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Advancing U.S. Seafood Production: Developing Hatchery Technology for Florida Stone Crab (Menippe mercenaria)

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ADVANCING U.S. SEAFOOD PRODUCTION: DEVELOPING HATCHERY
TECHNOLOGY FOR FLORIDA STONE CRAB (*MENIPPE MERCENARIA*)

By

Craig W. Purcell

A THESIS

Submitted to the Faculty
of the University of Miami
in partial fulfillment of the requirements for
the degree of Masters of Science

Coral Gables, Florida

December 2016

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(December 2016)

Advancing U.S. Seafood Production:
Developing Hatchery Technology for
Florida Stone Crab (*Menippe Mercenaria*).

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These experiments will be conducted in order to refine and implement reliable techniques for larval rearing of Florida stone crab (*Menippe mercenaria*). The aim is to secure the technology for production of large quantities of stone crab larvae for transfer to nursery growout environments to support domestic aquaculture production. Establishing a successful larval rearing protocol that maximizes total survivability and growth is essential for all aquacultures species. This experiment sought to define optimal larval rearing parameters from the first zoeal stage through the megalope stage (settlement). Four treatments, which included instar 1 *Artemia* (T1), decapsulated instar 1 *Artemia* (T2), enriched instar II *Artemia* (T3), and enriched rotifers (T3 and T4) were utilized to establish a proper feeding regime that maximizes larval growth and survival. Data revealed that survival through the zoea 3 stage of development (day 7) was higher when using enriched rotifers or decapsulated *Artemia*, and were not statistically different from each other. It was also shown that the fastest time (11 days) to reach megalope stage, as well as the highest survival of megalops (1122.50 ± 24.85) was obtained when crab larvae were fed decapsulated *Artemia* (T2) from zoea 1 through the megalope stage. Crab

larvae fed only instar I *Artemia* throughout the rearing experiment suffered the highest levels of mortality through the megalope stage. T4 which was fed only enriched rotifers, reached the zoea 5 stage, but never molted to the final megalope stage after 22 days. This trial showed that while enriched rotifers alone were an acceptable food prey during early stages of development, encapsulated *Artemia*'s small size increased nutritional profile was ideal for maximizing growth and survival.

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Chapter 1.0 Background

With human populations continuing to rise, industrialized fishing is no longer able to meet the growing global demand for seafood. Aquaculture production is increasingly seen as the only realistic alternative. The move towards aquaculture has been termed the “blue revolution” (Economist, 2003), and countries that are investing in aquaculture research and development are realizing enormous technical, social, and economic benefits. World aquaculture production attained another all-time high in 2014, at 73.8 million tons, with an estimated total value of US\$160.2 billion (FAO 2016). These staggering numbers have led the industry to surpass beef production. Although, some aquaculture practices have been criticized in the media for degrading marine environments, poorly utilizing resources such as fish meal and fish oil, and spreading and proliferating diseases and parasites (Naylor et al., 2000), these concerns are becoming less relevant as aquaculturists improve their protocols, and are also greatly outweighed by the potential benefit of filling the growing demand for high quality protein. By examining trends in human population growth and capture fisheries it becomes obvious that continued growth of aquaculture is essential and can compliment fisheries as an added value element of that industry.

The global human population has been growing at an increasingly fast rate in the past

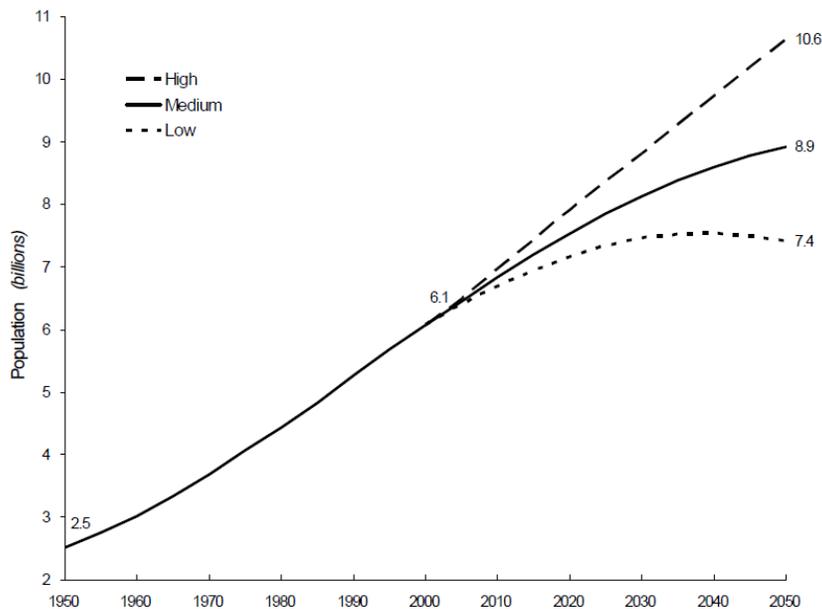


Figure 1: Global human population from 1950 to 2004 with low, medium and high fertility projections up to 2050 (ESA 2004)

century increasing from 2.5 billion people in 1960 to 6.8 billion in 2010 (Lester, 2011; ESA, 2004). Although a plateau is expected, this population growth will continue for at least a few decades leveling out to between 7 and 10 billion people (Figure 1; ESA 2004). The world will need to produce a huge amount of additional protein rich food to feed a healthy population of this size. This food will come from several sources. Poultry and pork production per capita has increased fairly steadily from 2.9 kg per person per year in 1960 to 13.4 kg per person per year in 2009 for poultry and 8 to 15.5 kg per person per year for pork over the same span (Lester, 2011). Beef production per person, however, reached a maximum of 11.1 kg per person per year in 1976 and has declined since to only 9kg (Lester, 2011). Aquaculture has shown the largest percent increase since 1960 rising from only half a kg per person per year to 7.7kg (Lester, 2011). Although all these sources of protein will be required, aquaculture has shown the greatest growth and has the most potential for future growth as it is the least restricted by three major limiting factors; land area, water resources and feed ingredients. All three factors are related as the

bulk of land and water requirements are those used to produce ingredients for feeds. In order to meet this growing demand of seafood consumption, it is important to study animals that present themselves as viable aquaculture species. One such species is *Menippe mercenaria*, commonly known as the Florida stone crab.

