The copepod/*Artemia* tradeoff in the captive culture of *Hippocampus erectus*, a vulnerable species in New York State

Thesis

Presented in Partial Fulfillment of the Requirements for the Degree of Master of Science
At Hofstra University

By

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2004

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Abstract

Seahorse populations worldwide are under increasing pressure from overexploitation and environmental degradation. Aquaculture may help to alleviate some of the pressure; however seahorse-culture attempts have met with mixed results and technological improvements are necessary before captive-bred seahorses can become an economically viable alternative. This study was undertaken to enhance technology for rearing the lined seahorse, *Hippocampus erectus*. In preliminary trials, it was demonstrated that the use of copepod-dominated wild plankton (CDWP) in the early diet of *H. erectus* can have a positive effect on survivorship. The primary goal of this investigation was to determine the minimum duration of copepod feeding necessary to achieve a significant increase in seahorse survivorship. In the experiment described herein, five broods of newborn *H. erectus*, each from a different set of parents, were separated into six tanks, each of which received CDWP for 0-5 days before being switched to a diet of enriched *Artemia fransiscanis*. Seahorses offered copepods for at least two days had a significantly greater survivorship than those offered copepods for 0 days or one day. Survivorship in the first brood was significantly greater than in broods 2-5. Seahorses in this experiment had a growth rate of approximately 0.07 cm/day. Between treatment groups with sufficient survivorship to support growth analyses, no differences in growth rate were observed; however there were significant differences in growth rates between broods. These results suggest that the use of wild plankton in the early diet of *H. erectus* may be a cost-effective means of maximizing survivorship and furthermore, that parental factors play a role in survivorship and rate of growth.

Introduction

Natural History

The family Syngnathidae is comprised of 52 genera and about 215 species. It includes the seahorses, pipefishes, sea dragons, and the pipehorse, an apparent morphological intermediate between seahorses and pipefishes (Nelson 1994). There are approximately 35 recognized species of seahorses, all belonging to the genus *Hippocampus* (Vincent 1996).

Seahorses are a group of highly specialized fishes characterized by a tubular mouth, an articulating neck, a prehensile tail, and rings of bony armor covering the entire body (Bigelow and Schroeder 1953). Locomotion is primarily by means of undulations or oscillations of the dorsal fin, whereas the pectoral fins serve in steering and lift generation (Fritzsche 1983). The anal fin has been greatly reduced, but may facilitate expulsion of feces, eggs, and fry (Gardner, pers. observ.). The pelvic and caudal fins have been lost entirely. One of the most extraordinary features of the family is their unique reproductive biology. Males have a specialized patch or pouch (marsupium) wherein eggs are deposited, fertilized, incubated, and possibly nourished for the entire gestation period (Fritzsche 1983). The exact physiological role of the marsupium remains under debate and there are no data to support the hypothesis of its nutritive function (Linton and Soloff 1964, Azzarello 1991), nevertheless it appears to be involved in osmoregulation of the fluid surrounding the developing embryos (Azzarello 1991, Carcupino et al. 1997).

Seahorses occur worldwide in tropical and temperate coastal marine waters. Their primary habitats are seagrass beds, mangroves, and coral reefs (Vincent 1996). Despite the extensive distribution of the genus, no single species is very wide-ranging and populations within species tend to be unevenly distributed. Seahorses may be locally abundant in some suitable habitats, but completely absent from others (Gardner unpubl. data, Vincent 1996). The narrow species ranges and patchy distributions are likely the result of seahorses' limited locomotory ability, low fecundity, and lack of a pelagic larval stage (Vincent 1996).

Exploitation

The unique morphology of seahorses has made them prominent figures in art, folklore, and mythology for thousands of years and probably is responsible for their popularity in the aquarium industry and curio trade. Seahorses are an important part of the global trade in live marine animals for the aquarium hobby, a $200 - to $330 - million - per - year industry (Wabnitz et al. 2003), less than 2% of which is supplied by aquaculture (Moe 2001). The greatest demand for seahorses, however, appears to come from traditional Chinese medicine (TCM). TCM is recognized by the World Health Organization as a viable health-care option and is practiced in China, Taiwan, Singapore, Japan, Indonesia, and ethnic Chinese communities worldwide. Seahorses are used to treat a variety of health problems including asthma, sexual dysfunction, and kidney disorders (Vincent 1996).

The economic growth of many Asian countries in the 1980s, coupled with a growing interest in alternative health-treatment options in western nations, fueled unprecedented sales in TCM products. The growth of this industry has contributed significantly to the near extinction of numerous plant and animal species. At least 430 species of threatened or endangered organisms are used in TCM, necessitating a largely illegal trade. The black-market nature of the industry makes it extremely difficult to monitor and regulate (Gaski and Johnson 1994).

In a report published by TRAFFIC International in 1996, Vincent provided the first and only detailed account of the world trade in seahorses. Her exhaustive research included investigations of customs reports as well as interviews with fishermen, pharmacists, and traders of seahorses at all levels throughout the global community. She found that seahorse consumption in China had increased 10 fold between 1986 and 1996 and cited China's surging economy and the decline of other fisheries as probable causes. At least 32 nations were found to be involved in seahorse trading. Consumption within Asian countries (excluding Japan, Korea, Malaysia, and Singapore – all believed to be important consumers) was calculated at about 45 tons of dried seahorses (approximately 16 million individuals) annually. This estimate only includes legal imports and does not consider trade routes through unofficial channels. The consensus among fishermen interviewed by Vincent (1996) throughout the Pacific is that seahorse populations appear to be declining. They also agree that they cannot catch enough seahorses to meet the demand.

Customs reports from various countries seemed to indicate that the United States is an important importer and exporter of live and dead seahorses, but no meaningful numbers for total US landings could be obtained. Of the 18 states wherein seahorses are known to occur, only Florida has attempted to record landings. Florida's Department of Environmental Protection reported a substantial increase in annual seahorse landings between 1990 (6,504 individuals) and 1994 (112,367 individuals) (Vincent 1996).

Several alarming facts have come to light as a result of Vincent's (1996) report: 1) the world trade in seahorses has still not been well quantified but it almost certainly exceeds 20 million individuals annually and is growing; 2) the sustainability of this fishery is virtually unknown, yet preliminary data suggest that they are being over-fished; and 3) population decline due to habitat loss and incidental by-catch in trawl fisheries has not been quantified, but it may easily exceed decline due to directed-effort harvesting.

The World Conservation Union (IUCN) currently lists three species of *Hippocampus* (*H. bargibanti, H. breviceps,* and *H. minotaur*) as endangered. Seventeen species, including *H. erectus*, are listed as vulnerable (IUCN 2000).

