Improvement of Culture Techniques

for the Seahorse *Hippocampus* sp.

Thesis submitted by

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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 ± 6.0 % and 78.3 ± 6.0%, respectively) than those receiving unenriched *Artemia* (48.3 ± 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^{o}C, 23^{o}C, 25^{o}C and 29^{o}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^{o}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.

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