The stone crab fishery includes Florida stone crab (*Menippe mercenaria*) and Gulf stone crab (*Menippe adina*). Together they generate significant revenue for Florida and serve as a source of cultural pride. The two species are found from Cape Lookout, North Carolina, southward and throughout the Gulf of Mexico to Yucatan, Mexico; Bahamas; Cuba; and Jamaica (Williams, 1965; Bert et al., 1978). Florida and gulf stone crabs are closely related species, readily interbreed, and are managed as one species by the Fish and Wildlife Committee (FWC) and the Gulf of Mexico Fishery Management Council). FWC estimate landings during the 2013-2014 stone crab season totaled almost two million pounds with a dockside value of around 25 million dollars (1,931,434 pounds and \$25,304,722 respectively). This fishery consistently ranks as one of Florida's most valuable, with a direct economic impact of roughly \$25 million dollars per year for the last fifteen years (See Figure 2). At a wholesale price of \$9 to \$30 per pound (depending on claws size), stone crab (*Menippe mercenaria*) offers great potential for commercial aquaculture.

1.1 Problem Addressed

Recent advances in hatchery and growout technology have led to the rapid development of the marine fish and shellfish aquaculture industry around the world. However, the U.S. has been slow to embrace this industry and is now lagging behind in seafood production. The U.S. currently imports over 90% of the seafood we consume, creating a \$10.4 billion

annual seafood trade deficit and incurring a foreign dependence comparable to that of oil products (NOAA, 2011). In the Gulf of Mexico and southeastern U.S., the high demand for Florida stone crab claws in the marketplace has brought the species under tremendous fishing pressure. Despite being federally managed for more than three decades, the fishery continues to experience overfishing. (FWRI, 2014). This is evident by increased fishing effort without the corresponding increase in landings over the past two decades. Additionally, the total landings and catch per unit effort have been declining since 2008 (FWRI, 2014). The 2013-2014 season witnessed a 31% decrease in landings (Figure 2) compared to both the previous five-year and thirty-year average (FWRI, 2014). At the same time, the retail price per pound and the total value of the annual catch are at record highs.

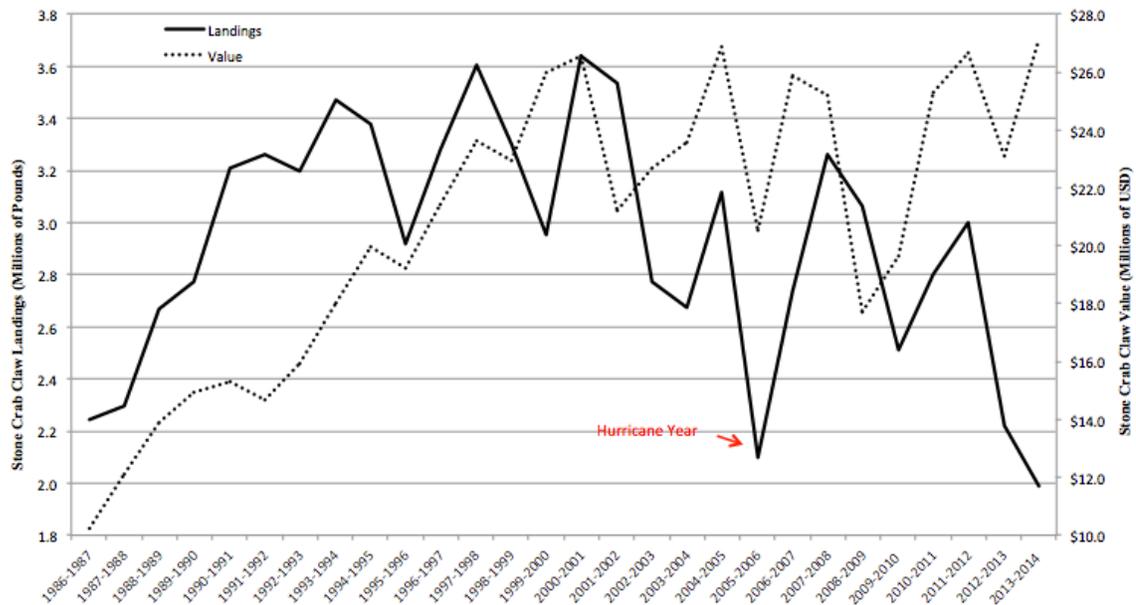


Figure 2: Stone crab landings and value per pound from 1986-2014 (FWRI, 2014).

Currently, aquaculture technology for stone crab is underdeveloped relative to other high value crustaceans around the world. The limited amount of published literature on this species focuses on life history and biological processes (Cheung, 1968; 1969; Ong and Costlow, 1970; Savage, 1971; Mootz and Epifiano, 1974; McConaughy et al., 1980; Lindberg and Marshall, 1984; Simonson, 1985; Gerhart and Bert, 2008; Krimsky et al., 2009). The technological protocols for producing commercial quantities of viable stone crabs in an inexpensive manner have yet to be developed and published. Much of the work exploring the culture of *Menippe spp.* was conducted in the 1970s and early eighties. The methodology employed during that time is rudimentary compared to modern aquaculture techniques. Therefore, the probability of successfully developing a scalable model for the aquaculture of stone crab is greater than ever before.

Early studies dating back to the 1950's provide vital information on the larval development of *M. mercenaria*. Hugh J. Porter's (1960) study gives detailed descriptions and illustrations of all the zoeal stages of the stone crab. This paper intricately describes and explains the changes in anatomy of each of the five zoeal stages (and rarely observed 6th stage), and explains that there is only one molt from one stage to the next. This study was the first to describe fully the early life stages of stone crabs. These illustrations and descriptions were extremely useful for this study in helping to identify the differences between each stage, thus giving an accurate measurement of growth and time in each stage. Furthermore, the study concluded that the highest survivability through zoea 5, was obtained with live newly hatched nauplii of *Artemia*, essentially laying the groundwork for further investigation into live feeds as a viable food source.