Hippocampus erectus

The lined seahorse, *Hippocampus erectus,* is one of three recognized seahorse species occurring in the western Atlantic. It inhabits shallow coastal waters from Nova Scotia to Argentina and reaches a length of at least 15 cm (Robins et al. 1986). Locally the lined seahorse can be found in eelgrass beds and around artificial structures in embayments along the south shore of Long Island and New York Harbor (Gardner unpubl. data). Although there is no commercial fishery for *H. erectus* on Long Island, local populations may be vulnerable for the following reasons:

- *H. erectus* has a typically low fecundity and patchy distribution.
- New York has a high concentration of tropical-fish dealers and a large Asian constituency, two of the most important consumers of seahorses.
- The large size and relatively smooth texture characteristic of *H. erectus* are the two most desirable characteristics of a seahorse by TCM standards (Vincent 1996).
- Crucial eelgrass habitat already has been lost to excessive coastal development, pollution, and blooms of the harmful chrysophyte, *Aureococcus anophagefferens*.
- Seahorse collection is neither monitored nor regulated in New York (J. A. Olin pers. comm.).

Aquaculture

Although seahorses, including *H. erectus*, have been spawned and reared in captivity for many years, they are notoriously difficult to culture, often suffering very high juvenile mortality (Vincent 1996). Despite these difficulties, a few investigators have experienced high juvenile survival in laboratory-rearing attempts (Correa et al. 1989, Wilson and Vincent 1998, Reyes-Bustamante and Ortega-Salas 1999), yet the reasons for these successes are poorly understood. Forteath (1997) claims to achieve 85% survival in rearing *H. abdominalis,* however he does not provide the technical details of his protocol. Scarratt (1995) estimated 70% survival 100 days after hatching, but did not count the fry on day 0. Additionally, her rearing system was probably too complex to be scaled up easily.

Newborn seahorses will feed only on live zooplankton and, to date, most rearing attempts have relied solely on the nauplii of *Artemia* spp. (Lunn and Hall 1998). The availability and convenience of *Artemia* cysts have made them extremely popular in aquaculture (Hoff and Snell 1999). Yet *Artemia* nauplii repeatedly have proven to be nutritionally deficient as a food source for larval and juvenile marine fishes compared to copepods, which have produced much better results in terms of growth, survival, and overall health (Kraul et al. 1991, 1993, Shields et al. 1999, Gardner 2000, Payne and Rippingale 2000). This is probably due to their relatively high levels of highly unsaturated fatty acids (HUFA).

Fishes, like other vertebrates, require three long-chain HUFAs, docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), and arachidonic acid (AA), for growth, development, and reproduction. These compounds play an important role in cell membrane structure and function and act as precursors to a group of highly biologically active hormones known as eicosanoids. Eicosanoids are involved in a wide range of physiologic functions and are produced in response to stressful situations. Unlike terrestrial vertebrates and most freshwater fishes, marine fishes are unable to synthesize these HUFAs from other molecules. DHA, EPA, and AA are therefore considered essential fatty acids in marine-fish nutrition. Recent research has demonstrated that because of competitive interactions between these fatty acids and between their chemical precursors and products, dietary ratios of DHA, EPA, and AA may be more important than actual levels. Therefore HUFA levels in aquaculture feeds and enriching products must be considered in relative terms rather than as absolute amounts. Furthermore, there may be considerable variation in HUFA requirements among marine fish species and ideal dietary levels and ratios remain unknown for most species (Sargent et al. 1999), nevertheless many marine species seem to benefit from a high DHA-to-EPA ratio (Kraul et al. 1991, 1993, Sargent et al. 1999). Naess et al. (1995) analyzed the fatty acid composition of the live feeds used in their experiment on first feeding of Atlantic halibut (*Hippoglossus hippoglossus)*, and found copepod-dominated wild plankton to have a DHA-to-EPA ratio ranging from 1.4 to 1.7, whereas unenriched and SuperSelco™ -enriched *Artemia* had DHA-to-EPA ratios of 0.1 and 0.5, respectively.

Although there are now a number of enriching products on the market intended to manipulate the levels and ratios of HUFAs in *Artemia* and other live foods, the use of copepods continues to produce better fish yields (Kraul et al. 1991, 1993, Shields et al. 1999, Gardner 2000, Payne and Rippingale 2000, Stottrup 2000). This suggests that either the ideal levels or proportions of HUFAs are not being attained through enrichment, or that there are other factors affecting growth and survival of fishes reared on copepods versus those reared on *Artemia*. The latter hypothesis is supported by an experiment conducted by Payne et al.

(1998) in which two groups of the pipefish *Stigmatopora argus* were reared on copepods containing either high or low HUFA levels. No difference in growth or survival was observed.

The use of copepods in aquaculture can be very expensive. Copepods do not reach nearly as high a density in culture conditions as other live foods. Therefore they require larger volumes of water and larger culture vessels. Shields et al. (1999) raised copepods for their experiment in a 150-cubic-meter outdoor tank, yet were only able to provide ~180 copepods per day to each of the 176 larval halibut (*Hippoglossus hippoglossus*) in their copepod treatment. The use of wild-caught copepods can eliminate this problem, but wild populations are subject to a high degree of fluctuation and their use can introduce parasites and fouling organisms into the rearing tank. Furthermore, the collection process can be labor-intensive, and if a boat is used to pull the plankton net, the cost of running the boat must also be taken into account. Moreover, inland facilities do not have the luxury of collecting wild marine copepods. In the 1970s, by using copepod-dominated wild plankton (CDWP), Moe and Young became the first and only people ever to rear the highly valued French angelfish, *Pomacanthus paru*, in captivity. However they never achieved commercial success, in part because of the high cost of collecting the copepods (Young pers. comm.).

Three preliminary seahorse-rearing trials were conducted at the Hofstra University Aquaculture Laboratory prior to the investigation reported herein. The purpose of these trials was to test some filtration designs and feeding regimens. All trials were conducted in standard 38-liter tanks. In trials 1 and 2, small air-driven foam filters were used. In trial 3, one end of the tank was sectioned off with 500-μm nylon netting, placed at an angle of 20° to the wall. The resulting compartment was filled with small, plastic beads, kept in motion with aeration to form a fluidized bed filter. In this tank, a 20-centimeter air diffuser was placed on the end with the filter compartment to prevent seahorses from being drawn into the netting. This method of aeration created a vertical circulation pattern in the tank that kept the newly hatched seahorses from becoming caught on the surface of the water as was experienced in trials 1 and 2. In each trial one liter of the marine chrysophyte *Isochrysis galbana* grown in standard seawater medium was added to the tank each day for the first 10 days. In the first trial nauplii of *Artemia salina*, enriched with Super HUFA (Salt Creek, Inc.), were offered as the exclusive food for an entire brood of *H. erectus*, estimated at 400-500 individuals. Super HUFA was chosen for its very high HUFA content. After 2 weeks 100% mortality was observed in this tank. In the second trial, copepod-dominated wild plankton (CDWP) was substituted as a live food for the first 2 weeks, after which time their diet was abruptly switched to enriched *Artemia salina*. At the end of the 60-day trial, 190 seahorses were counted. Initial brood size was not counted on day 0, so the percent survival could not be calculated. Nevertheless it is reasonable to say that survivorship approached 50%. In the third trial *Artemia franciscanis* was used rather than *A. salina* because it is smaller and has a higher HUFA content. Super HUFA was replaced by Ratio HUFA (also manufactured by Salt Creek, Inc.), which has a higher DHA-to-EPA ratio. In this trial CDWP was offered to a brood of similar size for only 3 days, followed by enriched *Artemia*. After 60 days, 214 seahorses were counted and moved into a 150-liter grow-out tank (Gardner 2003). The high survivorship achieved in this trial, in spite of the short copepod-feeding duration, was the inspiration for the following investigation.

