morrison, Dr Peter Ralph, Dr David Booth and all the other staff in Environmental Science for their assistance over the years.

I also need to thank my present colleagues at Hornsby Shire Council who have assisted in the full range of endeavors from interested support and encouragement to assistance with

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Finally and most importantly I wish to thank my family and my wife’s family, especially my wife Jane who has selflessly encouraged me to complete these studies for my personal development. I must also acknowledge the support of my brother and sister, Andrew and Bronwyn, and their extended families, and my mother Rosemary. My brother was also instrumental in the production of the larval video in the Appendix. Also thanks to Glyne and Patricia Morgan for the use of boats and motors and general encouragement over the years. Without this family support in many different ways this thesis could never have been completed.

Abstract

This thesis presents work outlining the development of the Blue Swimmer Crab (*Portunus pelagicus*) as a biomonitor of available cadmium and also presents a proposed method for the production of "soft-shelled crabs" utilising *P. pelagicus*.

The first experiments established that the Blue Swimmer Crab accumulates only cadmium in the hepatopancreas after being presented elevated levels of the metals Cd, Cu, Zn, As, Fe and Al via a food source, the mussel *Trychomya hirsuta*. Over eight weeks, crabs were fed a controlled diet to determine the accumulation of metals. Significant ($p = 0.05$) Cd accumulation was detected after four weeks of feeding. Food mussels were sourced from either "contaminated" or "uncontaminated" sites. Mussels from uncontaminated sites had an average Cd level of 0.07 µg/g wet weight and contaminated mussels had an average of 5.2 µg/g wet weight.

Having established that the crab accumulated cadmium it was then necessary to determine the animal's residency in a given area for it to reflect the available cadmium associated with a particular spatial scale. To do this it was necessary to test and develop a suitable tag for use in population studies. When applied as tested the standard "T" bar anchor tag (Hallprint Australia TBA-1, TBA-2), similar to the Floy anchor tag, was not successful for tagging *P. pelagicus*. A modified "T" Bar anchor tag, with the labeled barrel extending almost the complete length of the tag, was developed and data presented which show this tag to be superior for use with *P. pelagicus*.

The development of this tag then allowed the conduct of a series of population studies to assess the residence of *P. pelagicus* in estuarine areas. The distribution of *P. pelagicus* was found to equate with depth. This distribution results in a series of geographically distinct populations in the small bays along the Cowan Creek estuary during summer. Mark-recapture assessments in one of these bays, using the Weighted Mean Method over four consecutive capture release days, on two assessment periods, gave population estimates of 26.0 (±14.9) and 15.2 (±7.6). On two other sampling occasions during winter no crabs were captured suggesting the animals either move to deeper waters during colder months or lower their metabolism and remain in the sediment for these periods.

During the population assessment work late stage berried females in the study area raised the question of female crab movements relating to the release of egg masses. An initial survey of the plankton for *P. pelagicus* larvae suggested that the life cycle could be similar to other swimming crabs such as the Blue Crab, *Callinectes sapidus*, which moves to the mouths of estuaries and releases larvae on the out going tide. This information casts some doubt on the residency of berried females and their use as a biomonitor of available cadmium.

Previous work had established that this animal could accumulate cadmium and was at least resident on a scale of estuaries during summer months; the organism was then employed as a biomonitor of environmental cadmium levels. By examining the level of cadmium accumulation in the hepatopancreas of P. *pelagicus*, a number of New South Wales estuaries were assessed for potential contamination by this metal. Crabs assessed from Lake Macquarie, on the New South Wales central coast, were found to be significantly ($p<0.05$) higher in cadmium in the hepatopancreas than levels found in crabs in other estuaries. This result is consistent with other studies which reflect metal contamination of the Lake by a number of industrial and domestic sources. Thus proving the utility of the Blue Swimmer Crab as a biomonitor of available cadmium in estuaries.

Potential methods for the production of soft-shelled crabs were trialled using *P. pelagicus*. It was found that crabs held physically separated in a single aquaria, with both eyestalks ablated, resulted in the highest percentage of crabs moulting per unit time, (mean of 48% as opposed to 20% for controls). Crabs held as individuals, and completely isolated from others, again with both eyestalks ablated, resulted in the fastest mean days to moult when compared to crabs held in groups with double eyestalk ablation (19.0 days $^{+}$/- 2.4). The injection of moulting hormone, 30^{0}C temperatures, single eyestalk ablation and male only treatments were found to have little effect on the initiation of moulting in this species.

A method for the treatment and holding of *P. pelagicus* for the production of soft-shelled crabs is proposed.

Publications

Publications arising from this work;

McPherson R. G. (2002) Assessment of T Bar anchor tags for marking the Blue Swimmer Crab *Portunus pelagicus* (L.). *Fisheries Research*, **54**, 209-216.

McPherson R. G. and K. R. Brown (2001) The bioaccumulation of cadmium by the Blue Swimmer Crab *Portunus pelagicus* (L.). *Science of the Total Environment*, **279**, 223-230.

McPherson R. G. (1999) The assessment of T Bar anchor tags for marking *Portunus pelagicus*.
Blue Swimmer Crab Workshop, Marine Research Laboratories, North Beach, Perth.

McPherson R. G. and K. R. Brown (Submitted) The Blue Swimmer Crab, *Portunus pelagicus* (L.) as an indicator of cadmium contamination in estuaries, New South Wales, Australia. *Marine Pollution Bulletin*.

McPherson R. G. and K. R. Brown (Submitted) The application of a modified T Bar anchor tag for population assessment of the Blue Swimmer Crab, *Portunus pelagicus* (L.) in Cowan Creek on the Hawkesbury River Estuary. *Fisheries Research*.

McPherson R. G. and K. R. Brown (Submitted) The dispersal of *Portunus pelagicus* (L.) larvae in the Hawkesbury Estuary, New South Wales. *Austral Ecology*.

McPherson R. G. and K. Brown (Submitted) The induction of moulting in The Blue Swimmer Crab, *Portunus pelagicus* (L.). *Aquaculture*.

Other related publications;

Brown K. R. and **R. G. McPherson** (1992) Copper, lead and zinc contamination in the Sydney rock oyster, *Saccostrea commercialis*, (Iredale and Roughley) in New South Wales. *Science of the Total Environment*, **126**, 27-33.

McPherson R. G. and K. R. Brown (1992) Analysis of trialkyltin in estuarine sediments and oyster tissues.
Proceedings of the Eighteenth Annual Aquatic Toxicity Workshop, Ontario, Canada.

Brown K. R. and **R. G. McPherson** (1992) Study of copper and zinc levels in estuaries using the Sydney Rock Oyster as a biomonitor. *Proceedings of the Eighteenth Annual Aquatic Toxicity Workshop, Ontario, Canada.*

Brown K. R., **R. G. McPherson** and A. O'Grady (1988) Heavy metals in oysters from the Georges River, New South Wales. *Australian Marine Sciences Conference, Sydney University. pp 57-61 Wavelength Press, Sydney.*

Olson R. R., **R. G. McPherson** and K. Osborne (1988) In situ larval culture of the Crown of Thorns Starfish, *Acanthaster planci* (L.): Effect of chamber size and flushing on larval settlement and morphology. *Echinoderm Biology* pp 247-251.

Olson R. and **R. G. McPherson** (1987) Potential vs. realised larval dispersal: fish predation on larvae of the ascidian *Lissoclinum patella* (Gottschaldt). *Journal of Experimental Marine Biology and Ecology*, **110**, 245-256.

Guthrie A. C. and **McPherson R. G.** (1999) Restoring environmental values in a degraded catchment.
Environment Institute of Australia, National Conference.

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1 Introduction

1.1 Project aims

The main objective of this research was to establish the potential for the use of the Blue Swimmer Crab, *Portunus pelagicus*, as a biomonitor of the anthropogenic metal contamination of New South Wales estuaries. From this research an interest was developed in the moulting mechanism relating to this species and the potential for the development of a "soft-shelled" crab using *P. pelagicus*, thus enabling a value added seafood product.

A critical assessment of the literature pertaining to each section of this work is dealt with within each Chapter. This introduction will therefore deal with the following subject areas, as an understanding of each is necessary to interpret future Chapters;

- Species background (*P. pelagicus*) i.e. taxonomy, morphology, life history, growth and fishery.
- Principles for the development of a biomonitor, and
- The soft-shelled crab industry in the United States.

1.2 Taxonomy

The Blue Swimmer Crab has the following classification;

Phylum:	Crustacea
Class:	Malacostraca
Subclass:	Eumalacostraca
Order:	Decapoda
Infraorder:	Brachyura
Section:	Brachyrhyncha
Family:	Portunidae
Genus:	*Portunus*
Species:	*pelagicus*

The class Malacostraca contains the majority of well-known crustaceans, especially the edible species. Malacostraca have paired compound eyes, usually two branched (biramous) antennules and antennae, 8 pairs of thoracic walking legs (including up to three pairs of maxillipeds and five pairs of swimmerets (pleopods) on the abdomen. The female sexual openings, or gonopores, are located on the sixth thoracic segment or its appendages, while male gonopores are on the 8th segment or its appendages. Within this class are 3 sub classes: the Phyllocardia, the Hoplocardia and the Eumalacostraca. The sub class Eumalacostraca contains most of the large and commercially important crustaceans such as crabs.

The order Decapoda includes prawns, shrimps, rock lobsters and crabs, all having three pairs of maxillipeds and five pairs of thoracic walking legs which give the name, meaning 'ten footed'. Decapods are the largest crustaceans and support several major and minor fisheries in Australia.

The family Portunidae includes most of the crab species fished both commercially and by amateurs in Australian waters. *P. pelagicus* can attain large sizes and are renowned for their culinary properties. These crabs can usually be recognised by the broadly flattened last pair of legs, an adaptation to swimming. There are numerous species of Portunid

crabs, especially in tropical seas, with most in the genera *Portunus, Thalamita* and *Charybdis.*

The Blue Swimmer Crab, also known as, Blue Manner Crab, Sand Crab, Blue Crab and Sandy, has a broad and flattened carapace with nine teeth on each side, the last tooth being very pronounced as horns (Figure 1). The clawed legs are long, elongated and ridged. These crabs can grow up to 200 mm in carapace width. The colour is mottled blue on males and mottled brown on females, but the intensity and pattern of the colouration are very variable. The sexes can be distinguished by the previous colour markings, with the males developing larger claws and also having a narrower abdomen than the female (Plate 1). *P. pelagicus* is usually caught in bays and estuaries with soft bottoms, but is also trawled in offshore waters.

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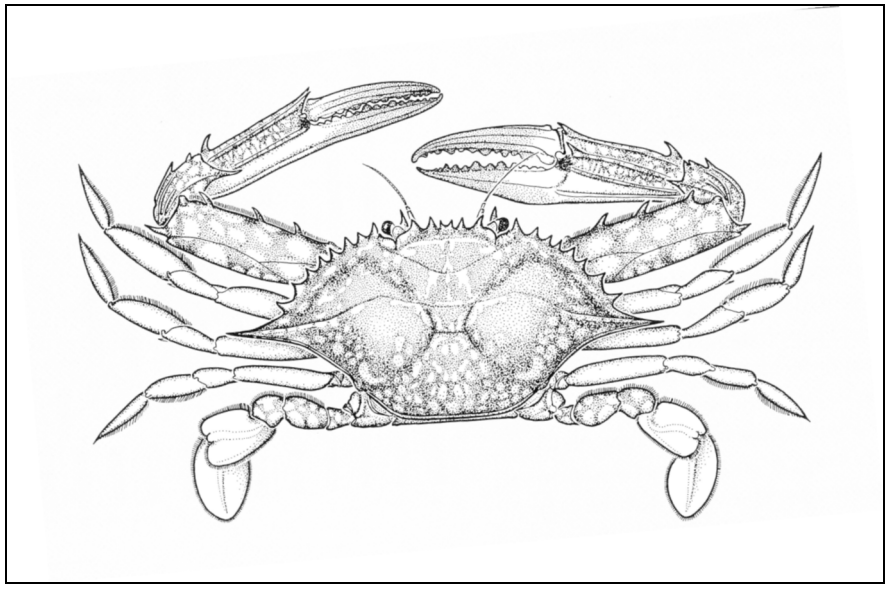
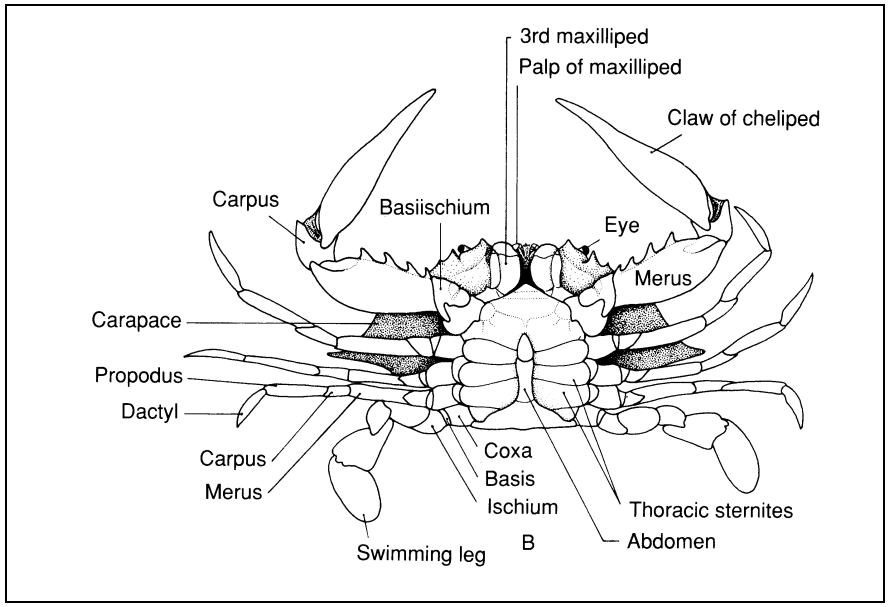
Plate 1. Male and female Blue Swimmer Crabs. *Note the narrow abdomen on the male.



A.



B.

Figure 1. Morphology of a swimming crab. A; Dorsal view (From Kailola *et al*. 1993). B; Ventral view (From Ruppert and Barnes 1994). * Note the paddle shaped swimming legs.

1.3 Distribution

The distribution of *P. pelagicus* is Australia wide (Figure 2) except for extensive areas of the southern coastline. Exceptions to this are the Spencer Gulf and Gulf St. Vincent in South Australia (Smith 1982, Potter *et al.* 1983, Kailola *et al.* 1993 and Bryars and Adams 1999). In Australia, Blue Swimmer Crabs inhabit coastal waters from Cape Naturaliste, in Western Australia, around the north of Australia to the south coast of New South Wales. They are also present around Lord Howe Island and in the warmer waters of the South Australian gulfs, as far south as Barker Inlet in Gulf St Vincent.

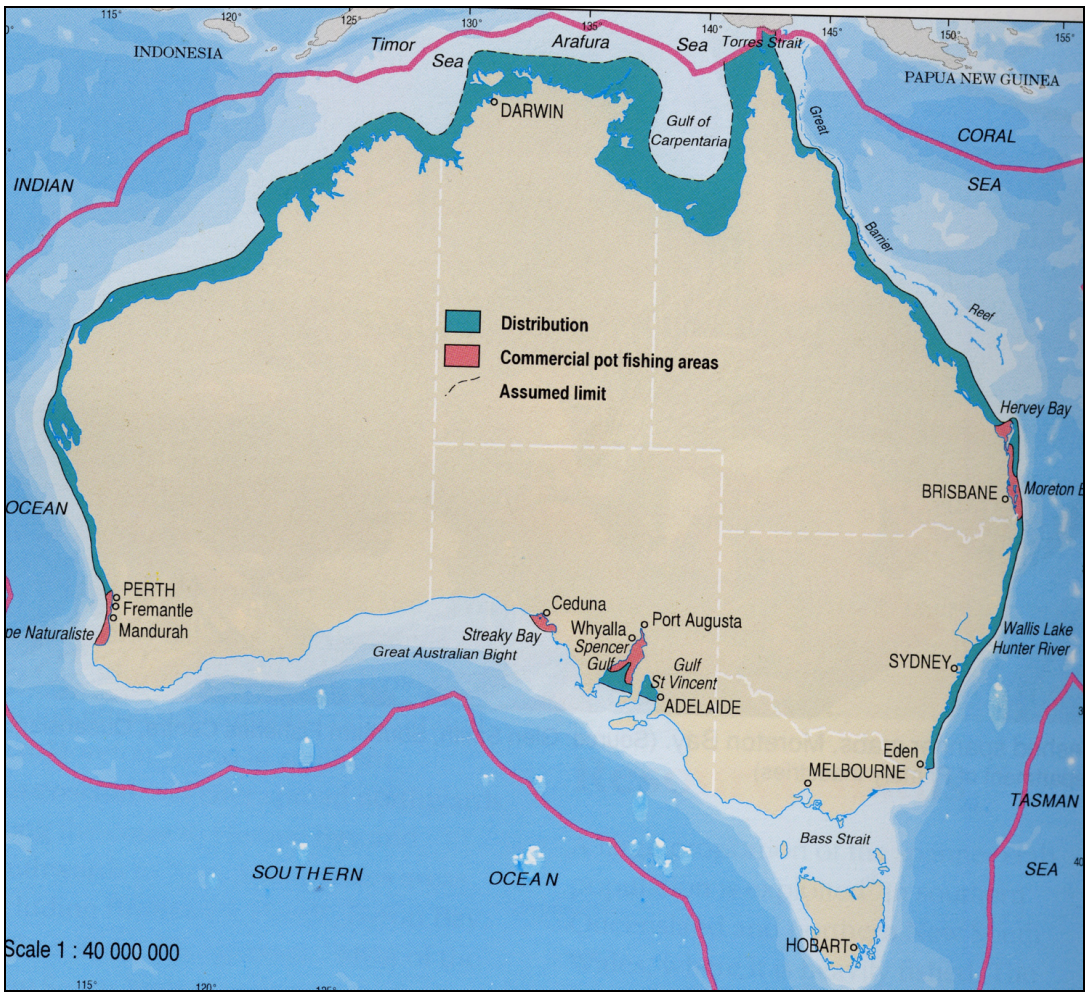


Figure 2. Blue Swimmer Crab distribution (From Kailola *et al.* 1993).

Blue Swimmer Crabs are distributed throughout the Indo-West Pacific region from East Africa to Japan, Tahiti and Northern New Zealand. They are also present in the Mediterranean Sea.

Blue Swimmer Crabs live in a wide range of inshore and continental shelf areas, including sandy, muddy or algal and seagrass habitats, from the intertidal zone to 50 m depth. Blue Swimmer Crabs move to deeper water as they age and in response to changes in water temperature and inshore salinity. Only juveniles live in intertidal habitats. Adults are generally found in salinities between 30 and 40 parts per thousand, though in upper Spencer Gulf (South Australia) both juveniles and adults are present in summer salinities of more than 45 parts per thousand. Female, egg bearing Blue Swimmer Crabs in Moreton Bay, Queensland, migrate to deeper, oceanic waters in the Bay to release their eggs.

Blue Swimmer Crabs are active swimmers, but when inactive they are usually buried in the bottom sediments, leaving only their eyes, antennae and gill chamber openings exposed (Kailola *et al.* 1993).

1.4 Life History

Blue Swimmer Crabs form breeding pairs and mating takes place during the late summer (January to March). Mature males moult some weeks before the maturing females; the mature male then carries a female clasped beneath him for 4-10 days before she moults. Mating occurs immediately after the female has moulted and when the shell is still soft. Males may mate with a number of females during the season (Kailola *et al.* 1993). Female crabs spawn up to two million eggs per batch, larger crabs producing more eggs than smaller crabs. Spawning takes place all year in tropical and subtropical waters although spawning females are more prevalent in the dry season (July to October) in the tropics (e.g. Gulf of Carpentaria) and in spring (August to October) in subtropical latitudes (e.g. Moreton Bay). In the temperate waters of New South Wales and South Australia, females are able to store sperm until spawning takes place in spring or summer (November to January) when there is a spawning peak. Female crabs may spawn several times during a season using the sperm from one mating, even as frequently as two or three times over a few months (Kailola *et al.* 1993). Blue Swimmer Crab eggs and larvae are planktonic. The eggs hatch after about 15 days at 24^0C water temperature (Figure 3).

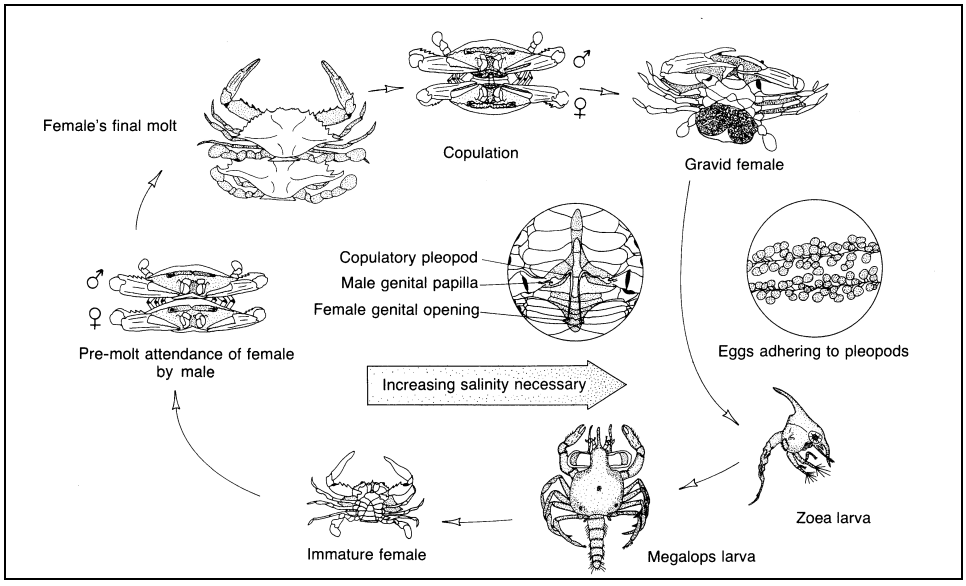


Figure 3. Life cycle of Portunid crab (From Villee *et al.* 1978).

The Blue Swimmer Crab larval phase consists of five stages (Figure 4, Note: *P. pelagicus* larvae have shorter anterior spines, otherwise the larvae in Figure 4 are similar to *P. pelagicus*). During the larval phase, the crabs may drift as far as 80 kilometers out to sea before returning to settle in shallow, inshore waters. Newly settled Blue Swimmer Crabs are about 15 mm in carapace width (Plate 2).

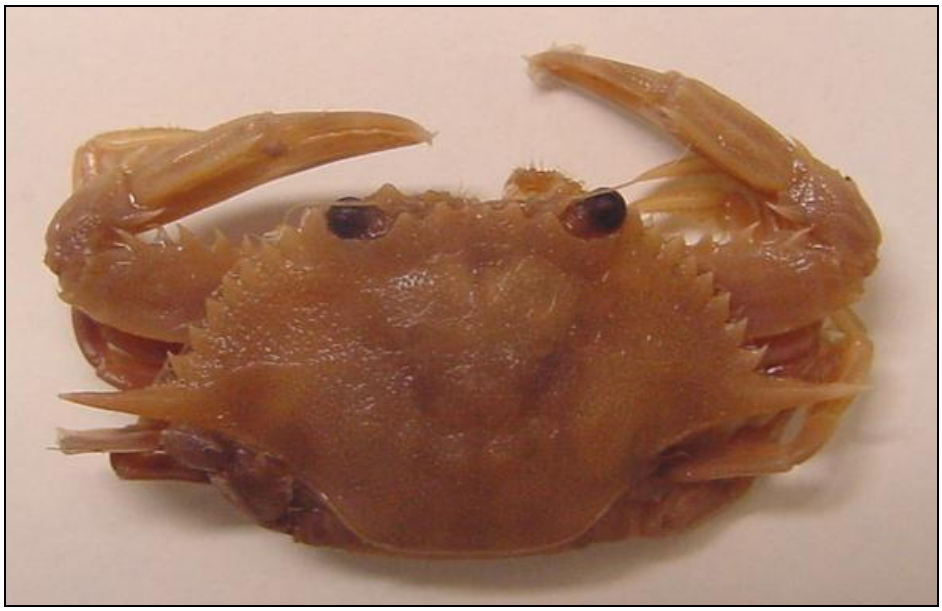


Plate 2. Newly settled crab, (*P. pelagicus*) approximately 15 mm across.

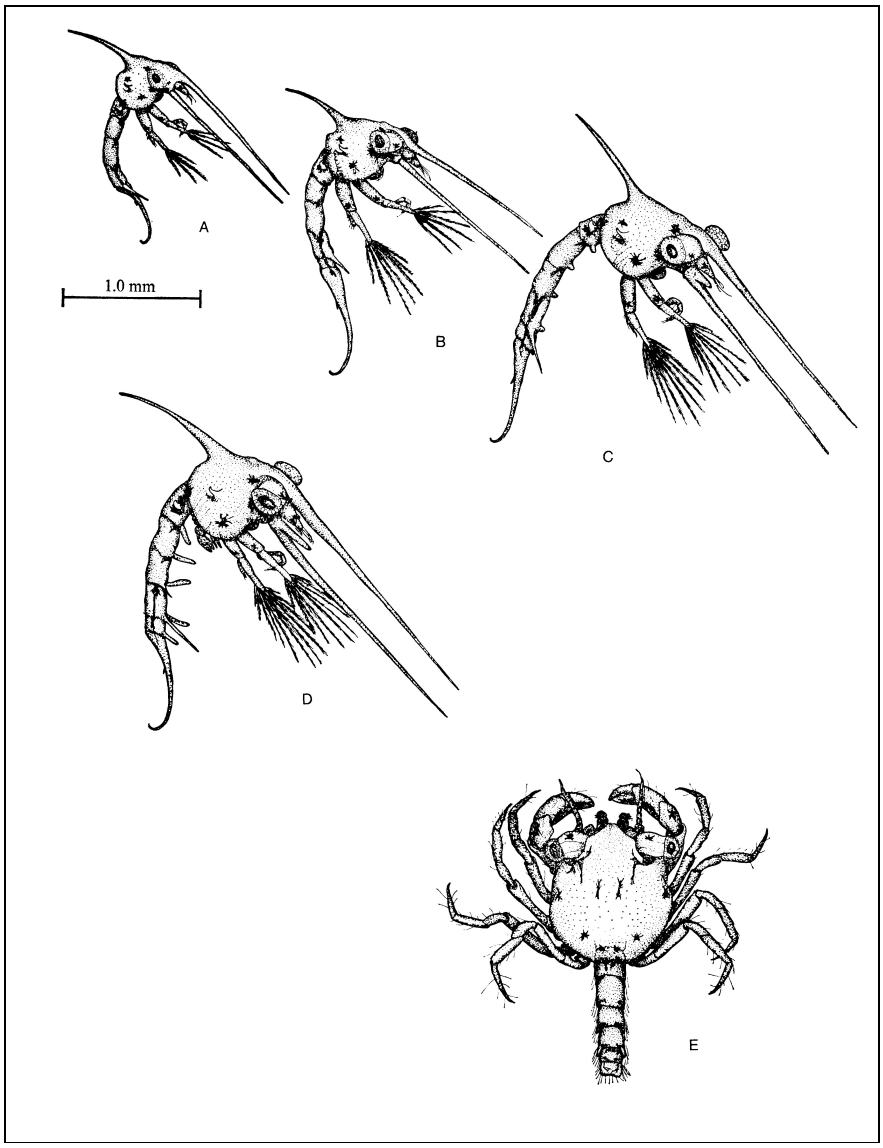


Figure 4. Larval phases of a brachyuran crab (not *P. pelagicus*). A-D, zoeal stages and E, megalops (After Ruppert and Barnes 1994).

Blue Swimmer Crabs usually moult a number of times within each of their several distinct life stages. Males and females grow at similar rates and reach similar maximum ages and sizes for given locations. The maximum recorded age of a Blue Swimmer Crab is three years (Kailola *et al.* 1993). The maximum size recorded in Australian waters is 218 mm in width. However, these crabs rarely reach 200 mm and in the Gulf of Carpentaria and South Australia they seldom reach 150 mm in width. Adult Blue Swimmer Crabs usually

weigh about 500 grams but have been recorded up to 1 kg (Kailola *et al.* 1993).

Blue Swimmer Crabs are sexually mature at about one year of age. The size at which sexual maturity occurs varies with latitudes and within individuals at any location. Males mature at 85 to 157 mm carapace width in the Peel Harvey Inlet, 80 mm and larger in the Gulf of Carpentaria, 95 to 150 mm in Moreton Bay, and 105 to 140 mm in South Australia (Kailola *et al.* 1993).

Blue Swimmer Crabs are bottom feeding carnivores and scavengers and they are most active in foraging and feeding at sunset. Their diet chiefly consists of a variety of sessile and slow moving invertebrates, including bivalve mollusks, crustaceans, polychaete worms and brittle stars (Ophiuroidea). Seagrasses (e.g. *Zostera* species) and algae may be eaten occasionally. In some localities, fish and squid discarded from prawn trawlers may be important sources of food.

Little is known of predation on Blue Swimmer Crabs but turtles, sharks, rays, larger fish and other crabs are probably the most common natural predators. Crabs are most vulnerable to predation immediately after moulting when movement is compromised by the soft exoskeleton (Kailola *et al.* 1993, Potter and de Lestang 2000).

1.5 Commercial fishery

Moreton Bay, Hervey Bay and other inshore areas of southern Queensland account for approximately half of the commercial catch of Blue Swimmer Crabs in Australia. Other important commercial grounds are along the New South Wales coast and include Wallis Lakes and the lower Hunter River. West Coast, Spencer Gulf and Gulf St Vincent in South Australia (Kumar 1997), and the Peel Harvey Inlet and Cockburn Sound near Fremantle and the Swan-Avon River near Perth in Western Australia are all important commercial grounds. Streaky Bay in South Australia periodically supports a small fishery.

Fishing takes place on off shore sand banks, in channels and deeper water up to 25m deep. Catches are highest from January to March (Summer). Blue Swimmer Crabs are caught in cylindrical wire traps or pots and folding traps, preferably baited with mullet. Hoop nets, drop nets and sunken crab gill nets are also used. Rakes and dab nets are used in very shallow water.

