JCU ePrints

This file is part of the following reference:

Holme, May-Helen (2008)
Towards development of a formulated diet for mud crab (Scylla serrata) larvae, with emphasis on lipid nutrition.
PhD thesis, James Cook University.

Access to this file is available from:

http://eprints.jcu.edu.au/2150

JCU
JAMES COOK UNIVERSITY

Towards development of a formulated diet for mud crab (*Scylla serrata*) larvae, with emphasis on lipid nutrition

Thesis submitted by
May-Helen Holme
January 2008

for the degree of Doctor of Philosophy in the
School of Marine & Tropical Biology
James Cook University

STATEMENT OF ACCESS

I, the undersigned author of this work, understand that James Cook University will make this thesis available for use within the University Library and, via the Australian Digital Theses network, for use elsewhere.

I understand that, as an unpublished work, a thesis has significant protection under the Copyright Act and;

I do not wish to place any further restriction on access to this work

_____ _____

Signature Date

STATEMENT ON SOURCES

Declaration

I declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any university or other institution of tertiary education. Information derived from the published or unpublished work of others has been acknowledged in the text and a list of references is given.

…….. ……

(Signature) (Date)

ELECTRONIC COPY

I, the undersigned, the author of this work, declare that the electronic copy of this thesis provided to the James Cook University Library, is an accurate copy of the print thesis submitted, within the limits of the technology available.

_____ _____

Signature Date

Statement of the Contribution of Others

Financial support for this study was provided by the AIMS@JCU Tropical Aquaculture Program and The Graduate Research Funding Scheme at James Cook University. Student support was provided by AIMS@JCU, Jasons Legat, Sparebak 1 SR-bank and Peder Smedvig Stipendiefond.

Mr. Ian Brock of the Queensland Department of Primary Industries for assistance with the chemical analyses conducted during this study.

Acknowledgements

They say a journey is always easier when you travel together, and looking back over the last four years I know I could not have completed this project without the help and support of a long list of people! With their encouragement, guidance and friendship this PhD-journey has been one of the most amazing, mind-blowing, exhausting and empowering experiences of my life, and I'd like to express sincere gratitude to those who helped make this thesis possible.

To my supervisor Professor Paul Southgate, who has taught me the importance of critical and individual thinking. Your wealth of knowledge and experience has been of enormous help. To my supervisor Dr. Chaoshu Zeng, who has taught me everything I know about mud crabs and larval rearing. Your dedication and commitment has certainly amazed me. I also want to acknowledge the support from my third supervisor, Dr. Mike Hall at the Australian Institute of Marine Science (AIMS). The use of your labs and equipment has been of great help. I also want to thank Dr. Dean Jerry for always putting things into perspective when the going has been tough. The conferences I've attended in Adelaide and Hanoi would not be possible without your support, and I'm tremendously grateful for your genuine interest in my progress.

I want to thank the staff at MARFU, John Morrison, Greg Duffin and Simon Wever, for all their help with technical challenges. I'm also very grateful to the people I've been working closely with in the crab-lab; Sarah, Nick, Jerome, Mal, Diyana, Heleena and Karen. I further want to acknowledge Dr. Nick Paul and Dr. Mark McCormick for their

help with statistical analysis, and Dr. Greg Smith for valuable comments on manuscripts.

Finally, I’m forever grateful to my friends and family. The enormous support and encouragement I’ve received during this project has been absolutely amazing. I’m extremely lucky to have so many very special people in my life, and I can honestly say that without you I’d never have the courage and drive to complete this project. You’ve really taught me how irreplaceable a strong and supportive network of colleges, friends and family really is, a lesson I’ll keep with me for life.

