Maternal and nutritional factors affecting larval competency in the spiny lobster, *Jasus edwardsii*.

By

Gregory George Smith

B.App.Sc (Hons)

Submitted in fulfillment of the requirements for the Degree of
DOCTOR OF PHILOSOPHY

University of Tasmania

Launceston, Australia

February 2004

Declaration and Authority of Access

I hereby declare that this thesis contains no material which has been accepted for the award of any other degree or diploma at any university, and to the best of my knowledge contains no paraphrase or copy of material previously published or written by another person, except where due reference is made in the text of the thesis.

Candidate's signature

Gregory George Smith

This thesis may be made available for loan and limited copying in accordance with the *Copyright Act 1968*.

Candidate's signature

Gregory George Smith

Abstract

The major impediment to closing the life-cycle of *Jasus edwardsii* Hutton (Decapoda: Palinuridae), a spiny lobster being assessed as a candidate for aquaculture, lies with larval rearing. Larval development in *J. edwardsii* phyllosoma is a complex and protracted phase taking in excess of 300 days in captivity. Often the initial phyllosoma competency (survival and growth) is poor and thought to be associated with parental influences. To improve survival in culture a method to assess larval competency at hatch was determined and environmental, physiological and nutritional influences on competency assessed.

The development of an activity test to assess larval competence involved short-term exposure of phyllosoma larvae to temperature and salinity stresses, responses monitored (stress indices) and later correlated with survival in starvation tests and in culture. The initial activity test (1 h in 23ºC at 10‰) was refined (1 h in 21ºC at 10‰) during an examination of nutritional influence on larval competency allowing the most competent larvae to be selected for culture, a significant advantage for any potential aquaculture species.

The effect of exposing broodstock to elevated temperatures (17 and 19ºC) during embryonic development was assessed in relation to the larval competence of newly-hatched phyllosoma. Elevated temperature provided out-of-season larvae by accelerating embryonic development by up to 2 months however; the larvae produced were smaller and less competent than those incubated under ambient conditions (9.5-13ºC). This was a significant finding as it ended the use of this technique to provide out-of-season phyllosoma larvae and stimulated research into alternate methods to provide phyllosoma at different times during the year.

Larval competence was also assessed in relation to broodstock physiology. Larger females produced larger phyllosoma, which demonstrated a greater potential for survival in culture, ascertained by stress indices and LD-50. Viable fecundity (phyllosoma number at hatch) in captivity was ≈ 45% of that expected from fisheries estimates and may indicate a reduction in fecundity associated with captivity, an area identified as requiring further dedicated studies. Size at onset of maturity was correlated with morphometric changes to male leg length and female abdominal segment width, providing non-destructive correlates for determining maturity in both males and non-ovigerous females.

The ability of juvenile *Artemia*, the predominant food source of cultured *J. edwardsii* phyllosoma larvae, to assimilate a range of enrichment products was assessed. The nutritional factors targeted were those credited with improving larval competency in crustaceans and included lipids and the development of a protocol for enrichment with ascorbic acid (AA). These live feed studies demonstrated that both lipid and AA (particulate form) are assimilated by juvenile *Artemia* (at >1% of *Artemia* dw for AA) concomitant with enrichment concentrations, and maintained with short-term starvation. The lipid assimilation ability of juvenile *Artemia* was

examined in relation to the loss of gut content, a common occurrence during the external mastication process of *J. edwardsii* larval feeding. Gut content did not make a significant contribution to total *Artemia* profiles shortly after the cessation of enrichment meaning any subsequent loss that may occur during feeding would not significantly alter *Artemia* nutritional profiles. It is considered that juvenile *Artemia*, like smaller metanauplii are powerful a tool for the delivery of specialized enrichment products.

Dietary influences on the maternal lipid and fatty acid digestive gland (storage), ovary (maturation) and tail muscle profiles were examined during starvation and ovarian maturation and their roles examined in relation to the production of competent larvae. The digestive gland lipid content was high (≈ 70% dw) and dominated by triacylglycerol (energy source); it showed evidence of utilization during starvation but not during ovarian maturation. The digestive gland fatty acid profile resembled that of the diet, a fact that could be used to identify preferred wild lobster diets, i.e., through the use of dietary lipid signatures. A potential method to ascertain a range of beneficial nutritional products that may assist larval competence in wild caught larvae. The ovary and tail muscle were resilient to dietary changes in lipid or fatty acid profile, with the tail muscle approximating that of newly-hatched phyllosoma. This study demonstrated that *J. edwardsii* are effective in obtaining their lipid requirements in spite of minimal dietary input and reduces the emphasis on lipids and fatty acids in the nutrition of *J. edwardsii* broodstock.