The preliminary trials suggest that although copepods are important in the early diet of *H. erectus*, using current *Artemia*-enrichment technology, it may be possible to achieve high survivorship by offering copepods for only a very short period. Considering the well-established importance of copepods as an early food for seahorses, and the costs associated with obtaining them, it would be useful to know the minimum number of days of copepod feeding necessary to achieve a reasonable rate of survival. A greater number of days on copepods seems to result in higher juvenile survival, but may also increase the overall cost of production.

The purpose of this investigation was to examine the copepod/*Artemia* tradeoff in the culture of *Hippocampus erectus* and to determine the minimum copepod-feeding duration necessary to achieve a significant increase in seahorse survival over *Artemia* feeding alone. The ultimate objective of this experiment is to take a step toward the development of a reliable, cost-effective protocol for the captive culture of *H. erectus.* This would enable aquaculture to be used as an economically viable alternative to wild harvesting and, if necessary, as a tool in future stock-enhancement initiatives.

Methods

The following experiment was repeated five times, each with a different brood from a different set of parents. Wild-caught broodstock animals were kept in 208-liter and 416-liter aquaria with undergravel and carbon filtration and were fed three times per day with a combination of the shore shrimp, *Palaemonetes pugio*, shredded squid, and previously frozen *Mysis relicta*. Broodstock tanks were lighted with fluorescent bulbs and kept on a 16-hour light photoperiod. Individual pairs were removed and placed in a 227-liter spawning tank with similar filtration.

A rearing system consisting of fourteen 38-liter aquaria with central filtration was constructed for the experiment (Fig. 1). Each tank was equipped with a divider of 500-micron nylon netting and a 20-cm air diffuser as described above, except that no plastic beads were used (Fig. 2). All water exchange took place behind the divider. The central filtration consisted of a foam prefilter, a 100-cubic-centimeter biological filter with plastic bio-ball media, a 60-watt UV sterilizer, and a protein skimmer. The biological filter, UV sterilizer, and rearing tanks were arranged in sequence, and powered by a 113-L-per-minute external pump. A 38-L-per-minute submersible pump within the 150-liter sump ran the protein skimmer. The system is capable of housing two broods simultaneously with two additional birthing tanks from which fry were removed and counted. Water-quality parameters were maintained as follows: pH: 8.0-8.3, $NH4^{1+}$: 0.0 ppm, $NO2^{1-}$: 0.0 ppm, $NO3^{1-}$: 0-20 ppm, Salinity: 25 ppt, Temperature 23-28°C.

Pregnant males were removed from the spawning tank and placed in a birthing tank within the rearing system until birth/hatching occurred. Only broods containing at least 600 individuals were used. One hundred individuals from a brood were placed in each of six aquaria and reared for 28 days, the approximate age at which weaning onto frozen diets is possible. One tank was fed only *Artemia* from day 1 to day 28 (treatment 1). Each of the other five tanks was fed CDWP for 1-5 days (treatments 2-6, respectively) before being switched to a diet of *Artemia* until day 28. This small range of CDWP-feeding duration was chosen based on the zero survivorship achieved in the first preliminary trial (0 days on CDWP), compared to the high survivorship achieved in the second and third (14 days and 3 days on CDWP, respectively).

Plankton was collected by hanging a 60-micron plankton net in a tidal current under one of several local bridges. After acclimating to room temperature, the plankton was strained through a 500-micron sieve to remove large plankters and debris. Cysts of *Artemia franciscanis* were decapsulated, hatched, and enriched with Ratio HUFA. Food organisms were maintained at 0.5/ml in all rearing tanks.

Isochrysis galbana was cultured in standard seawater medium according to the protocol outlined by Hoff and Snell (1999). For the first 5 days of each replicate *Isochrysis* was maintained at a density of approximately $2x10^5$ cells/ml in each rearing tank. The reasons for using microalgae were to maintain high water quality until the juvenile seahorses were old enough to resist the current generated by central filtration, and to serve as a nutritious food source for the zooplankton. *Isochrysis galbana* was chosen for its high HUFA content and the relative ease with which it can be cultured.

During the first 5 days, the fluorescent lights were kept on for 24 hours/day to prevent fluctuations in pH and dissolved oxygen that would be caused by intermittent photosynthesis on a regular photoperiod with such a high density of phytoplankton present. Beginning on day 6, all rearing tanks were connected to central filtration, lights were switched to a 16-hour light photoperiod, and *Isochrysis* was no longer added.

During the 28-day experimental period, a random sample of five juvenile seahorses was removed weekly so that the length of each seahorse could be measured. To minimize stress associated with taking these measurements, live seahorses and a small ruler were placed in a shallow petri dish with 20 ml of seawater, and photographed. Using Scion Image software, each photo was calibrated to the ruler and seahorse length was measured in centimeters by drawing a digital line from the coronet (head crest) to the tip of the tail.

Length data were plotted against time to determine the rate of growth in centimeters per day. Growth rates were calculated for each treatment using the least-squares regression method and compared between treatments using a repeated measures ANOVA in SPSS. The Student-Newman-Keuls test was used to compare growth rates between broods.

After 28 days all juveniles were removed and counted. Average survival was determined for each feeding regime and for each brood. Survivorship data did not meet the assumptions of standard parametric statistical tests, therefore nonparametric tests were employed. A Friedman test was performed to determine whether or not there were significant differences in survivorship. The Student-Newman-Keuls (SNK) test was adapted according to Glantz (2002) to allow for nonparametric data and was used to compare survivorship between the treatment groups and between broods.

Results

The use of copepods in the early diet of *Hippocampus erectus* appears to have a positive effect on survivorship (Fig. 3), but no effect on growth (Fig. 4). There was a significant difference in survivorship among treatment groups (Friedman test, $X^2_5 = 20.607$, $p = 0.001$) and among broods (Friedman test, $X^2_4 = 18.972$, $p = 0.001$). Seahorses offered copepods for at least two days (treatments 3-6) had a significantly greater survivorship (SNK test, $p < 0.05$) than those offered copepods for 0 days or one day (treatments 1 and 2). Survivorship in replicate 1 was significantly higher (SNK test, $p < 0.05$) than in replicates 2-5 (Fig. 5).