Abstract

Crabs of the genus *Scylla*, commonly referred to as mud crabs, are commercially important crabs with an Indo-Pacific distribution. *Scylla serrata* is the most widespread of the four recognized *Scylla* species, and it has traditionally been an important fishery in coastal communities throughout its range. In recent decades it has also become a targeted species for aquaculture, however, due to a lack of low cost formulated diets, mass production of mud crab seed stock is not reliable, and the majority of mud crab farms rely on juveniles caught in the wild. This has lead to a widespread seeds-stock shortage and over-exploitation of wild populations. Development of more effective hatchery techniques and more reliable production of juveniles is therefore considered critically important for sustainable growth of the mud crab farming industry in this region.

Current methods for *Scylla* hatchery production rely on live foods such as rotifers and *Artemia*. These prey species are costly to produce, have an inconsistent nutritional profile and lack certain nutritional components essential for normal growth of marine larvae. Development of a nutritionally optimised diet is therefore considered critically important, and recent research at James Cook University has shown that mud crab larvae readily accept microbound diet (MBD) particles. As the contents of such diets easily can be manipulated, MBD shows enormous potential as a tool for further specification of the nutritional requirement of mud crab larvae.

Lipids are required in crustacean diets as an important source of energy, essential fatty acids, sterols, phospholipids and fat-soluble vitamins. An appropriate supply of these

nutrients is particularly critical during larval development, where a series of important morphological, physiological and biochemical changes take place. On this basis, this thesis was designed to first collect and review all information on the nutritional requirements of *S. serrata* larvae published to date, and highlight areas where more research is required. Experiments were then conducted to fill gaps in our current knowledge on larval dietary lipid requirements. Some of the most important aspects of lipid metabolism; dietary cholesterol requirements, interaction between cholesterol and phospholipids, the optimum balance of dietary *n*-3 and *n*-6 fatty acids, as well as lipid class and fatty acid utilization by developing larvae have been addressed in separate chapters, with the aim to provide information assisting formulation of more appropriate species-specific MBD for *S. serrata* larval culture.

While previous experiments have demonstrated that megalopae of the mud crab can be cultured exclusively on MBD, the potential of MBD as a food source for zoea larvae of the same species has not previously been determined. The first experiment of this thesis was therefore set up to investigate the effect of partial and total replacement of live food with MBD for zoeal larvae, with the aim to determine the best suited larvae stage for further nutritional studies. Zoea III larvae were cultured communally at a density of 25 larvae L^{-1} and were fed either 100% live *Artemia* nauplii, 100% MBD or a 50%:50% combination of MBD and *Artemia* nauplii. Highest survival (66%) and development rate to the zoea IV stage were recorded for larvae fed the 50%:50% combination of MBD and *Artemia* nauplii. Some successful molts were also found among larvae fed exclusively on MBD, while total mortality was observed in the unfed control. The results indicate that the experimental MBD may contain certain beneficial nutrients lacking in *Artemia* nauplii, and that co-feeding the MBD with *Artemia* nauplii enhanced

larval survival and development. However, the findings also suggested that total replacement of live food with the experimental MBD will result in poor survival of zoea III larvae, and based on these data, the megalopa stage was chosen as the most appropriate larval stage for further studies.

The experimental MBD previously used for rearing *S. serrata* megalopa contained a high proportion of dried rotifers (38% of total diet dry weight). Although this work demonstrated that mud crab megalopa readily ingest and assimilate formulated diet particles, it did not reduce the reliance on live food cultures. Fundamental to the success of this study was therefore to replace the rotifer components with a commercially available protein source, and four marine animal meals (rotifer meal, *Artemia* meal, fish meal and squid meal) were evaluated based on larval survival, dry weight and carapace weight of newly molted first stage crabs (C1) and development time from megalopa to C1. Fifteen megalopae were reared individually in 250 mL aquaria, and survival of MBD-fed megalopae to C1 ranged from 46.7% to 60.0% with those fed MBD containing fish meal or squid meal showing higher survival than those fed MBD containing dried *Artemia* or rotifers. Larvae fed live *Artemia* nauplii showed the highest overall survival (80%), while none of the unfed megalopae survived to C1. There were no significant differences ($P > 0.05$) in the average time required for megalopae to reach the first crab stage when fed any of the four MBD, however, shortest development time was recorded for larvae fed live *Artemia* nauplii. The results indicate that squid meal is a suitable protein source for formulation of MBD for *S. serrata* megalopae, and this meal was used in MBD formulated for subsequent experiments.