Blue Swimmer Crabs form a significant proportion of the by catch of many prawn trawlers and in Queensland it has been estimated that between one third and one half as many crabs are caught in prawn trawls as are caught in the targeted pot fishery. Blue Swimmer Crabs are caught incidentally with rock lobsters and in finfish fisheries. Due to the seasonal nature of the fishery, Blue Swimmer Crab fishers usually engage in other fishing activities as well, such as catching the Spanner Crab (*Ranina ranina*) (Kailola *et al.* 1993).

Most of the crab marketed within Australia is as whole cooked crab or crab meat. There is a small export market for Blue Swimmer Crabs; this is mainly to Japan. The average 1991-92 price per kg at the Sydney Fish Market was A$4.86 (green) and A$5.54 (cooked) (Kailola *et al.* 1993). Commercial catch data for Western Australia recorded 738,882 kg of crabs landed in 1997/98 and 577,395 kg landed in 1998/99 (Fisheries of Western Australia commercial catch statistics (pers. comm.)). In South Australia commercial catch data recorded between 1990/91 and 1996/97 varied considerably with landed weights ranging between 425,000 kg and 655,000 kg (1995/96) (South Australia

Research and Development Institute commercial fisheries production statistics (pers. comm.)). In Queensland commercial catches were 244,000 kg in 1995, 406,000 kg in 1996, 273,000 kg in 1997 and 335,000 kg in 1998 (Queensland Department of Primary Fisheries (pers. comm.)).

In 2000/01 125,822 kg of Blue Swimmer Crab were taken by commercial fishermen in New South Wales. This had a commercial value of $982,753 (Appendix 9.3 for the last 10 years of New South Wales landings) (New South Wales Fisheries Commercial Fishing Database (pers. comm.)). Commercial catch and catch value for the last 10 years in New South Wales are depicted in Figure 5. This graph shows that the value per kilogram of crab does not vary markedly from year to year as opposed to the high variability in actual crab catch. Of the major estuaries supporting a Blue Swimmer Crab fishery in New South Wales, Wallis Lakes is by far the most significant (Figure 6).

In most states, regulations limit the types and quantity of fishing gear which can be used and the size and/or sex of crabs which may be taken. In Queensland, no females can be kept and only males over 150 mm carapace width can be taken. In New South Wales, it is not legal to take berried females or animals of less than 6 cm carapace depth. Arrangements for all states are listed in Table 1.

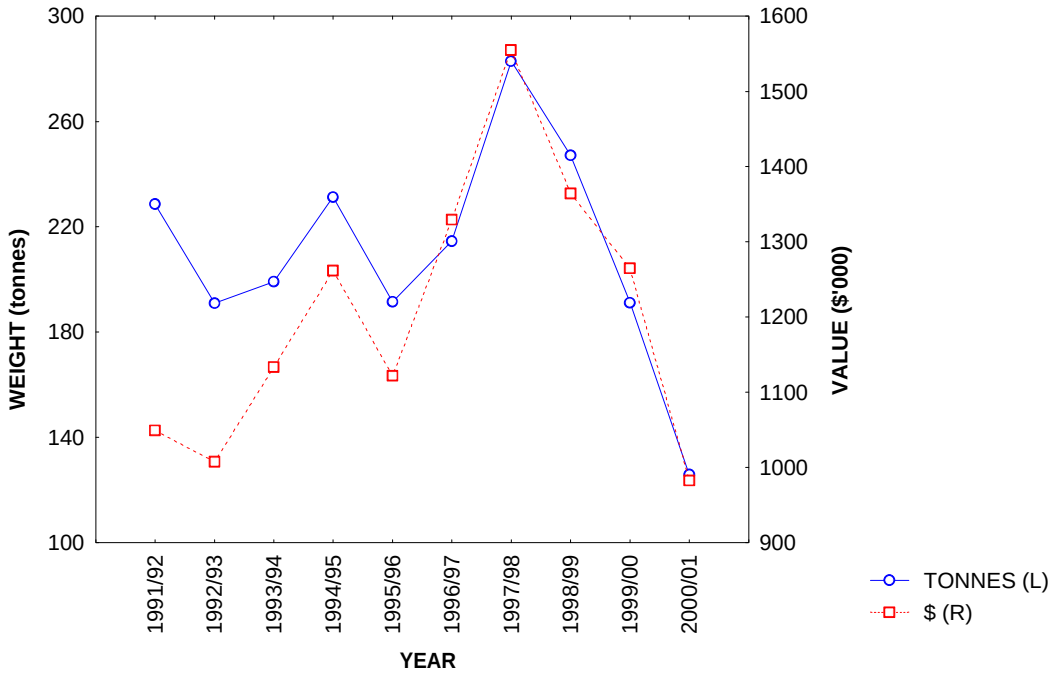


Figure 5. New South Wales Blue Swimmer crab landings for the last 10 years (New South Wales Fisheries Commercial Fishing Database).

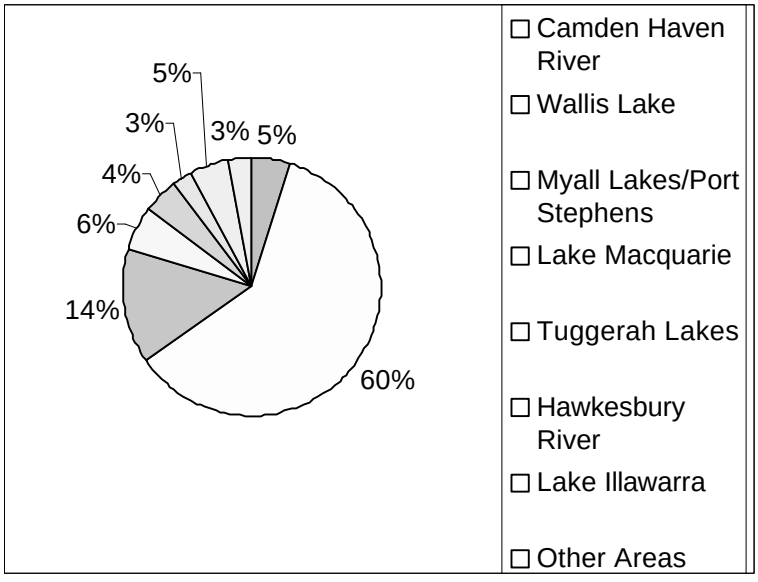


Figure 6. Main New South Wales estuaries for Blue Swimmer crab catches 2000/01 (New South Wales Fisheries Commercial Fishing Database).

Table 1. Summary table for management arrangements (Kumar 1997).

	South Australia	Western Australia	Queensland	Northern Territory	New South Wales
Size limits (min)	110mm base of spines	127mm point to point	150mm point to point	None	60mm - eve orbit to base
Bag limits	40/person/day 120/boat/day	24/person/day 48/boat/day 10 drop nets/boat	None	None	20/person/day
Fishing Gear	rake, dap, hoop, pot	pot, net	50 pots commercial 4 pots recreational	Drop nets, dillies Developmental license	10-20 pots commercial 1 pot-5 Witches hats recreational
Quota	520 tonne commercial	None	None	None	None
Closed Seasons	Nov/Dec Dec/Jan	Cockburn Sound - voluntary Geographe Bay - Dec/Jan	None	None	None
No. licenses	38	871	1064	None	200
Management Plan	No	For Cockburn Sound only	No	No	No
Protection measures	Berried females	None	Females	None	Berried females
Boat size limit	none	estuarine 6.5m	14m	<25m	None
Catch (t) comm. Catch recreational	650 ?	415 ?	400 ?	<100 kg ?	200 ?
License fee	$30,000 pot sector variable re quota, scalefishers	$890 or up to 2% GVP	$55	$100	$300

1.6 Recreational fishery

There are also fishing gear restrictions for recreational fishers. In New South Wales you are only allowed up to 5 Witches' Hats (Plate 3) and one rigid trap per person. Recreational fishers are also only allowed to take a maximum of 20 crabs per person per day.

The recreational fishery is substantial but is not well quantified in New South Wales (Kennelly 1997). Fishing occurs in much the same locations as the commercial fishery, although it is mainly targeted to the shallower inshore areas. Capture methods include pots, tangle nets (witches hats, dilly nets), rakes, dab nets, baited hoop nets and drop nets. There are few good statistics quantifying the catch levels for recreational fishers (Kailola *et al.* 1993).

Plate 3. Recreational fisher removing a crab from a tangle net (Witches Hat).

1.7 Moulting and growth

In many crustaceans, including barnacles, crayfish, and lobsters, moulting and growth continue throughout the life of an individual, although moults become further and further apart as the animal matures. Such crustaceans may live to be quite old, and some may become very large. In others, such as some crabs, moulting and growth cease with the attainment of sexual maturity or of a certain size or after a certain number of instars. The process of moulting has probably been investigated in more detail using crustaceans, especially decapods, than in any other arthropod group.

Moulting is virtually a continuous process in the life of a crustacean, 90 percent or more of the period between actual moults may be involved with concluding and preparatory processes associated with the preceding and future moults. This is especially true for species which moult year round. In species which moult seasonally, there is a rather definite intervening rest period during the intermoult. But even during the rest period food reserves are accumulated for the next moult.

Physiologists generally recognise four stages in the moult cycle: pro-ecdysis, ecdysis, post-ecdysis, and intermoult. The preparatory phase, or pro-ecdysis (or pre-moult), is marked by a continuing accumulation of food reserves and a rising blood calcium, probably resulting from the release of stored calcium from the hepatopancreas, and resorption of calcium from the cuticle. Eventually, the membranous layers and part of the calcified layers of the old exoskeleton are digested away by the epidermis. Resorption of calcium and the ingestion of the calcified layer are especially great in areas where splitting later occurs or where the old skeleton must be stretched or broken to permit extraction of a large terminal part of an appendage, such as a claw.

After separation of the old cuticle from the epidermis and the secretion of the new epicuticle and exocuticle, the animal is prepared for the actual brief process of ecdysis and usually seeks a protected retreat or remains in its burrow. As the body swells from the

uptake of water through the gills or midgut it quickly emerges from the old skeleton, which is commonly eaten later for its calcium salts. The precise mechanism of water absorption is uncertain, but the elevated salt level of the haemolymph probably establishes an osmotic gradient along which water enters. The amount of water absorbed may equal almost half of the pre-moult body weight.

During post-ecdysis, or met-ecdysis, the endocuticle is secreted, and calcification and hardening of the skeleton take place around the water-swollen body. As the new exoskeleton hardens excess body water is eliminated, and the soft tissues shrink away from the now slightly oversized exoskeleton to allow for tissue growth during the intermoult period. The animal remains in its retreat and does not feed during the first part of this phase.

The intermoult may be long or short, depending on whether or not the animal moults seasonally. Although the exoskeleton is completely formed, food reserves are accumulated for the next moult. The physiological processes involved in moulting are regulated by hormonal interactions that are essentially like those of insects.

The regulation of moulting hormones, and thereby the regulation of the actual moult cycle, depends on different stimuli operating on the central nervous system. In crayfish, which moult seasonally, day length is the controlling factor. In the crab, *Carcinus* spp, tissue growth is the controlling factor (Ruppert and Barnes 1994).

Limb loss is a common event in the life of many crustaceans, and in decapods limbs may even be self amputated as a defense mechanism. Severance often takes place along a pre-formed breakage plane, which runs across the basiischium, a proximal leg joint. Internally, there is a corresponding double membranous fold which is perforated by a nerve and blood vessels. When the limb is severed the membrane constricts around the perforations, and there is very little bleeding. In some species, severance can take place only if the limb is pulled either by the animal itself or by an outside force. However, as in most decapods

(except shrimp), autotomy is a unisegmental reflex. If a leg is caught or damaged by a predator, a reflex is set up, and an autotomiser muscle (one of the locomotor muscles), is stimulated to undergo extreme contraction, fracturing the limb along the breakage plane. Following severance and scab formation, a small papilla representing the new limb bud grows out from the stub. Growth then halts until the pre-moult period, where rapid growth and regeneration are completed. The new limb unfolds from a sac at the time of moulting, but at this moult the limb is not quite as a large as the original. If partially regenerated limbs are removed, then the moulting cycle can be delayed until new limb buds are formed.

Ruppert and Barnes (1994) contend that multiple amputations of limbs can induce moulting, but the pre-moult regeneration phase must first be completed.

1.8 Bioindicator development

What is an indicator? An indicator is something which can be measured in a pragmatic way which diminishes the need to measure a broader, more complex array of variables. The need to do this is obvious as it is not possible to measure everything, everywhere all the time.

Phillips (1977a) conducted a review of the literature looking at the use of bioindicators for the monitoring of trace metals in the marine and estuarine environments. At the time the matrices gaining attention included water, sediments, macro algae, bivalve mollusks and teleosts. Other organisms included barnacles, limpets and polychaetes, although these related to only one or two references per organism. In short, the use of crustaceans as bioindicators was in its infancy.

Phillips (1977a) provided a definition of a bioindicator and a list of characteristics that such an indicator should meet. He defined "*a bioindicator is an organism which may be used to quantify relative levels of pollution by the measurement of the toxicant concentrations in its tissues. Either the entire organism, or a part of it, or a single tissue (which may sequester metals from the rest of the organism) may be used*". Phillips (1977a) listed the ideal characteristics of such an indicator as follows:

- the organism should accumulate the pollutant without being killed by the levels encountered;
- the organism should be sedentary in order to be representative of the area of collection;
- the organism should be abundant in the study region;
- the organism should be sufficiently long-lived to allow the sampling of more than one year class if desired;
- the organism should be of reasonable size, giving adequate tissue for analysis;
- the organism should be easy to sample and hardy enough to survive in the

laboratory, allowing defecation before analysis (if desired) and laboratory studies of the uptake of metals;

- the organism should tolerate brackish water;
- the organism should exhibit a high concentration factor for metals, allowing direct analysis without pre-concentration;
- a simple correlation should exist between the metal content of the organism and the average metal concentration in the surrounding water; and,
- That all organisms in a survey exhibit the same correlation between their metal contents and those in the surrounding water, at all locations studied, under all conditions.

Some of these conditions have proved to be too restrictive as the development of bioindicators has progressed over recent years. The animal's sedentary nature, for example, is less of an issue if the potential movements of a motile organism are understood to a suitable level. Defecation is not such an issue if only certain organs are to be harvested. Improved analytical and sampling methods have meant that tissue mass is not the issue it once was. These criteria were formulated around the supposition that the major vector for input to the indicator would be via the water column and it is now understood that the vectors of diet, water column and absorption/adsorption are all important uptake pathways (Luoma 1983).

The further development of crustacean species as bioindicators of environmental metal levels has progressed substantially over the last 10 years, the majority of the quality work being driven by Rainbow and co-workers (Rainbow *et al.* 1993, Chan and Rainbow 1993a, Chan and Rainbow 1993b, Weeks and Rainbow 1993, Weeks and Rainbow 1994, Caparis and Rainbow 1994, Rainbow 1995a, Rainbow and Kwan 1995, Nugegoda and Rainbow 1995, Rainbow 1995b, Rainbow 1997a, Rainbow 1997b, Martin and Rainbow 1998a and 1998b, Rainbow *et al.* 2000a, Legras *et al.* 2000, Nassiri *et al.* 2000, Fialkowski *et al.* 2000, Rainbow *et al.* 2000b, Wang and Rainbow 2000, Rainbow and Blackmore 2001, Mouneyrac *et al.* 2001, Rainbow and Wang 2001,).

While this body of work has confirmed many of the required criteria for the development of bioindicators of environmental metals in crustacea, there have been relatively few attempts to utilise this information and apply it to "on the ground" monitoring programs. There are however some notable exceptions to this, Rainbow *et al.* (1993) utilised two amphipods (*Talorchestia quoyana* and *Orchestia tenuis*) in a biomonitoring study in New Zealand looking at whole body metal levels of copper, zinc and cadmium. These authors concluded that both amphipods were suitable biomonitors for use in New Zealand coastal waters. Fialkowski *et al.* (2000) also used an amphipod (*Talitrus saltator*) as a biomonitor to examine the levels of copper, zinc, iron, cadmium, lead, manganese and nickel around the Gulf of Gdansk, Poland with some success.

Further work carried out in the Gulf of Gdansk (Rainbow *et al.* 2000b) utilised the mussel *Mytilus trossulus* and the barnacle *Balanus improvisus*. The trace metals copper zinc, iron, cadmium, lead, manganese and nickel were assessed and both species were deemed to be suitable as biomonitors of these metals in this polluted area.

More recently, Rainbow and Blackmore (2001) used the barnacles *Balanus amphitrite* and *Tetraclita squamosa* to assess the levels of arsenic, cadmium, chromium, cobalt, copper, iron, lead, manganese, nickel, silver and zinc in Hong Kong coastal waters. Comparisons made with historical data sets from 1986 and 1989 make this the most comprehensive biomonitoring program utilising a crustacean bioindicator to date.

Rainbow (1995a) examined the general issue of the biomonitoring of heavy metal availability in the marine environment. This work promoted the use of metal accumulators in this role and also promoted the use of a suite of biomonitors to assess the source of the metal. For example, a mussel responds to dissolved and suspended metals and a deposit feeder responds to metals in the sediment. Rainbow (1995a) advocates the development of cosmopolitan species as biomonitors to enable comparisons between geographic areas. The Blue Swimmer Crab is such a cosmopolitan species with a distribution across the Indo-

West Pacific (Kailola *et al*. 1993).

No applied monitoring programs using crabs as biomonitors of metals could be found in the literature. There has however been a significant amount of work conducted on the ability of a range of crabs to bioaccumulate metals (e.g. Martin 1974, Jennings *et al.* 1979, Jennings and Rainbow 1979, Hopkin and Nott 1979, Davies *et al.* 1981, Sanders 1984, Rainbow 1985, Engle 1987, Balaji *et al.* 1989).

1.8.1 History of environmental indicator development

The development of physico/chemical indicators for aquatic ecosystems produced the first batch of "ecosystem health" measurements to be developed to assist in the monitoring and management of these systems. The current new generation of indicators is based on ecological processes and is only now being developed with some confidence. There is, however, ongoing debate relating to some of the proposed indicators and the methodologies promoted as "best practice" (Fairweather 1999).

In the development of these indicators ecologists need to be able to partition human induced effects on ecosystems and report on their scale and impact. To only report that the variability within the system is within the range of expected natural variability does nothing to address the potential long term degradation on the environment and the need to manage as best as possible mans impact on the environment. The further argument that the system must be "resilient" because of this large natural variability, so there is little need to take any action or implement particular controls on development, is equally flawed as in the majority of cases the time scales on which these systems have evolved means we do not have data on which to make such claims.

While it is apparent that all natural systems are, in some time scale, undergoing perpetual change through the processes of evolution and the effects of the biogeophysical environment, it is also apparent that the effect of man on his environment is more than

capable of influencing dramatic change on a global/spatial scale.

These principles make it important to collect information on our potential impact on the environment at the appropriate scales in both time and space (Underwood 2000).

Probably the most well known biomonitoring program utilising a bioaccumulator is the "Mussel Watch" program currently in operation, in various forms, around the world (Beliaeff *et al.* 1997, Cantillo 1998, O'Connor 1998, Tanabe 2000).

The development of an indicator needs to follow a series of logical steps; a summary of these is presented in Table 2 and generally follows that promoted by Fairweather (1999). It is considered that the work of Fairweather (1999) supercedes that presented by Cairns *et al.* (1993) who also dealt with the issue of developing indicators of ecosystem health.

Table 2. Sequence of steps needed for scientific development and validation of an indicator. From Fairweather (1999).

Step	Relevant questions arising
1. A monitoring need is perceived	*Is it recognised by scientists and/or managers?*
2. Hypothesised relationship	*What links an indicator to an impact?*
3. Scientific feasibility	*Is it worth persisting with?*
4. Experimental laboratory trials	*It performs under what range of conditions?*
5. Field validation	*What practical signal is recovered?*
6. Management-scale, onsite assessment	*How routinely implementable?*
7. Develop feedback loops	*What appropriate management responses?*
8. Protocols for implementation	*How best can it be disseminated and accessible?*
9. Verification	*How effective in use?*
10. Refinement	*How can it improve with advancing knowledge?*

While Table 2 was developed with the assessment of "ecological/estuarine health" indicators in mind the assessment of bioaccumulators as biomonitors for application to the estuarine environment is supported by similar principles. Fairweather (1999) also considers

the problem of miss-classifying sites in the search for pristine conditions, and warns of the need to have representative control sites.

Ward (2000) also deals with the problems associated with the development of indicators for reporting on Australia's marine and estuarine ecosystems. This author outlines a range of issues needing to be addressed to further develop such indicators, including, knowledge of the nature of ecosystems, scientific understanding of environmental issues, capacity of monitoring programs, data manipulation across spatial, temporal and taxonomic scales, case studies, reference sites, interpretive models and an established procedure for revising and updating indicators as new knowledge accrues.

This thesis deals with steps 1 to 5 of Fairweather's (1999) sequence and establishes the Blue Swimmer Crab as a biomonitoring tool which is useful for the routine management of estuaries across the Indo-West Pacific for the assessment of environmentally available cadmium contamination. Steps 6 to 10 will need to be assessed as the procedure is applied over time and knowledge of the methods advantages and disadvantages increases.

1.9 Soft-shelled crab production

The shedding of Blue Crabs (*Callinectes sapidus*) to produce the more commercially valuable soft-shelled form has been done for at least one hundred years in the United States (Warner 1994). Soft crab prices have consistently been much higher than the prices for hard-shelled crabs. In spite of the best efforts of many researchers, no one has yet developed a profitable system for the culture of crabs from eggs to marketable animals. The supply of soft-shelled crabs depends upon the ability of commercial fishermen to catch and recognise crabs that are nearing the moult of their old shell.

The Blue Crab is commonly found on the Gulf and Atlantic coasts of the United States. This crab moults as many as 25 times in its lifetime in order to grow. Before moulting, the crab forms a new soft shell under the existing exoskeleton. After casting off the old shell, the crab swells with water to a larger size and the new, soft shell becomes hard within 12 hours. The crab then begins to feed and add weight inside its new shell. Small crabs may moult every few days. As they become larger, the period between moults becomes longer. Female crabs do not moult after reaching sexual maturity. Soft-shelled crab shedding systems are designed to put near-moult crabs in a controlled environment, so they can be efficiently harvested during the few hours that the shell is soft.

For this industry to be profitable a reliable supply of crabs that are near shedding stage is required. These crabs are called pre-moult or "busters". Buster crabs must be obtained from the commercial harvest of wild Blue Crabs, either by catching them or buying them from crab fishermen. The Blue Crab fisherman must decide if it is worth the effort to grade crabs as they are caught and to separate and carefully handle the busters.

The production of soft-shelled crabs is labor intensive. Buster crabs placed in the shedding system must be graded, crabs within the system must be graded, and soft-shelled crabs must be removed and packaged. Buster crabs are graded according to how far they are from shedding. Crabs within the system are graded depending on the colour of the

perimeter of the last segment of the swimming legs. White-sign crabs are 7 to 14 days from shedding, pink-sign crabs are 3 to 6 days removed, red-sign crabs are 1 to 3 days removed, and cracked busters are within 24 hours of shedding. White-sign crabs should be graded every three days and crabs that have developed pink and red signs should be maintained separately. White-sign crabs are still feeding and cannibalize red and cracked crabs if not separated, white-sign crabs must be fed in order to moult successfully. Red-sign crabs must be checked once a day for the appearance of cracks, cracked busters must be checked every three to four hours.

Once a crab has moulted and has been allowed to expand to its full size, it is removed from the water immediately or its shell will start to harden. Soft shell crabs may be temporarily stored in a refrigerator and wrapped or packaged once a day. Some buyers also require that soft crabs be cleaned before packaging (Horst 1992).

Soft shell crab shedding systems fall into one of four categories:

- float cars;
- onboard flow through or open systems;
- land-based flow-through or open systems; and,
- closed or recirculating systems.

Float cars are floating wooden cages which hold the crabs in the water column. Onboard flow through or open systems are holding tanks installed on boats. These are either supplied with a constant source of water or are periodically flushed.

Land-based flow-through or open systems are fiberglass or fiberglass-coated wooden boxes, built as benches, (height convenient for the shedder). If space is at a premium, shedding boxes can be stacked. Water is drawn from a nearby estuary and pumped through the system, draining back into the estuary after one pass. If no suitable surface water is nearby, water from a saltwater well may also be used, if the salinity is correct. No part of this system that is in contact with the water is constructed of metal. The pump may or may

not be submersible. A $1^1/_2$ horsepower pump will handle three 1.2 x 2.4 m shedding boxes with a capacity of 800 to 900 crabs. The shedding boxes are located under a roof to keep rain and debris from entering the tanks, to provide shade, and for the comfort of the shedder. Particular attention is paid to the construction of the standpipe as this will determine the level of water in the tank. It should be designed so that the shedder can control the water level by changing pipes and allow the tank to drain. A hole, no larger than 6 mm is drilled in the standpipe 13 mm above the tank bottom to allow the tank to slowly drain in the event of a power failure. The small amount of water left will increase crab survival rates. When power is restored, the tanks automatically refill. The tanks can be made self-cleaning by inserting over the standpipe a longer, larger PVC pipe with notches cut from the bottom. This forces the standpipe to pick the water up from the bottom of the tank, effectively flushing dirt and other debris.

The advantages of a land-based flow-through or open system are:

- the shedder can aerate the water supply;
- the system does not have to be located immediately on the water;
- the shedder is protected from the weather, can use electric lights, and does not have to bend over the tanks;
- no crab losses are suffered during short power failures;
- the system is immediately operable at full scale since there is no filter to be conditioned; and,
- white-sign crabs may be fed in the system.

The disadvantages are:

- the system must be fairly near water (unless a well is used); and,
- silt or hydrogen sulphide in the water source may cause high mortalities.

Closed or recirculating systems are similar to land-based flow-through systems except that the same water is recirculated. Water is added only to make up for evaporation. Water leaving the shedding tanks flows by gravity into a filter system. The biological filter is

made up of shell material, limestone, sand, or plastic beads on which bacteria grow. These bacteria consume the ammonia that is given off as a waste product by the crabs (nitrification/denitrification). Without this, the ammonia would quickly build up to toxic levels and kill the crabs. From the filter, the water is pumped back to the shedding tanks. Three 1.2 x 2.4 m shedding boxes with two working filters one foot deep, 1.2 m wide and 2.4 m long will support 900 crabs at any one time, usually enough for a single crabber's production. It should be noted that Blue Crabs are smaller than the Blue Swimmer Crab.

The advantages of a closed or recirculating system are:

- poor environmental water quality does not affect the system;
- the system can be located anywhere; and,
- the shedder has good working conditions.

The disadvantages include:

- this system is more complex than the others;
- if the system is not carefully designed, a power failure can result in loss of either crabs or water;
- time is necessary to grow the bacteria before the system can be used at full capacity; and,
- white-sign crabs cannot be fed unless a sand or bead filter is used.

On the Atlantic coast, buster crabs are usually available each year from the middle of March through to October, with some variation caused by weather conditions. Peak production usually occurs in April or May with another smaller peak in September (Horst 1992). The vast majorities of pre-moult or buster crabs are caught with standard Blue Crab pots and are separated from the others during the course of fishing. Buster crabs are also harvested by other methods. Some are caught with shrimp trawls. In some localities fishermen catch buster crabs with dip nets from sea grass beds. Probably the most unusual method of harvesting buster crabs is with bushlines. The bushline is basically a heavy trot line with bundles of wax myrtle (shrub) instead of hooks. Crabs that are near moulting

enter the bushes apparently for protection. The crabber gently raises each bush and uses a large dip net to catch any crabs that fall from the bush when it is shaken. Almost all the crabs produced by this method are red-sign or cracked crabs.

The most important step in the shedding process is the proper handling of buster crabs after they have been caught and before they are put in a shedding system. This is especially true if the shedder has to buy the busters. The most successful producers immediately put busters in a separate crate filled with evergreen branches. The branches shade, cool, and cushion the crabs and prevent their points from damaging other crabs. They also calm them and prevent them from attacking each other. A crab with even a small hole in its shell will usually not shed successfully. When the buster crate is as full as desired, it is covered (leaving the bushes in), with wet burlap sacking material and placed out of the wind and the sun. The burlap covering must be kept moist. At the dock, the buster crate is handled and transported gently and not allowed to over heat. Crabs must be placed in the aquarium system as soon as possible. Careful handling can make the difference between a 50% shedding rate and a 90% rate. The minimum size requirements are different from those for hard crabs and vary from State to State in the United States (Horst 1992). An example of a newly moulted Blue Swimmer Crab is presented in Plate 4.

Soft-shelled crabs have now become a highly sought after delicacy across the globe due to the ability of the restaurant customer to consume the whole animal without the need to remove a shell and the unique taste of the dish (Appendix 9.1). Doyle's Restaurants in Sydney have, on a number of occasions, made contact expressing the belief that such a product would be in high demand in this country. It is currently very difficult to ship soft-shelled crab to Australia due to the excessive demand and high prices offered elsewhere in the world.

1.10 Moulting and bioaccumulation

A number of authors have examined the relationship between moulting and the bioaccumulation of metals in crustacea (Engel 1987, Engel and Brouwer 1991, Lasenby and Van Duyn (1992), Rao and Govindarajan (1992), Martin *et al.* (2000). These authors all ascribe a reduction in whole body metal burden to the moulting process.