Treatments 1 and 2 were not included in growth analyses because survivorship was too low to provide meaningful averages. Seahorses in this experiment had a growth rate of approximately 0.07 cm/day (SD = 0.0027, $r^2 = 0.89$ to 0.999, n = 4). No differences in growth rate were observed between treatments 3, 4, 5, and 6 (repeated measures ANOVA, $F_{3, 12} = 0.349$, $p = 0.791$) (Fig. 4); however there were significant differences in growth rate between broods (repeated measures ANOVA, $F_{3, 12} = 9.338$, $p = 0.001$) (Figs. 6 and 7).

Discussion

This investigation evaluated the effect of copepod feeding duration on survivorship and growth of *H. erectus* after 28 days of rearing. The data clearly demonstrate that the use of copepods as a first food for *H. erectus*, even for only a few days, can have a significant impact on survival, but not necessarily on rate of growth. Juvenile *H. erectus* offered CDWP for at least the first two days demonstrated significantly greater survivorship than those fed solely on enriched *Artemia* nauplii.

Survivorship

Treatment effect

Although the SNK test indicates two statistically distinct groups based on survivorship (those fed on copepods for ≤ 1 day, and those fed on copepods for ≥ 3 days), Figure 3 illustrates that, in general, survivorship increased with increased copepod feeding duration, at least until treatment 5 (4 days on copepods). In all but one replicate, survivorship appears to level off or decrease between treatments 5 and 6. A greater number of treatments would help to determine whether or not maximum survivorship, in fact, occurs at around 4 days of copepod feeding. It is possible that the apparent asymptote reached is incidental and that survivorship would continue to increase with increased copepod-feeding duration beyond 5 days. Additionally, repeating the experiment more than five times may strengthen the trend illustrated in Figure 4 and show significant differences between more treatments.

A correlation between survivorship and copepod feeding duration is not surprising considering the overwhelming evidence for the superiority of copepods as a first food for larval and juvenile marine fishes. Payne and Rippingale (2000) achieved significantly higher survivorship in seahorses (*Hippocampus subelongatus*) reared on copepods, as compared to those reared on *Artemia* enriched with Super Selco™. Kraul et al. (1993) conducted an experiment in which larval mahimahi, *Coryphaena hippurus*, were reared on *Artemia*, enriched with products containing a variety of HUFA ratios or cultured copepods, then were subjected to a physical stress. Copepod-fed larvae showed significantly lower stress mortality than any of the *Artemia*-fed groups.

Shields et al. (1999) raised two groups of halibut larvae, *Hippoglossus hippoglossus,* on two different feeding regimens to determine dietary effects on survival and various health indices. Both groups were fed newly-hatched *Artemia* from day 1 to day 9 post-first feeding (PFF). Beginning on day 10, one group was switched to *Artemia* enriched with reconstituted, spray-dried *Schizochytrium* sp. (a golden marine alga, sold commercially as Algamac 2000) and SuperSelco™. The other group was switched to the marine copepod, *Eurytemora velox*. The larvae were reared until day 71 PFF on their respective feeding regimens. Larvae fed on copepods had a significantly greater mean survival rate than *Artemia*-fed larvae. Additionally, the percentage of larvae undergoing complete, successful metamorphosis was significantly greater in copepod-fed larvae than in the *Artemia*-fed group.

While working to commercialize production of the Pseudochromidae, Gardner (2000) reared *Pseudochromis flavivertex*, *P. fridmani,* and *P. aldabrensis* in tanks with and without incidental blooms of the calanoid copepod, *Acartia* sp. Significantly greater larval survivorship was achieved in rearing tanks with copepods, than in tanks wherein only rotifers and *Artemia* were present.

The brood effect

The most surprising outcome of this investigation was the significant brood effect on survivorship. I decided to use a different set of parents for each replicate so that genetic factors could be ruled out as having been responsible for any trends resulting from the treatment effect. Although survivorship trends appear similar between broods, overall survivorship for the first brood was significantly greater than for all others. It is possible that this disparity is merely the result of a variation in genetic factors between parents affecting the health and survivorship of their respective offspring; however it is also possible that other health or fecundity factors may have come into play.

The wild broodstock was collected in late summer and the investigation was carried out between September and May. This temporal element of the experiment may have impacted the results in several ways. It has been demonstrated that *H. erectus* in the Chesapeake Bay are reproductively active from May to October (Teixeira and Musick 2001) and it is likely that *H. erectus* in New York have a similar (possibly shorter) breeding season. In the present study, the first two replicates began in September, followed by a 5-month period of reproductive inactivity. Replicates 3-5 commenced in March and April and these spawning events may have been induced prematurely by the unnatural conditions of temperature and photoperiod in the broodstock systems intended to approximate the summer breeding season. The abbreviated non-breeding season and absence of winter conditions may have interfered with season-dependent biological processes that regulate gamete activity, thereby compromising spawn quality and subsequently, juvenile health.

The sharp decline in survivorship between the first replicate and all the others may have resulted from a prolonged period in captive conditions. Water quality, diet, stocking density, exposure to pathogens and handling stress have all been associated with spawn quality (Bromage 1995). Stresses associated with confinement and handling have been shown to cause a reduction in sexual steroid levels (Sumpter et al. 1987), delayed ovulation, and reduced egg and sperm quality (Campbell et al. 1992) in male and female trout. Following a period of very high mortalities in the early larval stages of hatchery-reared gobies, Gardner (unpub. data) experienced a significant increase in survivorship after medicating the broodstock for a suspected infestation of internal protozoan parasites. The adult seahorses for this investigation were maintained at unnaturally high densities that may have enabled the proliferation of pathogens or exacerbated other captivity-related stresses, contributing to the degradation of spawn quality.

It is well established that the nutritional state of captive broodstock fishes can affect spawn quality. Watanabe and Kiron (1995) identified astaxanthin, vitamin E, and phospholipids containing DHA and EPA in the broodstock diet of red sea bream as being the most important determinants of egg quality. Furuita et al. (2000, 2002) found that levels of *n*-3 HUFA (EPA and DHA) and arachidonic acid in the broodstock diet of the Japanese flounder, *Paralichthys olivaceus*, can have a significant impact on spawn size, spawn quality, larval development, and larval survivorship. It is likely that there is some variation between species in the ideal levels and ratios of HUFAs and other dietary components for optimum reproductive success; however, as of this writing, these requirements have not been determined for seahorses.

The most commonly-used adult feeds for seahorses in research, public aquaria, and the aquarium hobby are adult *Artemia* sp. (live or frozen) and frozen mysid shrimps, usually the freshwater *Mysis relicta*. This is due largely to their widespread availability and seahorses' willingness to accept them. The obvious problem with using either of these crustaceans as a staple diet for seahorses is that they are not marine species and being isolated from ocean food webs, their respective nutritional profiles probably do not meet the needs of many marine fishes. Whereas marine fishes require a high (> 1.0) DHA-to-EPA ratio in their diets (Kraul et al. 1991, 1993, Sargent et al. 1999), *Mysis relicta* and unenriched *Artemia spp*. have approximate DHA-to-EPA ratios of 0.4 (CANTEST 1999) and 0.1 (Naess et al. 1995), respectively.