Cholesterol is an important sterol, serving as a precursor for many physiological compounds such as sex and molting hormones, adrenal corticoids, bile acids and vitamin D. As crustaceans are incapable of *de novo* production of cholesterol, dietary cholesterol is essential for optimum growth and high survival in crustaceans. Chapter 4 of this thesis reports on an experiment assessing the effects of dietary cholesterol levels on growth, development time and survival of mud crab megalopae. Five semi-purified, iso-nitrogenous and iso-energetic MBD containing cholesterol levels from 0.14% to 1.00% diet dry weight were tested, using fifteen individually reared megalopae for each dietary treatment. The results showed that megalopae from all treatments were able to metamorphose to the first crab stage, suggesting that the endogenous level of cholesterol in the basal diet (0.14%) was sufficient to meet dietary requirements. Highest overall survival (74.3%) was recorded for megalopae fed a diet containing 0.80% cholesterol, while the widest mean carapace width (3.53 ± 0.08 mm) and highest mean dry weight (21.11 ± 2.22 mg) were recorded for juveniles molting from megalopae fed live *Artemia*. No megalopae in the unfed control treatment metamorphosed into crabs. The average development time from megalopa to the C1 stage varied among the treatments, with megalopae fed live *Artemia* or MBD containing 0.20%, 0.40% or 0.80% total cholesterol showing relative shorter development time to C1; between 8.0 (± 1.46) days and 9.9 (± 1.37) days, whereas the longest development time was recorded for the megalopae fed diets containing 0.14% or 1.00% total cholesterol; 11 (± 1.45) days and 11 (± 1.24) days, respectively. Based on these findings, 0.80% total dietary cholesterol was suggested as optimum in semi-purified diets for *S. serrata* megalopa.

For some marine crustaceans, an interaction occurs between dietary cholesterol and dietary phospholipid. This has important implications for diet formulation, and to determine if this interaction is present in *S. serrata* larvae, the experiment reported in Chapter 5 was conducted to evaluate the effects of varying dietary levels of lecithin and cholesterol on growth, development and survival of megalopa. Six semi-purified MBD were formulated to be iso-energetic and iso-nitrogenous and to containing three levels of supplemental lecithin (0.0, 2.0 and 4.0% diet dry weight) and two levels of supplemental cholesterol (0.0 and 0.7% diet dry weight). The experiment was designed in the same manner as in Chapter 4, and the results showed a significant interaction between supplemental dietary lecithin and cholesterol for final mean dry weight of newly settled crabs. Highest survival (60%) was recorded for megalopae fed diets containing the highest levels of dietary lecithin regardless of whether diets were supplemented with cholesterol, and this rate of survival was identical to that of megalopae fed live *Artemia* nauplii. The experiment indicated that supplemental dietary cholesterol may not be essential for mud crab megalopae when fed a diet containing fish oil and sufficient levels of supplemental dietary phospholipids.

Most crustacean diets today are formulated to contain a 2:1 fish oil/corn oil ratio based on known requirements of penaeid prawns. As the results from the previous chapters indicated that the nutritional requirements of *S. serrata* larvae are different from those of other marine crustaceans, the experiment in Chapter 6 was designed to examine the optimum fish oil/corn oil ratio in semi-purified diets formulated for mud crab megalopae. Six iso-energetic and iso-nitrogenous MBD containing 6% total lipid were formulated to contain fish oil and corn oil either singly or in various ratios (0:1, 1:2, 2:1, 3:1, 1:0, 1:1), and each dietary treatment consisted of 20 individually reared megalopae.