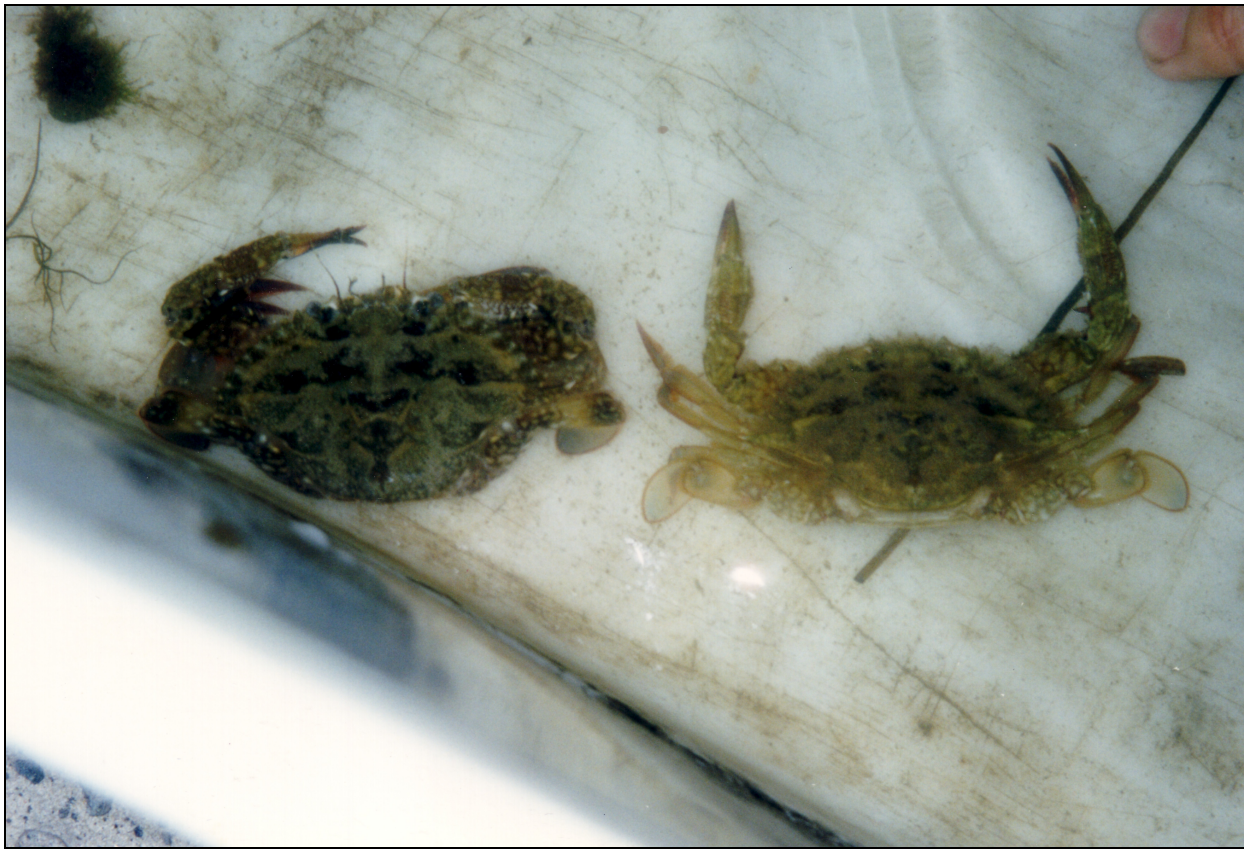


Plate 4. Just moulted Blue Swimmer Crab, old exoskeleton to the right of the photograph. * Note increased size of new crab (approx. 5.5 cm carapace width).

1.11 Research objectives

1.11.1 Objective One

There has never been a greater need for the development of monitoring tools to assist with the management of human impact on the environment. The fact that estuaries are “sinks” which take the waste from coastal development reflects the need to adequately conserve and protect these natural resources. Cadmium is a toxic metal associated with various development types and is indicative of development pressure in estuarine catchments. Cadmium can be an indicator of poor industrial and domestic catchment management and its presence reflects the need to better manage these land use types.

The use of such indicators has been on the whole poor, with many workers simply measuring metal levels in a range of species with no understanding of any given species potential as an indicator of any particular metal. Many researchers measure metal levels in a suite of different species and then make determinations on environmental condition with no consideration of the species ability to bioaccumulate the toxicant, its ecology or its physiologic condition (e.g. Batley 1987). In short they do not provide any information to support the claim that what they are measuring is indicative of the situation they are trying to assess.

The use of bioaccumulators as bioindicators has been well researched and has been applied with great success to a range of indicator species, such as mussels and oysters (Beliaeff *et al.* 1997, Cantillo 1998, O’Connor 1998, Tanabe 2000, Scanes 1993). The need however still exists to develop cosmopolitan species as bioindicators of anthropogenic metal inputs so as to enable the assessment of these impacts on a local, regional and international scale (McPherson and Brown 2001, Rainbow 1995a, Rainbow and Phillips 1993).

The Blue Swimmer Crab is such a cosmopolitan species and the information presented here will enable its utilization as a monitoring tool across the Indo-West Pacific.

1.11.2 Objective Two

With the pressure on domestic fisheries constantly increasing the need to value add already fished species makes sound economic and ecological sense.

The purpose of this work was therefore to use the existing Blue Swimmer Crab fishery to establish an industry revolving around the production of soft-shelled crabs for the restaurant trade. This required the development of suitable holding systems and the application of techniques capable of inducing a fast and synchronized moult in this species.

1.12 Objectives by chapter

The specific objectives of each chapter are listed below.

1.12.1 Chapter 2: The bioaccumulation of cadmium by the Blue Swimmer Crab *Portunus pelagicus* (L.).

Chapter 2 was a laboratory study designed as the first phase in the development of *P. pelagicus* as a biomonitor of environmental metal levels. A range of metals were presented to the animal and the accumulation of these metals in the hepatopancreas was assessed. Metals were presented in a naturally occurring food source, the hairy mussel, *Trichomya hirsute,* sourced from either a contaminated or non-contaminated site. Metal levels in this mussel had accumulated in the field due to natural processes.

1.12.2 Chapter 3: Assessment of T bar anchor tags for marking the Blue Swimmer Crab *Portunus pelagicus* (L.).

Having established in Chapter 2 that the animal was capable of accumulating cadmium with some relationship to time and metal level presented, it was necessary to establish the

potential range of the animal within an estuary and over time. To be able to assess populations a reliable tag had to be developed. Tagging an animal which moults periodically presents a range of unique problems. Such T bar anchor tags had been used on *P. pelagicus* in the past but in early tests these were found to cause very high mortalities and as such violated many assumptions required by population assessment models.
A modified T bar anchor tag was developed which reduced mortalities and allowed visual identification of individuals in a population.

1.12.3 Chapter 4: The application of a modified T bar anchor tag for population assessment of the Blue Swimmer Crab, *Portunus pelagicus* (L.) in Cowan creek on the Hawkesbury River estuary.

The tag developed in Chapter 3 was employed to conduct population assessments of *P. pelagicus* to better understand its movements within an estuary and assess its applicability as an indicator on the broad scale of estuaries and possibly within estuaries.
Small populations of animals within bays on Cowan Creek, on the Hawkesbury estuary, were assessed during both summer and winter using baited tangle nets.

1.12.4 Chapter 5: The dispersal of *Portunus pelagicus* (L.) larvae in the Hawkesbury estuary, New South Wales.

While conducting population assessments it was observed that a number of late stage berried female crabs were found in the bays well upstream in the estuary. To determine if the female crabs stayed in the bays to shed their eggs or might move down to the mouth of the estuary, as has been found in some other portunid crabs, plankton tows were conducted in a number of these bays.
This information added to the understanding of crab movements within the estuary and hence this animal's ability to be employed as a biomonitor of metal levels on an estuary wide scale.

1.12.5 Chapter 6: The Blue Swimmer Crab *Portunus pelagicus* (L.) as an indicator of cadmium contamination in estuaries, New South Wales, Australia.

Having established in earlier chapters that the Blue Swimmer Crab could accumulate cadmium and was resident within estuaries over the summer months, a method for the use of the animal as a biomonitor of estuarine cadmium metal levels was developed and trialed.

This involved sampling crabs from a number of estuaries along the New South Wales coast at the end of summer and comparing results of hepatopancreas cadmium levels to other studies conducted in these estuaries and to known land use patterns.

A method for the use of *P. pelagicus* as a biomonitor of available cadmium contamination in estuaries is presented.

1.12.6 Chapter 7: The induction of moulting in the Blue Swimmer Crab, *Portunus pelagicus* (L.).

Chapter 7 presents work on the development of recirculating holding systems for *P. pelagicus* with a view to the production of soft-shelled crabs.

A series of experiments (Phases 1-4) were then conducted on the manipulation of various parameters to induce the synchronous and timely moult of mature *P. pelagicus*. Factors such as eye stalk ablation, limb ablation, temperature, male crabs, smaller size class crabs, moulting hormone and individual versus group holding systems were trialed.

A method for the production of soft-shelled crabs is presented.

1.12.7 Chapter 8: Discussion

Describes the results and outcomes of this research in relation to the original objectives.

2 The Bioaccumulation of Cadmium by the Blue Swimmer Crab *Portunus pelagicus* (L.)

2.1 Summary

The Blue Swimmer Crab (*Portunus pelagicus*) accumulates increased concentrations of only cadmium in the hepatopancreas after being presented elevated levels of the metals Cd, Cu, Zn, As, Fe and Al via a food source, the mussel *Trychomya hirsuta*. Over eight weeks, crabs were fed a controlled diet to determine the accumulation of metals. Significant ($p = 0.05$) Cd accumulation was detected after four weeks of feeding. Food mussels were sourced from either "contaminated" or "uncontaminated" sites. Mussels from uncontaminated sites had an average Cd level of 0.07 µg/g wet weight and contaminated mussels had an average of 5.2 µg/g wet weight. The usefulness of presenting metals in this way to assess potential bioaccumulators is further considered against other presentation methods.

2.2 Introduction

The need to monitor environmental conditions in relation to trace metal contamination of aquatic systems has resulted in the development of bioindicators to assist in this complex task (reviews of Phillips 1977a and Luoma 1983). The focus of these studies has been the use of sediments and bivalve mollusks to assess environmental metal loads (e.g. Phillips and Yim 1981, Phillips 1985, Phillips and Rainbow 1988, Luoma 1989). By comparison, there has been much less investigation of the use of crustacean species as indicators of general environmental metal levels. Notable exceptions, however, include Bryan 1968, Martin 1974, Jennings and Rainbow 1979, Jennings *et al.* 1979, White and Rainbow 1982, Arumugam and Ravindranath 1983, White and Rainbow 1984, Ward *et al.* 1986, Engel 1987, Rainbow 1988, Nugegoda and Rainbow 1989, Rainbow and White 1989, Darmono and Denton 1990, and Florence *et al.* 1992.

The current study aims to assess the ability of the decapod crustacean *Portunus pelagicus* to accumulate metals from a potential food source via laboratory experimentation, and in turn assess the usefulness of this organism as a biomonitor of environmental cadmium levels.

Accumulation work of this nature has been dealt with by Bryan 1964, 1968, 1971, Davies *et al.* 1981, White and Rainbow 1982, Sanders 1984, White and Rainbow 1984, Rainbow 1985, Ahsanullah and Williams 1989, Balaji *et al.* 1989. Methods for the presentation of the metal to the test organism vary widely. The question of how well any particular presentation method reflects potential uptake/depuration in the environment is rarely considered. The observed variation is further complicated by the fact that the two major vectors for entry of the metal into the organism are via the surrounding solution or through the ingestion of food. This is excluding the adsorption of metals to the surface of the organism. As metal levels in aquatic marine environments can be extremely transient, it was decided to present the metals via a food source to better reflect potential accumulation/depuration in the field.

This methodology would also present further information relating to the possibility of biomagnification of the metals from one trophic level to another (Ward *et al.* 1986).

The method of presentation of the contaminant to the organism is also important from the point of view of the bioavailability of the metal to the test organism (Luoma 1989). It was postulated that by utilising a naturally occurring contaminated food source, the maximum potential for bioavailability would be realised.

Sanders (1984) found that concentrations of the metal contaminants copper and zinc in crab tissues (*Callinectes sapidus*) were significantly different between a contaminated site and an uncontaminated site. Sanders (1984) also assessed sediment and oyster tissue levels of copper and zinc and concluded that metal levels were highest in the tissues of crabs and oysters from sites adjacent to sources of domestic and industrial pollution. In contrast, a number of other studies have found the essential trace metals copper, manganese and zinc

to be regulated by decapod crustaceans (Bryan 1964, 1968, and 1971, White and Rainbow 1982 and 1984, Arumugam and Ravindranth 1983, Rainbow 1985, Bryan *et al.* 1986, Nugegoda and Rainbow 1989, Rainbow and White 1989).

As the contamination of a number of New South Wales estuaries by various metals has been identified as a threat to these environments, the development of future monitoring programs utilising biomonitors has become more relevant (Brown and McPherson 1991).

The present study aims to assess one of the basic questions associated with the assessment of any organism as a biomonitor of environmental chemical contaminants: is the contaminant taken up by the organism with some relationship to the level of the contaminant in the surrounding environment (Phillips 1977a, Luoma 1983)?

The primary aim of the work presented in this thesis was to determine if *P. pelagicus* (Barnes 1982, Kailola *et al.* 1993, Jones and Morgan 1994) can accumulate metals from a natural food source - the mussel *Trichomya hirsuta*, as this is one of the first steps in the development of the organism as a potential biomonitor (Fairweather 1999). The secondary aim, assuming this is so, was to determine if the level of metal accumulation in the crab increases with the metal content of the mussels.

Two groups of mussels were collected for the study – from Lake Macquarie downstream of a large zinc smelter, and from an area of Port Stephens devoid of significant industrial activity and with only minor urban development in the catchment.

The hypothesis then became that crabs fed mussels with elevated metal levels will accumulate metals to a greater extent than crabs fed mussels with background metal levels. These mussels originated from Lake Macquarie and Port Stephens respectively. Both of these water bodies are to the north of Sydney on the New South Wales coast.

2.3 Materials and methods

2.3.1 Food Source

Approximately 900 mussels were collected from Black Jack Point, Lake Macquarie and another 900 from Tanilba Point, Port Stephens. Three homogenates of 50 individuals were taken from each of the two 900 mussel groups and 3 sub samples from each analysed for the 13 metals listed below. The ability to detect a significant difference ($p = 0.05$) between metal levels in the mussels from the two areas determined which metals were analysed for in the crab tissues.

2.3.2 Crab Collection

Approximately 40 adult *P. pelagicus* were collected from the Hawkesbury River estuary just north of Sydney. All animals being 6.0 – 7.5 cm from middle anterior to middle posterior of the carapace. The legal size for fishing purposes in New South Wales is 6 cm. The collection was conducted with the assistance of a professional crab fisherman; animals were collected by traps set overnight and all crabs removed from the traps within a 2 hour period. Animals were then transported directly to the aquarium. The specimens were collected from as small a geographical area as possible in an attempt to reduce the variability of metal levels in individuals which may be influenced by point source inputs of contaminants. A random selection of males and females was collected. Of the 40 crabs, 10 were frozen ($^{-}18^{o}$ C) whole and later analysed for baseline metal levels, 12 were placed in aquaria and fed contaminated mussels (Lake Macquarie). Another 12 were placed in aquaria and fed uncontaminated mussels (Port Stephens) and extra crabs were kept to replace experimental crabs which may have perished during acclimatization to the aquaria. This experimental design was selected to assist in partitioning the effects of uptake of metal via solution and via ingestion of food and follows in principle the recommendations of Luoma (1983 and 1989).

2.3.3 Aquaria/Feeding

After an acclimatization period of 1 week in filtered estuarine water (salinity 34 ppt, constant temperature 25^{O}C), the 24 aquaria containing crabs were randomly assigned one of the two treatments, these being either fed "contaminated" or "baseline" mussels. There were no deaths during this period and crabs were not fed during this time. At feeding the mussels were opened with a clean stainless steel knife and dropped in the tank still in the shell. When all the tissue had been consumed the shell was removed. Crabs were fed enough mussels each day for 8 weeks to satisfy hunger (as indicated by a loss of interest in the food). Each animal was held individually in a recirculating aquaria utilising subsurface biological filtration with air lifts (Plate 5).

Plate 5. Holding tank with internal biological filter at Sydney Aquarium.

The water quality of the aquaria, dissolved oxygen, salinity, temperature and pH was assessed for each tank on a weekly basis and was always found to be within acceptable limits (Wedemeyer *et al.* 1976) and uniform across all tanks. These being particularly important as Nugegoda and Rainbow (1989) have shown the importance of salinity to the uptake of metal from solution. Holding experimental animals in this way reduces stress and the potential for mortalities. These techniques have been employed in the commercial aquarium industry for some time.

2.3.4 Analysis

At 2 weeks 3 crabs were sampled for analysis from each treatment, at 4 weeks 4 crabs were sampled and at 8 weeks the remaining 5 crabs were terminated from each treatment. This sampling strategy gave information on the time scale for possible accumulation and the number of crabs required for a statistically sound estimate of the "population". At sampling, crabs were frozen whole pending analysis. Prior to analysis, the hepatopancreas was removed from the crab using clean stainless steel dissecting instruments (Figure 7).

Metal levels in the hepatopancreas of each crab were then assessed by digestion and analysis by ICP/MS at the Australian Government Analytical Laboratories in Sydney. These laboratories hold national accreditation for the assessment of trace metals in biological samples (National Association of Testing Authorities).

The hepatopancreas was selected as the primary organ for analysis as various other studies (Hopkin and Nott 1979, Jennings *et al.* 1979, Jennings and Rainbow 1979, Davies *et al.* 1981, Rainbow 1988, Darmono and Denton 1990) had determined that there was significant potential for the accumulation of cadmium in this organ. Hopkin and Nott (1979) have also shown that metals could be stored in membrane bound granules in the digestive gland in the shore crab *Carcinus maenas*. Measuring metals in the hepatopancreas in isolation also has the advantage of not potentially confounding the results with the effects of gut contents (Chapman 1985). Differences between the treatments and the baseline metal levels enabled the assessment of the animal’s ability to

accumulate the metal levels in its tissues under these conditions. A simple t-test was applied to the results from each treatment; also a single factor analysis of variance was applied to the three data sets i.e. contaminated, uncontaminated and baseline metal levels for each metal type. Metals to be analysed in the mussel tissues included Cd, Cu, Zn, Pb, Se, As, Cr, Ni, Hg, Fe, Al, Ag, Co.

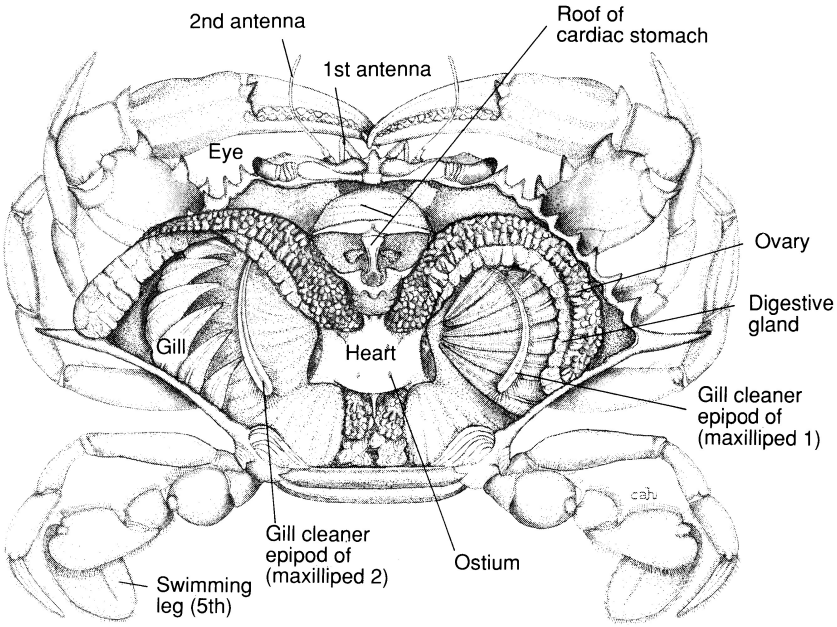


Figure 7. Internal anatomy of a Portunid crab. *Note digestive gland (hepatopancreas) (From Ruppert and Barnes 1994).

2.4 Results

There were no mortalities during the acclimatisation or experimental period. No animals moulted during this work, which was important as Engel (1987) had demonstrated that it was possible for metals to be lost during the moult process in the Blue Crab *Callinectes sapidus*. Jennings and Rainbow (1979) and White and Rainbow (1984) came to similar conclusions.

Table 3. Metals in *Trichomya hirsuta* homogenates from Lake Macquarie and Port Stephens (µg/g wet weight).

Metal	Port Stephens			Lake Macquarie			Standard Recovery (%)
	H1	H2	H3	H1	H2	H3	
cadmium	0.06	0.07	0.07	5.2	5.5	4.9	83
copper	1.5	1.7	1.4	2.3	2.3	2.0	
zinc	11	11	9.9	19.3	22	20	94
arsenic	1.7	1.8	1.9	5.0	5.2	4.9	83
aluminium	29.3	22.3	21.0	5.2	4.9	4.5	
iron	41.3	34.7	34.7	19.0	18.0	19.7	

H1, H2 and H3 correspond to replicate homogenate sub samples

There was a significant difference ($p = 0.05$) in metal content for each of the metals listed in Table 3, the remainder of the 13 metals analysed showed no significant differences in mussel tissue from the two areas. Cd, Cu, Zn and As were all higher in mussels from Lake Macquarie, while mussels from Port Stephens were higher in Al and Fe content. Al and Fe

occur naturally in the local environment. The elevated levels of these metals in Port Stephens mussels probably reflect the differing geology of the two areas.

Of the metals listed, only cadmium and arsenic are above the national Australian guidelines for metals in shellfish (Anon. 1983). Standards for some of the metals tested are included in Table 4.

Of the metals listed in Table 3, only cadmium showed a significant ($p = 0.05$) difference in hepatopancreas levels between the two treatments at week 8 (Table 5). The crabs fed mussels from Port Stephens did not accumulate cadmium to any extent when compared to the baseline crabs displayed in Table 4.

Table 4. Baseline hepatopancreas metal levels in the ten experimental crabs taken from the Hawkesbury River with some associated food standards (From Anon. 1983).

Metal	Concentration µg /g wet weight +/- sd	Food standards for human consumption in Australia
cadmium	0.09 +/- 0.06	0.2 mg/kg (crustaceans) 2.0 mg/kg (mollusks)
copper	24.4 +/- 10.4	70 mg/kg (mollusks) 10 mg/kg (all other foods)
zinc	27.1 +/- 9.3	1000 mg/kg (oysters) 150 mg/kg (other foods)
arsenic	1.85 +/- 0.48	1.0 mg/kg (inorganic for fish, crustaceans and mollusks)
aluminium	0.71 +/- 0.45	
iron	9.1 +/- 3.7	

Table 5. Cadmium levels in the hepatopancreas of eight week experimental crabs (µg/g wet weight) fed with mussels from either Port Stephens or Lake Macquarie.

Port Stephens	Lake Macquarie
0.05	2.0
0.09	2.4
0.11	1.2
0.03	2.1
0.02	2.2

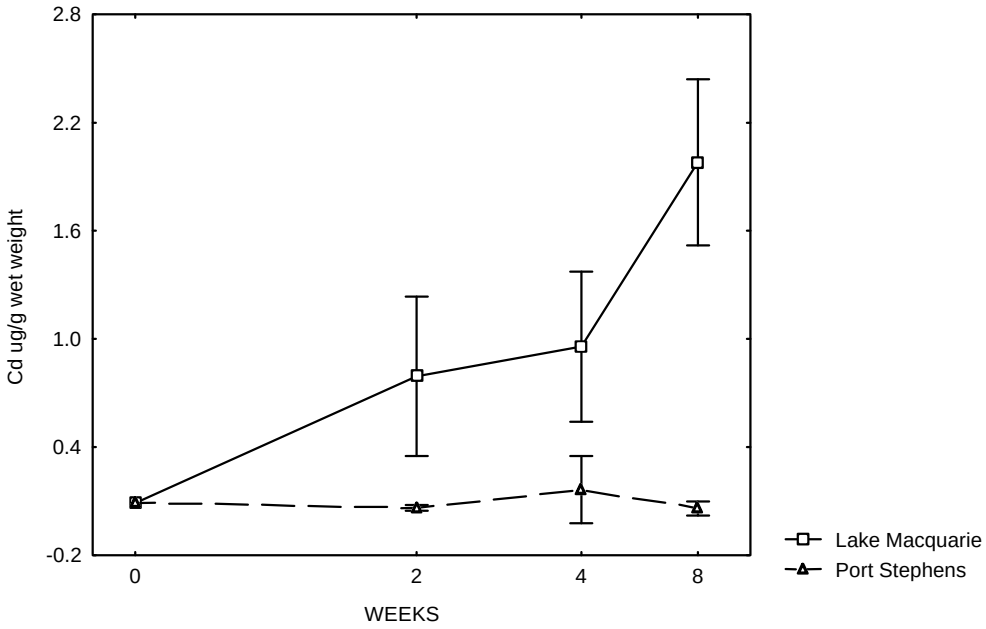


Figure 8. Cadmium accumulation in crabs fed mussels from either Lake Macquarie (contaminated) or Port Stephens (uncontaminated). At week two, n = 3, week four, n = 4 and week eight, n = 5. Weeks is a log scale.

By week 4 there was a significant difference in cadmium levels between the two treatments and this became greater by week eight (Figure 8). Means and error bars are +/- the standard deviation and calculated using the pooled variance. Figure 8 would also tend to indicate that accumulation may have continued post the week 8 sampling if the experiment had been continued. A single factor analysis of variance shows a significant difference ($p = 0.01$) between baseline, Port Stephens (8 week sampling) and Lake Macquarie (8 week sampling) crabs. There was no significant difference ($p = 0.05$) between the baseline and Port Stephens (8 week sampling) crabs.

While there was no significant difference ($p = 0.05$) between the treatments for arsenic as at week 8 (Table 6), sampling results would tend to indicate that a longer feeding period may have resulted in significant accumulation. Other than cadmium there were no significant ($p = 0.05$) differences between treatments for the other metals listed in Table 6.

Table 6. Metals in the hepatopancreas of crabs fed uncontaminated mussels (Port Stephens) and contaminated mussels (Lake Macquarie).

Week	Port Stephens Mean µg/g wet weight	Sd	Lake Macquarie Mean µg/g wet weight	Sd
Cd				
2	0.06	0.015	0.79	0.442
4	0.17	0.186	0.96	0.417
8	0.06	0.039	1.98	0.460
Cu				
2	24.3	10.0	26.7	6.7
4	19.0	4.3	29.0	12.7
8	24.8	10.0	24.0	6.3
Zn				
2	17.3	2.3	30.0	17.4
4	17.5	3.7	24.8	4.2
8	23.2	7.0	23.4	5.1
As				
2	1.70	0.30	1.70	0.15
4	2.20	0.53	2.40	0.57
8	2.02	0.78	3.76	1.17
Al				
2	0.31	0.04	0.42	0.08
4	0.33	0.12	0.39	0.17
8	0.33	0.19	0.23	0.07
Fe				
2	3.6	0.80	3.4	0.46
4	2.8	0.54	3.5	1.15
8	3.5	0.67	3.0	0.16

2.5 Discussion

Cadmium is an inorganic toxicant of great environmental and occupational concern which was classified as a human carcinogen in 1993 (Waalkes 2000).

Jennings *et al.* (1979) and Jennings and Rainbow (1979) conducted laboratory studies of the accumulation of cadmium in *Carcinus maenas* via either food or seawater. These authors attempted to assess the mass of metal entering the crabs via the food and determine some uptake efficiencies in the comparison of the importance of uptake from either of the two media. These authors quoted problems with the amount of food supplied to the crabs as confounding any conclusions concerning the comparison of uptake vectors. No such assessments were attempted in this work, not due to the amount of food supplied but due to the mechanism of feeding by *P. pelagicus*, that is, a significant amount of any food is dispersed into the water column by the mandibular action of the crab as it masticates the food prior to ingestion. Davies *et al.* (1981) compared the uptake of cadmium by the edible crab *Cancer pagurus* via either the water column or diet and determined the major uptake route to be diet.

When presenting the metal to the test organism, it is important that the laboratory methodology reflects as best as possible the likely matrix and physiological conditions which the potential bioaccumulator may face in the environment. These experiments have attempted to determine the uptake of cadmium from food alone by controlling for the effects of uptake from solution and presenting the metal to the potential bioindicator in a form which might be expected to be encountered in the environment.

The accumulation and depuration of metals by indicator organisms can be a species specific phenomenon. Other authors have examined the usefulness of using various crustacea as biomonitors of general metal pollution in aquatic environments (Phillips 1977b, Davies *et al.* 1981, Rainbow 1985) with varied results and recommendations. Darmono and Denton (1990) assessed the levels of cadmium in the hepatopancreas of the prawns *Penaeus merguiensis* and *P. monodon* in Northern Australia with reported values

in the range of 1.39 and 1.69 µg/g wet weight. These authors consider these results to reflect relatively uncontaminated environments and compare to the values found during this work of 0.09 +/- 0.06 (n = 10 to as high as 1.98 +/- 0.460 (n = 5) µg/g wet weight, in baseline crabs.

Some sessile and slow moving benthic invertebrates, such as mussels, have been shown to accumulate metals to significant levels (Phillips and Yim 1981). It has also been shown that the major dietary source for *P. pelagicus* is benthic invertebrates and that the organism itself spends much of its life buried in soft bottom sediments (Kailola *et al.* 1993). This information leads to the conclusion that possible vectors exist for the accumulation of some metals in the tissues of *P. pelagicus*.

Ward *et al.* (1986) assessed cadmium in whole *P. pelagicus* from the environment in an attempt to ascertain a relationship between whole body cadmium content and distance from a known point source. No significant relationship with distance could be found. A further conclusion was that there was no biomagnification of cadmium up the food chain.

The two questions to be answered before the utilisation of an organism as a biomonitor are therefore: does the organism accumulate/regulate the metal over some measurable scale and does the ecology of the organism lend itself to the monitoring of the environment in question? This study has shown that *P. pelagicus* will accumulate cadmium to levels (in the hepatopancreas) above those of background crabs over a 4 week period. Potter *et al.* (1983) has shown the species to be resident in an estuary over the summer months and perhaps for longer periods for female crabs.

2.6 Conclusion

A number of studies have randomly selected biota for analysis of metals in a given system and attempted to interpret the results to reflect the contaminated nature of the area. Studies such as those conducted here highlight the need to conduct controlled bioaccumulation trials prior to the conduct of such monitoring programs. This work has shown that *P. pelagicus* has the potential to be utilised as a biomonitor of cadmium pollution in the surrounding environment given the assessed bioaccumulative ability and the ecology of the species.

