



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

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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.

Certificate

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 Hassaplidakis, 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

field work and comments on the various manuscripts generated through the work. Special thanks go to Katie Clarke, Neil Keraunos, Anthony Collins, Dave Leggett, Jacqui Grove, Jamie Slaven, Lance Smith, Alasdair Guthrie and Stella Whittaker. Thanks also to Lyndal Wilson for drawing the “zipper crab” at the front of this thesis. I would also like to thank Hornsby Shire Council for the use of the Kangaroo Point laboratories to conduct the moult induction work.

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 $\mu\text{g/g}$ wet weight and contaminated mussels had an average of 5.2 $\mu\text{g/g}$ 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 \pm 2.4). The injection of moulting hormone, 30⁰C 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. R. 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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