This problem was addressed in the present study by feeding two marine species, squid and the marine shrimp *Palaemonetes pugio* to the broodstock, in addition to frozen *Mysis relicta*. Whereas this may have been an improvement over conventional captive seahorse diets, Teixeira and Musick (2001) found that adult *H. erectus* in the wild feed almost exclusively on amphipods. The high survivorship achieved in brood 1, which began within a few weeks of the collection of the broodstock, may be related to superior gamete quality resulting from nutritional reserves carried over from the wild. Similarly, the prolonged period on an inferior diet may have contributed to the decline in survivorship in subsequent broods. Reyes-Bustamente and Ortega-Salas (1999) and Wong and Benzie (2003) achieved very high survivorship in rearing experiments with *H. ingens* and *H. whitei*, respectively, however, in both investigations, juveniles were obtained from males that were collected, already pregnant, from the wild.

Growth

Because growth and survivorship are both commonly associated with overall health, one might expect a correlation between survivorship and growth, however no such relationship was observed in this experiment. No difference in growth was observed between treatment groups, however there were significant differences in growth rate between broods. It should be mentioned that growth was measured in terms of length alone, which may be a poor indicator of health. Although growth is often used as an index of health or fitness in studies on the biology (Grant and Brown 1999, Anderson and Neuman 2002) and aquaculture (Payne and Rippingale 2000, Copeman et al. 2002) of fishes, the convention is to measure growth and fitness in terms of weight, dry weight, or one of several condition indices such as lipid analysis (Shields et al. 1999) or condition factor (Anderson and Neuman 2002). Condition factor is a measure of the nutritional state or "well-being" of a fish and is calculated as $K = \text{weight}/(\text{Length})^3$ (Busacker et al. 1990). Perhaps one of these measures would have offered a more accurate picture of growth and/or fitness; however each of them was ultimately rejected for use in this investigation in favor of a technique that would not require killing the seahorses. Nevertheless, using more commonly-used measurement techniques, other investigators have found no relationship between growth and survivorship in similar nutrition studies.

For example, Kraul et al. (1993) found no difference in growth, measured in terms of wet weight, of larval mahimahi, *Coryphaena hippurus*, reared on a variety of feeding regimens, despite significant differences in stress mortalities between the treatment groups. Using wet weight and dry weight as measures, Shields et al. (1999) found no difference in growth rate of halibut larvae, *Hippoglossus hippoglossus*, reared on copepods versus those reared on *Artemia*, despite significant differences in survivorship and several health indices. Payne and Rippingale (2000) observed a significantly greater growth rate (in terms of length and weight) and survivorship in *Hippocampus subelongatus*, reared on copepods versus those reared on *Artemia*, however in their investigation copepods were offered for the duration of the experiment (15 days) and at a higher density than *Artemia* to correct for a difference in dry weight between the two feeds. In the present investigation food densities were equivalent in all tanks and diets were identical for 23 days of the 28-day experimental period.

Costs of copepod use

Approximately 3 hours/day were required to collect and process copepods, however considerably more effort may be required in a tropical climate where plankton abundance is typically lower, or in areas lacking the strong tidal currents characteristic of coastal embayments that made it possible to collect plankton for this experiment without the use of a boat. Natural fluctuations in copepod populations also can influence catch per unit effort and, consequently, cost. In addition, supplying wild-caught copepods to an inland aquaculture operation would be considerably more expensive and logistically complex; nevertheless, if only a 2-day supply is required to achieve a significantly greater yield, it may be cost-effective to do so.

Contamination is another factor that must be considered when using wild plankton in aquaculture. A number of pests were introduced to rearing tanks that received CDWP in this experiment. These included barnacles, hydroids, amphipods, gastrotrichs, and polychaetes.

The polychaetes were least abundant and appeared to be harmless. Gastrotrichs were observed crawling on the skin of seahorses. Although they were probably not parasitic, as most gastrotrichs are free-living, the affected seahorses were often observed scratching, suggesting physical irritation. When barnacles, hydroids, and amphipods reached high numbers, they offered strong competition for food. Hydroids were the most problematic of the contaminants. In addition to competing for food, they also are capable of stinging young seahorses. Hydroid stings are suspected of playing a role in the initiation of cutaneous *Vibrio* infections on seahorse tails in preliminary trials. Additionally, hydroids were the most difficult contaminant to remove. Whereas most of the other organisms were eliminated by simple mechanical means, such as siphoning or scraping, these methods appeared to stimulate reproduction by fragmentation when applied to hydroids. No known parasites or other disease organisms were associated with the use of wild plankton in this experiment, however this is a risk that must be considered.

Due to its relative availability, low cost, and high protein content, *Artemia* is probably the most widely-used live food in aquaculture. However, when used as an exclusive food source for juvenile *H. erectus*, lower survival can be expected than when used as a second food, following several days on copepods.

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Acknowledgments

I thank Dr. Eugene Kaplan for inspiring me with his undying enthusiasm, encouragement and also for financial support, Dr. Robert Johnson, Donald Axinn, and the Axinn Fellowship committee for financial support, Jill Olin, Donna McLaughlin, and John Maniscalco for their assistance in maintaining my seahorses and plankton cultures and for their technical support, Forrest Young for sharing his technical expertise, Tom Frakes and Aquarium Systems for their generous donation of synthetic sea salt, Penn Plax, Inc. for their generous donation of tank dividers, my family for their life-long support of my passion for marine biology, and of course, my committee: Dr. John Morrissey, Dr. Peter Daniel and Dr. Christopher Sanford for their patience, expertise and moral support, without which, this project would have been impossible.

Figure 1. Schematic diagram of rearing system (front view). (A) sump, (B) biological filter, (C) protein skimmer, (D) protein skimmer pump, (E) recirculating pump, (F) ultraviolet sterilizer, (G) return line, (H) PVC ball valve, (I) rearing tank, (J) drain line, (K) bypass valve, (L) bypass line, (M) drain/bypass convergence. Arrows indicate direction of water flow.