Survival, growth, development time to C1 and signs of molting death syndrome (MDS) were recorded daily, and carapace width and dry weight of newly molted crabs were measured immediately after molt. Megalopae from all dietary treatments successfully molted to C1; however, best survival (70%) was achieved by megalopae fed MBD containing a fish oil/corn oil ratio of 1:1. Megalopae fed MBD containing ratios of 3:1and 1:0 showed survival of 65%, while survival of megalopae fed either live *Artemia* nauplii or MBD containing a fish oil/corn oil ratio of 2:1 was 60%. Lower survival (55% and 35%) was recorded for megalopae fed MBD with ratios of 1:2 and 0:1, respectively. Significantly greater mean carapace width (3.51 ± 0.03 mm) and significantly higher mean dry weight (2.14 ± 0.14 mg) was recorded for crabs molting from megalopae fed live *Artemia* nauplii compared to those resulting from megalopae fed MBD. Results from this experiment indicate that the optimal fish oil/corn oil ratio is around 1:1 when oil is supplied at a level of 6% of total diet dry weight. The study further showed that complete replacement of fish oil with corn oil in the formulated diet resulted in a high occurrence of MDS-related mortality, indicating an essential dietary requirement for >C18 highly unsaturated fatty acids (HUFA), and a link between *n*-3 HUFA availability and the occurrence of MDS.

In nature the nutritional requirements of crab larvae are satisfied by the diversity and variability of natural prey and their broad nutrient contents. As the study of larvae in the wild is practically impossible, analysis of the biochemical changes occurring in the tissue during larval development in the laboratory has been used as an alternative method for elucidating information on the nutritional requirements of larvae. These principles have been applied successfully to studies of several crustaceans, but a complete record of changes in lipid and fatty acid profile during ontogenetic

development of mud crabs was not available. Comprehensive analysis of changes in dry weight, organic content, total lipid, lipid class and fatty acid composition in developing *S. serrata* larvae reared under standard hatchery conditions is reported in Chapter 7. An increase in mean dry weight (DW) was observed during larval development, from 12.11 ± 0.31 µg for newly hatched zoea I, to 1025.52 ± 87.11 µg for newly molted megalopae. The organic content of larvae ranged between 58.18 ± 2.37% (zoea I) and 70.16 ± 0.68% (zoea III) of sample DW, and statistical analysis indicated significant differences in the percentage organic content between the larval stages. On this basis the results relating to fatty acid composition of larvae were presented on a per unit ash free dry weight (AFDW) basis. The total fatty acid content of larval tissues increased from 22.89 ± 3.72 µg mg^{-1} AFDW in newly hatched zoea I larvae, peaking at 31.38 ± 18.30 µg mg^{-1} AFDW at zoea V before dropping down to 24.92 ± 13.56 µg mg^{-1} AFDW at the megalopa stage. During the first zoea stage, saturated fatty acids (SFA) and highly unsaturated fatty acids (HUFA), dominated the fatty acid profile, while monounsaturated fatty acids (MUFA) were less abundant. As the larval developed the HUFA were depleted, while an increase in MUFA was recorded during the zoea V and megalopa stages, suggesting that HUFA requirements go down during the later part of larval development. The fatty acid composition was relatively stable throughout larval development, dominated by palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1*n*-9), arachidonic acid (20:4-*n*-6, AA), eicosapentaenoic acid (20:5*n*-3, EPA) and docosahexaenoic acid (22:6*n*-3, DHA). A substantial increase in linolenic acid (18:3*n*-3, LNA) levels was recorded in zoea V larvae, a result probably linked to the elevated LNA content in enriched *Artemia* meta-nauplii used as food for later stage larvae.