3 Assessment of T Bar Anchor Tags for Marking the Blue Swimmer Crab *Portunus pelagicus* (L.)

3.1 Summary

Tagging is a critical technique when studying the population dynamics of any organism, in crustaceans the occurrence of periodic moulting further jeopardises the tagging process. When applied as tested the standard "T" bar anchor tag (Hallprint Australia TBA-1, TBA-2), similar to the Floy anchor tag, is not considered successful for tagging the Blue Swimmer Crab *P. pelagicus*. When assessed under laboratory conditions, the tag showed a 100% failure rate during moulting. The use of this methodology for population studies on *P. pelagicus* is not supported by data gathered by these laboratory based observations. A modified "T" Bar anchor tag, with the labeled barrel extending almost the complete length of the tag, has been developed and data are presented which show this tag to be superior for use with this animal. These results raise serious questions about data gathered by other studies utilising standard "T" bar anchor tags on *P. pelagicus*. The work also provides a better understanding of the mechanics of tag behavior during both the act of tag application, and over the moulting process.

3.2 Introduction

The commercial fishery for *P. pelagicus* in New South Wales produced 190,000 kg in 1996/97 (Fletcher 1998). While few data exist on the recreational pressure relating to this species it is assumed to be substantial (Kailola *et al.* 1993). In Australia *P. pelagicus* inhabits coastal waters from Cape Naturaliste in Western Australia around the north of the country to the south coast of New South Wales. It is also present around Lord Howe Island and in the warmer waters of the South Australian gulfs, as far south as Barker Inlet in Gulf St Vincent (Kailola *et al.* 1993). The stock structure of *P. pelagicus* populations in Australia is not well understood, and to further this more work was clearly needed on developing marking methods for the species (Thomson 1950, Baker and Kumar 1994, Kumar 1997). "T" bar anchor tags, similar to the Floy tags used elsewhere, had previously

been used. It was therefore decided to examine this past work and, if necessary, further develop the methodology.

Current management of the fishery is through regulations on fishing gear employed, and restrictions on size and sex of animals which can be landed. There are seasonal closures, and females in berry are protected in all states (Kailola *et al.* 1993). Current knowledge of the biology of this species has been presented by Potter *et al.* (1983) and Smith (1982).

Williams (1986) assessed plastic anchor tags to study migration and populations of *P. pelagicus*, and reported no short term effects on the crabs. However there were problems with the visibility of the tag post-capture. For this reason alone, Williams (1986) recommended against the use of this tag for population studies but also stated that the tag would be appropriate for short term migration studies as it was concluded that there was no problem that the tag would complicate the moulting process. The data presented to support this conclusion regarding moulting was inadequate due to poor experimental design, low numbers of replicates (only 3 tagged crabs ever moulted) and high mortalities of both control and treatment animals. Insufficient information was presented to allow an accurate reproduction of this study. This author did however recommend that long term studies to assess the impact of the tag on the moulting process should be conducted.

Much of the population/migration work presented on this species has been gathered by the use of this type of tagging methodology (e.g. Potter *et al.* 1987 and 1991, Kailola *et al.* 1993). Potter *et al.* (1987) found that the tags did contribute to significant mortalities but persisted with its use with no reference to the earlier study conducted by Williams (1986). The aim of this work was to more accurately assess the usefulness of "T" bar tagging systems over the short to medium term, especially with regard to the reaction of the crab to the presence of the tag during the moulting process. The need for this work was supported by Shiota and Kitada (1992) in a paper considering stock enhancement of the swimming crab *Portunus trituberculatus*. A series of slight modifications to the design and application of the tag were tested to improve its potential for use in population studies of

this species. The need for the work is also supported by Hurley *et al.* (1990) who assessed the effects of similar tags on the Snow Crab, *Chionoectes opilio.*

For a tag to be successful for use in population assessments on this animal it needs to have a negligible effect on the animal's physical well-being at application, during the moulting process and during the intermoult period. The tag must also have good visibility at capture, while not compromising survival potential or potential for recapture (Begon 1979).

The range of potential tagging systems for this species is quite large but many of the most likely techniques are either very expensive or require specialised equipment to sense the tag. Examples of these are the use of coded wire tags (CWT) and passive integrated transponder (PIT) tags (Laird and Scott 1978, Fitz and Wiegert 1992, Guy *et al.* 1996). The use of visual implant tags is worth considering in *P. pelagicus*, but the potential sites for their location would seem to be limited to under the membrane where the swimming legs join the thorax or possibly at the end of either swimming leg (Guy *et al.* 1996).

3.3 Materials and Methods

Plastic "T" bar anchor tags - Hallprint Australia TBA-1 and TBA-2, which are applied respectively with Monarch needle guns No. 3000 (fine) and No. 3030 (course), were tested to determine their usefulness in short term (days) to long term (months) population studies of mature *P. Pelagicus*. Mature crabs are those as defined by Campbell and Fielder (1986). Three trials were conducted, the first of a standard anchor tag as supplied by Hallprint Australia (TBA-1), the second of a modified tag (based on a TBA-2 anchor tag) and a third of a modified tag based on anchor tag TBA-1. Trials two and three also examined two different application points on the animal: in the suture between the carapace and the abdomen and in the dorsal membrane where the swimming leg joins the thorax. Each trial was run on a separate occasion.

3.3.1 First Trial - Standard Tagging Method

24 adult crabs were tagged using plastic anchor tags and a Monarch No. 3000 application gun supplied by Hall Print Pty Ltd., South Australia (TBA-1). This tag consisted of a 2 cm leader attached to a 3 cm labeled barrel which was orange in colour with black lettering (Figure 9). Of the 24 test crabs, 6 had the application gun inserted and removed but no tag was loaded in the gun so that these animals became the controls for the 18 animals with tags.

All animals used in these trials were collected from the Hawkesbury River, New South Wales, using witches hats (tangle nets) with fish baits. Test animals ranged in size from 5 to 9 cm carapace depth with a male/female ratio of approximately 4:1.

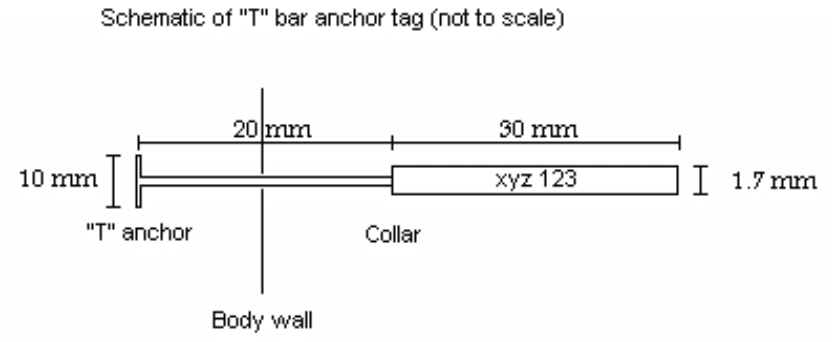


Figure 9. Standard "T" Bar anchor tag - First Trial.

Tags were inserted to the posterior suture of the carapace so that the "T" anchor of the tag (Figure 9 and 10 and Plate 6) resided in the middle of the muscle group associated with the left swimming leg in each tagged animal. The needle gun was thus inserted approximately halfway between the midline of the rear of the carapace and the line of the suture just above where the swimming leg attaches to the thorax. This was called a "carapace tag". The needle of the tagging gun was immersed in 100% isopropyl alcohol between each tag application to minimise the chance of infection. Prior to tagging, crabs were kept for an acclimatisation period of 1 week in filtered estuarine water (salinity 35 ppt, constant

temperature 22^{O} C). Crabs were held individually in aquaria with recirculating biological filtration. The trial was continued until the majority of crabs had been through at least one moult. Crabs were fed a sole diet of prawns (frozen bait type, no preservatives added) and were fed every 2 to 3 days; uneaten food was removed when interest in it had been lost. Ammonia, nitrate, nitrite, salinity, dissolved oxygen and temperature were monitored during the study and regular water changes were conducted on all tanks.

Other data collected at the time of tagging included body depth, width, wet weight and sex. No crabs were in the berried condition and only animals which were complete and in good condition were utilised in any of the trials.

Crabs were kept out of the water for a minimum time during tagging and data collection and all fed the next day as per the acclimatisation period. After each moult the animal was examined visually to assess moult success. Tissues surrounding the tag were examined at the end of each trial. This trial was run over 220 days.

3.3.2 Second Trial - Modified Tag and Application Point

This trial was conducted under the same general conditions as the first trial except that animals were held in groups of up to 10 in a single tank, but in individual mesh cells. Each tank was serviced by a single recirculating biological filter. A total of 41 crabs were assessed during this trial. Crabs were tagged using a modified TBA-2 tag, which is a coarser tag than that used in Trial one, and required application by the larger Monarch tagging gun Model No. 3030. The reason for using the coarser design was its ease of manufacture, the extension of the barrel of the tag closer to the "T" bar being more easily achievable by the tag manufacturer. The tag followed the general design depicted in Figure 10.

This tagging system utilises both a slightly coarser tag and a larger diameter and longer needle in the applicator gun. 17 individuals had the tag applied to the margin at the rear of the carapace as in the first trial (carapace tag). Another 17 had the modified tag applied

through the dorsal membrane at the junction of the left swimming leg and the thorax. The "T" section of the tag resided in the same muscle group as for the tags applied through the rear margin of the carapace, this type of tagging method being known as "swimming leg tagging". Another 7 control crabs had the needle gun alone inserted for each of the two application points (4 for the carapace tag and 3 for the swimming leg tag). The trial was run for 211 days.

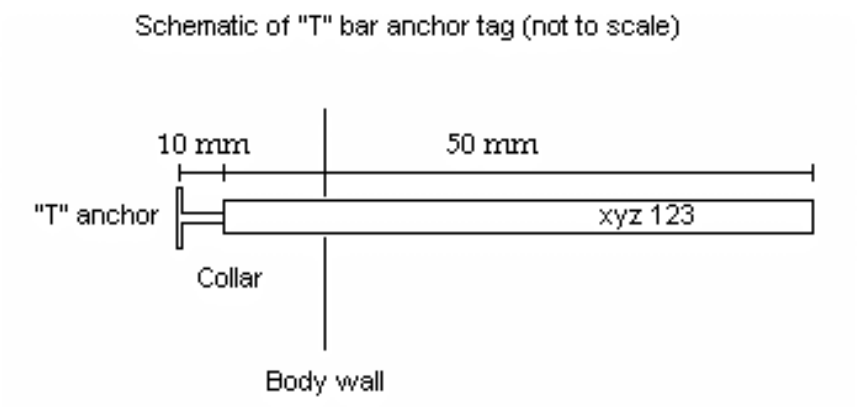


Figure 10. Modified "T" Bar anchor tag.

3.3.3 Third Trial - Modified Tag and Application Point

This trial was as per the second trial using identical tag design and application methods, only the size of the tag was changed (TBA-1). Tag dimensions were as depicted in Figure 10 and the application of the tag was by the same Monarch needle gun (fine) as utilised in the first trial (Model No. 3000). A modified fine tag was used in this trial in an attempt to eliminate the mortalities imposed during the second trial by the tagging operation itself. The diameter of the coarse tag leader was slightly larger than that of the fine tag.

A total of 39 crabs were assessed, with 15 tagged through the margin of the carapace, 15 through the swimming leg membrane with 9 controls (5 - carapace and 4 - swimming leg). After application, some tags needed to be further pushed in by hand after their application

by the needle gun. This was because the modified fine tag, with the shorter needle gun applicator, was sometimes not applied deep enough into the animal to ensure that the barrel of the tag was inside the shell of the individual. This was a quick and simple operation and tags remained in position for the duration of the experiment (Plate 6). This trial ran for 252 days.

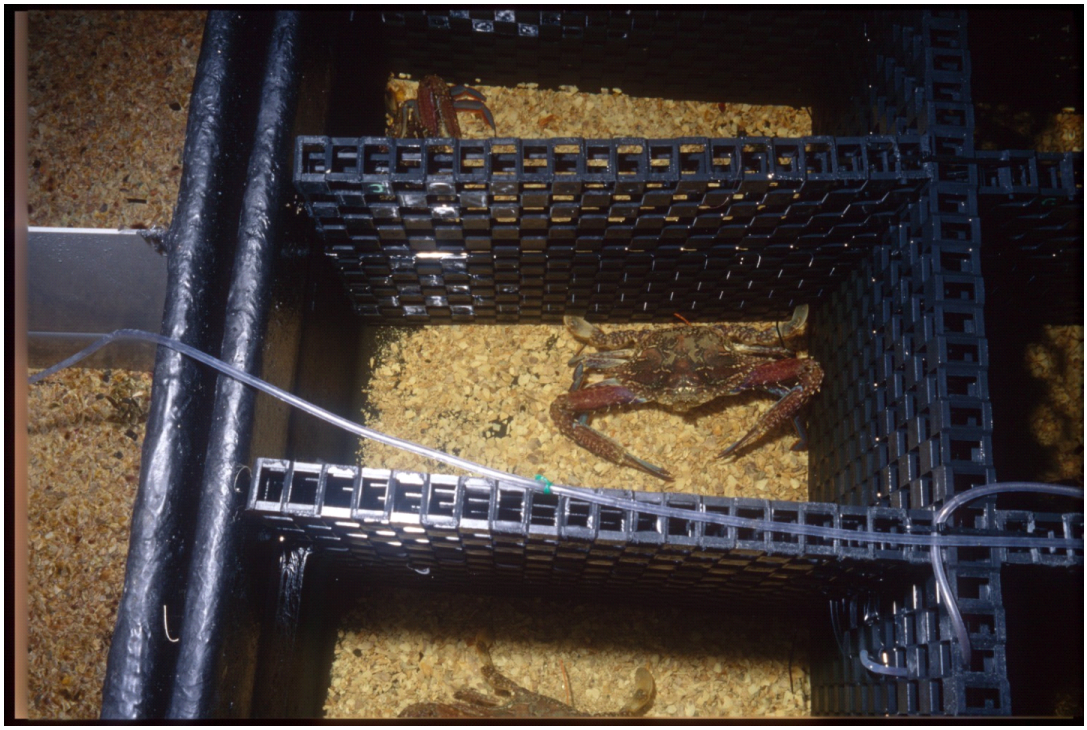


Plate 6. Crab (*P. pelagicus*) marked with modified "T" Bar anchor tag.

3.4 Results

Only one tag was lost from an individual in any of the three trials. Only one tag was observed to slip out of position during the study, and this was a swimming leg tag applied in the second trial. No behavioral changes were observed which could be attributed to the presence of the tag in any tagged individuals.

3.4.1 First Trial

Of the 24 experimental animals, 1 died shortly after being tagged, while all other mortalities were associated with the moulting process. By the conclusion of the trial, 14 of the 18 tagged crabs, and 5 of the 6 control crabs, had moulted. All the control crab moults were successful, that is, there were no mortalities and no crabs were deformed after the moulting process.

Table 7. Fate of the 14 tagged crabs which moulted during the first trial period.

Fate at moult	Number Affected	%	Days to moult
Died	6	43	7-166
Deformed	4	29	16-46
Threw tag	1	7	8
Tag moved	1	7	21
Shell Attached	2	14	18-49

Key

Died = perished either during the moulting process or within 48 hours of moulting

Deformed = loss of limbs/parts of limbs, disfigured limbs, gill filaments protruding from body cavity, loss of ability to move limbs, old shell attached to limbs.

Threw tag = tag left the animal and remained in the old shell.

Tag moved = tag migrated to be protruding from the exoskeleton from another position.

Shell Attached = tag remained in place and the animal was in a similar condition to premoult but the old shell remained attached to the animal via the tag.

The categories of died, deformed, threw tag, tag moved and attached are all assumed to invalidate the tag (Table 7). This tag was therefore unsuccessful on every moulting occasion. Most moults resulted in the old shell being caught on the collar of the tag and if the crab had not perished, the old shell was dragged along after the moulted animal (restricting its movements). All the dead crabs were dissected to assess possible infection due to the implanted tag, only one showed any sign of such infection (2 mm around entry point).

3.4.2 Second Trial

This trial was conducted over 211 days. Of the 17 animals with a "carapace tag", 4 (24%) died within a month of the application of the tag. A further 4 survived the trial but did not moult. Of the 8 animals which moulted, 6 moulted successfully (75%). One animal died inexplicably.

Of the 17 animals with a "swimming leg tag", 5 died within a month of tagging (29%). A further 4 survived the trial but did not moult. Of the 6 crabs which moulted only three were successful (50%). A further 2 animals died for no apparent reason.
All control crabs survived and the majority (86%) had moulted successfully by the end of the trial. There were a large number of mortalities due to the application of the tag itself, (i.e. within one month of tag application) possibly due to the use of the larger needle gun applicator and the possibility that the "T" bar of the tag may have been caught on a basal process within the thorax.

This larger carapace tag was also applied successfully to a single adult Mud Crab (*Scylla serrata),* although this individual did not moult during this experiment it may be that this larger tag would be worth examining in larger crustacean species.

3.4.3 Third Trial

Of the 15 tagged crabs for each of the swimming leg tags and the carapace tags a total of 11 and 9 moulted respectively for each group. Eight of the 9 control crabs moulted, all successfully. There was a 64% success rate for the swimming leg tag at moult and a 55% success rate for the carapace tag. There was no mortality associated with the tagging operation itself, i.e. within 4 weeks of the initial tagging operation. Autopsies on 6 tagged crabs which had both died at moult or survived the length of the experiment showed only a limited amount of brown connective tissue surrounding the entry area in either of the swimming leg or carapace tags, reflecting minimal infection at this site. No tags were determined to have been stuck in that position in the shell.

3.5 Discussion

While there is probably no such thing as the ideal tag (Laird and Scott 1978) it is however imperative that any worker attempting to conduct population or migration studies, using a particular tag, realise the limitations of that tag and make allowance for this in the final conclusions. For example Marullo *et al.* (1976) reported survival rates of 52% and 76% for prawns tagged with newly developed streamer tags. While some test animals had moulted during these trials the authors did not report on the success of the tag at moulting and it is possible that not all the test animals moulted, thus potentially over-estimating the survival rates. Somers (1987) and Buckworth (1992) subsequently based large scale field studies on this tag with no mention of survival rates. While low survival at moult is not as critical for migration studies, any studies focusing on growth or population structure will be influenced by such mortalities.

Prentice and Rensel (1977) were of the opinion that external tags were not satisfactory for use on prawns due to increased mortality, effects on behavior and loss at moult. For these reasons they examined coded wire tags in laboratory trials with some success.

Researchers at the Alaskan Department of Fish and Game have, however, successfully used Floy "Spaghetti" tags to mark the Golden King Crab, *Lithodes aequispinus* (Anon. 1998). This was due to the physical differences between Lithodid crabs and fused carapace crabs such as the Portunids. Lithodid crabs having an isthmus muscle joining the carapace and the abdomen, tags placed through this muscle can be retained through successive moults. Hill (1975) tagged the Mud Crab (*Scylla serrata)* with Floy FD67 anchor tags using a Dennison tagging gun. Tags were placed slightly off center at the junction of the carapace and the abdomen in the hope that this would reduce any effects of the tag at moult. Later, Hill (1982) used Floy FD68B anchor tags with a Dennison tagging gun again to tag *S. serrata*. While these workers attempted to test the effects of the tagging process itself on various-sized individuals they still did not assess the effect of the tag during moulting. Hyland *et al.* (1984) based a study of the potential movements of *S. serrata* on Hill (1975). While movement studies are less critical (also depending on the time frame) in terms of tag success at moulting the potential number of returns will be reduced to some extent.

Williams and Hill (1982) used anchor tags as per Hill (1975) to assess pot catches and population estimates of *S. serrata*. These authors concluded that, due to the bias of the capture methods (baited traps) and the cost of mark/release programs, such methods would not be applicable to population estimates of *S. serrata*. No assessment was made of the potential effect of the impact of this poorly tested tagging method on the survival of the crabs and hence the bias inflicted on the final population estimates. Smith and Jamieson (1991) used Floy T-Bar anchor tags to conduct mark-recovery population assessments over a twelve month period on *Cancer magister* (Dungeness crab). The tag was applied as originally tested in this work but no references were quoted in support. Results from this work would also have to be questioned given the results of the present study.

Melville-Smith (1987) used spaghetti type dart tags in various insertion points for field studies of the red crab *Geryon maritae* off South West Africa. While few details of the structure of these tags is presented this type of tag may have potential if its diameter is constant along the length of the tag and it is not of such a length so as to slow the moulting

process to any great extent. Results from this work (Melville-Smith 1987) were used to assess the movements of these animals, so effects of mortalities at moult would not be as important as if the tag were being used for population assessment. This same author did however use this tag later for population assessment of the same species (Melville-Smith 1988). Beyers (1984) used the same "T" Bar anchor tags assessed in this work, again on *G. maritae*, to conduct population assessments. The logic proposed for the use of this tag was based on the work conducted by Melville-Smith (1987) which used spaghetti tags and attempted only to assess crab movements. The results presented by Beyers (1984) would have to be considered seriously flawed in light of the present work.

Taylor *et al.* (1989) used traditional spaghetti tags tied around the carapace between the first and second walking legs (*Chionoecetes opillio*) to assess the speed with which soft-shelled crabs harden in the field. While this tag was probably effective for this application it would not have been successful for population work. In a more recent paper Chen and Kennelly (1999) used the same "T" Bar anchor tag assessed here to develop growth models for the Spanner Crab *Ranina ranina*. While it is not possible to estimate the effect of the tag on this species, data collected by these authors related only to the life histories of recaptured tagged individuals, thus lessening the effects of potentially high mortalities at moult due to the tag.

Anon. (1949) presents an excellent assessment of potential tagging methods for the Blue Crab, *Callinectes sapidus*, but only considers the problem of tagging terminal moult crabs which by their nature should not have the problems associated with other crab life history assessments.

One of the major reasons for the failure of the TBA-1 tag in this work is the assumption that by placing the tag in the suture at the rear of the carapace the tag is not penetrating the exoskeleton of the animal and that during the moulting process this part of the animal is the first to emerge from the old shell and so the tag will not complicate the moult (Hill 1975). The tag does, however, penetrate the exoskeleton because the shell of the abdominal flap extends up and under the rear of the carapace for a number of millimeters and it is not

possible to place the tag in a muscle group without going through this shell. The tag actually passes through three shell layers when placed in this position, the dorsal and ventral sides of the abdomen and the posterior of the thorax. Thus when the animal moults the leader attempts to pull the barrel of the tag into the body cavity prior to the body leaving the old shell, when this occurs the collar of the barrel catches on the old shell (Figure 9).

This phenomenon can result in a slowing of the moulting process to such an extent that the animal's exoskeleton begins to harden prior to all the body parts being released from the old shell. Limbs twisted in strange ways result in deformed appendages after moulting. The limb is sometimes caught in the old shell and has to be auto-excised. The stress resulting from this extended moulting process also produces increased mortalities. Hurley *et al.* (1990) observed similar mortalities and deformities in Snow crabs tagged in a similar way. These authors did not however attempt to discern the reasons for these results and thus did not attempt to modify the tag.

The second trial showed that the use of larger applicator gun (Monarch No. 3030) resulted in an unacceptable number of mortalities due to the tagging process alone, but also suggested that the modified tag did produce some success during the moulting process for both carapace and swimming leg tags.

The tag utilised in the third trial eliminated the problem of mortalities at tag application with no animals perishing during the first month of the experiment. This success means that the tag is now ideal for short term population work such as mark recapture experiments. The third trial resulted in a success rate at moulting of 55% for the carapace tag and 64% for the swimming leg tag. While not giving a 100% tag success rate due to the moulting process, this does supply calibration data which can be factored in to any population assessment utilising this tag (Hurley *et al.* 1990).

When applying the tag, the "T" bar needs to be in the swimming leg muscle but towards the median line of the animal, so as not to be through an exoskeleton process which

supports the muscle and is attached to the basal area of the thorax. These processes remain in the old shell after moulting and can stop the tag from leaving the old shell with the soft shell state animal.

It would seem that during laboratory trials testing anchor tags on *P. pelagicus,* most mortality occurs due to the tagging operation or during the moulting process. The success of a tag tested in this way will most likely be further reduced in the field due to influences of the tag on the animals and the visibility of the tag on recapture. It is also obvious from this work that the size of the applicator gun/needle is important when trying to limit the effect of tagging itself.

3.6 Conclusion

For a tag to be useful in any population or migration studies relying on public returns it must meet two basic criteria; it must not influence the subject to any discernible extent and it must be visible and easily read.

This study attempted to assess the first of these two issues and has demonstrated that the modified anchor tag, as presented here, is much more successful than the standard anchor tag, and has good potential for future field studies of *P. pelagicus*.

4 Application of a Modified T Bar Anchor Tag for Population Assessment of the Blue Swimmer Crab, *Portunus pelagicus* (L.) in Cowan Creek on the Hawkesbury River Estuary

4.1 Summary

The Blue Swimmer Crab, *P. pelagicus*, is an important commercial and recreational species on the east coast of Australia. There is unfortunately little information available on its distribution and abundance in this region. To assist in the management of this fishery a series of mark-recapture experiments were conducted to determine the distribution and abundance of *P. pelagicus* for the Cowan Creek area. The method presented utilises a modified "T" Bar Anchor tag which proved both successful in its application and low in resource allocation. The distribution of *P. pelagicus* was found to equate with depth. This distribution results in a series of geographically distinct populations in the small bays along the estuary during summer.

Mark-recapture assessments in one of these bays, using the Weighted Mean Method over four consecutive capture release days, on two assessment periods, gave population estimates of 26.0 (±14.9) and 15.2 (±7.6) respectively. On two other sampling occasions during winter no crabs were captured suggesting the animals either move to deeper waters during colder months or lower their metabolism and remain in the sediment for these periods.

4.2 Introduction

The status of *P. pelagicus* in the Hawkesbury River, on the New South Wales coast, is not well understood. The species supports both an important commercial and recreational fishing industry around almost the entire country with its distribution covering all but the southern coastline. The crabs life history includes both an estuarine and off shore component. Crabs form breeding pairs during late summer and mating occurs while the female is in the soft shell state. In temperate climates the female will store sperm until spawning takes place in spring or summer (November to January). Eggs hatch after approximately two weeks and the larvae (zoea) then spend an indeterminate planktonic larval phase off shore. Juvenile crabs return to estuaries and settle at about 15 mm carapace width where they inhabit intertidal and just sub-tidal areas until becoming adults. They are then primarily bottom feeding carnivores predating mainly on slow moving benthic invertebrates.

Much of the information on the animal's life history has in the past been sourced from other states (Kailola *et al.* 1993, Potter *et al.* 1983 and Smith 1982). To further our knowledge of the distribution of *P. pelagicus* in this estuary a combination of trapping and mark-recapture experiments were conducted in Cowan Creek which is a major tributary of the Hawkesbury River estuary.

Cowan Creek is a typical flooded river valley with a series of minor catchments entering from either side along its length. This results in a deeper main creek (> 20m) with more shallow bays along its length. Each of these small bays has a sandy alluvial delta entering at its headwaters from a freshwater creek. The catchment is protected as National Park and there is no commercial fishing in the area.

Population assessment was proposed to follow two methods. The first phase was a range finding experiment to determine the crab's distribution in the Cowan Creek estuary. This was followed by a series of mark-recapture experiments based on the modified "T" bar anchor tag described previously.

The two major goals of this work were therefore to use the tag in a field application and then subsequently to gain a better understanding of the distribution and abundance of this species in relation to environmental variables and to support the improved management of both the commercial and recreational fisheries across the region.

Population estimates using mark-recapture methods are now a routine part of fisheries management with the major questions to be answered to complete such studies being which tag to utilise (Nielsen 1992) and what statistical model to apply to any particular question.

Population assessments of this type have in the past proved questionable in their reliability due to the violation of some or all of the assumptions associated with a particular model. The most successful methodology to date, relating to crab population assessment, would seem to be that developed for the Blue Crab, *Callinectes sapidus* by Fitz and Wiegert (1992). The only problem with this method being the resource costs involved due to a marking method utilising microwire tags.

One of the more common models employed is the Jolly-Seber estimate (Jolly 1965, Seber 1965). The Jolly-Seber model was used by Hightower and Gilbert (1984) to estimate the population of fish in a reservoir. These authors concluded that the model was reliable with low sampling intensities (approx. 0.01) only if the population size was greater than about 5,000 individuals. These workers also attempted to test the effect of tag loss on these estimates by double tagging but in the end concluded this was not a successful strategy and recommended the use of more reliable tags. Hightower and Gilbert (1984) also looked at the effects of handling mortalities on estimates and determined a 20% mortality rate for their methods. Arnason and Mills (1987) found similar major problems associated with high handling mortalities and developed a test to detect these biases. Arnason and Mills (1981) also looked at the Jolly-Seber model and the errors resulting from tag loss and developed formulae to allow for projected tag loss.

Pollock and Mann (1983) modified the Jolly-Seber model to allow for age dependant survival and outline the import bias this factor can have in population estimates. Cone *et al.* (1988) gave an excellent assessment of how population estimates can be flawed by violations of the six assumptions associated with the Jolly-Seber model (and the Peterson estimate). They promoted the careful validation of the assumptions of mark-recapture models as a prerequisite to the acceptance of numerical estimates of wild populations. The selection of population estimation models was linked to the decision as to whether the population could be considered as closed or open given Seber (1986) suggested the Jolly-Seber model was more appropriate for use with open populations.

Another likely method proposed by fisheries scientists was the Fisher-Ford model of population assessment (Begon 1979). This model was most appropriate when there are low sampling intensities (< 12%), low survival rates (around 0.5) and for relatively small population sizes (< 1000).

Schwarz and Taylor (1998) compared the use of a 'stratified' to a 'pooled Petersen estimate' of pink salmon (*Oncorhynchus gorbuscha*) in the Fraser River in Canada. These authors looked at the potential bias inflicted by the violation of the assumptions of equal catch ability and mixing of tagged and untagged fish. The work also highlighted potential problems associated with an acute or chronic increase in mortality or differential movement of tagged fish compared with untagged fish.

There have been very few attempts by researchers to apply mark-recapture methods to the assessment of crustacean populations in general. There have been even fewer attempting to assess crab populations. This is probably a function of the problems associated with the production of reliable tags. Given the success of the previously described tag, a means for population assessment is now feasible.

