Nursery Protocols for the Rearing of the Brown Shrimp, *Farfantepenaeus aztecus:* Effects of Stocking Density and Salinity

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ABSTRACT. Recreational saltwater fishing in coastal areas of the United States of America is partly dependent on the availability of live bait for anglers. One bait species commonly used is the brown shrimp, *Farfantepenaeus aztecus*. Three experiments were conducted with eight-day-old postlarvae (PL_8) to evaluate the effects of stocking density and water salinity on survival, growth, and feed conversion ratio (FCR) of *F. aztecus*, in nurseries. In the first experiment, the effects of stocking density were evaluated for a period of 11 days. Post-larvae (PL) were stocked in to round tanks (760 L) at densities of 30, 40, 50, and 60 PL/L with four replicates per treatment. There were no significant differences ($P > 0.05$) in final mean weight, survival, and estimated FCR among treatments. In the second experiment, shrimp were stocked into 159-L square polyethylene tanks at four densities (1.0, 1.9, 7.9, and 13 PL/L) and maintained for 12 days. Shrimp reared at low densities grew significantly larger and had significantly lower FCR than those stocked at

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higher densities. There were no significant differences in survival. In the third experiment, PL were stocked at two salinities (16 ppt and 32 ppt) with six replicates per salinity. Three of the tanks at each salinity were then treated with EDTA (3 ppm). Fourteen days after the start of the experiment, sub-samples of shrimp from each treatment were weighed and returned to the tanks. Salinity in the 32 ppt treatment was then gradually decreased to 16 ppt during a 24 hour period and the shrimp reared for an among treatments at the end of the 14 day grow-out periadditional seven days. There were significant differences in mean weights od (32 ppt, 10.1 mg; 16 ppt, 7.2 mg). At the end of the experiment, the use of EDTA did not seem to have an effect on production. However, shrimp subjected to late acclimation to the lower salinity were significantly smaller than those acclimated at stocking (11.8 mg vs. 21.2 mg). This reduced weight is presumably due to acclimation stress resulting in a lag in growth. The high salinity treatment resulted in significantly higher survival (32 ppt, 51.3%; 16 ppt, 44.4%), while the FCR was significantly lower (32 ppt, 1.75; 16 ppt, 2.08). Present results indicate that nursery systems, if properly utilized and correctly managed, could assist in the development of a viable bait-shrimp aquaculture industry.

KEYWORDS. Bait shrimp, Brown shrimp, nursery, salinity, EDTA

INTRODUCTION

Recreational saltwater fishing is a major industry in coastal areas of the United States of America. The United States Fish and Wildlife Service estimates that 9.1 million anglers spent 8.4 billion dollars on saltwater fishing trips and equipment in the U.S. in 2001 (USDOI 2001). The ability of the coastal region to provide positive fishing experiences to anglers is dependent on various factors such as proper habitat and fish populations, hotels, boat launching areas, and the availability of suitable bait during fishing season.

Live shrimp is a favorite food for many of the game fish (e.g. red drum, *Sciaenops ocellatus*; flounder, *Paralichthys sp.*; and spotted seatrout, *Cyanoscion nebulosus*) in the southern states of the USA (McKee 1986; Samocha et al. 1999). Since shrimp destined for the live bait market are traditionally captured in estuaries, availability of shrimp for bait is dependent on seasonal and environmental conditions (Benfield and Downer

2001). Presently, the bait shrimp supply along the Gulf coast cannot keep up with demand. A survey in Texas showed that demand for shrimp could only be met by bait shops for two months out of the year. Retailers have indicated an increase in the demand for live shrimp in recent years without a concurrent increase in supply (Gandy et al. 2001).