There have been multiple studies examining the effects of temperature and salinity on survival and developmental rates of several species of Brachyura (Costlow and Bookhout (1962, 1971), and Costlow, Bookhout and Monroe (1960,1962, 1966). In 1970, Ong et al. conducted larval rearing experiments with *M. mercenaria*, raising larvae at various salinities and temperatures. Salinity of 30 ppt and temperature of 30°C resulted in the highest survivability for stone crab larvae, as well as a faster rate of larval development. High survivability (60-72%) at these parameters as well as fast growth to the megalope and settlement stages, clearly suggests stone crab as a viable species for commercial production and needs to be more thoroughly investigated. Similarly, Brown et al. (1992) examined the impact of salinity and temperature on the development of stone crab larvae and juveniles. Stone crab larvae and juveniles were reared in captivity and their responses to varying temperature and salinity levels were recorded. Observation of the larval and juveniles' development showed considerable tolerance of fair levels of salinity and temperature, with optimal growth and survival at 30-35 ppt salinity, and 30°C. This study was performed at very low densities (non-commercial stocking densities), with no varied feed regimes, but has given valuable information on ideal environmental parameters for stone crab larval rearing.

A number of previous studies have investigated the application of aquaculture to other crustacean species at the commercial level. Efforts to produce mass quantities of high value marine crustaceans, particularly the swimming crab (*Portunus pelagicus*), and blue crab (*Callinectes sapidus*), have been extremely successful (Takeuchi, 2000; Zmora et al., 2005; Zohar et al., 2008). Dwindling swimming crab populations in Japan led to the development of major crab hatchery programs that boasted production of approximately

50 million juveniles per year (Takeuchi, 2000). The Zohar Lab in the Department of Marine Biotechnology (DMB) at the Institute of Marine and Environmental Technology (IMET), University of Maryland, Baltimore County (UMBC) has developed a technology to mass produce blue crabs (*Callinectes sapidus*) in captivity. This technology was the foundation for subsequent work to develop a hatchery-based stock enhancement strategy for *C. sapidus* in the Chesapeake Bay, as well as for related programs to study crab reproduction, growth and development, endocrinology, molting and disease. Ruscoe et al. (2004) established a commercially viable larval rearing protocol for mud crabs (*Scylla serrate*) using various treatments consisting of different combinations of enriched rotifers and *Artemia*, with megalope survival reaching as high as 83%.

Other studies conducted on crustacean larvae emphasize the importance of enriched rotifers for successful larval development. In recent years, enrichment of live feeds to increase the nutritional efficiency has significantly contributed to larval survival and quality (Sorgeloos and Leger, 1992). However, studies on this aspect of crab larval rearing are very limited (Levin and Sulkin, 1984). Hamasaki et al. (1998) suggested that in mass seed production of the swimming crab, *Portunus trituberculatus* the enrichment of rotifers should be a routinely carried out to improve the quality of the rotifers and to deliver crucial early developmental n-3 unsaturated fatty acids (n-3HUFA). It has been well documented that n-3HUFA are essential for the growth and survival of marine crustaceans (Watanabe et al., 1978; Rainuzzo et al., 1989; Hamasaki et al., 1998). As the larvae develop and molt to subsequent stages, their increased metabolic activity results in an increase in energy requirements. Frank et al. (1975) attributes the high mortality of mud crab (*Rhithropanopeus harrisi*) between stages III and IV to the failure of “normal

diets” such as enriched rotifers to meet this increased energy demand. Thus, it is imperative that other diets such as decapsulated *Artemia* (instar I) nauplii and enriched *Artemia* (instar II) nauplii be included in this study.

Enrichment diets for *Artemia* can be specifically formulated, as was done in the study by Kannupandi et al. (2003). For this study, accelerated growth and increased survival was observed due to the availability of n-3 long chain polyunsaturated fatty acids (PUFA) that are present in the enriched *Artemia* diet through cuttlefish liver oil. The study also concluded that PUFA’s are most essential for better growth and survival of the brachyuran crab, *Callinectes lucifera*. Although enriched *Artemia* has many advantages, some of the enrichment products can be difficult for larvae to digest, which may increase mortality (Kannupandi et al., 2003). For this reason, decapsulated *Artemia* must also be investigated as a viable food option.

Decapsulated cysts contain 30%-40% more energy as compared to freshly hatched nauplii, and their volume is 30% smaller, meaning that the nauplii can be offered to crustacean larvae at an earlier life stage (Vanhaecke et al., 1983). This increased nutrition is contributed to the process of decapsulation itself, in which the chorion of the *Artemia* cyst is burned off completely. This allows the embryo inside to expend less of its highly nutritional yolk-sac when “bursting” from the cyst, which in turn is consumed by the larvae. Furthermore, the decapsulation process disinfects the cysts, removing bacteria that can hide in the porous chorion.

Suprayudi et al. (2002), noted that increased survival of larval mud crab (*Scylla serrate*), was best achieved when a combination of rotifers and *Artemia* were used. The first experiment of two consisted of 10 treatments in in which initial rotifer feedings were

shifted to *Artemia* nauplii at the zoea 1, 2, 3, 4, and 5 stages. The other 5 treatments were similar to the first 5, except rotifers and *Artemia* were continuously added together at each stage (co-fed). Survival was maximized when *Artemia* was added at the 3rd stage of development. For this reason, it is important to test a treatment that utilizes a combination of both *Artemia* and rotifers.

The experiments in this study will focus on the nutritional demands of zoeal stone crab, from zoea 1 to the megalope stage. Additionally the study will develop improved techniques for increased larval survivability and growth. The techniques will be based on the innovative and highly successful intensive methods developed by the Institute of Marine and Environmental Technology (IMET), in combination with methods developed by UM for use with the larviculture of other marine species, (Benetti, 1997; Benetti et al., 2002; Watanabe et al., 2005; Benetti et al., 2008a). Developing intensive hatchery protocols for *Menippe mercenaria* is the first, critical step in ushering the species into commercial production.

Chapter 2.0 Methods and Materials

2.1 Broodstock

According to Cheung (1969), molting is determined by a combination of light intensity and temperature. Immediately after reaching sexual maturity both light intensity and temperature in nature initiate ovary development. However, development after sexual maturity is most likely favored by temperature alone. *M. Mercenaria* continue to grow after sexual maturity, unlike *Callinectes* in which growth halts following sexual maturity (Truit, 1939). These findings help explain the spawning season of May through October, with the height of the spawning season occurring in the warm summer months of August and September. During these months in 2015, gravid adult females were collected from the wild by hand from Biscayne Bay and maintained communally in a 1000 l plastic tank filled with filtered, aerated seawater. Only one collection trip was necessary as a single female can produce from four (Porter, 1960) to six (Binford, 1913) egg masses or "sponges" during a single mating season. Cheung (1969) reported an average of 4.5 spawns per molt. Filtered, ambient water for this flow-through tank system first goes through sand, bio, and UV filtration, and immediately through a 10micron sock before entering the tank. Daily water temperature, salinity, and dissolved oxygen (D.O.) were recorded for each 24-h period. The 1000L tank is located inside a temperature controlled, indoor facility at the University of Miami's Experimental Fish Hatchery (UMEH). The facility allowed for a managed light/dark sequence of approximately 10/14 h, and was maintained throughout the project. All female adult crabs were fed excess pieces of Spanish sardines (*Dussumieria elopsoides*), Northern mackerel (*Scomber scombrus*), and

California lolongya squid (*Grimalditeuthis bonplandi*) each morning, and any uneaten food was syphoned out of the tank. PVC fittings were placed in the tank to mimic the function of natural habitat crab holes.