A study to ascertain the benefit of supplementary AA during broodstock maturation found that ovarian AA concentration was easily saturated, and in the absence of supplementation was sequestered from the tail muscle, the first time this has been considered as a significant AA store. There was minimal AA utilization during embryonic development, no improvement to broodstock fecundity or phyllosoma competency suggesting that there are no reproductive or larval competency benefits to providing supplementary AA to broodstock. In contrast, the delivery of mega-doses of AA to newly-hatched phyllosoma via juvenile *Artemia* resulted in large increases to larval AA content (161 – 2250 μg g^{-1}) and concomitant increases in larval survival (74%). This is the first time *Artemia* enrichment protocols have resulted in an improvement to larval survival.

This thesis has identified factors which contribute to the production of competent phyllosoma larvae; a dietary mix of live and pellet feed is sufficient to supply the lipid and AA requirements of broodstock, large females producing large eggs and phyllosoma should be targeted as broodstock, while incubation should be conducted under conditions of ambient temperature. The development of an activity test is an effective tool that provides a quick and easy determination of larval competency at hatch, capable of enhancement by feeding with juvenile *Artemia* supplemented with a particulate form of AA. Adoption of these procedures should ensure the most competent larvae are available to improve the success of phyllosoma larval rearing in the spiny lobster *J. edwardsii*.

Acknowledgments

I sincerely thank my university academic and research supervisors, Drs. Peter Thompson, Arthur Ritar and Danielle Johnston as well as my CSIRO research advisors Dr. Malcolm Brown and Mr. Graeme Dunstan.

Dr. Peter Thompson provided me with initial guidance in the planning stages of my project, with sound advice on lipid and fatty acid biochemistry as well as some experimental design components of my research plan. Due to his acceptance of a position outside of the university our supervisor/student role was prematurely curtailed however, I am grateful for the initial assistance he provided.

Dr. Danielle Johnston enthusiastically took up his role, she provided a different insight into lobster physiology and the processes required to fulfill the role of a research student in an emerging field. I would like to thank her for perusing the many drafts that have crossed her desk during the course of my studies and the perspective that she brought to the project.

Dr. Arthur Ritar provided continuity throughout the project as my Research/co-supervisor. I had daily contact with Dr. Ritar; I would like to thank him for his thoroughness and accessibility, with ready advice and assistance when required. I would like to thank him for passing on his knowledge of lobster broodstock and larval biology as well as the intricacies of the English language through thoughtful editing of draft manuscripts.

My association with the CSIRO marine laboratories was a rewarding one, as well as providing me with a supplementary scholarship to assist with living expenses, for which both myself and my family thank you, they provided use of their workplace for biochemical sample analyses. I would like to thank all those that made my work at the CSIRO ML an enjoyable experience, in particular Dr. Malcolm Brown for guidance in the extraction and processing of ascorbic acid samples, and for helpful advice and suggestions in the perusal of draft manuscripts.

Mr. Graeme Dunstan provided endless advice and assistance in deciphering lipid and fatty acid trends, reading manuscripts and providing me with encouragement when there were so many fatty acid tables that they took up a whole wall of his office.

During my PhD I had a close association with a number of other CSIRO ML staff and students not associated with my project. I would like to make special mention of Dr. Peter Nichols, Dr. Peter Mancuso and Professor Rick Phleger, on sabbatical from the University of San Diego, for passing on some of their knowledge of biochemistry to me. Thanks to Danny Holdsworth who ran the GC facility and Mina Brock who ran the LC facility during my studies. Thanks to Matt Nelson who gave support as a student working in a similar field.

I gratefully thank all the staff who have worked at the Marine Research Laboratories (MRL) during my studies, some gave assistance and advice in live feed preparation, system construction and environmental parameters; Craig Thomas, Ed Smith, Justin Ho, Bill Wilkinson, Joan Van Drunen, Alan Beech. While others perused manuscripts; Drs. Brad Crear, Piers Hart and countless others gave moral support and encouragement; Richard Davis, Ross Goldsmith, Anna Overweter and Drs. Steve Battaglene, Dave Morehead and Jenny Cobcroft.

Special mention must be made of Mark Grubert and Peter Lee whom I shared an office, as well as many of the personal trials and tribulations, which contribute to the production of a thesis and the journey that is involved in a Ph.D.

Thanks to my parents and in-laws for endless phone calls, support and assistance when required. Lastly but certainly not least of all my heartfelt thanks to my immediate family, my wife Michell who worked long hours to help keep our heads above water and our children Brodie, Casey, Lachlan and Dana for endless support and perseverance in the face of impossible deadlines, postponed holidays, weekend work, domestic disruptions during the building of our new home and for seeing me constantly glued to the computer screen. And lastly the prophetic words of our 4 yr old daughter, Dana “Mum do you know dad is going to be a doctor when he grows up”.

Table of contents

List of tables

List of figures

List of Plates