With the resources available and the level of importance placed on the need for accuracy of the result, the use of a simple population assessment model, such as the Weighted Mean Method or the Simple Peterson Estimate (Begon 1979) is proposed for this work. Begon

(1979) outlines that the major difference between the Peterson estimate and the Weighted Mean estimate is that the Weighted Mean estimate utilises data collected over several days. The Weighted Mean model assumes as much as the most restrictive Peterson estimate: that the population is closed, and has neither births nor deaths. Its only advantage over the Peterson estimate is that it accumulates data over several days. This advantage can be considerable. In studies where there are very few captures, and, application of the more sophisticated models is not possible, the next decision is that of whether to use several Peterson estimates or to use a weighted mean.

The Weighted Mean method is proposed due to the assumed small “closed” standing population, the fact that only adult animals are being assessed and the territorial nature of this species. The Weighted Mean method (Begon 1979) is particularly useful in situations where mark-recaptures are very low due to the assessment of small populations and the need for only qualitative population estimates.

4.3 Methods and materials

4.3.1 Mark-recapture assessment

Using information gathered from a pilot trapping exercise (unpublished data); tangle nets were deployed on a 10m grid pattern to a depth of 10m in Waratah Bay to estimate the abundance of adult crabs in the area (Figure 11). Adult crabs being those greater than or equal to 6 cm in depth measured from midway between the eyes to the middle of the posterior of the carapace. The Weighted Mean Method (Begon 1979) for mark-recapture population assessment was utilised by conducting four days of consecutive mark-recapture analysis using tangle nets as described above.

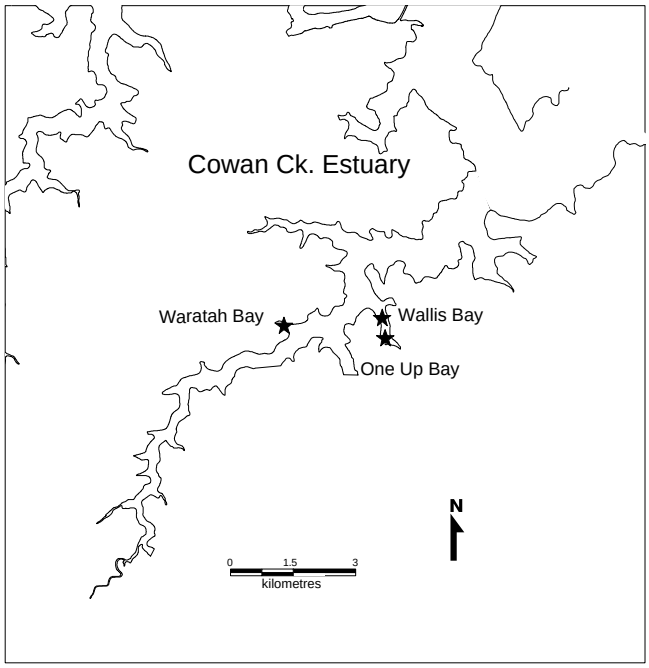


Figure 11. The Cowan Ck. Estuary, New South Wales, Australia (Latitude 151.1966º E, Longitude -33.6146ºS).

The captured crabs were marked with the modified “T”-bar anchor tag (Hallprint Australia) (Plate 7). Tagging consisted of removing the animal from the net, placing a black cloth soaked in water over the carapace and applying the tag with a needle gun to the suture at the rear of the carapace. Data collected included tag number, carapace width from spine tip to spine tip, carapace depth, sex, position within the bay, date, time, tide, weather and any additional comments relating to crab condition. Tags utilised in this work were consecutively numbered and included the words ‘reward and a contact phone number’. Nets were deployed at 1200 noon on each sampling day and retrieved at two hourly intervals until approximately 1800 hrs.

Plate 7. Field tagging operation.

4.4 Results

4.4.1 Mark- recapture assessment

Data presented in Tables 8 and 9 show the results of two of the four mark-recapture exercises conducted during summer periods (December 1998 and March 2000). The two other assessments, conducted in winter, resulted in no crab captures.

Table 8. First mark-recapture exercise – Waratah Bay summer 1998. Weighted Mean method (Begon 1979).

Day (*i*)	1	2	3	4
n (*i*)	-	8	5	4
m (*i*)	-	1	1	2
r (*i*)	3	8	5	-
M (*i*)		3	10	14
M(*i*)n(*i*)		24	50	56

Where n (*i*) = the number of individuals caught on day i.
m (*i*) = the number of marked individuals caught on day i.
r (*i*) = the number of marked individuals released on day i.

$$N = \frac{\sum Mi\ ni}{(\sum mi) + 1}$$

$$= 26$$

Standard error = 14.9

Table 9. Second mark-recapture exercise – Waratah Bay summer 2000. Weighted Mean Method (Begon 1979).

Day (*i*)	1	2	3	4
n (*i*)	-	6	5	3
m (*i*)	-	1	2	2
r (*i*)	3	6	5	-
M (*i*)		3	8	11
M(*i*)n(*i*)		18	40	33

N = 15.2

Standard error = 7.6

When applied to these data, the Simple Peterson Estimate, with an initial capture/release and one more capture (marked individuals < 10), gave population estimates of 16.7 and 15.7 respectively.

The next most likely model for population estimates to be applicable to these data would be the Triple Catch Method where individuals were captured, marked and released on day one, then captured and released on days two and three. However, as there were no Day 2 marks captured from the first experiment the results are in doubt as it is not possible to determine a standard error for any population estimate. The second experiment did however collect enough data to apply to the Triple Catch Method and gave a population estimate of 15.75 ± 16.6. The standard error does however raise some questions about the validity of this result.

While these sampling exercises did not have many recaptures this was due mainly to the low standing population in each bay. The low population numbers have however resulted in a very high sampling intensity (0.4) with up to 40% of that population being marked during the exercise.

On only one occasion during these experiments did a member of the public ring to say they had captured a marked individual in Waratah Bay (Plate 8). This being due to two factors, the low intensity of recreational effort in the area and the relatively low numbers of marked individuals.

Plate 8. Waratah Bay, Cowan Creek at high tide. * Note seagrass patches to the right of the photograph and the shallow area to the left.

4.5 Discussion

The Weighted Mean Model is a slight variation on the Simple Peterson Estimate with all the same assumptions. These being that, all marks are permanent, marks and animals are unaffected by being captured, there is no death/emigration effect, all individuals have an equal chance of being caught, marking does not effect the chance of death or emigration, assumes no deaths or emigrations or no births or immigrations. The Weighted Mean Method is conducted over several days as opposed to only one for the Peterson estimate thus increasing confidence in the results.

In terms of the assumptions applicable to these models, adult *P. pelagicus* are highly territorial and cannibalistic (personal observation), this will limit the effects of immigration and emigration (of adults) over such a short time frame for sampling. The effect of the tag has been assessed in laboratory experiments to be negligible over the short term (McPherson 2001). For long term population assessments it would be necessary to apply a correction factor to any model applied to allow for the approximate 40% mortality occurring due to the moulting process. The probability of moulting occurring in adult *P. Pelagicus* of this age over such a short time frame was only small and no recaptured animals had moulted during these assessments. A population assessment of approximately 15 individuals in the bay gave a density of $0.01/m^2$ in bay waters less than 10 m deep.

Few studies have attempted the assessment of crab populations by mark-recapture methods. Drummond-Davis *et al.* (1982) used baited traps to estimate the population density of the rock crab *Cancer irroratus* in kelp beds in Nova Scotia. These researchers marked the crabs by engraving a number on the carapace. No consideration was given to the effect of the tagging process on the animal or of the effect of moulting on the population estimates derived from the use of the Jolly -Seber model. Indeed, these authors admitted that the assumptions required for the use of the Jolly-Seber model were not fully met and hence relied on the results from another model to support their conclusions. Due to the potential loss of tags during moulting this work was confounded without even considering the other assumptions required for the use of any population models.

Diaz and Conde (1989) also used baited traps for population work on the mangrove crab *Aratus pisonii*. Again the majority of the assumptions for the estimation of population size were violated with the main flaw being the use of an adhesive carapace tag which did not account for moulting, which would have been of particular concern here due to a sampling regime which occurred over a two year period.

The effect of tagging on mortality has been considered rarely but is an important assumption in any population assessment model. Brewis and Bowler (1983) used the model of Jolly (1965) to assess a population of the freshwater crayfish, *Austropotamobius pallipes* in an aquaduct. The problems associated with mark visibility with the use of branding as a tagging procedure were well understood and allowed for, but there was never any assessment of the handling/tagging effects on the mortality of this animal. The use of the Jolly model was appropriate in this case due to the large population numbers (> 5,000 individuals).

This study utilised visual identification tags due to the lower resource costs associated with the mark and recapture process. These increased costs include the costs involved in the purchase of sophisticated tag sensing equipment and the additional resources associated with the recapture process which are reduced when using public return programs.

A potentially more accurate, but more costly, tagging method is the use of coded microwire tags (Krouse and Nutting 1990 and Fitz and Wiegert 1992). Krouse and Nutting (1990) used microwire tags in juvenile American Lobsters with some success but found that in laboratory trials tag retention at first moult ranged from 52 to 86%. Hurley *et al.* (1990) found similar results associated with microwire tags used on the Snow crab *Chionoecetes opilio*.

Fitz and Wiegert (1992) used coded microwire tags to mark the Blue Crab *Callinectes sapidus* and then applied the data to the Jolly-Seber mark-recapture model (Jolly 1965, Seber 1965). Prior to this field work these authors conducted laboratory trials of the tagging technique and found tag retention rates of between 98 and 100% through multiple

moults, along with other data, these results helped to address the assumptions required for application of the Jolly-Seber model and would seem to place this methodology as the preferred procedure for mark-recapture studies of swimming crabs, if resources allow.

Issues which could be associated with the use of tangle nets (witches hats) as sampling units for this species include the possibility of multiple captures (no exclusions), this occurred on a number of occasions with up to three animals being captured during one trap deployment, the advantage of this method in this instance is however, that individuals could not attack each other during confinement as they were bound by the net. This is not the case when using other types of baited traps.

There is the concern that the animals could become trap shy even over short periods, this would seem not to be the case as during one part of the sampling the same crab was captured in the same net on three occasions in roughly the same area within the bay.

These results also lead to the conclusion that individuals do not move far over the short term and is supported by the observation that they are very territorial in captivity. Another concern is that trap addiction could bias results; this however can not be addressed from the results presented here. Equally trap avoidance could be a problem but with the multiple capture of individuals during this work trap avoidance is not considered to have influenced the results to any great extent.

The development of this methodology will enable such population studies to be completed without the allocation of vast resources, but with some confidence in the tagging methods employed and the subsequent population estimates gained. The results presented have shown that small “closed” populations can be assessed even when the number of mark-recaptures is relatively low.

The work has also shown that *P. pelagicus* is resident in this estuary for at least the summer months. It is not known however if the animal goes to ground over winter or moves to deeper water as is suggested by Kailola *et al.* (1993).

This work has also shown that the abundance of *P. pelagicus* in this estuary is very low in absolute numbers and that the current ban on commercial fishing is probably justified. Some additional controls may also be required to manage the local recreational fishery.

4.6 Conclusion

The modified T bar anchor tag has been proven to be a useful tool for the assessment of Blue Swimmer Crab populations.

The residency of Blue Swimmer Crabs over summer, in this estuary, supports their use as a bioindicator, at least on the scale of “between estuaries”, and that they could even be utilised for this purpose on the scale of “within estuaries”.

5 The Dispersal of *Portunus pelagicus* (L.) Larvae in the Hawkesbury Estuary, New South Wales

5.1 Summary

Only limited work has been conducted on the Blue Swimmer Crab, *P. pelagicus*, in New South Wales waters and little information exists on the local life cycle of this animal. An initial survey of the estuarine plankton for *P. pelagicus* larvae suggests that the life cycle could be similar to other swimming crabs such as the Blue Crab, *Callinectes sapidus*, where gravid (berried) females move to the mouths of estuaries prior to releasing their eggs.

Previous studies have shown that the maximum rate of tidal assisted movement in this species is in the order of 23 m min^{-1}, this would enable an individual to potentially move to the mouth of Cowan Creek over two ebb tide periods, i.e. less than 24 hrs.

5.2 Introduction

Studies of *P. pelagicus* larval life cycles conducted in other parts of Australia support similar findings from overseas (e.g. relating to Blue Crab, *Callinectes sapidus* larvae, Epifanio 1995, Johnson and Perry 1999) showing a larval life cycle offshore with post larvae (megalopae) recruiting into coastal estuaries (Kailola *et al.*, 1993, Williams 1982).

During adult population assessment studies of *P. pelagicus*, in the bays along Cowan Creek, to the north of Sydney, it was identified that a number of captured females were carrying very late developmental stage egg masses (Plate 9). These being grey/black in colour compared with the newly laid yellow/orange egg masses. This information suggested that there could be significant numbers of larval crabs in the water column associated with these adults and the bays in which they were being captured. This also had implications in relation to the residence of crabs in a given area and hence their usefulness as a biomonitor of available metals.

An alternative hypothesis was that there would be few larvae in these upstream bays and this would support the contention that gravid females move downstream towards the mouth of the estuary to release their eggs.

Plate 9. Berried *P. pelagicus* female (late development stage egg mass).

Provenzano *et al.* (1983) showed Blue Crab (*Callinectes sapidus*) larvae at the mouth of Chesapeake Bay were in the neuston layer immediately after hatching and that the release of larvae was determined to coincide with the beginning of a night time ebb tide. While larvae were released mainly during the night, significant numbers were always found in surface waters during either the night or daytime sampling runs. Larval numbers being in the order of 100's to 1000's per 10 m^3.

Lochman *et al.* (1995), on the other hand, found *C. sapidus* zoea were equally abundant on ebb and flood tides but were more abundant during the day than at night. Provenzano *et al.*

(1983) did not support the theory of vertical migration of this species and maintained that larvae were found rarely at the bottom of the water column.

DiBacco *et al.* (2001) contend that vertical migration "has been well documented as a behavioural mechanism of brachyuran larvae that are actively exported from or retained within estuaries." These authors used field studies to validate a hydrodynamic model which predicted that larval migratory behaviour was necessary to transport larvae out of bays. Garrison (1999) examined the zoea from three families of brachyuran crabs in Chesapeake Bay and found significant vertical migration.

Provenzano *et al.* (1983), Epifanio (1995) and Johnson and Perry (1999) all support the concept of the migration of berried females to the mouth of the estuary prior to the hatching of the larvae as this would explain the highly synchronised peak in larval numbers during ebb tides.

The processes of emigration and immigration of crab larvae to estuarine habitats have been dealt with by Christy and Morgan (1998) who maintain that during their study, larval recruitment was not uncoupled from production with similar numbers leaving and returning to the estuary through time. The important point not dealt with by these authors is that were the larvae from these estuaries in the first place? If they were not then production and recruitment might be considered uncoupled. Todd (1998) makes the same point that pelagic larvae must be considered as uncoupled between production and recruitment and that many marine populations should be considered demographically "open".

Hovel and Morgan (1999) examined the impact of ultra violet radiation on crab larval survivorship and determined that while some doses of radiation could be lethal, larvae which tended to stay in surface waters during their initial dispersion stages had likely evolved mycosporine-like amino acids and chromatophores to screen harmful UV radiation.

This work supports the contention that the larval life cycle of *C. sapidus* is primarily offshore. This contention is also supported by Queiroga (1996) and Drake *et al.* (1998).

General turbidity of specific habitats was also found to be a significant factor in larval survivorship. The above studies support daytime sampling of surface waters for *P. pelagicus* larvae in the Hawkesbury estuary to determine the larval life cycle in this region.

The present study therefore undertook a series of plankton tows to determine the presence or absence of crab larvae in these upstream bays to supply information on the potential movement of gravid females within the estuary. The information gained would then assist in the design of future monitoring programs using the Blue Swimmer Crab as a biomonitor of available cadmium contamination.

5.3 Methods and Materials

To test the hypothesis that there might be significant numbers of larvae in the water column, and hence better understand the potential movement of gravid females, a series of plankton tows (samples) were conducted along the estuary while continuing to capture berried females in a number of these small bays. Five minute surface tows (at approximately 2 knots), using a 100 micron plankton net (40 cm aperture and 100 cm length), were conducted in random bays within Cowan Creek over two days. Each plankton tow therefore sampled approximately 11 m^3 of surface water. Cowan Creek is a major estuarine tributary of the Hawkesbury River estuary just to the north of Sydney on the Australian east coast (Figure12).

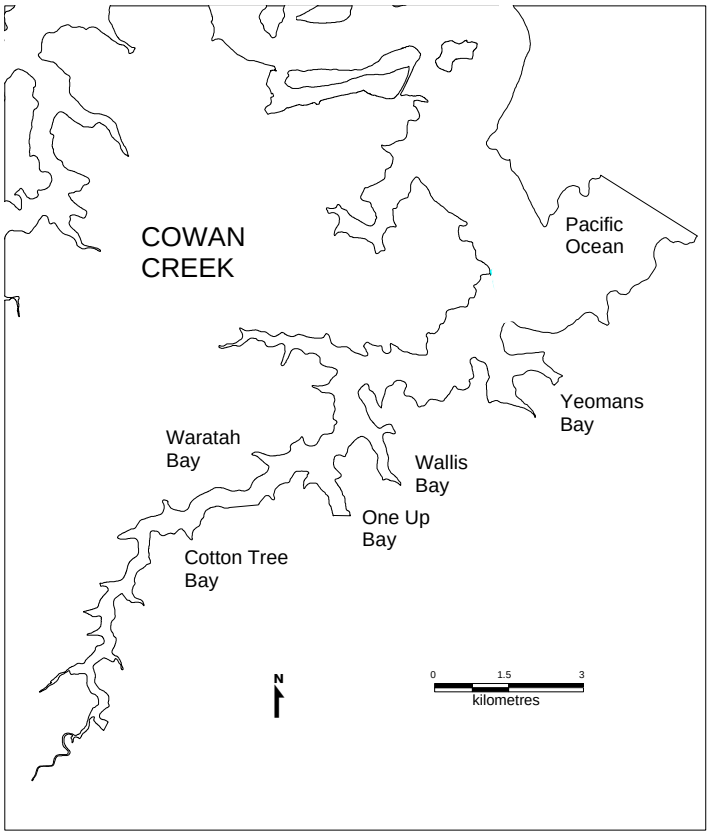


Figure 12. The Cowan Creek estuary (Latitude 151.1966º, Longitude -33.6146º).

Bays assessed were Cotton Tree Bay, 4 samples, Waratah Bay, 3 samples, Wallis Bay, 3 samples, Yeomans Bay, 4 samples, and One up Bay, 2 samples (Figure 12). The tows were all done during the run up/high tide over the sandy intertidal areas in the small bays of Cowan Creek. The tows were conducted in the middle of each bay and where appropriate, in the middle of each channel associated with that bay. Weather conditions were calm and dry during the sampling.

5.4 Results

All samples were sorted using stereo microscopes and 100 micron sieves to determine the presence of larval crabs. Samples were referenced against first stage zoeal larvae of *P. pelagicus* which had been collected from females which had released larvae in laboratory aquaria (See Appendix 9.2 inside back cover for a VCD produced during the production of reference larvae).

Of the 16 samples sorted only 4 zoeal crab larvae were found and one megalopa larva. Larvae were found only over sand flats and in Waratah Bay, Wallace Bay and Yeomans Bay. The zoeal larvae, when compared with the reference larvae, were identified as *P. pelagicus*. Due to a lack of reference material for the megalopa larva it was not possible to positively identify the specimen.

5.5 Discussion

These results are in contrast to those of Provenzano *et al.* (1983) who found hundreds to thousands of larvae in similar volumes sampled at the mouth of Chesapeake Bay.

Johnson and Perry (1999) determined that prior to moulting in brackish waters of the upper estuary, Blue Crab larvae, (within the Mississippi Bight), were released by females from the mouths of coastal bays on an ebbing tide. Positively buoyant larvae remained in surface waters for 30 – 50 days prior to development into megalopae and subsequent migration back into coastal estuaries using vertical migration in the water column and tidal

synchronisation. This vertical migration could be influenced by turbulence (Welch *et al.* 1999). Xie and Eggleston (1999) modelled the effects of wind and density induced circulation patterns on larval transport (including blue crab larvae) in North Carolina waters. These authors determined that physical processes could explain much of the observed variation in larval recruitment to nursery grounds within estuaries. Paula *et al.* (2001) considered that re-invasion of estuarine habitat by brachyuran megalopae related firstly to cycles of tidal amplitude and secondly to off shore wind stress.

Natunewicz *et al.* (2001) examined the transport of newly hatched *C. sapidus* and concluded that buoyancy as influenced by estuarine outflows was also a significant larval transport forcing factor. Epifanio and Garvin (2001) supported this contention.

While a number of researchers have considered the exchange of larvae between estuaries via a pelagic larval life stage (DiBacco and Chadwick 2001) there appears to be no research regarding the potential exchange of adult *P. pelagicus* between estuaries.

New South Wales commercial catch data gives some information to support such a possibility. The incidental Blue Swimmer Crab catch in offshore trawls in 2000/01 was 20,733 kg (Appendix 9.3). These trawls operate several kilometres offshore and crabs occupying this zone would seem to have the potential to move between estuaries.

The very small number of larvae encountered in the water column during this work might be explained by a lack of sampling intensity to encounter larvae. This is however considered unlikely as 11 m^3 of water were sampled on each of 16 plankton tows during the study and the large number of berried females resident in the bays would have meant high numbers in the surface waters if these animals were releasing larvae in the area. A more likely scenario is that even though the berried females were at such a late stage of larval development, they migrate to the mouth of the estuary before releasing their eggs. This is a distance of approximately 10 kilometres. Gribble and Thorne (1998) used ultrasonic tags to mark *P. pelagicus* in Southern Queensland and determined that the maximum rate of tidal assisted movement was 23 m min^{-1}, this would enable an individual

to potentially move to the mouth of Cowan Creek over two ebb tide periods (i.e. less than 24 hrs).

Salinities in these bays are not significantly different to those encountered in the downstream marine environment. This would mean crabs are being driven more by the need to successfully disperse larvae rather than by some salinity gradient. The disadvantages and advantages of such larval life cycle strategies have been dealt with by Pechenik (1999).

If it is considered that *P. pelagicus* larvae may follow similar life cycle strategies to those observed for *C. sapidus* then the results of the sampling can be explained in either of two ways. Either the berried females remain in these upstream bays and release their zoea at night on the ebb tide or they migrate to the mouth of the estuary prior to larval release. Even if the larvae are released at night they could not all be flushed out of the area in a single tide and this sampling would still have encountered significant numbers in the water column.

5.6 Conclusion

Even though berried females are found at a very late stage of brood development, the fact that very few larvae were encountered during this sampling supports the theory that females migrate down stream prior to larval release. The question still remains, where do the berried females move to in the downstream environment prior to releasing their eggs? Further adult population assessments and larval sampling in the mouth of the estuary are required to examine this question.

6 The Blue Swimmer Crab *Portunus pelagicus* (L.) as a Biomonitor of Cadmium Contamination in Estuaries, New South Wales, Australia

6.1 Summary

Having established the ability of *P. pelagicus* to accumulate cadmium, the organism was then employed as a biomonitor of environmental cadmium levels. By examining the level of cadmium accumulation in the hepatopancreas of *P. pelagicus,* a number of New South Wales estuaries were assessed for potential contamination by this metal. A method is proposed where intermoult, non-berried crabs of a standardised size range are collected from commercial fishermen at the end of summer. Cadmium load is then determined in the individuals hepatopancreas, and comparisons made between estuaries to assess potential anthropogenic cadmium contamination on the scale of estuaries or bays. Crabs assessed from Lake Macquarie, on the New South Wales central coast, were found to be significantly ($p<0.05$) higher in cadmium in the hepatopancreas than cadmium levels found in other estuaries. This result is consistent with other studies which reflect metal contamination of Lake Macquarie by a number of industrial and domestic sources. The Hawkesbury River estuary, on the other hand, was found to be relatively uncontaminated by cadmium. This result is also supported by other studies looking at cadmium contamination in this estuary. The importance of such background work is discussed in relation to the development of bioindicators of environmental contaminants.

6.2 Introduction

As the contamination of a number of New South Wales estuaries by various metals has been identified as a threat to these environments (Batley 1987, Scanes 1993), the development of future monitoring programs utilising bioindicators has become more relevant (Brown and McPherson 1991).

Fairweather (1999) outlined a sequence of ten steps needed for the scientific development and validation of an indicator (See Introduction Chapter). The work presented in this chapter follows these steps and is the final "field validation" (Step 5) in the development of the Blue Swimmer Crab as a biomonitor of available cadmium.

While numerous research projects have focused on the ability or otherwise of crustaceans to accumulate metals, very few have taken the further step of attempting to apply this knowledge to some type of environmental monitoring program, or the development of the particular species as a bioindicator of environmental condition. A number of studies have however, attempted to measure cadmium levels in crustacean tissues from various environments and then to relate these measurements to the contaminated nature of these areas.

Harris and Santos (2000) for example assessed the cadmium contamination in tissues of the mangrove crabs *Ucides cordatus* and *Callinectes danae* from "polluted" and "unpolluted" sites in Brazil. Although these authors quote a range of studies to demonstrate the ability of decapod crustaceans to accumulate cadmium, little evidence is presented to support the contention that these species are in fact bioindicators of environmental cadmium levels. Not only is information lacking on the ability of the species to accumulate cadmium through either water, diet or sediment vectors through time, but the animals ecology has not been considered nor its physiological state at the time of sampling. Physiological state, such as position within the moult cycle, maturity of ovaries, starvation and stress have all been shown to be important to the ability of various decapods to accumulate cadmium (Bondgaard *et al.*, 2000, Styrishave *et al.*, 2000).

Harris and Santos (2000) found elevated cadmium levels in the hepatopancreas of *C. danae* from an "unpolluted" site which they were unable to explain. The contention would be that the sole assessment of metal levels in tissues of species at various sites does not establish that the species in question can be utilised as an indicator of any given metal. See reviews by Luoma (1983) and Phillips (1977a). Sastre *et al.* (1999) examined the bioaccumulation of heavy metals in *Callinectes* spp. and found this to be highly variable between tissues, sexes, sites and species.

McPherson and Brown (2001) showed that from a range of anthropogenically sourced metals, the Blue Swimmer Crab, *P. pelagicus,* could accumulate cadmium from its diet and store this in the hepatopancreas with a direct relationship to the period of exposure (over an eight week period). This finding is also supported by an assessment on another portunid crab, the Blue Crab, *Callinectes sapidus* by Brouwer *et al.* (1995).

Jennings *et al.* (1979) and Jennings and Rainbow (1979) conducted laboratory studies on the accumulation of cadmium in *Carcinus maenas* via either food or seawater. This work attempted to assess the mass of metal entering the crabs via the food and determine some uptake efficiencies to compare the importance of uptake from either of the two media. These authors quoted problems with the amount of food supplied to the crabs as confounding any conclusions concerning the comparison of uptake vectors. Davies *et al.* (1981) compared the uptake of cadmium by the edible crab *Cancer pagurus* via either the water column or diet and determined the major uptake route to be via the diet.

Experimental work attempting to model cadmium uptake in biota has mainly considered the vectors of water column and sediments. Warren *et al.* (1998) and Hare and Tessier (1996) supported the use of the water column as the most relevant vector. Modeling the accumulation of cadmium via the diet has not received any significant attention to date.

The level of contaminant accumulation is only the first step in the development of any bioindicator of environmental condition. Once having established a relationship between

the organism and the parameter of interest, it must also be established that the ecology of the organism lends itself to the role of bioindicator. As *P. pelagicus* is a particularly motile organism, how resident it might be in any given area would impact heavily on the relevance of accumulated metals.

From studies of the animal's ecology (Kailola *et al.* 1993 and Jones and Morgan 1994) it can be seen that *P. pelagicus* is resident in estuaries, at least in temperate climates, during the summer months. It is probably even more resident on the scale of bays and tributaries within estuaries during summer periods (Chapter 4). This is due to the need for suitable benthic habitats and the highly territorial/cannibalistic nature of this swimming crab. The use of this species as a bioindicator is also supported on a trophic level with the diet of *P. pelagicus* consisting mainly of soft bodied benthic infauna some of which have also been confirmed to be accumulators of a range of metals.

The principle of the trophic transfer of metals by crabs was examined by Nott and Nicolaidou (1994) who demonstrated the transfer of cadmium from snails to hermit crabs in a marine food chain. The concept of the trophic transfer of cadmium has also been examined by Wallace *et al.* (1998). These authors examined the transfer of cadmium between an oligochaete and an omnivorous shrimp. The major finding of this work was that even though the oligochaete was resistant to the effects of the cadmium, from a toxicity viewpoint, there was still significant transfer of cadmium, in a bioavailable form, to the predatory shrimp.

Thus, with a good knowledge of the potential bioindicators ecology and relationship to the contaminant in question, a simple sampling program can be developed to further establish the significance of using the proposed indicator. The current research project was designed to apply this knowledge to the design of a monitoring program which would attempt to assess on the scale of "estuaries" which areas in New South Wales might be contaminated with cadmium. These results would then be compared with the current understanding of the level of cadmium contamination of these areas.

Experimental work was therefore based on the hypothesis that crabs from estuaries known to have a higher cadmium load will display significantly higher cadmium levels in their hepatopancreas than crabs from less contaminated estuaries. The known relatively contaminated estuary is Lake Macquarie and the known relatively uncontaminated estuary is the Hawkesbury River. Two other estuaries of unknown cadmium contamination were also assessed, these being Port Stephens and Wallis Lakes (Figure 13).

6.3 Materials and methods

P. pelagicus were collected from Wallis Lakes, Port Stephens, Lake Macquarie (North) and the Hawkesbury River estuaries on the east coast of Australia (Figure 13) All animals were > 6.0 cm from middle anterior to middle posterior of the carapace. No berried females or soft-shelled animals were selected for analysis. The legal size for fishing purposes in New South Wales is 6 cm. The collection was conducted by purchasing crabs caught by professional fishermen from fishing cooperatives associated with each of the estuaries in question. All crabs had been caught less than 24 hours prior to purchase and had been kept on ice for the duration. A random selection of males and females was collected. A random selection of sizes greater than 6 cm was also collected although this depended on availability from the fishing cooperatives.