A solution to inconsistent shrimp supplies may be to culture shrimp for bait. A survey conducted by Gandy et al. (2001) found that 74% of bait shop operators said they would buy farm-raised shrimp if supply is consistent and shrimp are of good quality. The benefits of farm-raised live bait-shrimp to supplement wild catch and reduce fishing pressures on wild populations has attracted a great deal of attention (Gandy and Samocha 2001). A supply of cultured shrimp could decrease the amount of trawling activity conducted in estuaries. Moreover, shrimpers could benefit from cultured shrimp if wholesale prices were adjusted to allow them to buy the cultured product, and still make a profit when they sell to a bait shop. More importantly, a commercial bait-shrimp operation could provide some consistency to a sporadic supply during periods of great demand. Based on a survey conducted in Alabama by Mays (2003), demand for bait shrimp exists on a year-round basis. Hence, if a farm-raised bait-shrimp industry is to be developed it should be designed for year-round production in order to maintain a consistent supply. If such a system is to be developed, it will most likely require the use of nursery systems.

Because fishing with live bait risks releasing animals into the wild, only an indigenous specie may be used (exotic species would present ecological problems) (Johnson 1998). Therefore, we decided to evaluate the feasibility of culturing the Gulf brown shrimp, *Farfantepenaeus aztecus*, for use as bait in Alabama. Although culture techniques of penaeid shrimp are well documented, information regarding the culture of Gulf brown shrimp is limited. Furthermore, since Alabama has a limited access to full-strength seawater, commercial farms are most likely to utilize brackish water. Consequently, modified culture protocols commonly used for various penaeids were evaluated for Gulf brown shrimp. Three experiments were designed to investigate the use of a nursery system to rear post-larval (PL) shrimp in Southern Alabama. The objectives were to evaluate the effects of stocking density and salinity acclimation, on the growth and survival of Gulf brown shrimp PL, reared in a covered nursery facility.

MATERIALS AND METHODS

The present work was conducted at the Claude Peteet Mariculture Center, Gulf Shores, Alabama. The first and third experiments were conducted in an outdoor, shaded, semi-closed recirculating system consisting of a series of 16 circular polyethylene tanks (795 L), each equipped with a center drain, and two air diffusers. Water volume (760 L) in each tank was maintained by an internal stand pipe (75 cm) surrounded by an external screened pipe (250 um mesh netting). Water was recirculated between tanks and a biological filter (one for each salinity tested) at a rate of 6 L/minute/tank. The second experiment was conducted in a series of 159-L square polyethylene tanks all connected to a biological filter. All tanks were equipped with submersible air diffusers. Eight day old Gulf brown shrimp post-larvae (PL_8) were purchased from Lone Star Farms, Corpus Christi, Texas[1] for all experiments. Feed regimens for all experiments were similar to each other (Table 1). Upon stocking, and for the subsequent three days, the PLs were offered newly hatched *Artemia* nauplii (INVE Americas, Inc., Salt Lake City, Utah) at a rate of 100 nauplii/PL (day 1) and 50 nauplii/PL (day 2 and 3). From day-1 to day-7, the PLs were offered PL Redi Reserve (Zeigler Bros, Inc., Gardners, Pennsylvania) then crumbled shrimp feed (40% protein, Burris Mill & Feed, Inc., Franklinton, Louisiana) mixed with PL Redi Reserve (day 8-10) or alone after day 11. Dry feeds were offered four times a day. Daily ration was calculated based on decreasing feed rates that ranged from 25%-15% per day. Shrimp biomass was estimated based on intermittent sub-samples and an assumption of 100% survival.

Experiment 1

Marine water (32 ppt) was passed through a sand filter to remove debris, organic matter, and unwanted organisms, then pumped into the tank-system where it was disinfected with sodium hypochlorite (approximately 10 ppm chlorine). Two days later, the water was tested for residual chlorine, treated with sodium thiosulfate, and fertilized with inorganic fertilizers (1.68 L of 10-34-0 and 420 mL of 32-0-0). The system was then inoculated with phytoplankton (*Chaetoceros* sp.; Reed Mariculture, Inc., San Jose, California).

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[1] Use of trade or manufacturer's name does not imply endorsement.

TABLE 1. Typical feeding regimen utilized during nursery trials.