When subjected to starvation, crustaceans go through distinct phases of biomass degradation. This process is useful for identification of key fatty acids, and Chapter 8 reports on the changes in lipid and fatty acid metabolism observed in zoea V and megalopa subjected to feeding and starvation, respectively. The objective was to gain a better understanding of fatty acid utilization, and to identify possible changes in lipid requirements during the two last larval stages, which again may be linked to the high incidence of MDS during these larvae stages in mud crab hatcheries. Larvae were reared following established hatchery protocols, and newly molted zoea V and megalopae were subject to 4 days of feeding or starvation, and larval tissue was sampled for lipid analysis. An additional 6 day starvation treatment was incorporated for the megalopa stage as a response to the longer duration of this larval stage compared to the zoea stages. The results showed that fatty acids had an important role as an energy store in starved larvae, and the fatty acid content of larval tissue was reduced by 71.93% and 72.96% for zoea V and megalopa, respectively, during the 4 day starvation period. Further depletion of stored fatty acids was not, however, observed when megalopae were starved for another 2 days, indicating that other nutrient sources are utilized for energy when larvae are subject to prolonged starvation. Fatty acids from the polar lipid fraction dominated larval tissue, while fatty acids from the neutral lipid fraction were preferentially metabolized for energy during food deprivation. Depletion of both SFA, MUFA and HUFA was observed among unfed larvae, and in accordance with the findings in the previous chapter, depletion of EPA, DHA and AA indicated a possible decreasing requirement of these fatty acids during later larval development. Fed larvae, on the other hand, maintained more stable fatty acid levels, and they were able to sequester a range of fatty acids from live *Artemia.* Comparison of the lipid profile of

starved and fed larvae highlights an inability for *de novo* synthesis of both C18 fatty acids such as LNA, and HUFA like DHA, EPA and AA.

This project utilised integrated methodology to study lipid requirements of mud crab larvae. Growth trials were the most optimal way of specifying nutritional requirement, while biochemical analysis was used to gain a deeper understanding of the underlying mechanisms behind growth promotion. The findings have significantly enhanced our current understanding of dietary lipid requirements and utilization in mud crab larvae, which will have significant implications for the development of a nutritionally appropriate MBD for *S. serrata* larval culture in the future.

Table of Contents

Statement of Access……i
Statement of Source……ii
Electronic Copy Declaration…… iii
Statement of the Contribution of Others……iv
Acknowledgments……v
Abstract……vii
Table of Contents……xvi
List of Tables……xx
List of Figures……xxiii

Chapter 1. General Introduction
1.1 Mud crab farming; the past, the present and the future……1
1.1.1 Mud crab farming in the past and at present ……2
1.1.2 Mud crab farming in the future; constrains and prospects ……3
1.1.3 The mud crab market……4
1.2 A review of aspects influencing the use of formulated diets in *Scylla serrata* hatcheries, and current knowledge of nutritional requirement of *S. serrata* larvae
1.2.1 Problems associated with use of live food in larval culture……5
1.2.2 Development of a formulated diet for hatchery production of S. serrata…7
1.2.2.1 Types of food particles……7
1.2.2.2 Binding agents……10
1.2.2.3 Particle size……11
1.2.2.4 Considerations in development of formulated larval diets……12
1.2.2.5 Digestibility and enzyme activity in *S. serrata* larvae ……14
1.2.2.6 Use of formulated diets to investigate nutritional requirements.17
1.2.3 The nutritional requirements of S. serrata larvae……18
1.2.3.1 Protein……19
1.2.3.2 Lipids and sterols……23
1.2.3.3 Carbohydrates……31
1.2.3.4 Vitamins and minerals ……34
1.2.4 Conclusion……37
1.3 Aims of this study……37

Chapter 2. General Materials and Methods
2.1 Collection and conditioning of broodstock……39
2.2 Eye-stalk ablation……40
2.3 Larval rearing……41
2.4 Production of live food
2.4.1 Microalgae ……45
2.4.2 Rotifers……45
2.4.3 Artemia spp……46

2.5 Preparation of microbound diets (MBD)……... 46
2.6 Experimental design: zoea III feeding experiments……...47
2.7 Experimental design: megalopa feeding experiments ……...48
2.8 Lipid analysis……49