All female crabs that were nearing the end of egg incubation (noted by the darkish brown color of the egg mass located on their abdomen), were removed to a glass aquaria hatching tank with flow through, ambient water supply. This water was filtered as previously mentioned by the main filtration system, and then put through additional cartridge filtration (down to 1micron) and UV sterilization. The hatching aquarium is divided by glass into 5 distinct tanks, each with their own ports where water can enter and exit. Hatched zoeae 1 (Z1) could exit with the tank and into a harvester with a 64micron mesh that contained the zoeae within it, while water and particles smaller than 64 microns were able to drain out of the system.

Newly hatched zoeae are strongly phototactic, and a strong LED light was used to obtain only the zoeae that congregated towards the concentrated light beam. These captured zoeae were then transferred to a bucket with a known volume of 12 l, uniformly mixed, and counted volumetrically.

2.2 Rearing Facilities

This experiment was conducted at UMEH, using the same modular 18- tank (not all tanks used for the trial) flow through system located inside a controlled facility (Figure 3). The 18 rearing vessels used are 55-l cylindrical-conical tanks filled with 50-l of ambient seawater. Again, this system was supplied water that is primarily filtered as mentioned, and again passed through additional cartridge filtration (down to 1 micron) and UV sterilization. Water levels in each tank were maintained at 50-l by an external “tree” that



Figure 3: 18 -tank flow through system used for stone crab larval rearing.

can be bypassed with a valve for draining the tank. All tanks are fixed in the center with a 300micron meshed “banjo” style standpipe, the tops of which are halfway below the water’s surface. These standpipes are designed to eliminate the surface tension of draining water that could otherwise trap zoea against the mesh. The base of each standpipe was fitted with an air diffusion ring, connected to individual air nozzles, which supplied desired aeration. Identical

water nozzles supplied each tank with a 1000% exchange throughout the experiment.

2.3 Stocking Larvae

Stocking density of larvae is a critically important variable in larviculture (Hitzfelder et al., 2006) and has a direct effect on the survival (Benetti et al., 2008a) and growth rates (Alvarez-Gonzalez et al., 2001). Larvae for each tank were stocked at 100 larvae/L (5000 larvae/tank) on the day of hatching (0 days post-hatch (dph)).

2.4 Live Feeds

All rotifers (*Brachionus spp*) used in this study are mass cultured at UMEH. Maintained rotifers are fed a special UMEH diet consisting of a unique combination of microalgae

(*Nannochloropsis spp*) and yeast. Each morning, approximately 50 million rotifers were transferred from the main culture tank, into a 55-l black cylindrical-conical tank with heavy aeration for a 24-hr enrichment period. The rotifers were enriched with Reed Mariculture's OnesStep (Table 1) micro algae paste (2ml/million rotifers) and taurine (4g/L), and harvested each morning by draining through a UM designed, submerged rotifer harvester with a 64-micron mesh. The seawater used for rinsing was filtered to 0.35 microns and UV sterilized. Following a thorough rinse, rotifers were concentrated to approximately 2,800/ml into a 32-L cylindrical cooler. Ice bottles were utilized to lower the temperature of the rotifers in the cooler to approximately 14°C. Rotifers were then fed to appropriate tanks (treatment specific) at a rate of 15/ml, 3 times/day.

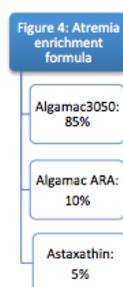


Figure 4: Percent composition of *Artemia* enrichment ingredients.

Artemia (INVE, AF grade: 430 micron) used in these experiments were varied and include decapsulated *Artemia*, newly hatched instar nauplii, and enriched instar nauplii II. All decapsulated *Artemia* cysts were treated in a sodium hydroxide and chlorine solution to remove the outer chorion, and kept refrigerated in a 1000 ml plastic container. Instar I nauplii were ran through a magnet gun shortly following hatch to remove polarized cysts, and rinsed with filtered seawater. Instar II enrichment consisted of a specially designed UM combination (Figure 4, Table 2). Taurine was also included at 2g/ L of water in the enrichment tank.

Table 1: Rotifer enrichment biomass composition.

Rotifer Enrichment	
Reed Mariculture OneStep Algae Paste	
Proximate Analysis	Amount
Protein	67.0%
Fat	11.3%
Fatty Acids (mg/g)	
DHA	17.0
EPA	12.0
ARA	1.9

Table 2: Artemia enrichment biomass composition.

Artemia Enrichment					
Bio-Marine Alagamac 3050		Reed Mariculture N-Rich Plus		Bio-Marine AstaRose	
Proximate Analysis	% weight	Proximate Analysis	% weight	Proximate Analysis	% weight
Protein	17.6	Protein	32.0	Astaxanthin	1.0
Fat	56.2	Fat	44.0	Protein	17.0
Carbohydrate	15.9	Carbohydrate	15.0	Fat	38.0

Ash	8.2	Ash	9.0	Ash	1.0
Moisture	2.1	DHA (% of lipids)	41.0	Carbohydrates	40.0
Calories (c/100g)	640	ARA	1.0	Moisture	3.0
		EPA	2.0		
		Total HUFAs	45.0		
		Dry weight Biomass	9.0		

2.5) Larval Feeding Protocols

This study used the established feeding protocol for larval blue crab (Zamora et al., 2005, Zohar et al, 2008) and previous work conducted at UMEH with live feeds for marine finfish (Benetti, 1997; Benetti et al., 2002; Watanabe et al., 2005; Benetti et al., 2008a). All treatments were tested against a control consisting of newly hatched (instar I) *Artemia* nauplii. Treatments used and quantities of feeds for this study are described in Table 1, and all rotifers and *Artemia* were enriched with commercially available products. All treatments were administered 3 times per day (9:00AM, 12:30PM, and 5:00PM respectively). In order to assess the performance of stone crab raised in this study, performance metrics such as total larval survival (zoea 1-5), larval growth (time to and in each zoeal stage), and total megalope survival were quantified.

