

JCU ePrints

This file is part of the following reference:

Chang, Marshall C. (2000) *Improvement of culture techniques for the seahorse *Hippocampus* sp.* Masters (Research) thesis, James Cook University.

Access to this file is available from:

<http://eprints.jcu.edu.au/10341>



**Improvement of Culture Techniques
for the Seahorse *Hippocampus* sp.**

Thesis submitted by

Marshall C CHANG BA *Calif.*, USA

In April 2000

**for the research degree of Master of Science
in Aquaculture
within the school of Marine Biology and Aquaculture
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I understand that, as an unpublished work, a thesis has significant protection under the Copyright Act and;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

Or

I wish this work to be embargoed until

Or

I wish the following restrictions to be placed on this work:

Signature

23 march 2010

Date

ACKNOWLEDGEMENTS

The author would like to first thank my supportive family and the many volunteers of the project. Many thanks to Paul Hough and Reef HQ for their support, study facility and seahorses. The author would also like to extend gratitude to the many people who have contributed to the direction and execution of the project including: Paul Southgate, JCU; Tom Bowling, Domestic SeaLife; Rob Cirocco, JCU advanced analytical center; Rob Mayer, DPI, QLD; Mark McCormick, JCU; Ian Brock at DPI, QLD; Laura Castell, JCU; and Sara Lourie, Project Seahorse. The author would also like to acknowledge the JCU Department of Marine Biology and Aquaculture and the Great Barrier Reef Marine Park Authority for support through research grants.

ABSTRACT

The culture requirements of broodstock and juvenile seahorse *Hippocampus* sp. were investigated in four experiments: (1) the influence of dietary fatty acids on growth and survival of juvenile seahorses; (2) the effects of varying dietary fatty acid content on reproductive performance of broodstock and juvenile quality; (3) the effects of ambient calcium level on growth and survival of juvenile seahorses; and (4) the combined effects of temperature and salinity on the growth and survival of juvenile seahorse.

In the first experiment, three commercially available fatty acid enrichment emulsions (DC Selco, DC DHA Selco and DC Super Selco) were used to enrich *Artemia* nauplii fed to juvenile seahorses. The emulsions varied in their *n*-3 highly unsaturated fatty acid (HUFA) composition. Total *n*-3 HUFA content ranged from 200-450 mg g⁻¹ while levels of eicosapentaenoic acid (EPA, 20:5*n*-3) and docosahexaenoic acid (DHA, 22:6*n*-3) ranged between 47-220 mg g⁻¹ and 80-190 mg g⁻¹, respectively. Survival and growth of seahorses at the end of the 30-day growth trial were greater in treatments receiving enriched *Artemia*. Seahorses receiving *Artemia* enriched with DC DHA Selco and DC Super Selco showed significantly ($p < 0.05$) greater mean survival ($71.6 \pm 6.0\%$ and $78.3 \pm 6.0\%$, respectively) than those receiving unenriched *Artemia* ($48.3 \pm 6.0\%$). Mean standard length was also significantly greater ($p < 0.05$) for fry fed DC DHA Selco and DC Super Selco enriched *Artemia* (20.2 ± 0.3 mm and 19.7 ± 0.3 mm, respectively) compared to those fed unenriched *Artemia* (18.1 ± 0.3 mm). The results show that dietary *n*-3 HUFA are essential for optimal growth and survival of *Hippocampus* sp. and, based on the fatty acid compositions of the enriched *Artemia* used in this study, a level of dietary DHA

supporting optimal growth and survival was indicated to be greater than 9.3 mg DHA g⁻¹ DW.

In the second experiment, unenriched and enriched (DC DHA Selco) *Artemia* (Prime *Artemia* cysts, Great Salt lakes USA) were used to improve the nutritional quality of pelagic schooling shrimp, *Acetes sibogae*, to determine the effectiveness of nutritional enrichment on the fecundity and fertility of breeding seahorses. Six pairs of seahorses were fed either enriched or unenriched *Acetes* for a period of 45 days. Mean standard lengths, weights and number of newborns were counted for each clutch and fatty acid analysis was conducted on newborn seahorses and *Acetes* diets. Dietary quality of *Acetes* was effectively improved by feeding with enriched *Artemia*; (n-3) / (n-6) HUFA level increased from approximately 5:1 in the unenriched treatment to 7:1 in the enriched treatment and the DHA/EPA ratio increased from 0.76 in the control treatment to 0.92 in the enriched treatment. As a result, the weight at birth of newborn seahorses was significantly increased, demonstrating the importance of HUFA's in *Hippocampus* sp. broodstock diet.