Data collected included length, from anterior to posterior of the carapace and width from spine tip to tip on the carapace using vernier calipers. The sex and general physical condition of each animal was also recorded.

Prior to analysis, the hepatopancreas was removed from the crab using clean stainless steel dissecting instruments and frozen. Metal levels in the hepatopancreas of each crab were then assessed by nitric acid/hydrogen peroxide microwave digestion and analysis by ICP/MS at the Australian Water Technology Laboratories in Sydney (USEPA method 3051 and USEPA method 6029). These laboratories hold national accreditation for the assessment of trace metals in biological samples (National Association of Testing Authorities).

Analysis of variance was conducted on the four data sets to determine similarities between the areas and then compare these to existing information relating to the known extent of cadmium contamination of these environments.

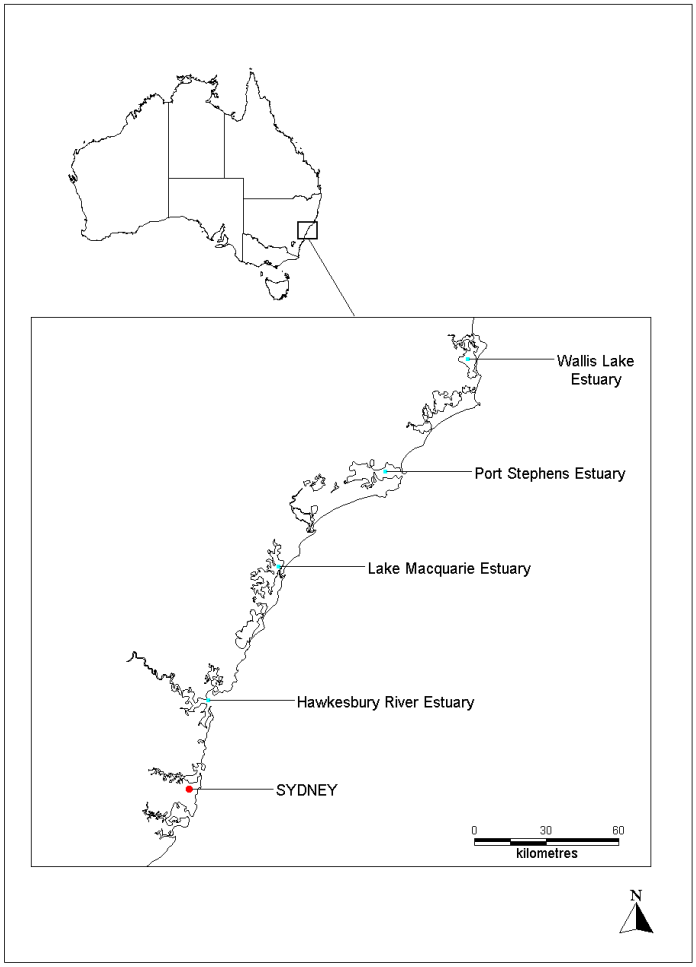


Figure 13. Estuary locations on the east coast of Australia.

6.4 Results

Table 10. Cadmium in *P. pelagicus* from four New South Wales estuaries (mg/kg wet weight).

Port Stephens	Wallis Lakes	Lake Macquarie	Hawkesbury River
1.4	0.02	0.29	0.03
0.09	0.04	1.06	0.04
0.62	0.03	0.73	<0.01
0.63	<0.01	**0.39**	0.02
0.21	<0.01	**0.30**	0.04
2.18	0.04	0.54	<0.01
0.03	0.03	0.56	0.01
0.11	0.29	**1.52**	0.04
0.12	0.01	1.39	0.03
1.88	0.23	0.33	<0.01
0.04	0.07	0.38	0.02
1.76	0.01	0.35	<0.01
		1.07	<0.01
		0.19	0.07
			0.05

***** Bold results represent crabs greater than 8.0 cm carapace depth.

* Detection limit = 0.01 mg/kg

An initial single factor analysis of variance grouped Lake Macquarie and Port Stephens and also grouped Wallis Lakes and the Hawkesbury River (Table 10). These differences were significant at $p < 0.05$. The Lake Macquarie and Port Stephens results were elevated when compared to those for Wallis Lakes and the Hawkesbury River.

Further analysis has found a significant correlation between size and Cadmium level ($p < 0.05$) with larger animals storing higher levels of cadmium in the hepatopancreas.

This correlation was found to bias the initial analysis as there were proportionally more of the larger crabs (> 8.0 cm) in the Port Stephens sample. Crabs greater than 8.0 cm were then removed from the analysis across all estuaries. This resulted in Port Stephens, Wallis Lakes and the Hawkesbury River being grouped as not significantly different ($p < 0.05$) with Lake Macquarie being significantly more contaminated by cadmium than any of the other estuaries ($p < 0.05$).
There was no relationship found between sex and hepatopancreas cadmium level ($p < 0.05$).

6.5 Discussion

The significant correlation ($p<0.05$) between size and metal load in this crab shows the need to standardise the selection of sizes when conducting these surveys. Crabs greater than 8.0 cm have an increased likelihood of elevated cadmium levels in the hepatopancreas independent of location. All crabs sampled during this work were adults ranging from 6.0 to 9.0 cm carapace depth, from middle anterior to middle posterior of the carapace. The Port Stephens sample contained proportionally more large crabs than the other estuaries. For this reason animals with a carapace depth 8.0 cm or greater were removed from the analysis across all estuaries.

The relatively high values for cadmium in the hepatopancreas of crabs sourced from Lake Macquarie are not surprising due to the significant amount of work completed to date on the levels of metals found in a range of matrix types across the lake (Batley 1987, Scanes 1993, Weimin *et al.* 1993, Peters *et al.* 1997 and Kirby *et al.* 2001). Cadmium has been particularly prominent as being well above background levels in the northern areas of the lake (Batley 1987 and Scanes 1993). This lake has a well developed catchment including industrial, residential and recreational land uses. Worthy of note is a zinc smelter which has been in operation for more than 100 years at the northern end of the lake and the effluent and fly ash from a number of coal fired power stations is also discharged into the lake waters. As Lake Macquarie has been identified before as being polluted by cadmium, the relatively high result from the present study helps to verify the use of P. *pelagicus* as an

indicator of cadmium contamination. Cadmium levels in the hepatopancreas of *P. pelagicus* assessed in this system are also comparable to the levels found in the hepatopancreas of the Horseshoe Crab, *Tachypleus tridentatus*, from polluted Japanese coastal waters (Kannan *et al.* 1995).

The Hawkesbury River estuary, on the other hand, has extremely low levels of cadmium in the hepatopancreas of *P. pelagicus*. These levels are also consistent with the body of information relating to the Hawkesbury River and metal contamination in general (Hardiman and Pearson 1995). This estuary is essentially a drowned river valley with good mixing and tidal flushing in the lower estuary. Some of the smaller bays and tributaries have been shown to have slightly elevated localised metal levels (Hardiman and Pearson 1995) but these are not being assessed on the scale of "estuaries" being sampled here as commercial fishing targets the main river. There is, however, no reason why this type of design could not be developed by the use of a targeted sampling design on the scale of "smaller bays".

Lake Macquarie and the Hawkesbury River estuary were not only selected for analysis on the basis of the presence of *P. pelagicus*. The significant amount of research aimed at the metal contaminants in both systems presented an opportunity to further examine the validity of the use of this bioindicator. Port Stephens and Wallis Lakes, on the other hand, have much less data concerning the assessment of metal contamination. Neither of these systems has much in the way of catchment development which might suggest a source of anthropogenically derived cadmium in the local environment.

The levels of cadmium in the hepatopancreas of crabs sourced from Wallis Lakes and Port Stephens are noted to be very low and comparable to those from the Hawkesbury River. An initial examination of the catchment land use types for these areas shows mainly light agriculture and forest with relatively few urban settlements and few obvious major cadmium sources. The low cadmium levels in crabs from these two estuaries again support the contention that there is little metal contamination in these systems.

The results from this work further add to the current knowledge of the potential for the use of this crab as a bioindicator of environmental cadmium levels. The methods developed here can thus be utilised in future environmental monitoring programs which suspect cadmium contamination on a scale of between or within estuaries and can be employed across the natural distribution of this crab.

6.6 Conclusion

The work completed during this chapter has successfully progressed, to a field validation stage (Fairweather 1999), the development of the Blue Swimmer Crab as a biomonitor of available cadmium in estuaries. Future work will apply the methods developed to monitoring over an extended time frame and the further improvement of this technique.

7 The Induction of Moulting in the Blue Swimmer Crab, *Portunus pelagicus* (L.)

7.1 Summary

Potential methods for the production of soft-shelled crabs were trialled using the Blue Swimmer Crab, *P. pelagicus*. It was found that crabs held physically separated in a single aquaria, with both eyestalks ablated, resulted in the highest percentage of crabs moulting per unit time, (mean of 48% as opposed to 20% for controls).
Crabs held as individuals, and completely isolated from others, again with both eyestalks ablated, resulted in the fastest mean days to moult when compared to crabs held in groups with double eyestalk ablation (19.0 days $^{+}$/- 2.4). The injection of moulting hormone, 30^{0}C temperatures, single eyestalk ablation and male only treatments were found to have little effect on the initiation of moulting in this species.
A method for the treatment and holding of *P. pelagicus* for the production of soft-shelled crabs is proposed.

7.2 Introduction

The Atlantic Coast of the eastern United States supports a unique fishery/aquaculture industry which has existed for more than 100 years; this is the soft shell crab industry which revolves around the Blue Crab, *Callinectes sapidus* (Horst 1992,Warner 1994). Breeding populations extend from the Atlantic and Gulf coasts of Florida to Long Island. This industry is based on the concept that the proximity to moult of this crab can be gauged by a change in colour of the margin around the last segment on either swimming leg. This ability to detect how close an animal is to moulting has enabled a “shedding” industry to develop where fishermen bring in crabs in a near moult condition, which are then held using various aquaculture techniques, prior to collection immediately post the moulting process. This results in the production of soft-shelled crabs for commercial markets. Soft-shelled crab prices are usually much higher than prices obtained for hard shelled crabs.

The premoult crabs are called “busters” and require careful handling to avoid high mortalities (10% to 50%). Colour change in the swimming leg of crabs is graded in the following way; white-sign crabs are 7-14 days from shedding, pink-sign crabs are 3 to 6 days from shedding and red-sign crabs are 1 to 3 days from shedding, “cracked busters” (shell has split) are within 24 hours of shedding. White-sign crabs are checked every 3 days and kept separately from pink and red-sign crabs as they are still feeding. Red-sign crabs are checked once a day and cracked busters are checked every three to four hours. Once a crab has shed its shell, and been allowed to expand to its full size, it is removed from the water to prevent the shell hardening. Buster crabs are usually available from Spring through to Autumn (Horst 1992).

The existence of this industry/process led to the concept of applying similar methods to other species of crab to also produce a “soft-shelled” product. *C. sapidus* is a member of the Portunidae (swimming crabs) spending their adult life in estuaries with an off shore larval life cycle. There are two commercially caught swimming crabs in Australian waters which are similar to *C. sapidus*. These are the Blue Swimmer Crab, *P. pelagicus*, and the Mud Crab, *Scylla serrata.* As the Blue Swimmer Crab has a greater spatial distribution and availability in temperate waters than the Mud Crab it was selected as the test species for this work.

As the rearing of *P. pelagicus* through its whole life cycle is still only in its formative years (Kailola *et al.* 1993) it was decided that value adding to the commercial catch by the production of soft-shelled crabs would be a goal for this research. The future development of ‘whole of life cycle rearing’ of this species will better facilitate the prediction of a premoult condition.

Unlike *C. sapidus*, *P. pelagicus* does not undergo any obvious colour change leading up to moulting in eastern Australia. This inability to predict a pre-moult condition makes the application of the soft shell crab methodology, as employed in the United States, more difficult.

In crustaceans, cycles of post embryonic growth and development are characterised by the moulting process, where the old exoskeleton is shed and replaced. This moulting process is regulated by the endocrine system. The cellular events necessary for moulting are stimulated by steroid hormones termed ecdysteroids. Ecdysteroids are secreted by paired cephalothoracic Y organs (Umphrey *et al.* 1998). Y organs are subject to negative regulation by a neuropeptide, known as the Moult Inhibiting Hormone (MIH) (Chang *et al.* 1987). MIH is synthesised in a cluster of eyestalk neurosecretory cells, the X organ, and released from an adjacent Neuro hemal organ, the sinus gland. Thus, a generally accepted model suggests MIH inhibits Y organs during much of the moulting cycle, and the moulting sequence is initiated when MIH secretion diminishes or stops. Amino acid sequence data places MIH in the crustacean hyperglycaemic hormone family (Umphrey *et al.* 1998). Umphrey *et al.* (1998) determined that brachyuran MIH is a 78 amino acid peptide containing six cysteines, and that its primary structure is highly conserved.

Eyestalk ablation, therefore, can induce precocious moulting, while sinus gland, eyestalk extract or purified MIH suppresses the secretion of ecdysteroids by Y organs in vitro, and delays moulting when injected in vivo. It is however possible that MIH is not the sole regulator of ecdysteroid synthesis (Lee *et al.* 1998). Based on the above, a general model for crustacean moult control suggests that MIH suppresses the synthesis of ecdysteroids during much of the moulting cycle (i.e. during post moult and intermoult stages), and that a moulting sequence is initiated (i.e. the animal enters pre moult) when MIH secretion diminishes or ceases (Lee *et al.* 1998).

The release of hormones and the electrical activity of X organ neurons are regulated by environmental and endogenous influences, such as light and dark and circadian rhythms. These influences appear to be mediated by a host of neurotransmitters/modulators. Each of these mediators' acts upon a definite ionic substrate to regulate X organ cell activity (See review of X organ cell function by García and Aréchiga 1998).

A range of parameters has been shown to play a role in the moulting process in crustaceans (Table 11).

Table 11. References for Crustacean moulting factors.

Hormonal control	Wittig and Stevenson 1975, Chang *et al.* 1976, Chang and O'Connor 1978, Armstrong and Stevenson 1979, Dall and Barclay 1979, Chang and Bruce 1981, Chang *et al.* 1987, Tamone and Chang 1993, Sefiani *et al.* 1996, Spaziani *et al.* 1997, Charmantier and Charmantier-Daures 1998, Cooper and Ruffner 1998, Lee *et al.* 1998, Umphrey *et al.* 1998 Chang *et al.* 1999, Spaziani *et al.* 1999, Okumura 2000, Subramoniam 2000, Spindler *et al.* 2001
Eyestalk ablation	Keller and Schmid 1979, Chang and Bruce 1980, Rahman and Subramoniam 1989, Sagi *et al.* 1997, Nakatsuji *et al.* 2000, Stella *et al.* 2000, Meade and Watts 2001
Temperature	Winget *et al.* 1976, Twibell *et al.* 1998, Chang *et al.* 1999, Mezquita *et al.* 1999
Salinity	Guerin and Stickle 1997, Perry *et al.* 2001
Dissolved oxygen	Chittleborough 1975, Clements *et al.* 1999
Holding density	Cobb *et al.* 1982, Chittleborough 1975
Photoperiod	Chittleborough 1975, Waddy and Aiken 1999
Tidal rhythms	Zeng *et al.* 1999
Diet	Chittleborough 1975, Sheen and Wu 1999, Vega-Villasante *et al.* 1999, Sheen 2000
Calcium/protein/metabolism	McWhinnie *et al.* 1972, Dall and Smith 1978, Babu *et al.* 1985, Coblentz *et al.* 1998, Terwilliger 1999, Wheatly 1999, Zou and Fingerman 1999, Mykles *et al.* 2000
Limb loss/damage	Chittleborough 1975, Durica *et al.* 1999
Habitat type	Shirley *et al.* 1990

After synthesising this information (Table 11), suitable holding systems were developed for *P. pelagicus* and a series of trials conducted which manipulated a number of these parameters in an attempt to influence moult frequency in this species.

7.3 Materials and Methods

7.3.1 General

All experiments were conducted in a temperature controlled laboratory sited on the Hawkesbury River estuary at Brooklyn, just to the north of Sydney. A constant air temperature of 25 ^{0}C (water temperature of 23-25 ^{0}C) was maintained for all experiments. The room was illuminated by florescent lights which were on a timer to be on for 12 hrs and off for 12 hrs with no incident external light sources (Dall and Barclay 1977). Aquarium tanks were made from black fiberglass and were approximately 1 m by 1 m by 40 cm deep. Water depth was controlled with a 30 cm standpipe. There were 12 of these tanks fitted into the laboratory. Each tank utilised an external recirculating biological filter with surface spray bars to increase oxygenation of the water. The biological filter consisted of a 40 L reservoir tub, a 40 L shell grit filled tub, covered with 3 cm of filter wool, and a 200 L per hour in-line pump (Figure 14).

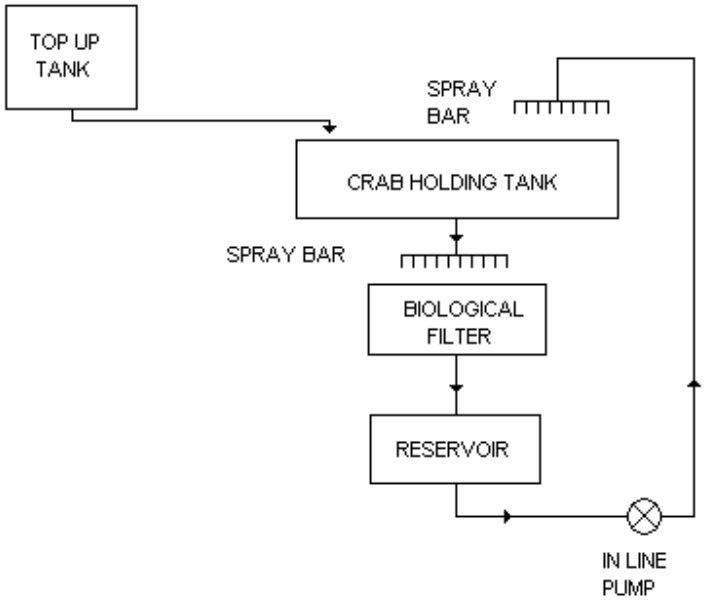


Figure 14. Flow diagram showing configuration of holding tank and biological filtration system.

The tanks had dividers fitted which produced 10 equal sized cells in each fiberglass tank. These dividers were black PVC mesh with a mesh size of approximately 1 cm by 1 cm; this allowed water to move freely between cells but restricted crab contact. A thin layer of shell grit was placed on the floor of all tanks as a calcium source to aid in the moulting process. For each experiment the 12 tanks were randomly divided into groups of three and these represented the treatments for each experiment (See Plate 10).

Plate 10. Holding tanks. * Note individual holding tank on the left and flow through cells on the right. The filter, reservoir and pump can be seen under the tank.

Water was drawn from the estuary at high tide during dry weather to attain water of the appropriate/constant salinity, approximately 25 ppt. This was stored in a 2000 L polyethylene tank and used as make up water for the tanks as necessary due to evaporation and or water quality. 50% water changes were conducted every week.

General water quality was assessed on a weekly basis (pH, nitrites, nitrates, ammonia and salinity). In Phase One, crabs were fed commercially available shucked mussel meat (*Mytilus edulis*) and during all other work, crabs were fed local (Hawkesbury River) bait prawns (*Metapenaeus macleayi*). These prawns were frozen only and had no preservative added to them. Crabs were fed to satiation every two days. All animals were fed the same food type, i.e. mussels for Phase 1 and prawns for Phases 2 to 4.

Mussels were selected as a feed item in the first experiment due to the knowledge that bivalves make up a significant proportion of this animals diet in the wild. Prawns were utilised in subsequent experiments due to their higher sterol content and the increased capacity to improve growth rates in these crabs (pers. comm., Sydney Aquarium staff).

Experimental crabs were sourced from the Hawkesbury River estuary. Crabs were caught in the lower estuary using tangle traps (Witches hats) baited with mullet (*Mugil cephalus*) fillets or with the help of local commercial fishermen who use mullet baited wire mesh box traps. Crabs used in these experiments ranged in size from 4 cm carapace depth, measured from the centre anterior to the centre posterior of the carapace, (6 cm is the legal size under local fisheries legislation) to 9 cm. Experimental crabs were acclimatised in the laboratory tanks for one week prior to the commencement of any experiment in case there were ever any mortalities due to capture and handling.

Carapace depth and width were measured by vernier callipers at capture. Sex and condition of each animal were also assessed at capture. This included the loss of limbs and any growth on the shell. Only hard shelled crabs in good condition with all limbs present were used in these experiments. A random selection of sexes was utilised depending on availability.

Every two weeks the wet weight of each individual was measured. Crabs were checked every 24 hours for general mortalities (i.e. of unknown reason), mortalities due to the moulting process, successful moults and to inspect for discarded food which might overload the biological filter. Information collected during all experiments included, days to moult, weight change, size change and mortalities. Mortalities due to moults were classified as deaths.

All the appropriate animal ethics approvals were gained for the completion of this work. Animals surviving to the end of any given experiment were released to the same area of the river from which they were captured. If an animal perished for any reason during the experiments it was replaced so as to keep the density of animals in the tanks constant through time. Most experiments ran until the majority of crabs had moulted.

7.3.2 Phase 1

The first series of experiments consisted of three treatments and a control. These treatments were, single eyestalk ablation, increased temperature (30 ^{0}C) and male crabs only between 6 and 7 cm.

After a week of acclimatisation, the eyestalk was removed using stainless steel dissecting equipment. The eyestalk was lifted out of the orbital socket with fine tweezers and cut off with fine dissecting scissors as close to the base of the orbit as possible. Prior to ablation, animals were removed from the tanks with a gloved hand and held for a minimum time out of the water. Half the animals had the right eyestalk ablated and the other half had the left eyestalk ablated. Animals treated in this way commenced normal feeding the day after the procedure.

For the temperature treatment, each of the three replicate tanks had immersion water heaters installed to the water reservoir tank of the biological filter. The water temperature

was increased slowly from 25^0C to 30^0C over a 24 hr period; crabs had previously been acclimatised in these tanks at 25^0C as above.

The final treatment involved the sorting of field caught crabs into males only of carapace depths between 6.0 and 7.0 cm.

The three control tanks had none of these treatments applied. The duration of these trials was 67 days.

7.3.3 Phase 2

The next series of experiments built on the results of the first trials (Phase 1) and involved, double swimming leg ablation, individual holding, hormone injection and double eyestalk ablation. Crabs in the 4 to 5 cm size class were used in these experiments to increase the probability of moulting.

The double swimming leg ablation treatment involved the removal of both appendages from the posterior of the animal. This was done using stainless steel dissecting equipment with a scalpel to cut off the limb at the suture closest to the carapace on the limb where it was observed that auto excision occurred if the animal were stressed during capture and held only by this limb. The animal's ability to "throw" limbs in this way is a common defence mechanism in Crustacea (Ruppert and Barnes 1994).

The individual holding treatment involved the modification of each of the three treatment tanks. The flow through mesh cells (10 in each tank) were removed and replaced with solid PVC sheet which was fixed in place using aquarium grade silastic. This resulted in the construction of six water tight individual cells. The single external biological filter was then replaced with six subsurface internal biological filters. Crabs held in this way could not "sense" any other crabs. A single crab was placed in each of the cells resulting in six crabs per replicate tank with three tanks.

The double eyestalk ablation treatment was conducted in the same manner as in the Phase One experiments with the major difference being that both eyestalks were removed during Phase Two.

In the hormone treatment, crabs were injected with the ecdysteroid (Goodwin *et al.* 1978) 20-hydroxyecdyesone (Crustecdysone, Hampshire and Horn 1966) (Sigma Chemicals, HPLC Grade) at an application rate of 2 ug/g of wet body weight. Injections were made with a 50 uL syringe through the flexible membrane on the dorsal side of the joint between the left swimming leg and the carapace. Injections were conducted on days 0 and 2. The solvent used for injection was seawater:ethanol 9:1 (v/v) which was filtered down to a 0.45 µm pore size using a Millipore disc filter. This method follows that described by Dall and Barklay (1977), who worked on the induction of ecdysis in the Western Rock lobster *Panulirus longipes*. The duration of these trials was 87 days.

7.3.4 Phase 3

This series of experiments again built on the previous work and involved the following treatments; density/double eyestalk ablation, density/no eyestalk ablation, group/double eyestalk ablation and group/no eyestalk ablation. All experimental animals used in this trial were between 4 and 6 cm carapace depth.

The two density treatments involved holding crabs in the PVC cells (6 tanks with 6 individually held crabs per tank). Three of these tanks had both eyestalks ablated as per the Phase Two experiments and three had no eyestalks removed. An improved ablation technique was also employed for these experiments and is discussed further in the Results Section.

The other six tanks continued to hold the crabs in the flow through cells (10 crabs per tank, i.e. group treatment). Three of these tanks had both eyestalks ablated and the other three did not. This resulted in four different treatments. The duration of these trials was 67 days.

7.3.5 Phase 4

The final series of trials repeated the Phase Three experiments only using crabs in the size range of from 6 to 7 cm carapace depths (greater than the legal size of 6 cm). The duration of these trials was 45 days.

7.4 Results

All moults occurred during the 12 hrs of lights out.

Figure 15 shows the incremental size classes associated with *P. pelagicus*. It would seem that the terminal moult is around the 9 cm size class. Figures 15 and 16 also reflect the wet weight variability for any given size class. This demonstrates the need for the animal to "grow into" the new exoskeleton.

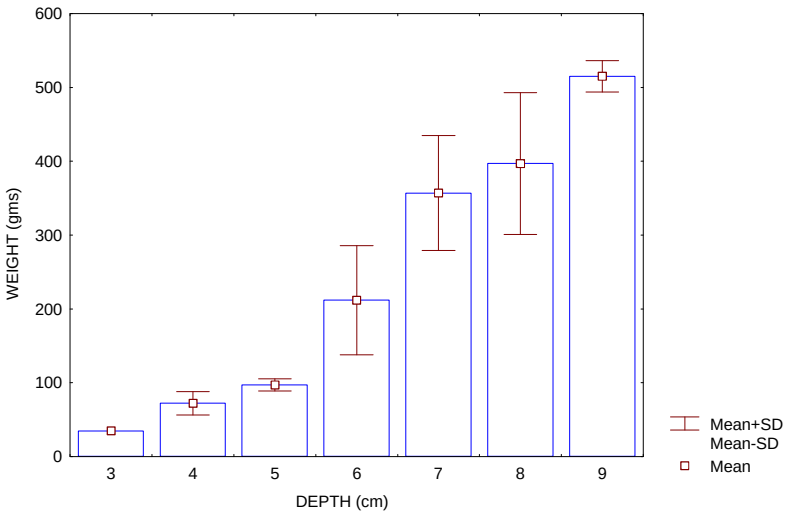


Figure 15. Mean wet weight (g) of the various size classes (carapace depth) for all experimental animals (n=428).

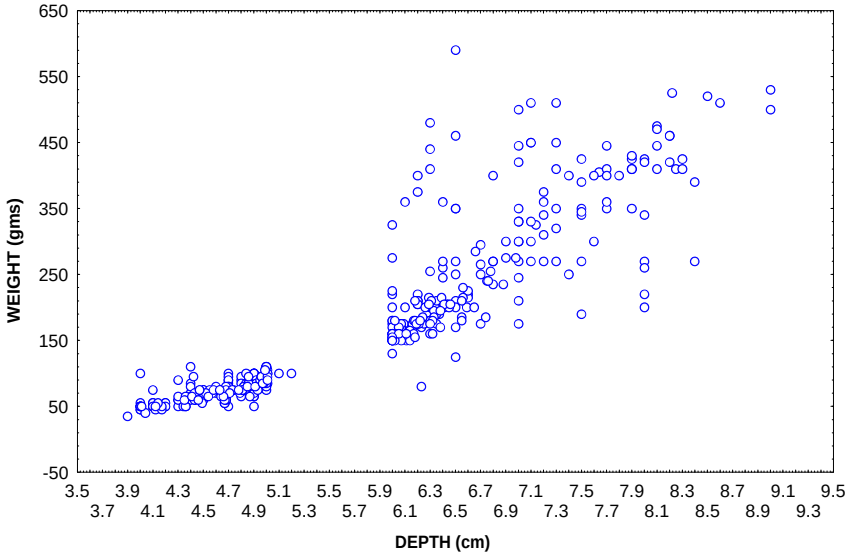


Figure 16. Wet weight (g) versus carapace depth for all experimental animals (n=428).

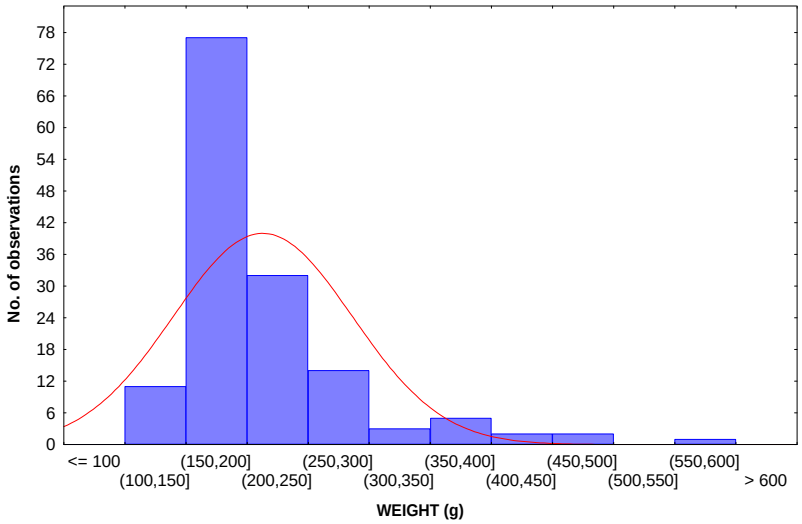


Figure 17. Weight frequency distribution for crabs between 6 and 7 cm carapace depth (wet weight, n = 147).