Table 3: Each treatment for this experiment is described. All treatments were administered 3X day in the quantities listed below.

Treatment	Enriched Rotifers (15/ml)	Instar I <i>Artemia</i> Nauplii (0.5/ml)	Decapsulated <i>Artemia</i> Cysts (0.5/ml)	Enriched Instar II <i>Artemia</i> Nauplii (0.5/ml)
1 (Control)		Z1-megalope		
2			Z1-megalope	
3	Z1-megalope			Z3-megalope
4	Z1-megalope			

2.6) Data Collection

Growth was observed by the intermolt period between zoeal stages. As soon as molting was observed (molts clumped on the surface and were easily identified), the previous stage's time period was terminated. The days to megalope parameter was determined as soon as one megalope was seen in any given tank.

Day 7 total survival was determined through a volumetric count. 5 random samples from each tank (with increased aeration) were extracted with a 200mL container, and total number of zoeae was counted with the average of all counts being recorded.

Final megalope survival data was collected for each tank 2 days after the initial molting to megalope was observed. This was decided because molting to the megalope stage did not occur all at one particular time, rather it was a gradual process lasting about 1-1.5 days. After the 2-day period, water was drained from each tank and all megalope were counted individually on a mesh screen and the total number of megalope was recorded. No animals in T4 survived to megalope, and complete mortality across all 4 tanks occurred at day 22.

2.7) Data Analysis

Normality of water quality parameters were tested using a Shapiro-Wilk test: temperature ($p < 0.001$), dissolved oxygen ($p < 0.001$), and salinity ($p < 0.001$). Because water parameter data do not follow a normal distribution, a nonparametric Kruskal-Wallis test was utilized.

To determine any statistical differences among the treatments as they relate to survival, a One-way Analysis of Variance (ANOVA) was used. Prior to ANOVA, normality was verified through Shapiro-Wilk test ($p > 0.05$) and homogeneity of variance was verified through Levene test ($p > 0.05$) for all treatments. All ANOVA assumptions were met with survival data, and significant differences were observed. A Tukey post-hoc analysis was then performed to determine differences between treatments. All results are reported as means \pm the standard error (SE). The program utilized for statistical analysis was IBM SPSS (22.0) and CRAN R-Project.

2.8) Results

Water quality parameters were maintained within the suitable levels for stone crab, *Mennippe mercenaria*. The temperature ($28.78^{\circ}\text{C} \pm 0.05$) of the experimental units as well as dissolved oxygen ($6.44 \text{ mg/l} \pm 0.02$) and salinity ($31 \text{ ppt} \pm 0.06$) data did not meet the criteria for normality (Shapiro Wilks, $p < 0.001$), indicating nonparametric tests are necessary. Therefore, a Kruskal-Wallis H test was executed in order to determine if each parameter was significantly different between treatments (T1, T2, T3, T4). The test concluded that all treatments received approximately the same temperature ($p = 0.8699$), dissolved oxygen ($p = 0.7459$), and salinity ($p = 0.1000$) throughout the trial.

A Levene's test was then used to assess the homogeneity of variances for survival on day 7 and for total megalope survival. Day 7 total survival data ($p = 0.775$) and total megalope survival ($p = 0.229$) were determined to have equal variances.

Normality of survival data at day 7 and total megalope survival for each treatment were tested using a Shapiro-Wilk test. The test confirmed that all survival data for each treatment was normally distributed ($p > 0.05$). One-way ANOVA were run to determine whether significant differences were present in total survival at 7-days post hatch, and again for total megalope survival. There were significant differences between treatments for day 7 total survival ($F(3,12) = 127.998$, $P < 0.001$) and also for total megalope survival ($F(2,8) = 312.613$, $P < 0.001$).

Differences between treatments were assessed by Tukey's post-hoc analysis. Day 7 survival analysis concluded that only T1, which received instar 1 *Artemia* ($p < 0.001$), was significantly different than T2, T3, and T4 (Figure 5). Survival for T1 was significantly lower than the other treatments receiving rotifers (T3 and T4) or decapsulated *Artemia* (T2). There were no significant differences in survival between T2, T3, and T4 on day 7.

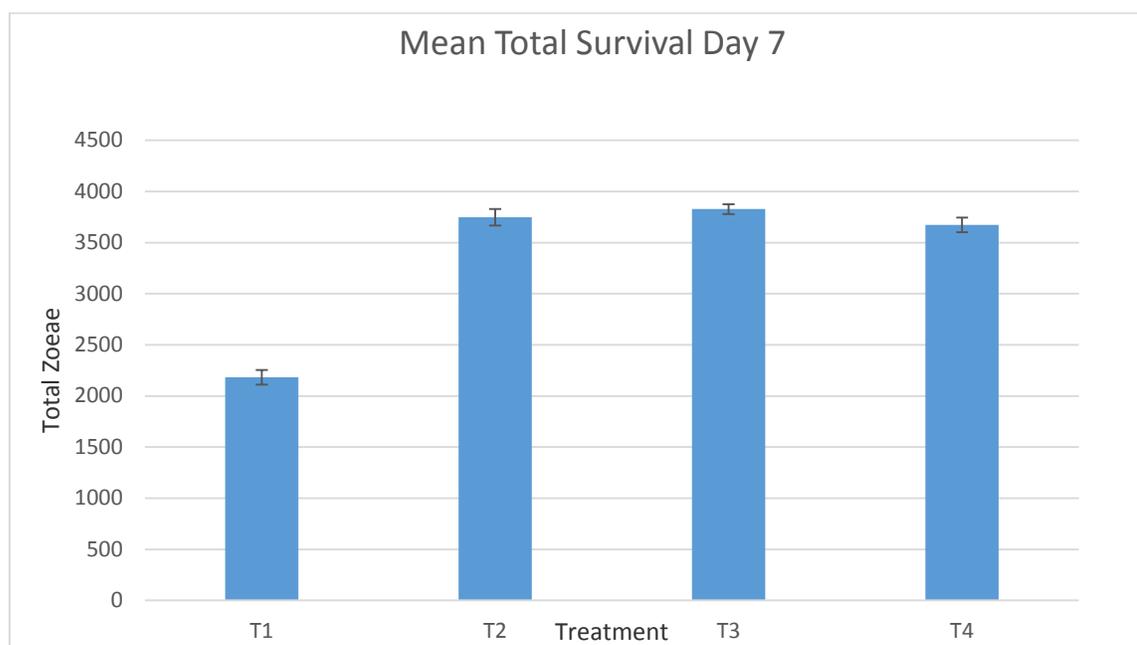


Figure 5: Total survival shown for each treatment at day 7 with standard error.