In the third experiment, juvenile seahorses were subjected to four levels of ambient calcium to determine the effects of varying calcium levels on growth and survival during a 30-day period. Concentrations of calcium tested were: 489 ± 15.43 ppm, 520.83 ± 11.62 ppm, and 583 ± 10.21 ppm in the Low, Medium, and High treatments, respectively. Natural seawater was used as a control treatment with a calcium concentration of 432.67 ± 13.44 ppm. Under these treatments, final survival (% ± SE) ranged from 56.67 ± 12.03 % to 66.67 ± 12.03 %, with no significant differences observed between treatments. Final mean dry weights (mg ± SE) of juvenile seahorses in the treatments ranged from 10.67 ± 0.53 to 11.09 ± 0.82 with no

significant differences observed between the treatments. It was concluded that increasing ambient calcium levels above levels found in natural seawater did not significantly affect the growth or survival of juvenile seahorses examined in the study.

In the final experiment, a 4 x 4 factorial analysis of temperature and salinity combinations was investigated to determine their individual and combined effects on survival and growth of juvenile *Hippocampus* sp. Seahorses were stocked in sixty-four 7 L buckets at a density of 4 seahorses per bucket. Mean length, dry weight and survival were compared after 28 days at temperatures of 20°C, 23°C, 25°C and 29°C and salinities of 20 ppt, 25 ppt, 30 ppt and 35 ppt. All seahorses were fed DC DHA Selco enriched *Artemia* to satiation. Growth significantly increased with temperature; however, salinity had no significant effect on growth. At all salinities tested, a water temperature of 20°C was lethal for juvenile *Hippocampus* sp. Survival was generally improved at lower salinities (20 ppt and 25 ppt). The combined effects of temperature and salinity significantly affected growth but not survival. These results indicate that growth of *Hippocampus* sp. during the newborn-juvenile stage may be maximized through appropriate adjustment of water temperature and salinity.

TABLE OF CONTENTS

List of Figures	10
Signed Statement on Sources	11
CHAPTER 1	
1.1 Introduction	
1.1.1 General.....	12
1.1.2 Reproduction.....	12
1.1.3 Diet and nutrition.....	14
1.2 Market	16
1.3 Conservation and Aquaculture	18
1.4 Research Aim and Approach	
1.4.1 Dietary and nutritional requirements.....	20
1.4.2 Environmental conditions.....	20
CHAPTER 2-Methods and Materials	
2.1 Study Area and Duration	22
2.2 Taxonomy and species description	
2.2.1 Confusion of seahorse taxonomy.....	22
2.2.2 Species description.....	22
2.3 Culture Techniques and Materials	
2.3.1 Broodstock system design and husbandry.....	26
2.3.2 Rearing protocol for newborn and juveniles.....	26
2.4 Measuring Standard Length of Juveniles	27
CHAPTER 3-Effects of Varying Dietary Fatty Acid Composition on Growth and Survival of Seahorse, <i>Hippocampus</i> sp., Juveniles	
3.1 Introduction	
3.1.1 Requirements of fatty acids by larval fish.....	29
3.1.2 <i>Artemia</i> as a food source for larval fish.....	29
3.1.3 Aims of the experiment.....	30
3.2 Materials and Methods	
3.2.1 Preparation of <i>Artemia</i>	30
3.2.2 Seahorse culture conditions.....	31
3.2.3 Data collection and analysis.....	32
3.2.4 Fatty acid analysis.....	32
3.3 Results	
3.3.1 Survival.....	33
3.3.2 Growth.....	35
3.3.3 Fatty acid content of <i>Artemia</i>	37
3.3.4 Fatty acid content of seahorses.....	37
3.3.5 Correlations.....	38