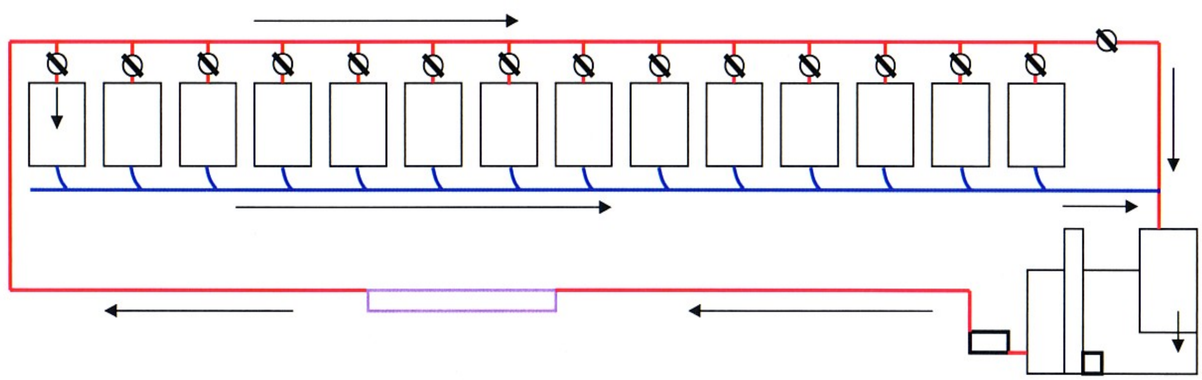


Figure 2. Schematic diagram of rearing tank (side view). (A) return line, (B) divider (500-micron netting), (C) air diffuser, (D) drain line. Arrows indicate direction of water flow.

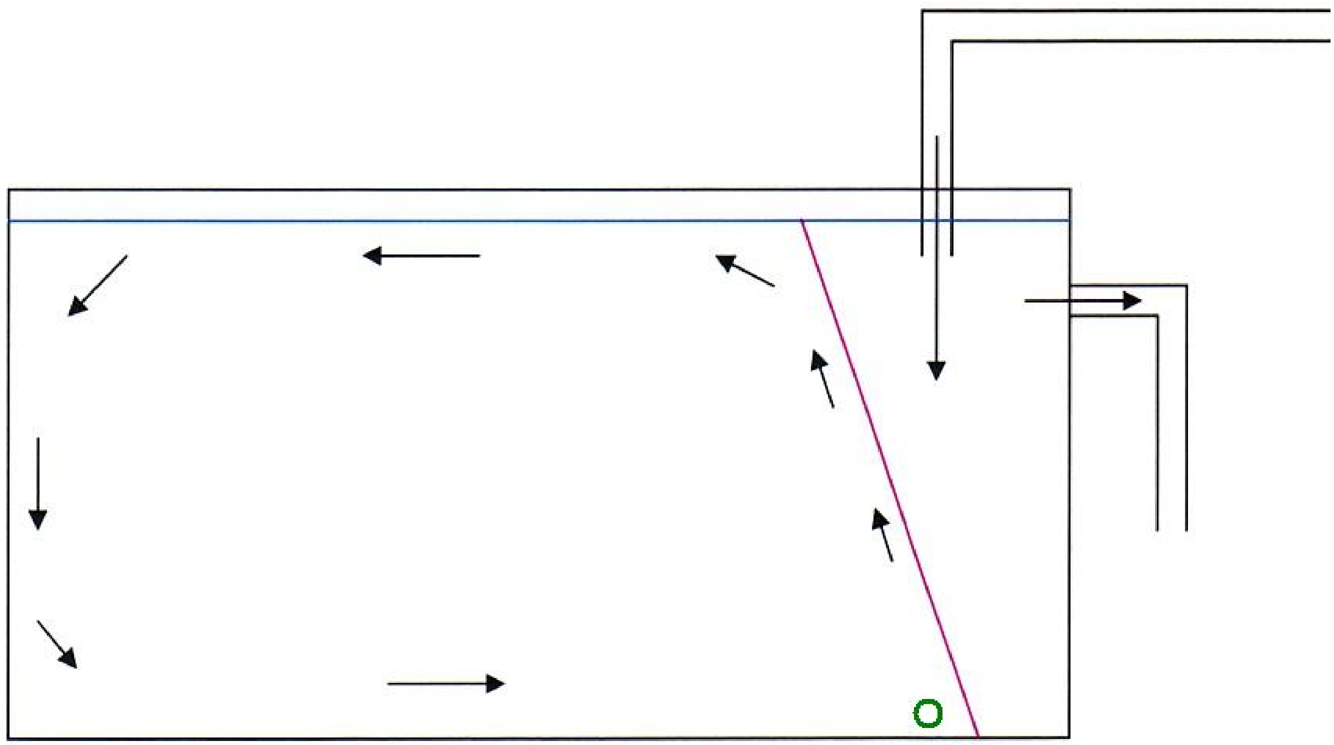


Figure 3. Bar Chart of survivorship plotted against copepod-feeding duration (treatment).