For final measurements, there were significant differences between T1, T2, and T3 in survival to megalope (Tukey's test, $p < 0.05$) with T2 performing significantly better than other treatments (Figure 6, $p < 0.001$). T3 was receiving a combination of rotifers and *Artemia* and performed slightly better than T1, which was fed unenriched *Artemia* only. Treatment 2, which had the highest survivorship, was fed decapsulated *Artemia*. No tanks in T4 reached the megalope stage and therefore were not included in the final megalope

Tukey post-hoc analysis (Table 2). Furthermore, tank 3 in T1 completely crashed on day 9 of the experiment, and was therefore not part of the final survival analysis.

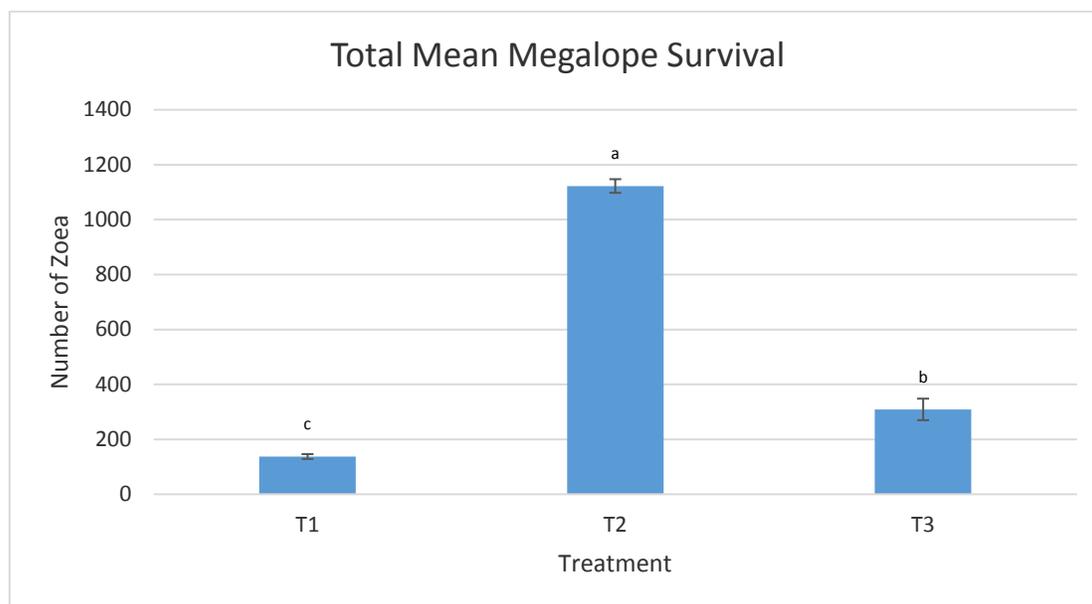


Figure 6: Total megalope survival shown for each treatment with standard error. Treatment 4 not included as no zoea in treatment molted to megalope stage. Different superscript above individual treatment bars indicates significant differences ($P > 0.05$).

Table 4: Survival of stone crab (mean \pm SE) at day 7, and at final MG count. Values with different superscript in the same column are significantly different (Tukey's test, $P < 0.05$).

Treatment	Day 7 Total Survival	Total Megalope Survival
T1	2182.50 \pm 71.95 ^b	137.33 \pm 8.84 ^c
T2	3746.25 \pm 80.97 ^a	1122.50 \pm 24.85 ^a
T3	3827.50 \pm 48.41 ^a	309.00 \pm 39.16 ^b
T4	3673.75 \pm 72.27 ^a	-

In combination with exhibiting the lowest survivorship, T1 also took more time to reach the megalope stage (Table 3). This lagged development began at the second molt and extended throughout the duration of the experiment. The onset of megalope development for T1 occurred on day 15, later than all other treatments except T4, which never achieved the megalope stage. All zoeae in T4 reached stage 5 of development, but all animals were dead by day 22. Overall, T2 performed the best with the highest survivorship, and the shortest amount of days to reach the megalope stage (11 days).

Table 5: Observed intermolt period (days) at successive developmental stages of stone crab larvae.

Treatment	Zoeal Stage					Total days to reach MG
	Z1	Z2	Z3	Z4	Z5	MG
T1	2.5	3.0	2.5	3.5	4.0	15.0
T2	2.5	2.5	2.0	2.0	2.0	11.0
T3	2.5	2.5	2.0	3.0	3.0	13.0
T4	2.5	2.5	2.0	5.0	>12	-

Although an ANOVA was performed because it passed the normality test, a pairwise proportionality test was also executed as the proportional survival data itself is in fact truncated on the upper end. This means it theoretically violates the assumption of

normality because it is not possible to have symmetric error distributions in close proximity to the upper asymptote. This test was run with Day 7 total survival data and shows significant differences in survival between each tank, and thus between treatments. Although there are some significant differences between tanks within each treatment, the pairwise proportionality test agrees with the results of the ANOVA that overall treatment 1 is highly significantly different than treatments 2, 3, and 4 (Table 4, Figure 7).

Table 6: Pairwise Proportionality Test for Day 7 total survival stone crab data. Tanks 1-16 were individually compared against each other. Highlighted cells indicate significant relationship ($P < 0.05$).