Chapter 3. The use of microbound diets (MBD) as total or partial replacement of live feed for zoea III larvae, and specification of an optimal protein source in MBD for megalopae
3.1 Introduction……51
3.2 Materials and methods
3.2.1 Preparation of experimental MBD…… 53
3.2.2 Exp. 1.Replacement of live food with MBD for zoea III ……..….54
3.2.3 Exp. 2.Use of defined MBD in rearing of megalopae ……..…….55
3.2.4 Statistical analyses……..……...55
3.3 Results
3.3.1 Exp. 1.Replacement of live food with MBD for zoea III……56
3.3.2 Exp. 2.Use of defined MBD in rearing of megalopae…….....58
3.4 Discussion……61

Chapter 4. An assessment of the dietary cholesterol requirement of *Scylla serrata* megalopae using semi-purified diets
4.1 Introduction…….……...67
4.2 Materials and methods
4.2.1 Preparation of experimental MBD ……68
4.2.2 Statistical analysis……..70
4.3 Results
4.3.1 Survival from megalopa to the first crab stage……..70
4.3.2 Carapace width of newly metamorphosed first stage crabs.. ……71
4.3.3 Dry weight of newly metamorphosed first stage crabs …….……72
4.3.4 Development time from megalopa to first stage crabs……...72
4.4 Discussion…….…….73

Chapter 5. Assessment the dietary cholesterol and phospholipids requirements of megalopae using semi-purified microbound diets
5.1 Introduction……..……..77
5.2 Materials and methods
5.2.1 Preparation of MBD……...78
5.2.2 Experimental design……. ……79
5.2.3 Statistical analysis……..…….79
5.3 Results
5.3.1 Diet compositions……...80
5.3.2 Dry weight of newly metamorphosed first stage crabs…… 81
5.3.3 Carapace width of newly metamorphosed first stage crabs ……..83
5.3.4 Survival from megalopa to the first crab stage……..84
5.3.5 Development time from megalopa to first stage crabs ……..84
5.3.6 Total lipid and fatty acid compositions of MBD……85
5.4 Discussion…….…….87

Chapter 6. Survival, development and growth response of mud crab megalopae fed semi-purified diets containing various ratios of fish oil:corn oil ratios
6.1 Introduction……..……..92
6.2 Materials and methods
6.2.1 Experimental design and preparation of experimental MBD…..……..94
6.2.2 Statistical analysis……..94
6. 3 Results
6.3.1 Survival and incidence of molting death syndrome……95
6.3.2 Mean carapace width and mean final dry weight ……97
6.3.3 Development time from megalopa to first stage ……....98
6.3.4 Fatty acid compositions of fish oil and corn used in diet formulation ….99
6.4 Discussion…… ……100

Chapter 7. Ontogenetic changes in lipid and fatty acid composition of mud crab larvae
7.1 Introduction…….105
7.2 Materials and methods
7.2.1 Sampling of larvae and live food. ……...107
7.2.2 Measurement of dry weight and ash free dry weight of larvae……108
7.3 Results
7.3.1 Dry weight, ash-fee dry weight, weight increase and total tissue lipid…108
7.3.2 Total lipid and lipid class composition……109
7.3.3 Major fatty acid ……...112
7.4 Discussion……...115

Chapter 8. Effects of starvation on lipid class and fatty acid profile of zoea V and megalopae larvae of mud crab
8.1 Introduction…….123
8.2 Materials and methods
8.2.1 Experimental dietary treatments …….124
8.3 Results
8.3.1 Major fatty acids……..125
8.3.2 Total fatty acid and lipid class composition in fed and starved larvae……127
8.4 Discussion……...129

Chapter 9. General Discussion
9.1 General discussion……. ……..135
9.2 Future directions…….. ……143