Day	Feed type	Feed rate (% biomass)
1	*Artemia* [a]	100/PL
2	*Artemia* [a], PL Redi Reserve [b]	50/PL, 25
3	*Artemia* [a], PL Redi Reserve [b]	50/PL, 25
4-7	PL Redi Reserve [b]	25
8	PL Redi Reserve [b], #0 Crumble [c]	25
9	PL Redi Reserve [b], #0 Crumble [c]	15
10	PL Redi Reserve [b], #0 Crumble [c]	15
11-15	#0 Crumble [c]	15
16	#0 Crumble [c], #1 Crumble [c]	15
17	#0 Crumble [c], #1 Crumble [c]	15
18	#0 Crumble [c], #1 Crumble [c]	15
19-onwards	#1 Crumble [c]	15

[a] INVE Americas, Inc., Salt Lake City, Utah.
[b] Zeigler Bros, Inc., Gardners, Pennsylvania.
[c] Burris Mill & Feed, Inc., Franklinton, Louisiana.

Upon receipt of the shrimp, PLs were acclimated to the temperature and salinity of culture water and concentrated in a 56-L tank, from which five 60-mL grab samples were taken after thorough mixing. Shrimp density was estimated and the PLs were stocked based on volumetric counts into the round tanks at four densities (30, 40, 50, and 60 PL/L) with four replicate tanks per treatment. Shrimp were offered feed as described above (Table 1). Post-larval growth was determined every three days by weighing a sub-sample of PLs from each tank using an analytical scale (0.01 mg). The number of PLs in the sub-sample were then counted into another container. The mean weight calculated for each tank was then used to compute the appropriate feed regimens and estimate growth and mean weights. Temperature and dissolved oxygen were monitored twice daily using a YSI 55 dissolved oxygen meter (Yellow Springs Instruments, Yellow Springs, Ohio). Salinity was determined using a conductivity meter, total ammonia-nitrogen (TAN) using methods described by Boyd (1979), nitrite-nitrogen using the PLN code test kit from LaMotte (Chestertown, Maryland), and pH using a handheld pH meter. The shrimp were harvested after 11 days and survival, growth, and feed conversion were estimated.

Experiment 2

The second experiment was conducted in the square tanks. Marine water (28 ppt) was passed through a sand filter and chlorinated. Two days later it was de-chlorinated and the tanks were stocked with Gulf brown shrimp PL. Sixteen tanks were stocked at densities of 1.0, 1.9, 7.9, and 13 PL/L with four replicate tanks per treatment using volumetric counts as previously described. Shrimp were maintained for twenty days as described for the previous experiment and offered feed as described in Table 1. A sub-sample of the shrimp from each tank was weighed every three days. At harvest, survival, growth and feed conversion were estimated.

Experiment 3

The 16 circular tanks were prepared and filled as in Experiment 1. However, internal standpipes were cut to a height of 61 cm, resulting in an operational volume of 600 liters in each tank. Six tanks were filled with 32 ppt seawater, and six tanks with 16 ppt water. In order to control for effects of heavy metals that could have been present in the water, three tanks from each salinity treatment were treated with 1.8 grams (3 ppm) of EDTA after system disinfection. Upon receipt, PLs were acclimated to the salinity and temperature of the water in the holding tank (32 ppt) and counted. Approximately half the PLs were stocked into the high-salinity (32 ppt) treatment tanks, while the remaining PLs were acclimated over a five-hour period to the lower salinity before stocking. Stocking densities were 24 PL/L in the high salinity and 25 PL/L in the low salinity tanks. Shrimp were offered feed according to the regimen shown in Table 1. Growth and water quality were monitored as previously described.

On day fourteen, a random sample of shrimp (> 30 shrimp per sample) was taken from each tank to determine mean weights. The PLs in the high salinity tanks were then slowly acclimated to 16 ppt using fresh well-water over a period of 24 hours in preparation for pond stocking. On day 22, PLs were harvested, counted, weighed and stocked into grow-out ponds. Survival, growth, and feed conversion were estimated.

Statistical Analysis

Results were analyzed using SAS 8.02 (SAS Institute, Inc., Cary, North Carolina). Data from each trial was analyzed using one-way and two-way analysis of variance to determine if significant differences ($P \leq$

0.05) existed among treatment means. Student-Newman-Keuls multiple comparison test (Steel and Torrie 1980) was utilized to determine differences among treatment means.