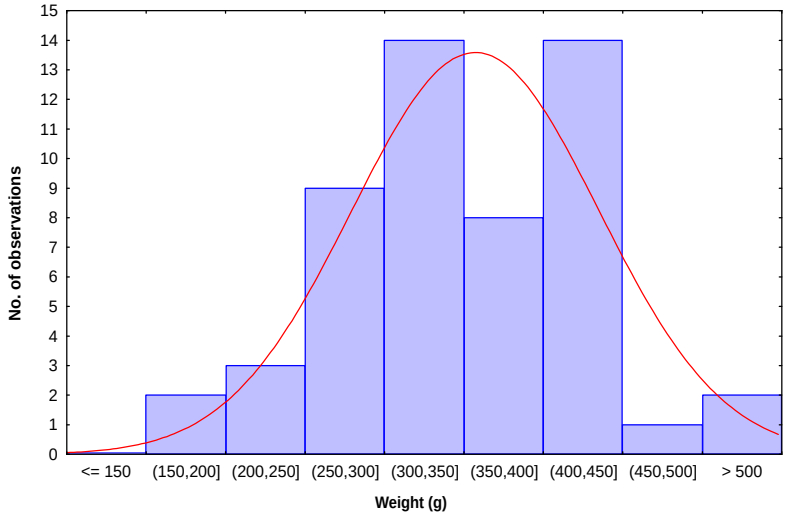


Figure 18. Weight frequency distribution for crabs between 7 and 8 cm carapace depth (wet weight, n = 53).

Figures 17 and 18 shows the weight frequency distributions for the two most common size classes (6-7 cm and 7-8 cm).

A correlation of days to moult versus start weight was performed for crabs in the 6-7 cm size class. This is using wet weight as an indicator of intermoult stage and presumes that heavier crabs in the same size class will moult sooner than lighter crabs. There was no significant correlation ($n = 29$, $r = -0.0324$, $p<0.05$). This result is being effected by the treatment types which brought forward the time to moult of the lighter crabs. There were not enough moults in the control treatments to perform this correlation. It is logical to assume that for any given size class, the heavier the crab, then the increased likelihood of moulting. In the 6-7 cm size class weights of experimental crabs range from approximately 100 g to 450 g and the 7-8 size class range from 150 g to about 500 g.

Table 12. Phase one data, experiment ran for 67 days (30 animals per treatment). Note: individual crab numbers, first number represents tank no. (1 to 12) and second letter represents the individual in that tank (A to J).

Temperature treatment (30°C)									
Deaths	Days	Depth (cm)	Weight (g)	Sex	Moults	Days	Depth (cm)	Weight (g)	Sex
1A	67	7.1	320	M	1E	22	6.5	210	M
1B	59	6.9	275	M	12F	18	6.5	170	M
1C	59	7.6	405	M					
1D	47	8.2	525	M					
1F	62	7.2	310	M					
1I	49	8.2	410	M					
1J	55	7.9	410	M					
12C	35	6.5	250	M					
12E	27	9	500	M					
12H	53	7	330	M					
Single eyestalk ablation treatment									
Deaths	Days	Depth (cm)	Weight (g)	Sex	Moults	Days	Depth (cm)	Weight (g)	Sex
4A	29	8.2	440	M					
4B	1	8	360	M					
4G	60	8.4	500	M					
7I	2	8.3	425	M					
Males 6-7 cm treatment									
Deaths	Days	Depth (cm)	Weight (g)	Sex	Moults	Days	Depth (cm)	Weight (g)	Sex
3G	47	7	340	M	3B	54	6.1	200	M
6G	54	7	420	M	3F	12	7	300	M
Control treatment									
Deaths	Days	Depth (cm)	Weight (g)	Sex	Moults	Days	Depth (cm)	Weight (g)	Sex
5E	59	7.1	510	M					
8I	41	7.6	400	M					

The Phase 1 experiments resulted in very few moults (Table 12). The temperature treatment (30^0C) resulted in the death of one third of the experimental animals, mainly towards the end of the nine week experimental period. Single eyestalk ablation resulted in no moults and four deaths. Control animals and males 6-7 cm also resulted in few moults and no reduction in time to moult. 71% of control crabs gained weight during the experiment. Average weight gain was 35 $^{+}$/- 10.5 g per animal.

Table 13. Phase two data, experiment ran for 85 days (18 animals for the density treatments, others 30 animals per treatment). Note: individual crab numbers, first number represents tank no. (1 to 12) and second letter represents the individual in that tank (A to J).

Double swimming leg ablation treatment									
Deaths	Days	Depth (cm)	Weight (g)	Sex	Moults	Days	Depth (cm)	Weight (g)	Sex
3A	1	4.8	90	M					
5I	1	4	45	M					
9C	1	4.5	75	M					
5H	1	4	45	F					
9B	3	5	95	M					
9F	3	4.1	50	M					
9E	32	4.9	100	F					
3E	36	4.2	50	F					
5A	36	4.9	80	F					
5C	39	5	100	M					
3B	43	4.3	50	F					
5D	43	4.9	50	M					
5J	43	4.7	85	M					
3J	45	4.7	50	F					
9J	53	5	105	F					
5G	53	5	110	M					
9G	58	4.7	90	M					
9A	58	4.7	70	F					
9I	69	5	105	M					

Table 13. Continued.

Density treatment									
Deaths	Days	Depth (cm)	Weight (g)	Sex	Moults	Days	Depth (cm)	Weight (g)	Sex
7C	43	4	50	M	1B	37	4.9	60	F
7E	56	5	95	F	7F	42	4.5	75	M
7B	77	4.9	95	F	7D	45	4.1	55	F
					1D	49	4.2	55	M
					7A	57	4.6	75	M
					11C	71	5	100	M
					11D	71	4.9	85	F
					7B	68	4.9	95	F
					11A	78	4.4	110	M
Double eyestalk ablation treatment									
Deaths	Days	Depth (cm)	Weight (g)	Sex	Moults	Days	Depth (cm)	Weight (g)	Sex
6F	32	4.8	75	F	6C	39	4.8	95	M
12A	1	5	90	M	2A	39	4.7	75	F
2B	1	4.7	70	M	12H	42	4	50	M
12E	1	4.6	80	F	6I	43	5	100	M
2J	1	4.9	65	F	12C	43	5	105	F
2C	3	4.7	70	M	2H	43	4.5	60	M
2F	3	5	95	M	6G	43	4.4	85	F
6E	36	4.9	100	F	6J	45	5	100	M
12D	39	4.8	65	M	12J	53	5	110	F
6A	39	4.1	55	M	12F	68	5	100	M
6C	39	4.8	95	M					
2H	43	4.5	60	M					
6D	43	5.1	100	F					
6J	45	5	100	M					
2D	46	4.9	85	F					
6B	54	5	105	F					
12G	58	5	95	F					
12H	64	4	50	M					

Table 13. Continued.

Hormone treatment									
Deaths	Days	Depth (cm)	Weight (g)	Sex	Moults	Days	Depth (cm)	Weight (g)	Sex
8C	26	4.7	75	M					
8D	29	4.9	100	M					
8B	32	4.2	55	M					
4C	35	4.9	95	M					
4A	42	4.9	70	M					
4I	42	4.9	90	M					
8F	43	4.7	75	M					
10A	43	4.9	95	F					
4J	43	4.4	65	F					
8G	44	4.5	65	M					
10I	46	5	105	F					
10H	46	4.5	75	F					
8J	47	5.2	100	M					
8A	50	5	100	F					
4F	50	4.7	100	M					
10F	50	4.9	90	F					
4D	50	5	95	F					
8E	53	4.7	90	M					
8I	54	4.4	80	M					
10E	58	4.5	70	M					
4E	58	4.1	50	M					
10A	58	4.9	95	F					
10C	69	4.9	90	F					

The double swimming leg ablation treatment in the Phase Two experiments resulted in a 63% mortality rate with no moults. The density treatment resulted in three deaths and nine moults (50% of animals moulted) with a mean days to moult of 57.6 $^{+}$/- 5 days. The double eyestalk ablation treatment was conducted on the assumption that during the single eyestalk ablation treatment in Phase One (no moults) the second eyestalk took up the entire function of controlling the moult process. While the percentage of moults went up with the

double eyestalk ablation to 33% in Phase Two, the number of mortalities also increased to 60%. Mean days to moult for the double eyestalk ablation was 45.8 $^{+}$/- 2.8 days. The hormone treatment resulted in a 77% death rate and no moults.

Table 14. Phase three data, experiment ran for 67 days (18 animals for the density treatments, others 30 animals per treatment). Note: individual crab numbers, first number represents tank no. (1 to 12) and second letter represents the individual in that tank (A to J).

Density/double eyestalk ablation treatment									
Deaths	Days	Depth (cm)	Weight (g)	Sex	Moults	Days	Depth (cm)	Weight (g)	Sex
					1B	15	5	100	F
					1C	15	5	85	F
					1F	19	4.6	75	M
					5A	30	4.5	55	F
					5B	29	4.7	75	M
					5D	17	5	80	M
					5E	12	4.8	80	F
					9E	15	4.8	100	F
Density/no eyestalk ablation treatment									
Deaths	Days	Depth (cm)	Weight (g)	Sex	Moults	Days	Depth (cm)	Weight (g)	Sex
3E	37	4.9	80	M	3B	26	4.7	80	F
					3E	37	4.9	80	M
					7C	24	4.9	80	F
					7D	29	4.9	80	M
					11D	17	5	95	F
					11C	18	4.1	55	F
					11B	17	5	95	F

Table 14. Continued.

Group/double eyestalk ablation treatment				
Deaths	Days	Depth (cm)	Weight (g)	Sex
Moults	Days	Depth (cm)	Weight (g)	Sex
4E	18	4.8	70	M
4A	18	5	100	F
6A	15	4.9	85	M
6B	22	4.6	70	F
6C	22	5	80	M
6D	22	4.7	60	F
6E	22	6.2	80	F
6F	25	4.9	80	F
6G	25	4.8	75	F
6H	25	4.3	50	M
6I	25	4.7	65	F
6J	29	4.7	55	F
12E	24	4.5	75	M
12C	24	5	85	F
12J	24	5	105	M
12I	24	4.9	95	F
Group/no eyestalk ablation treatment				
Deaths	Days	Depth (cm)	Weight (g)	Sex
Moults	Days	Depth (cm)	Weight (g)	Sex
2D	10	4.4	70	M
2F	12	5	95	F
2B	12	5	105	M
8H	18	4.5	65	M
8I	18	4.9	75	F
10I	19	4.5	75	M
10H	22	4.7	60	F
10F	27	5	100	M
10C	32	4.7	75	M

The major variation between Phase Three experiments (Table 14) and the previous experimental phases was an improvement in the eyestalk ablation technique. A sterile technique was employed with dissecting instruments washed in iso propyl alcohol and flamed between each ablation. The operation was conducted in a room with reduced light and a damp cloth was covering the animal at all times. The operation was conducted as quickly as possible and the animal was returned to the water. This modification in technique resulted in a dramatic reduction in mortalities associated with this treatment. Mortalities in all treatments were reduced to almost zero.

None of the treatments were significantly different in time to moult apart from the density/double eyestalk ablation treatment which was faster than the group double eyestalk ablation treatment with a mean days to moult of 19 $^{+}$/- 2.4 days ($p = 0.08$). Days to moult and percent moulted are summarised in Table 15.

Table 15. Summary of descriptive statistics for Phase 3 experiments.

Treatment	Mean days to moult	Std. Error	Percent moulted
Density/Double eyestalk ablation	19	2.4	44
Density/no eyestalk ablation	24	2.8	39
Group/double eyestalk ablation	22.8	0.8	53
Group/no eyestalk ablation	18.9	7.3	30

Table 16. Phase Four data, experiment ran for 45 days (18 animals for the density treatments, others 30 animals per treatment). Note: individual crab numbers, first number represents tank no. (1 to 12) and second letter represents the individual in that tank (A to J).

Density/double eyestalk ablation treatment									
Deaths	Days	Depth (cm)	Weight (g)	Sex	Moults	Days	Depth (cm)	Weight (g)	Sex
					1B	15	6.3	215	M
					1E	32	6	160	F
					5A	17	6	155	F
					9A	8	6	160	M
Density/no eyestalk ablation treatment									
Deaths	Days	Depth (cm)	Weight (g)	Sex	Moults	Days	Depth (cm)	Weight (g)	Sex
					7E	8	6.4	215	M
					7D	15	6.3	210	M
					11B	25	6.3	205	M
					11D	21	6.1	160	F
					11E	41	6.7	265	F

Table 16. continued.

Group/double eyestalk ablation treatment									
Deaths	Days	Depth (cm)	Weight (g)	Sex	Moults	Days	Depth (cm)	Weight (g)	Sex
4I	21	6.4	190	F	4C	41	6.1	170	M
6C	16	6	160	F	4E	17	7	275	M
6H	14	6.2	160	M	4G	34	6.2	155	F
					6D	44	6.4	205	M
					6G	36	6.6	215	F
					6H	36	6.2	160	M
					6I	36	6.2	180	F
					12B	44	6	155	F
					12D	30	6.2	180	M
					12E	8	6	150	M
					12F	16	6.3	175	F
					12H	41	6.9	235	M
					12I	34	6.8	240	F
Group/no eyestalk ablation treatment									
Deaths	Days	Depth (cm)	Weight (g)	Sex	Moults	Days	Depth (cm)	Weight (g)	Sex
2A	25	6.3	180	F	8D	30	6.2	175	F
2C	17	6.3	180	F	8F	25	6.1	150	F
10F	34	6.7	185	F	8I	17	6.6	210	F

The Phase Four experiments duplicated exactly those conducted in Phase Three but were only conducted for 6 weeks as opposed to 9-10 weeks for the previous experiments and utilised 6-7 cm deep size class crabs (Table 16). The shorter experimental period reflected the rate at which crabs moulted in the previous phase and the use of the larger size class crabs (legal size in New South Wales) was to confirm legal size crabs could be used in any future soft shell crab production process.

Days to moult in the Phase Four experiments was significantly faster in the Density/Double Eyestalk ablation treatment ($p = 0.04$) with a mean days to moult of 18 $^{+}$/- 5.0 days compared with the Density/No eyestalk ablation treatment (Table 17). The group/ no eyestalk ablation are essentially the control treatment.

Table 17. Summary of descriptive statistics for Phase 4 experiments.

Treatment	Mean days to moult	Std. Error	Percent moulted
Density/Double eyestalk ablation	18	5	22
Density/no eyestalk ablation	22	5.5	28
Group/double eyestalk ablation	32.1	3.2	43
Group/no eyestalk ablation	24	3.8	10

If the days to moult data are pooled for both the Phase 3 and Phase 4 experiments, as the treatments were essentially the same, there is a significant difference ($p = 0.009$) between the individual (density) and group treatments for double eyestalk ablation. Mean days to moult for the individual treatment was 18.7 $^{+}$/- 2.2 days. Group/double eyestalk ablation was 27.0 $^{+}$/- 1.7 days. There is also a significant difference ($p = 0.029$) in the pooled data for the group treatments comparing eyestalk ablation and no eyestalk ablation with the no eyestalk ablation treatment being faster. The Group/Double eyestalk ablation treatment consistently resulted in a larger percentage of animals moulting in all experiments (Tables 16, 17 and 18).

Table 18. Summary of descriptive statistics for pooled Phase 3 and Phase 4 experiments.

Treatment	Mean days to moult	Std. Error	Percent moulted
Density/Double eyestalk ablation	18.7	2.2	33
Density/no eyestalk ablation	23.2	2.7	33
Group/double eyestalk ablation	26.9	1.7	48
Group/no eyestalk ablation	20.2	2.1	20

7.5 Discussion

The recirculating aquarium systems developed for this work were capable of holding *P. pelagicus* indefinitely. This resulted in no significant mortality for all control treatments.

The concept that heavier crabs of the same size might moult sooner was examined in this work but no significant relationships could be found. This may have been influenced by the various treatments which contributed most to the numbers of moulted crabs i.e. double eyestalk ablation and individual (density) or group holding methods. More work needs to be done in this area as the theory that heavier crabs of the same size should moult sooner would seem to be a logical assumption. In support of this assumption Zhou *et al.* (1998) used wet weight change as a growth index for the King Crab *Paralithodes camtschaticus*.

The temperature treatment (30^0C) was obviously too hot as even though most crabs survived to almost the end of the experimental period, there was then significant mortality. A temperature of 30^0C can be observed for short periods at high tide on the sand flats adjacent to the major habitat locations on the Hawkesbury estuary, and it is known that *P. pelagicus* moves onto these sand flats to feed. This was the basis for the selection of this temperature treatment. All other experiments were conducted at approximately 25^0C with almost no mortalities in the final experimental phases. A temperature of around 28^0C would probably be optimal for the keeping of adult crabs being cultured for the soft shell product. Twibell *et al.* (1998) found 25^0C to be optimal for the induction of moulting, with double eyestalk ablation, in the crayfish *Orconectes virilis*. In contrast to the results reported here Winget *et al.* (1976) found temperatures of 30^0C significantly increased moult frequency in the Blue Crab, *C. sapidus*.

The single eyestalk ablation treatment was based on the large amount of literature which shows the pivotal role played by the X and Y organs located in the eyestalks of arthropods (Table 11). This treatment in the Phase One experiments resulted in only mortalities and had no influence on the induction of moulting. It was therefore postulated that the high mortalities must be a function of the ablation technique itself and that the loss of the single

eyestalk was being compensated for by the remaining eyestalk. A similar reaction has been found by Meade and Watts (2001) who found that the crayfish *Cherax quadricarinatus* also compensated for the loss of only one eyestalk in this way.

A common handling technique employed by professional fishermen is to lift *P. pelagicus* by either of the swimming legs; this minimises, but does not eliminate, the chance of being bitten. Periodically animals "throw" this limb when held in this way. It was theorised that if an animal lost both of these limbs then its movements would be hampered significantly and thus the need to regenerate them might bring on an early moult (Chittleborough 1975). This treatment however resulted in high mortalities and did not induce moulting in any way. The shock associated with the removal of both limbs was probably the overriding factor in this instance. A number of animals were, however, observed to begin the development of fluid filled membranous sacks containing limb buds.

The Phase Two experiments were the first to explore the idea that animals held individually (density treatments) might moult differently to those held in individual cells but which could sense the other crabs in the same recirculating tank (group treatments). This first experiment resulted in enough moults to suggest that further experiments might give some interesting results even though there were a small number of mortalities.

The trial of the double eyestalk ablation treatment was first conducted in the Phase Two experiments. While this treatment resulted in a significant increase in the number of crabs moulting (50%) it also resulted in an unacceptably high level of mortalities (63%). To address this all subsequent treatments involving eyestalk ablation used an improved dissection technique which helped to almost eliminate mortalities in these trials.

The injection of 20 hydroxyecdyesone in the hormone treatments resulted in the majority of animals throwing (auto excising) all of their limbs subsequent to their deaths. This is in contrast to the results of Dall and Barclay (1977) who had some success with a similar technique to induce moulting in the Western Rock Lobster, *Panulirus longipes*. These

authors did, however, report a certain number of autonomies of appendages and mortalities in some treatments.

The Phase Three and Phase Four experiments further explored the results for moult induction gained for the individual holding and eyestalk ablation treatments in Phases One and Two. The Phase Four experiments were a replication of Phase Three but Phase Four was only conducted for six weeks as opposed to nine to ten weeks for the previous experiments. This was because the mean time to moult from the Phase Three experiments was down to only three to four weeks. When analysed separately, both groups (Phases 3 and 4) of experiments showed that animals held individually with both eyestalks ablated were faster to moult than animals treated in the same way but held in groups which could sense each other (but still held individually). Previous aquarium holding of *P. pelagicus* had shown that massive mortalities resulted from holding this species in groups of more than two or three per square meter where individuals could fight with each other. The species is cannibalistic and extremely territorial (personal observation).

The other major finding was that animals held in groups (but able to sense each other), with the double eyestalk ablation treatment, always resulted in a higher percentage of animals moulting during the experimental period.

To increase the confidence in these results, data for time to moult was pooled for both the Phase Three and Phase Four experiments. This again showed that crabs held in isolated individual tanks, with both eyestalks ablated, moulted faster than crabs which were held in a single tank (group treatment). 18.7 $^{+}$/- 2.2 days for individuals and 26.9 $^{+}$/- 1.7 days for the group animals. It is interesting to note that in the group double eyestalk ablation treatments for Phase Three, all of the ten crabs in one of the three replicate tanks moulted. This happened again, but to a lesser extent, for the same treatments in Phase Four with six out of the ten crabs in one tank moulting. This may just be coincidence but it might also reflect that pheromones emitted by moulting crabs of either sex might influence others in the group to moult. Dunham (1978) reviewed the literature relating to sex pheromones in crustaceans and concluded that there was some evidence to support the case that portunid

crabs utilised such a pheromone. There seems to have been little research in this area over recent years. It does however seem logical that a female crab might emit some form of chemical attractant to let the males know they are ready to moult and mature enough to mate. Males will mate with females in the soft-shelled state only and will hold the female beneath him until the female moults and then mate with her. This does not explain the increased moulting of crabs which can sense each other in a single tank.

There is also the possibility that the moulting hormone, 20 Hydroxyecdyesone, or a related compound, plays a dual role as a sex pheromone. See Subramoniam (2000) for a detailed review of the relationship between ecdysteroids and reproduction. The concept of a pheromone relating to moulting and/or reproduction is however not considered by Subramoniam (2000). Spindler *et al.* (2001) has reviewed the molecular mechanisms relating to moulting hormones, such as those involved in reproduction and metabolism, but again did not consider the possible role in pheromone activity.

Another possibility is that crabs in the holding tanks which are moulting might be expelling enough moulting hormone in their waste so as to trigger other animals in the tank to moult. This could cause a chain reaction where as increasing numbers of crabs undergo a moult more hormone is introduced to the tank water, so inducing even more crabs to moult.

The cannibalistic nature of the species resulted in the original theory that animals held individually might be more inclined to moult. This pretext is not supported by the group moulting results.

While the economics of the production of soft-shelled crabs using these methods has not been examined here it would seem physically possible to collect and hold legal sized (6 cm deep) *P. pelagicus* in recirculating aquaria. Then by holding crabs in groups, but separated by mesh, and ablating both eyestalks, almost half the animals will moult in approximately three weeks (Plate 11). The use of heavier crabs for the 6 to 7 cm size class and the holding

of crabs in temperatures up to approximately 28^0C might also assist in the induction of moulting.

The problem of how to establish where any given animal might be within the intermoult stage could be assessed by looking at the shell hardness. Hicks and Johnson (1999) developed a shell hardness tester for use on Dungeness Crabs (*Cancer magister)* in Alaska. Similar methods might also be able to be applied to Blue Swimmer Crabs to assist in the selection of pre ecdysis animals for moult induction/soft shell crab production. Another indicator of proximity to moult was the observation during this work that animals would, on most occasions, "go off" food 3 -5 days before moulting (Vega-Villasante *et al.* 1999).

Eyestalk ablation may even result in the increase of the size increment at moult. This was shown for larval American lobsters (*Homarus americanus*) and is postulated to be due to the enhanced hydrostatic stretch of the tegument at ecdysis (Charmantier and Charmantier-Daures 1998).

Perry *et al.* (2001) has shown that by lowering the calcium level in seawater, the length of time a Blue Crab will remain in the soft-shelled state can be extended. With the subsequent economic benefits for the shedder. The influence of lowered calcium on the time to moult or the number of crabs moulting was, however, not explored in this work.

7.6 Conclusion

While not proved economically viable the methods presented here show that it is physically possible to produce soft-shelled crabs in a value adding exercise using legal sized *P. pelagicus* (> 6cm). Crabs can be induced to a synchronous moult of approximately 3 weeks by the application of double eyestalk ablation and the holding of the animals in groups in flow through mesh cells.
The development of better methods to establish where in the intermoult stage an animal might be will further improve the selection of crabs for moult induction.

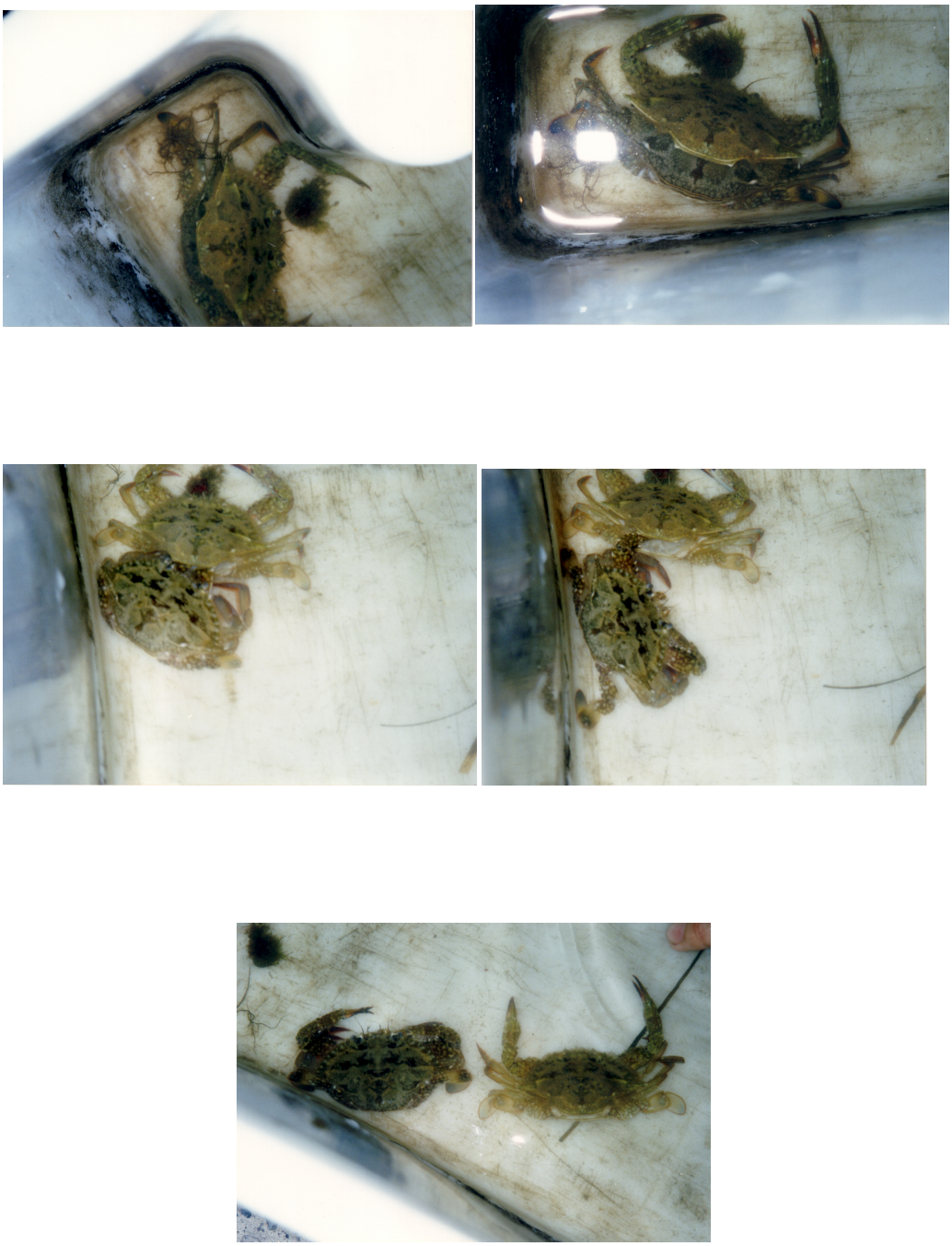


Plate 11. Moulting sequence for *P. pelagicus*. * Note increased size of new crab (approx. 5.5 cm carapace width).

8 General Discussion

8.1 Objectives

The Blue Swimmer Crab is widely known as a commercial and recreational fishing species. Significant catches of this animal make it one of the preferred seafood species in the country; indeed its distribution enhances this reputation as it can be found almost all-around the country except for stretches of the southern coastline and Tasmania. The Blue Swimmer Crab is a decapod crustacean in the same Order as lobsters and prawns. The swimming crabs (Portunidae) of which the Blue Swimmer is a member, are represented commercially and recreationally in Australia by the Blue Swimmer Crab, the Sand Crab (*Ovalipes australiensis*), the Coral Crab (*Charybdis feriata*) and the Mud Crab (*Scylla serrata*), both the Blue Swimmer and the Mud Crab are the favored table species.

The life history of this animal includes an offshore larval stage with female crabs migrating to the mouths of estuaries to release their eggs into coastal embayments (Potter and de Lestang 2000). The juvenile crabs return to the estuaries to settle in protected habitats such as seagrass beds.

As in all other Crustacea, Blue Swimmer Crabs need to moult to grow. This process is complex and is mediated by a range of environmental and physiological parameters. An understanding of the ecology and physiology of the Blue Swimmer Crab is necessary for the development of the species as a bioindicator and for its application towards the production of soft-shelled crabs.

The main objective was to examine the potential for the establishment of the Blue Swimmer Crab as a biomonitor of anthropogenic metal contamination in New South Wales estuaries. The secondary objective was the examination of the possibility of the production of soft shell crabs for commercial applications.

These objectives have both been successfully completed and have resulted in the development of the Blue Swimmer Crab as a biomonitor of environmentally available cadmium and also the establishment of a method whereby the Blue Swimmer Crab could be used as the basis for the production of soft-shelled crabs. This soft shell crab methodology has not as yet been proven economically.

8.2 Principles for the development of a biomonitor

The use of indicators for the monitoring of environmental condition is developing rapidly. The need for these indicators has been made more poignant over the last 10 years due to our increasing aspirations concerning the management of the environment. A range of management intervention technologies are now applied on a regular basis and are touted as "best management practice" in a range of published material in both journals and the grey literature. Even though many of these management programs are promoted as best environmental practice, a great many have little scientific data to support them (See review by Walmsley *et al.* 2001).

Environmental managers concerned with the area of catchment management require the development of indicators to assist with the identification of potential impacts from a range of land use types. A suite of different chemical pollutants can be associated with the common land use types found in the greater Sydney region. These include urban, rural, developing rural to urban and industrial land use. Chemical contaminants from these land use types include nutrients, pesticides and metals.

This thesis deals with the development of an indicator species (biomonitor) for the assessment of anthropogenically sourced metals from a catchment into estuarine receiving waters. The development of the Blue Swimmer Crab as such a biomonitor has followed the principles outlined in the published literature (Phillips 1977a, Fairweather 1999). These principles can be summarised as follows;

- The organism being used as a biomonitor must accumulate the metal with a

quantifiable relationship to the surrounding environment,

- The test organism must not be affected in its ecology or physiology by the accumulation of the metal,
- The organisms ecology needs to be adequately understood, and
- The organism's physiology also needs to be adequately understood.