References……146

Appendix 1

Holme, M.H., Zeng, C, Southgate, P.C., 2006 Aquaculture 257: 482-490

Appendix 2

Holme, M.H., Zeng, C, Southgate, P.C., 2006 Aquaculture 261:1328-1334

Appendix 3

Holme, M.H., Southgate, P.C., Zeng, C., 2007 Aquaculture Nutrition 13: 413-423

Appendix 4

Holme, M.H., Southgate, P.C., Zeng, C., 2007 Aquaculture 269: 427-435

List of Tables

Table 1.1 Formulated diets previously tested as live food replacement for various crustacean species……..……8

Table 1.2 Examples of dietary protein levels typically used in formulated diets for crustacean juveniles and larvae……..……...20

Table 1.3 Summary of dietary lipid levels used in previous nutritional studies with crustacean larvae……..……...24

Table 1.4 Examples of cholesterol levels previously used in nutritional studies with crustacean juveniles and larvae…….....……28

Table 1.5 Examples of phospholipid levels previously used in nutritional studies with crustacean juveniles and larvae…….……..30

Table 1.6 Dietary carbohydrate levels previously used in experimental formulated diets for crustacean larvae and juveniles……..……31

Table 2.1 Standard rearing conditions used for culturing mud crab larvae ……44

Table 3.1 The composition (% dry weight) of the basal experimental diets used in feeding experiments with *Scylla serrata* zoea III and megalopae. ……54

Table 3.2 Percentage survival to zoea IV stage and mean ± SE intermolt duration of zoea III larvae of *Scylla serrata* fed varying proportions of microbound diet and live *Artemia* nauplii. Different superscripts in each column indicate significant differences between means (P <0.05).……58

Table 3.3 Percentage survival of *Scylla serrata* megalopae to the first crab stage and mean ± SE development time (days) when fed live *Artemia* nauplii and four experimental microbound diets. Different superscripts indicate significant differences between means (P <0.05).……59

Table 4.1 The composition (% dry weight) of the experimental microbound diets used in feeding experiments with *Scylla serrata* megalopae. Formulation based on Sheen (2000), Genodepa et al.(2004) and the results from Chapter 3 of this thesis.……..69

Table 4.2 Percentage survival, mean ± SE final dry weight, carapace width and development time for juvenile *Scylla serrata* molted from megalopae fed semi-purified diets containing different levels of dietary cholesterol. ……72

Table 5.1 Formulation (% dry weight) of experimental MBD used in feeding experiments with megalopae and their analysed total cholesterol, total phospholipid and total lipid contents……...80

Table 5.2 Two-way ANOVA results, testing interaction between lecithin and cholesterol on the dry weight (mg) and carapace width (mg) of newly molted first stage crabs fed 6 different MBD formulated to contain three levels of supplemental lecithin and two level of supplemental……. …….82

Table 5.3 Mean ± SE carapace width and development time of newly molted first stage crabs resulting from megalopae fed microbound diets containing various levels of supplemental dietary lecithin and cholesterol……83

Table 5.4 Fatty acid composition (% of total fatty acid) of the six experimental microbound diets containing different level of supplemental cholesterol and lecithin…….....……86

Table 6.1 Formulation (% dry weight) of experimental MBD fed to megalopae...96

Table 6.2 Mean ± SE carapace width and mean ± SE dry weight of newly settled first stage crabs (C1), and mean development time from megalopa to C1 for *Scylla serrata* fed semi-purified diets containing various fish oil:corn oil ratios. ….. …….99

Table 6.3 Fatty acids profile (% of total fatty acid) of the fish oil and corn oil used in formulation of microbound diets fed to megalopae …….100

Table 7.1 Mean ± SE dry weight (DW), ash free dry weight (AFDW), dry weight increase from one larval stage to the next (WI), organic content and total lipid content of developing *Scylla serrata* larvae fed rotifers and enriched *Artemia* meta-nauplii……108