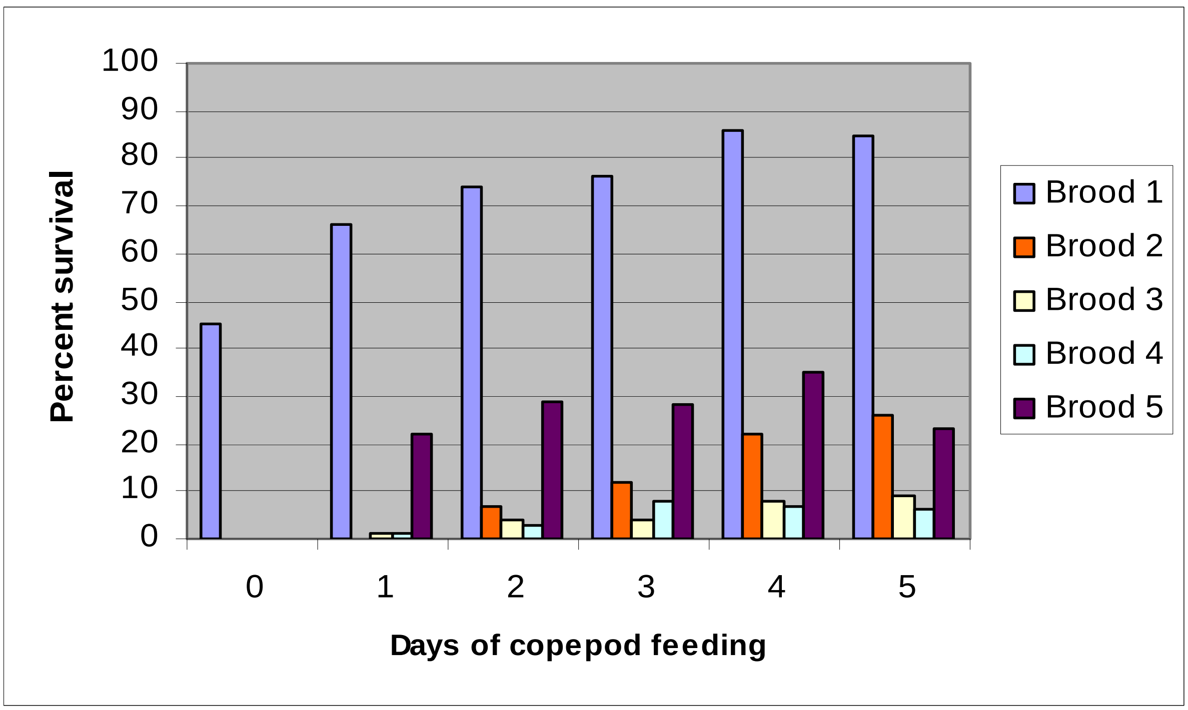
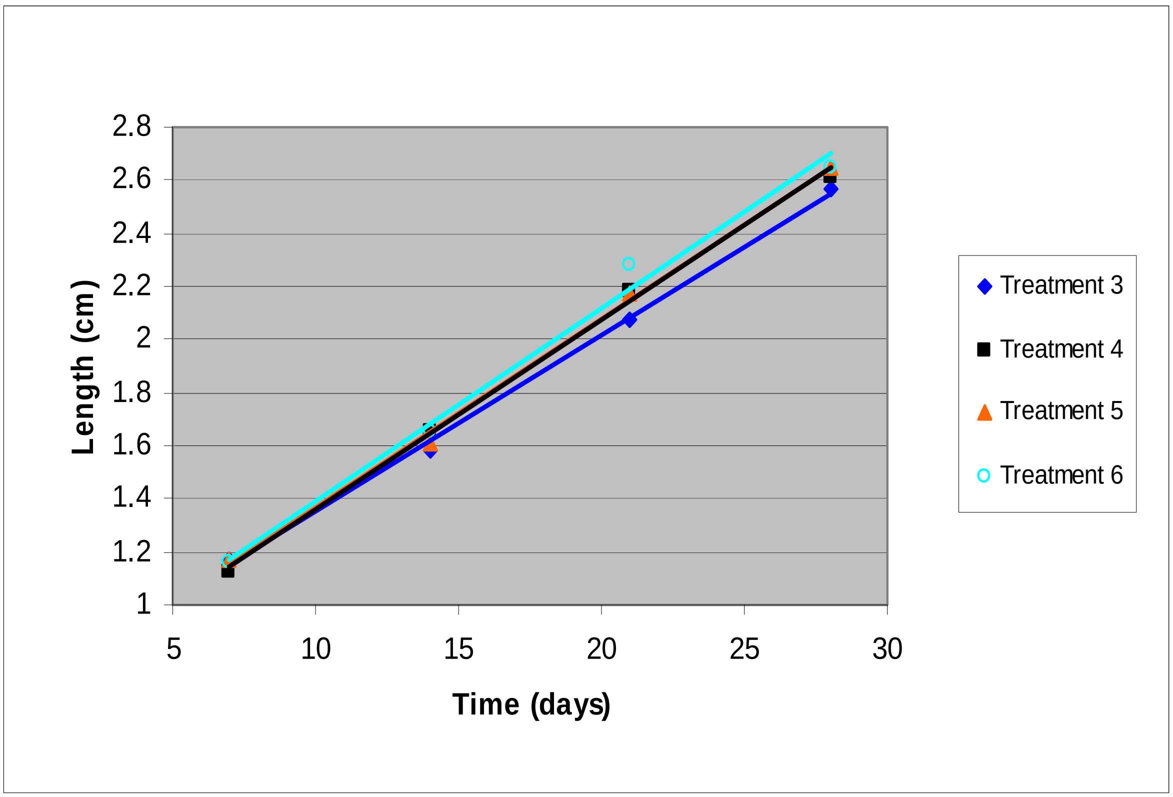

Percent survival
100
90
80
70
60
50
40
30
20
10
0
0
1
2
3
4
5
Days of copepod feeding
Brood 1
Brood 2
Brood 3
Brood 4
Brood 5

Figure 4. Growth curves by treatment (least squares regression) for treatments 3-6. Treatments 1 and 2 were not included in growth analyses because survivorship was too low to provide meaningful averages.



2.8
2.6
2.4
2.2
2
1.8
1.6
1.4
1.2
1
5
10
15
20
25
30
Length (cm)
Time (days)
Treatment 3
Treatment 4
Treatment 5
Treatment 6

Figure 5. Bar chart of mean survivorship, based on 6 treatments, plotted against brood number. Broods are coded with date (MDDYY format), followed by brood (B) number. Error bars indicate standard deviation.