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
2	0.0002	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	0.54094	2.60E-10	-	-	-	-	-	-	-	-	-	-	-	-	-
4	1	0.00079	0.25977	-	-	-	-	-	-	-	-	-	-	-	-
5	<2e-16	<2e-16	<2e-16	<2e-16	-	-	-	-	-	-	-	-	-	-	-
6	<2e-16	<2e-16	<2e-16	<2e-16	0.00755	-	-	-	-	-	-	-	-	-	-
7	<2e-16	<2e-16	<2e-16	<2e-16	0.30913	1	-	-	-	-	-	-	-	-	-
8	<2e-16	<2e-16	<2e-16	<2e-16	0.00023	6.40E-15	6.30E-11	-	-	-	-	-	-	-	-
9	<2e-16	<2e-16	<2e-16	<2e-16	0.03974	1	1	3.10E-13	-	-	-	-	-	-	-
10	<2e-16	<2e-16	<2e-16	<2e-16	0.00311	1	1	8.50E-16	1	-	-	-	-	-	-
11	<2e-16	<2e-16	<2e-16	<2e-16	1	9.50E-05	0.014	0.01452	0.00084	3.00E-05	-	-	-	-	-
12	<2e-16	<2e-16	<2e-16	<2e-16	0.1167	1	1	4.80E-12	1	1	0.00374	-	-	-	-
13	<2e-16	<2e-16	<2e-16	<2e-16	0.30913	1	1	6.30E-11	1	1	0.014	1	-	-	-
14	<2e-16	<2e-16	<2e-16	<2e-16	1	0.01651	0.54094	8.10E-05	0.0821	0.00741	1	0.22934	0.54094	-	-
15	<2e-16	<2e-16	<2e-16	<2e-16	0.11347	1.50E-09	2.40E-06	1	3.50E-08	3.00E-10	1	3.10E-07	2.40E-06	0.05632	-
16	<2e-16	<2e-16	<2e-16	<2e-16	8.30E-05	1.00E-15	1.30E-11	1	5.40E-14	<2e-16	0.00703	9.00E-13	1.30E-11	2.70E-05	1

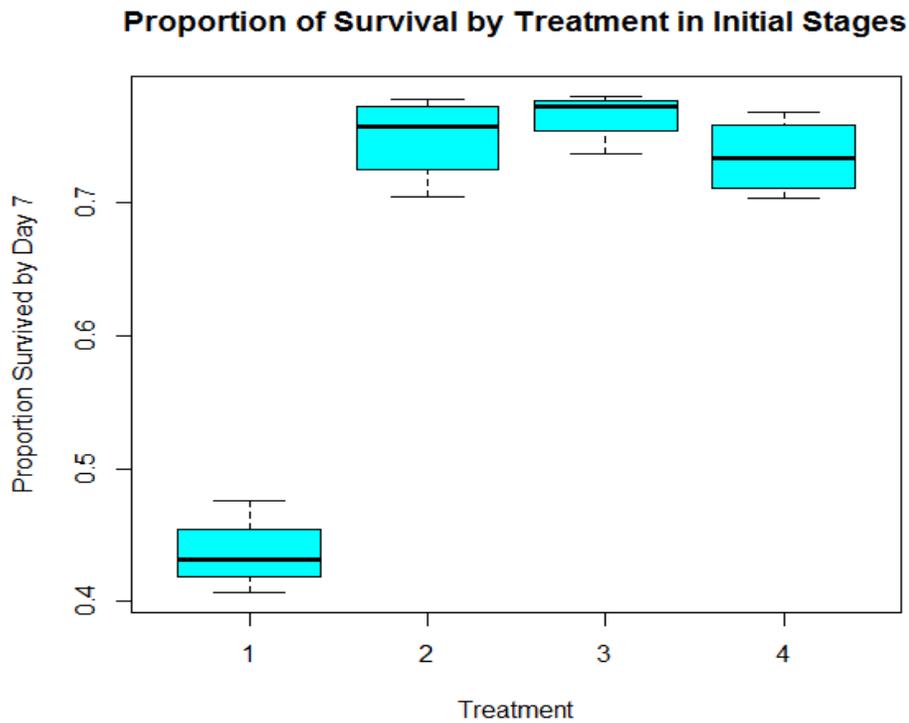


Figure 7: Boxplot shows the proportions of survival (with mean and SE) at day 7 of the experiment across all treatments.

Exponential decline in survival was modeled using a Gompertz style equation

$$N(t) = N_0 \exp^{-\exp(a-bt)}$$

where, $N(t)$ is the population abundance at time t , N_0 is the initial population abundance, t is the time in days, a is a scalar, and b is the rate of decline. Starting values and parameter estimates are presented in Table 6. A visual representation of the results from the statistical analysis displaying survivorship over time are presented in Figure 8.

Table 7: Values of initial abundances and parameter estimates within the Gompertz Equation.

Treatment	N_0	a	b
T1	5000	-15.567	-2.196
T2	5000	-3.311	-0.287
T3	5000	-3.476	-0.302

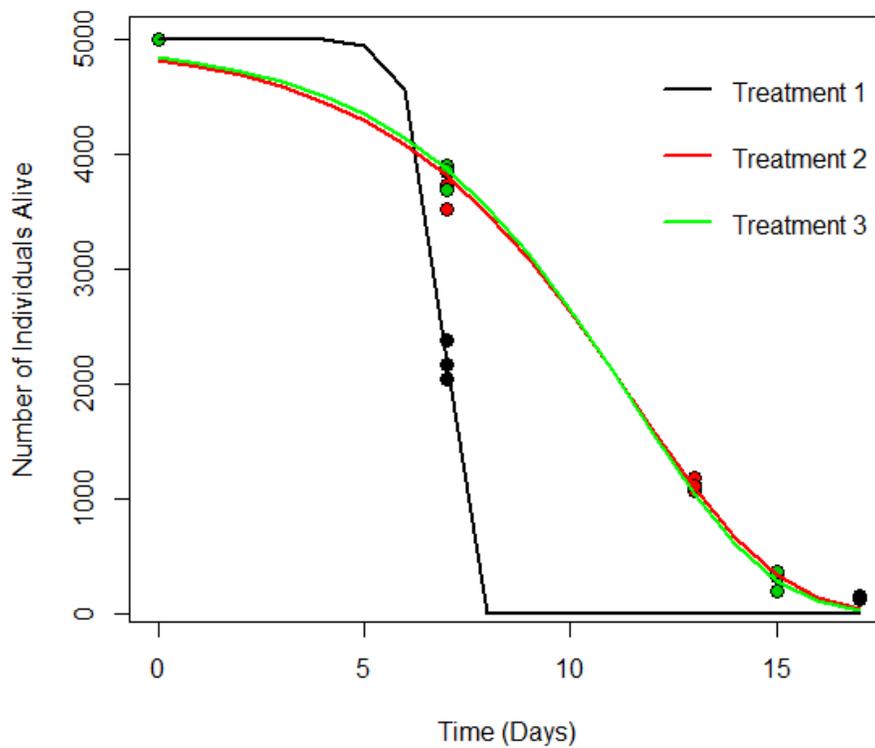


Figure 8: Gompertz growth curves applied to the survival and intermolt periods of stone crab larvae.