Table 7.2 Fatty acid composition (µg mg^{-1} ash free dry weight) of *Scylla serrata* larvae (mean ± SE; n = 2). Included in the table are the fatty acid composition of the food sources; rotifers, AF Specialty *Artemia* nauplii and enriched *Artemia* meta-nauplii presented as µg mg^{-1} dry weight (- indicates <1% of total fatty acid).……110

Table 7.3 Ontogenetic changes in fatty acid composition of the polar lipid fraction (µg mg^{-1} ash free dry weight) of *Scylla serrata* larvae (mean ± SE; n = 2) (- indicates <1% of total fatty acid)……..111

Table 7.4 Ontogenetic changes in fatty acid composition of the neutral lipid fraction (µg mg^{-1} ash free dry weight) of *Scylla serrata* larvae (mean ± SE; n = 2) (- indicates <1% of total fatty acid)……...112

Table 8.1 Fatty acid composition (µg mg^{-1} ash free dry weight) of fed and starved *Scylla serrata* zoea V and megalopae. Samples were collected immediately after molt and following 4 days of feeding or starvation. An additional 6 day starvation treatment was included for the megalopa stage (- indicates <1% of total fatty acid)……..126

Table 8.2 Changes in fatty acid composition (µg mg^{-1} ash free dry weight) of the polar lipid fraction in fed and starved *Scylla serrata* zoea V and megalopae. Samples were collected immediately after molt and following 4 days of feeding or starvation. An additional 6 day starvation treatment was included for the megalopa stage (- indicates <1% of total fatty acid)…….128

Table 8.3 Changes in fatty acid composition (µg mg^{-1} ash free dry weight) of the neutral lipid fraction in fed and starved *Scylla serrata* zoea V and megalopae. Samples were collected immediately after molt and following 4 days of feeding or starvation. An additional 6 day starvation treatment was included for the megalopa stage (- indicates <1% of total fatty acid)……..129

Table 9.1 Schematic overview of the results and implications of this study……137

List of Figures

Fig. 1.1 Structure of microencapsulated diets and microbound diet……..……..9

Fig. 2.1 Eyestalk ablation of female mud crab……..41

Fig. 2.2 Development of *Scylla serrata* eggs from spawn till hatch……….42

Fig. 2.3 Development of *Scylla serrata* larvae from zoea I to the first crab stage……..43

Fig. 3.1 Daily percentage **(**± SE) survival of zoea III larvae fed diets containing four different levels of MBD and live *Artemia*……..57

Fig. 3.2 Proportional number of megalopae and juveniles present in each of the six dietary treatments from day 6 to day 11 of Exp.1……60

Fig. 3.3 Mean (± SE) carapace width of first stage crabs metamorphosed from megalopa fed MBD containing different nutrient sources……..61

Fig. 4.1 Survival (%) of *Scylla serrata* from megalopae to the first crab stage when fed microbound diets containing different levels of cholesterol. ……..71

Fig. 5.1 Dry weight of *Scylla serrata* first stage crabs molted from megalopae fed six different microbound diets formulated to contain three different levels of supplemental lecithin (0.0, 2.0 and 4.0%) and two different levels of supplemental cholesterol (0.0 and 0.7%). Means with different superscripts are significantly different (P <0.05). ……..82

Fig. 5.2 Percentage successful metamorphosis to first stage crabs of *Scylla serrata* megalopae fed either live *Artemia* nauplii or one of six microbound diets supplemented with different levels of dietary lecithin and cholesterol.…… ……...85

Fig. 6.1 Percentage successful metamorphosis from megalopae to the first crab stage, when fed either live *Artemia* nauplii or one of six MBD containing different ratios of fish and corn oil……..97

Fig. 7.1 Changes in levels (g 100g $^{-1}$ fatty acid) of linolenic acid, eicosapentaenoic acid, docosahexaenoic acid, linoleic acid and arachidonic acid in developing *Scylla serrata* larvae fed rotifers, *Artemia* nauplii and enriched *Artemia* meta-nauplii……...114