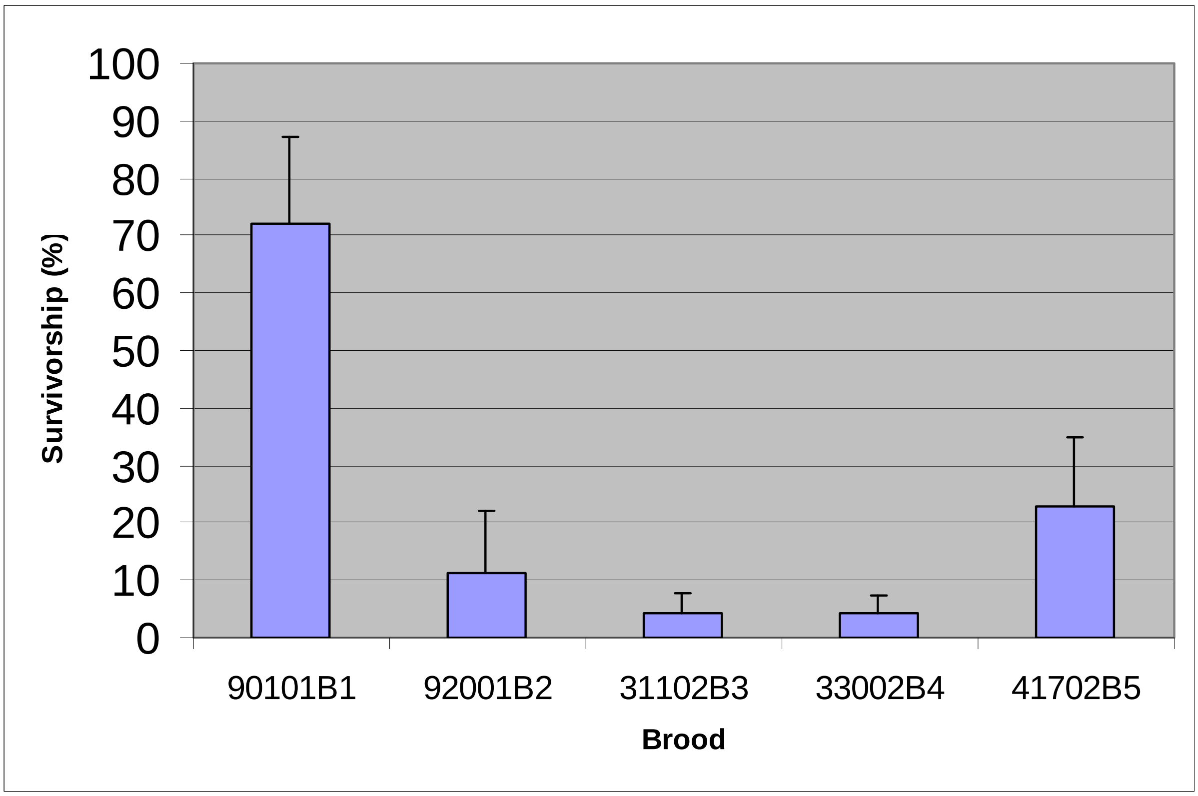
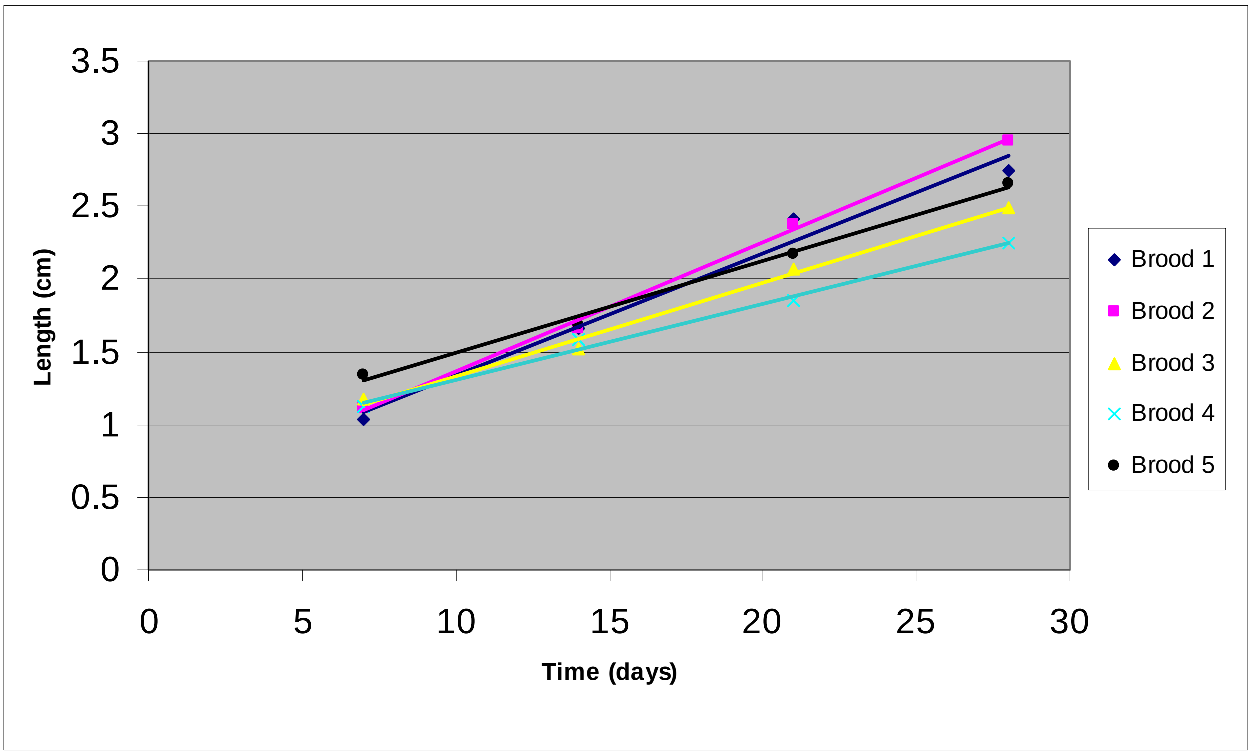
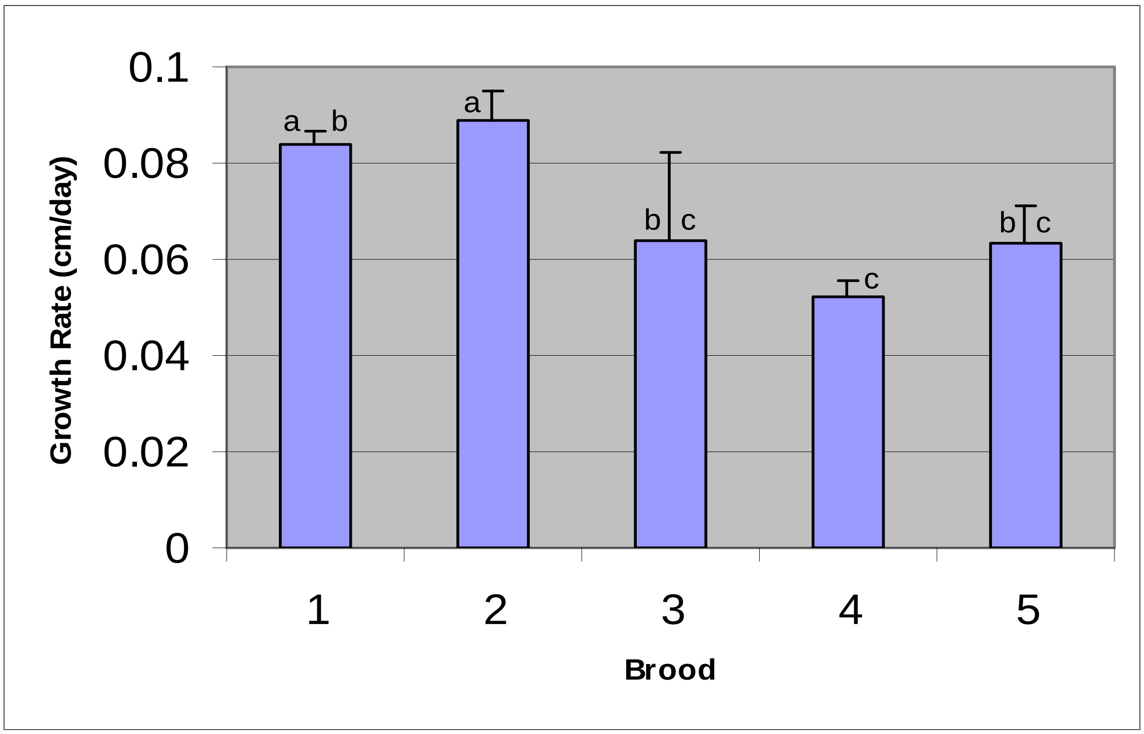

100
90
80
70
60
50
40
30
20
10
0
Survivorship (%)
90101B1
92001B2
31102B3
33002B4
41702B5
Brood

Figure 6. Growth curves by brood (least squares regression) for treatments 3-6. Treatments 1 and 2 were not included in growth analyses because survivorship was too low to provide meaningful averages.



3.5
3
2.5
2
1.5
1
0.5
0
Length (cm)
0
5
10
15
20
25
30
Time (days)
Brood 1
Brood 2
Brood 3
Brood 4
Brood 5

Figure 7. Bar chart of mean growth rate for each replicate (brood). SNK post-hoc test for significance groupings denoted by the letters a, b, and c. Error bars indicate standard deviation.


0.1
0.08
0.06
0.04
0.02
0
Growth Rate (cm/day)
a b
a
b c
c
b c
1
2
3
4
5
Brood