3.4 Discussion.....	38
---------------------	----

CHAPTER 4-Improvement of Pelagic Schooling Shrimp (*Acetes sibogae*) as a Broodstock Diet for *Hippocampus* sp. and the Subsequent Effects on Larval Quality and Fatty Acid Content

4.1 Introduction	
4.1.1 Lipids in broodstock nutrition.....	41
4.1.2 Diet of broodstock seahorses.....	42
4.1.3 Research aim.....	42
4.2 Materials and Methods	
4.2.1 Live feed treatment of <i>Acetes</i> fed to broodstock.....	43
4.2.2 Broodstock culture conditions.....	44
4.2.3 Data collection and analysis.....	44
4.3 Results	
4.3.1 Reproductive performance of broodstock fed different diets.....	45
4.3.2 Fatty acid content of <i>Acetes</i>	46
4.3.3 Fatty acid content of newborn <i>Hippocampus</i> sp.....	47
4.4 Discussion	49

CHAPTER 5- Effect of Increased Ambient Calcium Levels on Growth and Survival of Newborn Seahorse *Hippocampus* sp.: A preliminary study

5.1 Introduction	
5.1.1 Calcium requirements of fish.....	52
5.1.2 Calcium requirements of seahorses.....	53
5.2 Materials and Methods	
5.2.1 Seahorse rearing conditions.....	53
5.2.2 Methods for the determination of alkalinity and calcium levels.....	54
5.2.3 Preparation of calcium supplemented seawater.....	55
5.2.4 Experimental design, data collection and analysis.....	55
5.3 Results	56
5.4 Discussion	56

CHAPTER 6-Combined Effects of Temperature and Salinity on Survival and Growth of Juvenile Seahorse, *Hippocampus* sp.

6.1 Introduction	
6.1.1 The physiological effects of temperature and salinity on fish.....	59
6.1.2 Background of current species and previous studies.....	60
6.1.3 Experimental aim.....	60

6.2 Materials and Methods	
6.2.1 Broodstock spawning and newborn rearing conditions.....	61
6.2.2 Experimental design and materials used.....	61
6.2.3 Statistical methods.....	62
6.3 Results	
6.3.1 Survival of juvenile seahorse reared under different temperature/salinity regimes.....	63
6.3.2 Growth of juvenile seahorse reared under different temperature/salinity regimes.....	65
6.4 Discussion.....	67
CHAPTER 7-General Conclusion.....	71
References.....	75
Appendix.....	88

List of Figures:

- Figure 1-1: Developing seahorse *Hippocampus* sp. during endogenous yolk absorption.
- Figure 2-1: Adult male *Hippocampus* sp. examined in study
- Figure 2-2: 1200-L pond used to house broodstock seahorses
- Figure 2-3: Seven-day old seahorse measured using video image processing procedure.
- Figure 3-1: Percent survival of *Hippocampus* sp. juveniles fed unenriched *Artemia* or those enriched with three enrichment preparations.
- Figure 3-2: Changes in standard length of *Hippocampus* sp. juveniles fed unenriched *Artemia* or those enriched with three enrichment preparations.
- Figure 6-1: Mean (\pm SE) percent survival of juvenile seahorse *Hippocampus* sp. at 28 days reared under different temperature and salinity combinations.
- Figure 6-2: Response surface estimation of percentage survival of *Hippocampus* sp. juveniles at 28 days reared under different temperature and salinity combinations.
- Figure 6-3: Final mean (\pm SE) weights (mg g^{-1} DW) of seahorse *Hippocampus* sp. juveniles 28 days reared under different temperature and salinity combinations.
- Figure 6-4: Mean standard length (mm) and standard errors of seahorse *Hippocampus* sp. juveniles 28 days reared under different temperature and salinity combinations.
- Figure 6-5: Response surface estimation of standard length of *Hippocampus* sp. juveniles at 30 days reared under different temperature and salinity combinations.
- Figure 7-1: Significant results of this study and how they contribute to the major goals and key areas of seahorse culture

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.