RESULTS

Water quality in the three experiments remained within acceptable ranges throughout the project. Temperature averaged above 27°C, dissolved oxygen remained above 4.5 mg/L, pH was approximately 7.7 at all times, and total ammonia and nitrite nitrogen were below toxic levels (Table 2).

No significant differences were observed in final mean weight (7.42-8.89 mg), survival (54.0-60.5%), or FCR (0.65-0.84) among the four stocking densities evaluated in Experiment 1 (Table 3). Although mean final weight appears to be greater in the low density treatment than in the other treatments, no statistical differences were observed.

In the second experiment, there were no significant differences in survival among treatments (Table 4). However, shrimp reared at low densi-

TABLE 2. Temperature, salinity, dissolved oxygen (D.O.), pH, total ammonia-nitrogen (TAN), and nitrite-nitrogen observations from the nursery trials. Numbers are displayed as mean±standard deviation and minimum/maximum values (below in parentheses).

	Nursery I	Nursery II	Nursery III
Temperature (°C)	27.7±1.31 (25.4, 31.7)	27.8±0.73 (26.8, 29.3)	27.4±1.19 (24.6, 29.7)
Salinity* (ppt)	30.68±0.46 (30.2, 32)	28.0±0.15 (27.4, 28.1)	32.9±1.10/16.7±0.14 (31, 34.2)/(16.4,16.9)
D. O. (ppm)	5.80±0.60 (5.0, 6.5)	7.65±0.79 (6.1, 8.2)	6.96±0.90 (4.6, 9.7)
pH	7.88±0.88 (7.7, 8.2)	7.7±0.6 (6.9, 8.2)	7.7±0.2 (7.3, 7.9)
TAN (ppm)	16.64±9.99 (0.1, 24.9)	0.19±0.15 (0.19, 0.29)	1.99±1.49 (0.4, 5.6)
Nitrite-nitrogen (ppm)	0.025±0.05 (Nd, 0.1)	Nd (Nd, Nd)	0.31±0.33 (Nd, > 0.8)

* Two different salinities were utilized in the third nursery trial.
Nd–Not detectable.

TABLE 3. Production results from a 11-day shrimp nursery trial usin g *Farfantepenaeus aztecus* postlarvae stocked at four densities. FCR is the estimated feed conversion ration = feed input/biomass harvested.

Treatment	Final mean weight (mg)	Survival (%)	FCR
30 PL/L	8.89	60.3	0.65
40 PL/L	7.42	60.5	0.80
50 PL/L	7.53	59.5	0.79
60 PL/L	8.04	54.0	0.84
P-values	0.27	0.82	0.12

TABLE 4. Production results from a 20-day nursery trial using Farfantepenaeus aztecus postlarvae (1.5 mg initial weight) stocked at four different densities. FCR is the estimated feed conversion ratio = feed input/biomass harvested. PSE = Pooled standard error.

Treatment	Final mean weight (mg)	Survival (%)	FCR
1.0 PL/L	87.2a	55.0a	2.1a
1.9 PL/L	70.0a	41.5b	2.9ab
7.1 PL/L	84.3a	32.0bc	2.5ab
13 PL/L	69.3a	23.2c	3.6b
PSE	5.77	2.98	0.26

ties grew significantly faster than shrimp reared at high densities and had a significantly lower FCR.

In the third experiment, the supplementation of EDTA to culture waters at 3 ppm had no effect on survival, growth or FCR of the shrimp (Table 5). Results were therefore pooled by salinity and analyzed. After 14 days of nursing, PLs were significantly larger in the high salinity treatments (10.1 vs. 7.2 mg). At day 14, the salinity was reduced to 16 ppt in the high salinity treatment to acclimate the PL to pond conditions and then harvested at day 22. At the conclusion of the nursery period (day 22) shrimp reared at 16 ppt had lower survival (44.4% vs. 51.3%) and higher final weights (21.2 mg vs. 11.8 mg) than those reared initially at 32 ppt.