2.9) Discussion

For the first time, growth and survival of stone crabs was analyzed in commercial stocking densities with various live food prey organisms. Organisms which are introduced to a larval rearing system must be easily obtained and digested by the stone crab larvae. These prey have different sizes, digestibility, swim speeds, and nutritional profiles that greatly effect larval growth, health, feeding ability and ultimately survival. The main goal of this experiment was to establish a larval rearing protocol that produced the highest amount of megalope, while minimizing the length of time to that stage. The results demonstrate that when only either decapsulated instar 1, or enriched rotifers (T3,T4) are fed through zoea 3,

- a. survival was higher than when fed unenriched instar I *Artemia* alone (T1) and
- b. survival was not significantly different from each other (T2, T3, T4) .

Baylon et al (2001) noted that zoea 1 *S. serrata* larvae more easily captured highly dense, slower swimming rotifers than *Artemia*. The fast movement of instar one *Artemia* in T1 likely made it a more difficult prey item to capture at younger zoeal stages. The data in this experiment suggests that T2, T3, and T4 which utilized smaller prey items, was more easily captured due to smaller size and presumably slower swimming speed. Using either decapsulated *Artemia* or enriched rotifers had no significant difference in survival at day 7 (T2, T3, T4). The instar I *Artemia* treatment (T1) sustained losses greater than 40% by day 7. As well as influencing survival, the lack of a smaller prey item in T1 during the first zoeal stages slowed the development process beginning as early as Z2. Another

possible explanation of treatment 1's poor performance may be nutritionally related. Kobayashi et al (2000) suggested that DHA is important for the growth of mud crab larvae, and is known to be in very low concentrations in newly hatched *Artemia* nauplii (Evjemo and Olsen, 1997). Feeding an enriched instar II could be a possible solution to this issue, however a Z1 would most certainly have difficulties catching this larger prey item, and enrichment products can be difficult to digest, especially during early zoeal development. However, the fact that the decapsulated instar I treatment (T2) performed very well contributes heavily to the argument that poor larval survival and development up to day 7 is most likely a prey capture problem rather than a nutritional issue, as was similarly noted with mud crab larvae (*Squilla serrata*) by Ruscoe et al., 2004.

The presence of rotifers and decapsulated *Artemia* significantly improved survival of stone crab larvae in early stages up to Z3. However, when enriched rotifers were used alone (T4) past Z3, survival and growth began to fall. As the larvae develop and molt to subsequent stages, their increased metabolic activity results in an increase in energy requirements. Frank et al. (1975) attributes the high mortality of mud crab (*Rhithropanopeus harrisi*) between stages III and IV to the failure of "normal diets" such as enriched rotifers to meet this increased energy demand. This was evident when T4 development slowed during the zoea 4 stage, where 5 days were spent in this intermolt period, which was 1.5 days longer than all other treatments. Furthermore, T4 spent over 12 days as Z5 and never molted to the megalope stage. At day 22, all tanks in T4 were completely empty of surviving larvae and thus were not included in the total megalope survival analysis.

Total megalope survival was analyzed for treatments 1, 2, and 3. The results concluded

that all treatments were significantly different from each other, with T2 performing the best (22.45% survival) and T1 performing the poorest (2.75% survival). Using a nutritionally superior, smaller prey like decapsulated *Artemia* (T2) throughout the larval rearing process not only increases survival, but also increases growth. T2 reached the megalope stage a full 2 days before any other treatment.

Treatment 3, which was fed enriched rotifers through Z3 and began a co-feeding regime with enriched instar II at Z4, had 6.18% total megalope survival. Treatment 3's poor survival can most likely be attributed to visual prey confusion, as stone crab zoea are passive feeders. Ruscoe et al., 2004 concluded that decrease in survival of *S. serrata* zoea that were co-fed enriched rotifers and *Artemia*, "could not be due to a lack of food, but may have been due to visual confusion of the larvae resulting in decreased rotifer consumption." The larvae in T3 likely were consuming nutritionally inferior rotifers, which delayed growth to megalope by 2 days before T2, and had detrimental effects on survival. When co-feeding with rotifers and *Artemia*, future studies should consider the complete elimination of rotifers after the zoea 3 stage of development in *M. mercenaria*.

Treatment 1's significantly low survival and growth is likely due to its stunted performance during the earlier zoal stages, and the ingestion of a nutritionally inferior prey item (unenriched instar I). As the larvae in T1 molted to more advanced stages, they were able to catch and ingest the instar 1 nauplii more easily. The instar 1 alone was sufficiently nutritious enough for a small number of Z5 to molt to the megalope stage.

Total megalope survival across all three treatments could have been higher if all larvae were transferred to separate “settlement tanks” as soon as megalope were observed in any given tank (Zmora et al., 2005). Once Z5 settle to the megalope stage, they need a tank where they can easily settle to the bottom and hide from each other to avoid cannibalism. Cannibalism can be reduced through the use of various screens and substrates once the larvae are transferred at lower densities, to settlement tanks (Zmora et al., 2005). This experiment was limited by the amount of tank space, thus resulting in megalops remaining in the larval rearing tanks. These conical tanks were set up with the intent of keeping the larvae suspended in the water column. Furthermore, megalopes in these tanks could easily find Z5 and consume them before they molted or during the molting process.

2.10) Conclusion

The results of this experiment showed that enriched rotifers and decapsulated *Artemia* nauplii were equally acceptable for sustained growth and survival through Z3, but rotifers could be removed from the regime as early as Z3. Furthermore, a constant diet of decapsulated *Artemia* (T2) through the megalope stage was shown to be the most effective treatment for significantly higher growth and survival for stone crab. T2 not only resulted in the highest megalope survival, but also, reached megalope two days before T1, and T3 (see Figure 5). Further studies should examine the using enriched rotifers in early developmental stages, and introducing decapsulated *Artemia* nauplii at progressive stages, and examine growth and survival. Through the development of a suite of aquaculture technologies tailored to stone crab, this experiment has encouraged the

growth of a valuable marine aquaculture industry in the southeast U.S. and demonstrated an efficient protocol for advanced larval rearing techniques.

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