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The development of a biomonitor as a catchment management tool also needs to be established through a sequence of logical deductive reasoning. Having identified the need for the development of such a monitor, test species need to be selected on the basis of previous scientific knowledge, and then the species ability to perform the function of a biomonitor needs to be assessed. This assessment must be conducted initially in the laboratory, under a range of conditions, and then to be validated in the field. The use of the biomonitor on a management scale is then assessed and feedback loops developed to trigger appropriate catchment management responses. Having established a monitoring program utilising a particular biomonitor, an assessment can then be made of how effective the process has been, and include the subsequent refinement of the methodology (Fairweather 1999).

8.3 Bioaccumulation of metals

The first step, therefore, in the development of the Blue Swimmer Crab as a biomonitor of environmental metal levels was to establish its ability to accumulate metals with some relationship to the surrounding environment. Given that there are a number vectors by which metals can accumulate in Crustacea it was decided that, after assessing the literature, the vector of diet would be utilised for this work as opposed to the use of metal accumulation via either the water column or the sediments. Each of these matrices (vectors) is obviously interlinked with the movement of metals between water, sediment and biota occurring through a range of complex interactions.

The presentation of metals to the Blue Swimmer Crab via the diet was supported by a detailed understanding of the animal's ecology and feeding habits (e.g. Wu and Shin

1997). This enabled the development of a manipulative experiment utilising "contaminated" and "uncontaminated" mussels from two estuaries on the New South Wales north coast. The nominated contaminated estuary was Lake Macquarie where significant industrial activity has resulted in previous studies detailing the contaminated nature of the estuary in relation to trace metal pollution. This enabled the collection of the Hairy Mussel (*Trichomya hirsuta*) from intertidal areas of Lake Macquarie and its use as a food item. These mussels were found to contain elevated levels of the metals cadmium, copper, zinc, arsenic, iron and aluminium. Of these metals only cadmium was found to accumulate in the hepatopancreas through time (McPherson and Brown 2001).

A similar result has recently been reported by Schuwerack *et al.* (2001) for the South African River Crab (*Potamonautes warreni*). These authors, however, considered that there was a higher cadmium accumulation in the gills. In complete contrast to the work presented here, Al-Mohanna and Subrahmanyam (2001) have reported increased levels of copper and zinc in the hepatopancreas of *P. pelagicus* from Kuwait City and relate these levels to the effect of the Gulf war oil spill. Their work is however an example of a poorly designed experiment confounded by potentially flawed reference site selection. These authors did not include cadmium in their analytical procedures.

8.4 Population assessment

Having established in laboratory trials that the Blue Swimmer Crab could accumulate cadmium in its hepatopancreas, the next step in the development of the animal as a bioindicator of available cadmium was to determine its residence time over a particular area. The fact that this organism is highly motile, and not sedentary as is the case for many of the previously developed bioindicators, it was necessary to establish how representative of metal contamination this organism might be on any given spatial or temporal scale.

While a significant amount of information on the ecology of this crab was available from other studies in both Australia and the Indo-West Pacific, very little was understood concerning the population dynamics of Blue Swimmer Crabs in New South Wales. Indeed

much of the population data collected elsewhere in both Australia and around the world was based on the use of “T”-bar anchor tags initially developed for use on fish populations.

To understand the potential movements of the Blue Swimmer Crab in New South Wales estuaries it was obviously necessary to utilise some form of tagging technology. As previously stated the common tagging method for the species in this country had previously been the use of “T”-bar anchor tags. While these tags had been used on a number of occasions in the past, little data existed supporting the use of this methodology.

Potter *et al.* (1991) used a Floy “T”-bar anchor tag for field assessments of *P. pelagicus* and concluded that these tags were not suitable for growth studies on this species. They did however quote moulting rates which were flawed due to the unknown mortality at moult.

The fact that this animal needs to moult to grow, and that an assessment of how close any individual is to moulting is difficult, the use of the T-bar anchor tag as a marking methodology needed to be carefully assessed, as this technique had the potential to interfere with the moulting process.

8.5 Tag development

It was determined from these trials that the existing T-bar anchor tag marking method was completely inappropriate and caused significant mortalities and deformities in individuals during both the act of tagging and then subsequently during the moulting process.

It was therefore necessary to develop a new method which allowed for the visual identification of individuals and minimized the need for high-tech sensing equipment, such as those needed for micro wire tagging systems. The use of a visual identification tag is a more resource efficient methodology, as the tag can be labeled in such a way as to offer a reward for information regarding the subsequent capture of the animal by both commercial and recreational fishers, thus improving recapture efficiency.

The development of a modified T-bar anchor tag reduced the incidence of mortalities and provided a tool for the subsequent evaluation of the movement and population estimates for *P. pelagicus*.

8.6 Mark-recapture experiment

To better understand the potential movements of *P. pelagicus,* the modified "T"-bar anchor tag was utilised in a number of mark-recapture experiments on the Hawkesbury River estuary. These field experiments were conducted with a view to learning more about the potential movements of this species within an estuary and also to confirm previous studies conducted in other parts of Australia, on the distribution and abundance of this animal. The mark-recapture experiments confirmed that the Blue Swimmer Crab was resident in the Hawkesbury River estuary during much of the summer period and that this time scale was adequate to facilitate the accumulation of cadmium, which had previously been confirmed in laboratory trials to be significant over a four to eight week period. While it was confirmed that these animals are resident on a scale of bays within estuaries a conservative approach was applied to the use of the animal as a bioindicator, and further work with the species was based on the conclusion that *P. pelagicus* was resident during summer months on a scale of estuaries. These mark recapture experiments did however suggest that during summer, populations of *P. pelagicus* can be resident in bays within estuaries, future catchment management monitoring procedures could be developed on this basis.

Gribble and Thorne (1998) used ultrasonic tags to mark *P. pelagicus* in Southern Queensland. Results from Gribble and Thorne (1998) confirm the findings that *P. pelagicus* displays a medium term residency within a temporary foraging area. Area size varied but averaged 80 m^2 +/- 74 m^2, n = 6. These results compare favorably to the estimate of approximately one individual per 100 m^2 as determined by the use of the modified T-bar anchor tag in Chapter 4. Such a conclusion is significant as these findings help to understand the potential range of this crab and hence its value as an indicator of a particular spatial scale.

8.7 Movement of berried females

The potential movement of berried female crabs was questioned during these mark-recapture experiments as a number of late stage berried females were captured in these bays. The literature from other parts of Australia suggested that berried female crabs moved to the mouths of estuaries to release their eggs. The fact that the egg masses on a number of these berried females were in a very late developmental stage, that is they were a dark grey black colour, suggested that these females might be releasing eggs in the bays where they were being captured. To test this hypothesis a number of plankton tows were conducted in the surface waters of these bays. Very few larvae were encountered during this work and it was concluded that in all probability late stage berried females were moving out of the bays to the mouth of the estuary to release their eggs.

This information results in there being some doubt as to the residency of berried females within estuaries, for this reason berried females were subsequently exempted from sampling designs attempting to use Blue Swimmer Crabs to assess the extent of cadmium contamination in New South Wales estuaries.

8.8 Biomonitor field trial

It has thus far been established that;

- *P. pelagicus* accumulates cadmium in its hepatopancreas via its diet and,
- *P. pelagicus* is at least resident on a scale of estuaries during summer periods in New South Wales.

To further investigate the ability of this species to be a biomonitor of available cadmium, it was necessary to conduct a field based monitoring trial. To do this a number of estuaries along the New South Wales coast were selected as potential monitoring areas. Some of these estuaries were selected due to the range of literature covering metal levels in those

estuaries and other estuaries were selected on the basis of the need to know more about the existing metal contamination of these areas.

Crabs were collected from fishing co-operatives associated with each of these estuaries along the New South Wales coast. Hepatopancreas cadmium levels were assessed for these crabs and the metal levels obtained compared to those from the known literature covering these same areas. Following some manipulation of data, a good relationship was found between the results presented by the biomonitor and the results presented in the literature concerning which estuaries were contaminated with metals and which estuaries were not.

Recent Crustacean biomonitors reported in the literature have concentrated on the use of barnacles as indicators of available metals. For example Blackmore (1996 and 1999) employed *Balanus amphitrite* and *Tetraclita squamosa* as biomonitors of metal pollution in Hong Kong coastal waters. Elevated cadmium levels were found at some sites. Wang *et al.* (1999) examined the trophic transfer of zinc to *B. amphitrite* and Ruelas-Inzunza and Paez-Osuna (2000) found barnacles to be better concentrators of cadmium than oysters or mussels.

8.9 Proposed methodology for the use of *P. pelagicus* as a biomonitor of available cadmium in an estuarine environment

Proposed procedure for the use of the Blue Swimmer Crab as a biomonitor of environmental cadmium;

1. Formulate a suitable question; e.g. is cadmium present in "X" estuary at higher than background levels?
2. Determine the presence of *P. pelagicus* for the estuaries in question.
3. Sampling is conducted towards the end of the summer period.
4. Only adult crabs between six and eight cm carapace depths are sampled.
5. Only sample hard shell intermoult crabs.
6. No berried females are collected for analysis.
7. Male and female crabs can both be included in the analysis.
8. Crabs can be collected from local fishing co-operatives or captured for the purpose.
9. Sample 10-20 individuals per estuary.
10. Crabs can be sampled on a scale of estuaries or bays.
11. The hepatopancreas is dissected from the crab within 24 hours of sampling.
12. The hepatopancreas can be frozen pending analysis.
13. Cadmium levels in the hepatopancreas are analysed by ICP-MS.
14. Experimental design can utilise the selection of impacted and non impacted sites (controls) (e.g. ANOVA).
15. Depending on management initiatives the monitoring program can be implemented annually or at multiple years.

8.10 Interaction of moulting and metal partitioning in crustacea

Rao and Govindarajan (1992) examined the trophic transfer of copper and zinc between a diatom (*Skeletonema costatum*), a bivalve (*Donax cuneatus*) and the prawn *Penaeus indicus*. These authors found copper and zinc to be primarily stored in the exoskeleton and theorized that moulting was a potential method of detoxification.

As reported by a number of other researchers Martin *et al.* (2000) studied ink particles injected into decapod crustaceans and found that these particles were sequestered within nodules in the gills. When these animals moulted the nodules were discarded with the old exoskeleton, this would appear to be a method of detoxification.

Lasenby and Van Duyn (1992) examined the consumption and accumulation of zinc and cadmium in the shrimp *Mysis relicta.* These authors found moults to be 5 to 8 times higher in cadmium than whole body concentrations. That is 21 – 35% of cadmium taken up by this species was eliminated via moulting.

The leading researcher in this field has been Engel (e.g. Engel 1987, Engel and Brouwer 1991) who has examined the partitioning of metals during the moulting process in the crab *C. sapidus*. This author has found metallothionein to be involved in the synthesis of hemocyanin and that metals (e.g. copper and zinc) are decreased significantly at moult. This decrease was not associated only with losses ascribed to the exoskeleton but to physiological processes post moulting involving the metallothionein protein.

This information leads to the conclusion that moulting is an important mechanism for the detoxification of metals in decapod crustaceans and needs to be considered when developing a biomonitor of available metals.

8.11 Soft-shell crab production

The production of soft-shelled crabs on the Atlantic coast of the eastern United States is based on the natural process of moulting in arthropods. In effect soft shell crabs are not "produced" they are only an incidental part of the life cycle of these animals. The process of moulting is in itself interesting from an evolutionary perspective, as arthropods as a group have been so successful. Given that crustaceans are a very diverse group within the arthropods (> 20,000 species), it is interesting that such a moulting strategy can be so successful in an evolutionary sense.

To grow the animal needs to moult, during the period leading up to moulting the animal ceases to feed and needs to seek out hiding places to undergo the moulting process. Once moulting is initiated the animal is unable to move and the process of extracting itself from the old exoskeleton may take many minutes. In the case of the Blue Swimmer Crab once the animal has extracted itself from the exoskeleton it must remain motionless in this position for tens of minutes to hours until the exoskeleton is hard enough to allow movement. This is due to the fact that the outer shell is an exoskeleton and the musculature depends on this exoskeleton to allow movement, for this reason the animal is not physically capable of moving until the shell is sufficiently hardened.

As the Blue Swimmer Crab will need to moult between 15 and 20 times in its lifetime this is 15 to 20 times when it finds itself completely defenseless for a significant period. Mortalities during these periods must be high, but the group continues to be one of the most successful on the planet.

The other complexity of the moulting process is the physical extraction of the crab from the old exoskeleton. An examination of the old exoskeleton after moulting reveals a number of physical structures remaining in the old shell. These basal processes, or pinnacles of shell protruding internally from the old exoskeleton (apodemes), are structures on which muscles and organs attach to keep the correct internal spatial orientation. Even the old gill coverings remain in the discarded exoskeleton. How the animal physically

extracts itself from around these processes is difficult to comprehend. It is safe to say that despite a large amount of work having been completed which is contributing to an understanding of moulting there are still many aspects of this process which require further study.

8.12 Holding systems

The holding systems developed here are based on the general design of a closed recirculating aquarium, with a suitably sized biological filter and a water delivery system specifically designed to be as simple as possible, with a minimum potential for breakdown, but also one which will maximise the oxygenation of the water and the removal of ammoniacal waste. Two improvements could be made to the design, which were not needed for this series of experiments, these include the addition of a foam fractionation column to the biological filter and modifications to the stand pipe design which would cause the tank to self drain in the event of a power failure. These design modifications were not needed during these experiments and it was found that careful monitoring through the day, and the prompt removal of excess food reduced the need to remove protein scum (foam fractionation column) and the constant presence of laboratory workers meant that power failure was not an issue. If this methodology were applied to a commercial setting both modifications would probably become necessary.

The holding systems developed therefore comprised a black fiberglass holding tank, capable of holding up to 10 individual *P. pelagicus* in separate mesh cells. The tank measured approximately 1 m by 1 m by 40 cm deep (water depth 30 cm). The biological filtration system was placed under the tank and consisted of a 40 L water reservoir with a 40 L biological filter containing shell grit, a 10 cm layer of filter wool was placed across the top of the shell grit.

A 200 L/hr in-line pump takes water via the stand pipe to a spray bar positioned above the filter wool, the spray bar ensures the even distribution of ammonia laden water across the top of the filter media, this results in the maximum treatment through this media in relation

to surface area of shell grit which comes in contact with the water. The success of the water treatment system depends on the biofilm on the surface of the shell grit which facilitates the nitrification/denitrification process and results in the removal of ammoniacal waste. Water drains to the bottom of this filter media and enters the holding reservoir. From here the in-line pump moves the water via a 25 mm PVC line back onto the surface of the tank where another spray bar, designed to increase water circulation in the holding tank and to increase oxygenation of the water, again adds water to the aquaria. Additional oxygenation of the water can be supplied by the use of separate aeration systems. The water temperature of these tanks was controlled using a standard air-conditioning unit with an air temperature of 25^0C; this resulted in a water temperature within the tanks of between 23 and 25^0C. Various recirculating systems are employed in the Chesapeake Bay soft shell crab industry (Messick and Kennedy 1990).

The development of this holding system has enabled the indefinite aquaculture of this species in areas remote from estuarine waters. It has also provided a platform on which to further develop a soft shell crab industry utilising the Blue Swimmer Crab.

8.13 Moult induction

This work sought to develop techniques which might be useful for the production of soft shell crabs as a value added product for commercial sale. While not proved on a commercial basis the potential for the production of soft shell crabs has been established at a technical level. It should be realised that just by holding Blue Swimmer Crabs in an appropriate holding system will eventually result in the animals moulting during the course of normal growth. This however will take some time under “normal” conditions so a range of parameters were investigated which were postulated could result in the synchronisation of moulting across a number of individuals and also to decrease the time to moult for a group of individuals.

The control of moulting in crustacean species is extremely complex on both a physiological and environmental level. A range of parameters relating to growth and

development has been shown to be important in the moulting process; these include temperature, salinity, dissolved oxygen, photoperiod, tidal rhythm, and limb loss and habitat type. Calcium and protein metabolism and the density of animals have also been shown to be important in the control of moulting. At a physiological level the moulting process is controlled by the endocrine system. Cellular processes required to initiate moulting are stimulated by steroid hormones called ecdysteroids. These substances are secreted by the Y organs which are in the eyestalks of crabs, and which are subject to regulation by a neuropeptide known as the Moult Inhibiting Hormone (MIH). Moult inhibiting hormone is produced in the X organ, and released from the adjacent sinus gland. The generally accepted model for hormonal moult control is that MIH inhibits the Y organ during much of the moulting cycle and the moulting sequence is initiated when MIH secretion diminishes or stops. A range of external environmental factors, similar to those mentioned above, will influence the production of MIH.

This information lead to the conclusion that eyestalk ablation could be used as a method to induce precocious moulting in the Blue Swimmer Crab. Other parameters which were postulated to have the potential for the induction of precocious moulting included hormone injection, increased temperatures, male only treatments and the effect of various densities in holding systems. Initial experiments focused on the ablation of only a single eyestalk on an individual. This proved to have little effect and subsequent experiments were more successful with double eyestalk ablation. The assumption therefore became that with only single eyestalk ablation the remaining eyestalk took over the complete function of MIH production.

Dall and Barklay (1977) claimed some success with the induction of moulting in decapod species using the injection of the moulting hormone, 20-hydroxyecdyesone. In *P. pelagicus* the procedure resulted in only massive mortalities and the auto excision of limbs. The application rates used in Chapter 7 were as described by Dall and Barklay (1977). The results from these authors are in some doubt as no other workers have reported a similar result i.e. induced moulting.
Moult induction experiments conducted during this work, using 20-hydroxyecdyesone did

however only use the single application rate and did not explore further other methodologies for varying hormonal loads. There may be some value in repeating this work using a range of concentrations and application methods in the future.

Winget *et al.* (1976) had some success with use of increased temperatures (30^0C) to induce moulting in the Blue Crab, *Callinectes sapidus*. When this temperature was applied to adult *P. pelagicus* this resulted in only increased mortalities towards the end of the nine week holding period. The success of Winget *et al.* (1976) in the use of this temperature and the observation that Blue Swimmer Crabs are known to move on to sandbanks covered at high tide in the Hawkesbury Estuary, which can attain temperatures in excess of 30^0C, it was postulated that the species could tolerate this temperature and it might have some significant affect on moulting. This however was not the case and the conclusion to be drawn from these experiments is that while *P. pelagicus* might tolerate these temperatures for a short period they are not sustainable. Further experiments, however, looking at the effects of thermal shock on *P. pelagicus* may also have some value in the induction of moulting. During all of these experiments crabs were held at 25^0C. Temperatures as high as 27 to 28^0C would also probably assist with the induction of moulting using the techniques developed here.

The most successful results from this series of experiments revolved around moult induction utilising double eyestalk ablation and the group holding of *P. pelagicus*. The individual holding of *P. pelagicus* was also interesting in that while it did not cause a higher percentage of animals to moult in any given experiment it did however cause the animals to moult faster than control crabs. Forty eight percent of crabs held in groups in flow through cells (with both eyestalks ablated) moulted during the experimental period. Only 20% of crabs held in the same way with no eyestalk ablation moulted (controls) ($p < 0.05$). Crabs treated the same way, but held individually, moulted faster than those held as a group, 18.7 $^{+}$/- 2.2 days as opposed to 26.9 $^{+}$/- 1.7 days ($p < 0.05$).

8.14 Production of soft shell crabs

The proposed method for the production of soft shell crabs therefore revolves around the use of the technique of double eyestalk ablation and the holding of individual crabs in flow-through mesh cells. The technique of double eyestalk ablation needs to be conducted in an aseptic manner to minimise mortalities, early experiments which did not utilise such techniques resulted in significant mortalities and the survivorship increased dramatically when instruments used during the ablation technique were washed in isopropyl alcohol and flamed between ablations. Stella *et al.* (2000) had similar success with the estuarine crab *Chasmagnathus granulata* moulting more frequently with double as opposed to single eyestalk ablation.

The holding of individual crabs in adjacent mesh cells results in the same water flowing from one crabs cell to the other; this would seem to play some role in the induction of moulting in this species. There is very little in the literature concerning the effects of pheromones on the life history of *P. pelagicus* (Dunham 1978), these experiments suggest that pheromones may play an important role in both reproduction and moulting due to the fact that synchronised moulting among individuals was significantly greater for crabs held in the one water mass as compared to crabs held individually and unable to sense other crabs.

8.15 Proposed method for the production of soft shell crabs

Proposed procedure for the use of the Blue Swimmer Crab in the production of soft shell crabs;

1. Mature *P. pelagicus* of a legal size (> 6 cm carapace depth) are collected from a commercial fishery.
2. Only complete, undamaged crabs are collected.
3. Crabs are transported to holding aquaria as quickly as possible, depending on transport methods and water quality control during transport.
4. Crabs can be held in recirculating aquaria utilising biological filtration.
5. Holding temperature of between 25 and 28^0C.
6. The lighting system is set to 12 hrs on and 12 hrs off.
7. A diet of prawns or mussels and prawns can be utilised.
8. Crabs are held individually in adjacent mesh cells with flow-through systems.
9. Foam fractionation and emergency power failure systems can be included.
10. Crabs should be acclimatised for at least one week prior to treatment.
11. Conduct the double eyestalk ablation treatment aseptically.
12. Crabs should be checked every couple of hours especially during the period of lights out.
13. Crabs are removed post moulting to avoid the shell hardening.
14. Crabs can be processed (cleaned) and frozen or processed and refrigerated.

This process will result in approximately 50% of crabs held moulting within a three-week period.

8.16 Further studies

Future major investigations arising from this work would include:

- The application of the proposed biomonitor methodology to a long term monitoring program for further refinement;
- A commercial pilot trial of the soft shell crab methodology to determine its economic viability.

Future more minor investigations could include:

- Plankton and adult female movement studies to determine larval export mechanisms from New South Wales estuaries;
- Estuary wide population mark recapture studies to develop population estimates using the modified T-bar anchor tag;
- An investigation of the potential existence of a pheromone involved in the moulting process in *P. pelagicus*;
- A study examining a range of different moulting hormone application rates to induce moulting in *P. pelagicus*;
- Studies examining the holding of *P. pelagicus* at temperatures of 26 to 28 ^{0}C to determine the effect on moult induction.

9 Appendix

9.1 Recipe

Sautéed Soft-shell Crabs with Garlic Butter (Stein 1999)

I first tried sautéed soft-shell crabs in St Michaels, a little town on the shores of the Chesapeake Bay in America. It was a bit early in the season but I went into a restaurant called The Crab Pot and asked if by any chance they might have some soft-shell crabs, and the waitress said, 'Go into the kitchen and ask Eric'. So I did, and he'd just got some in a box and had just had an order for two portions, the first of the season. I explained to him who I was and he said, 'Why don't you cook some?' So I did.

serves 4

8-12 soft-shell crabs
100 g (4 oz) plain flour
I tablespoon shrimp-boil seasoning (see p. 199) or Old Bay seasoning mix
3 tablespoons *Clarified Butter* (see p. 239)
100 g (4 oz) butter, at room temperature
3 garlic cloves, crushed
1 tablespoon lemon juice
2 tablespoons chopped parsley
Salt and freshly ground black pepper

method

To prepare the crabs, rinse them first in cold water. Cut off the eyes and mouth with scissors - cut straight across the face, about 5 mm (1/4 inch) Just behind the eyes. Push your finger into the opening and hook out the stomach, a small Jelly-like sac. Then turn the crab over and lift up and pull off the little tall flap. Finally, lift up both sides of the soft top shell in turn and pull out the dead man's fingers (the gills).

Sift the flour, shrimp-boil seasoning, I teaspoon of salt and some pepper on to a plate. Dredge the crabs well in the flour, then pat off the excess.

Heat the clarified butter in a large frying pan. Fry the crabs in batches over a moderate heat for 2 minutes on each side, until lightly browned. Shake any excess butter off as you remove them from the pan. Keep warm while you fry the rest.

Add the rest of the butter and the crushed garlic to the pan and allow it to sizzle for a few seconds. Add the lemon Juice, then throw in the parsley and some seasoning. Spoon the butter over the crabs and serve immediately.

9.2 Larval video

See inside back cover

9.3 Blue Swimmer Crab data - Reported New South Wales commercial catch (kg) and value of catch ($) by area by year for the last 10 years.

Note: Ocean Zone 1: Tweed Heads to Ballina; Ocean Zone 2: Ballina to Arrawarra; Ocean Zone 4: South West Rocks to Forster; Ocean Zone 5: Forster to Swansea and Ocean Zone 6: Swansea to Botany Bay

Reference: New South Wales Fisheries Commercial Fishing Database

	1991/92		1992/93		1993/94	
Area	Weight (kg)	Value ($)	Weight (kg)	Value ($)	Weight (kg)	Value ($)
Ocean Zone 1	4,440	24,593	1,568	8,825	2,754	16,079
Ocean Zone 2	6,969	33,510	3,308	20,591	1,632	9,182
Ocean Zone 4	1,998	9,608	636	3,790	2,053	12,063
Ocean Zone 5	21,327	106,884	4,332	25,922	8,398	46,241
Ocean Zone 6	11,097	55,511	2,085	12,532	3,832	21,251
Camden Haven River	11,432	48,958	19,801	110,642	9,432	57,609
Wallis Lake	89,191	384,356	84,803	472,050	82,643	449,928
Smiths Lake	750	3,070	1,718	9,831	7,725	40,692
Myall Lakes/Port Stephens	28,371	135,945	20,934	124,693	23,001	132,470
Hunter River	8,847	45,254	5,736	33,160	7,301	43,575
Lake Macquarie	16,464	68,815	11,826	63,407	5,912	34,581
Tuggerah Lakes	2,676	12,777	3,367	17,339	47,323	234,886
Hawkesbury River	14,237	69,053	14,791	88,522	15,695	89,897
Port Jackson	856	4,209	1,132	7,164	1,974	11,699
Botany Bay	3,639	17,981	1,679	9,597	1,536	9,134
Lake Illawarra	2,382	10,014	16,512	95,667	3,930	18,190
Other Areas	3,939	18,842	4,891	29,502	6,142	34,306
Total	**228,614**	**1,049,381**	**199,118**	**1,133,234**	**231,281**	**1,261,781**

	1994/95		1995/96		1996/97	
Area	Weight (kg)	Weight (kg)	Value ($)	Weight (kg)	Value ($)	Value ($)
Ocean Zone 1	3,305	2,216	12,410	3,313	27,186	18,146
Ocean Zone 2	6,187	3,216	18,825	1,991	14,793	38,167
Ocean Zone 4	2,101	1,528	9,165	2,411	17,458	12,524
Ocean Zone 5	7,259	9,129	55,673	9,739	70,188	42,016
Ocean Zone 6	2,978	3,433	20,469	1,505	10,902	17,576
Camden Haven River	7,551	1,612	9,822	2,197	12,700	37,803
Wallis Lake	99,229	92,800	531,322	85,681	526,311	510,432
Smiths Lake	5,650	1,250	7,188	1,144	5,946	22,797
Myall Lakes/Port Stephens	22,822	32,110	198,262	32,305	224,597	132,894
Hunter River	1,790	3,200	20,144	2,192	12,519	8,227
Lake Macquarie	9,925	5,783	33,805	8,342	47,562	50,420
Tuggerah Lakes	844	17,824	102,260	50,876	272,411	3,855
Hawkesbury River	6,858	11,154	66,239	5,219	36,427	37,107
Port Jackson	181	1,163	7,021	154	969	953
Botany Bay	1,583	1,686	9,574	2,000	14,836	10,959
Lake Illawarra	8,886	59	388	156	810	41,628
Other Areas	3,819	3,308	19,189	5,358	33,628	22,110
Total	**190,968**	**191,469**	**1,121,755**	**214,582**	**1,329,244**	**1,007,614**

	1997/98		1998/99		1999/00	
Area	Weight (kg)	Value ($)	Weight (kg)	Value ($)	Weight (kg)	Value ($)
Ocean Zone 1	904	5,396	4,530	28,422	3,947	27,560
Ocean Zone 2	2,509	15,789	4,281	25,243	5,993	41,476
Ocean Zone 4	3,449	19,379	5,970	35,755	4,648	32,161
Ocean Zone 5	6,714	39,298	10,805	67,016	6,985	46,792
Ocean Zone 6	1,342	9,240	1,590	9,332	1,795	12,394
Camden Haven River	18,308	88,899	8,129	49,517	3,518	22,028
Wallis Lake	108,198	576,234	104,114	535,828	86,030	548,519
Smiths Lake	706	4,175	666	3,293	713	4,883
Myall Lakes/Port Stephens	58,441	321,136	40,943	241,157	22,213	155,623
Hunter River	4,578	22,718	1,367	7,567	323	2,189
Lake Macquarie	12,010	70,600	9,374	51,022	9,609	64,027
Tuggerah Lakes	37,094	213,856	10,241	53,289	7,665	49,682
Hawkesbury River	8,676	50,971	9,777	59,416	4,660	32,699
Port Jackson	1,440	7,369	2,381	15,305	1,323	8,720
Botany Bay	3,909	25,878	3,700	24,213	1,435	10,281
Lake Illawarra	415	2,077	15,521	77,451	23,067	157,236
Other Areas	14,232	81,635	13,771	80,639	7,264	48,776
Total	**282,923**	**1,554,651**	**247,156**	**1,364,465**	**191,186**	**1,265,048**

	2000/01	
Area	Weight (kg)	Value ($)
Ocean Zone 1	5,706	47,188
Ocean Zone 2	9,258	76,118
Ocean Zone 4	1,998	15,890
Ocean Zone 5	2,808	22,416
Ocean Zone 6	963	7,645
Camden Haven River	4,935	38,231
Wallis Lake	63,672	492,188
Smiths Lake	632	5,432
Myall Lakes/Port Stephens	15,245	120,108
Hunter River	81	634
Lake Macquarie	5,803	45,847
Tuggerah Lakes	4,537	32,791
Hawkesbury River	2,647	21,599
Port Jackson	672	5,159
Botany Bay	310	2,518
Lake Illawarra	4,919	35,695
Other Areas	1,637	13,292
Total	**125,822**	**982,753**

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