TABLE 5. Response of Farfantepenaeus aztecus post-larvae to two salinities (32 ppt and 16 ppt) and the use of EDTA (with (w) and without (w/o)). Fourteen days after stocking, shrimp at the high salinity were acclimated over 24 hours to the lower salinity and maintained for an additional seven days.

		Day 14	Day 21		
Salinity (ppt)	EDTA	Mean Weight (mg)	Mean Weight (mg)	% Survival	FCR
32	w	8.82	11.69	48.2	1.79
32	w/o	11.47	11.85	54.4	1.71
16	w	6.93	17.99	43.5	2.10
16	w/o	7.48	24.42	45.3	2.07
P-values	EDTA	0.09	0.22	0.21	0.74
	Salinity	0.007	0.005	0.04	0.07
	EDTA*Salinity	0.24	0.25	0.48	0.89
Pooled by Salinity	Salinity	Mean Weight (mg)	Mean Weight (mg)	% Survival	FCR
	32	10.1	11.8	51.3	1.75
	16	7.2	21.2	44.4	2.08
P-values		0.011	0.005	0.04	0.04

DISCUSSION

There are many justifications for the use of nursery systems in shrimp aquaculture. Nurseries can help extend limited growing seasons (Sandifer et al. 1988). They also allow the stocking of larger and hardier PLs into grow-out ponds and decrease the grow-out time in the ponds (Samocha and Lawrence 1992; Peterson and Griffith 1999). Indoor nursery systems utilize space more efficiently, requiring less land and water than traditional ponds (Mock et al. 1977; Samocha and Lawrence 1992; Losordo et al. 2001). Nurseries can also provide biosecurity in a time when disease is of major concern (Peterson and Griffith 1999). Furthermore, nursery systems offer more control over inputs, protection from predation, as well as better observation of feed consumption, growth, and water quality (Samocha and Lawrence 1992; Peterson and Griffith 1999). Indoor nursery systems can allow for temperature and salinity manipulations because of the enclosed area and lower volumes of water in the system. The development of a bait shrimp industry will most likely occur in coastal areas where land costs are high and production will probably be geared towards year round outputs; hence, nursery systems will most likely be utilized in the production schedule.

Survival in Experiments 1 and 3 were low compared to reported results for Gulf brown shrimp (Samocha et al. 1999), Pacific white shrimp, *Litopenaeus vannamei* (Samocha and Lawrence 1992; Williams et al. 1996; Davis and Arnold 1998; Samocha et al. 1999), and for blue shrimp *L. setiferus* (Mock et al. 1973; Davis and Arnold 1998; Samocha et al. 1999). This could be partially attributed to high stocking densities but it is more likely due to site specific water quality problems. The site for which this research was conducted typically has variable survival that is often low for nursery trials (Garza 2001; Padgett 2003). This was thought to be due to the possibility of heavy metals present in the water. However, since the addition of EDTA did not enhance survival in Experiment 3, there appears to be other reasons for variable survival at this site. Padgett (2003) also treated his water with EDTA, but observed survivals of approximately 41% after a 21-day nursery period.

The lack of a response, in terms of shrimp growth and survival, to the high densities tested in Experiment 1 are similar to those observed by Zelaya et al. (2004) working with *L. vannamei*. They reported that growth and survival of *L. vannamei* were not influenced when shrimp were reared at densities similar to those of the present work. As these densities are considerably higher than those typically used in nurseries, it was presumed that the lack of response was because the lowest density tested was beyond the point of a density dependent response. Hence, in the second experiment, lower densities were tested. The results of Experiment 2 reveal a significant effect of stocking density on weight gain and feed conversion but not on survival. These results may indicate that there is a density dependent response up to a given threshold after which there are minimal effects on growth and survival.

Although shrimp stocked at the lowest density appear to have survived better than shrimp stocked at the highest density (87.2% vs. 69.3%), results were not statistically significant. Conversely, PLs reared at low desities grew significantly faster than those reared at high desnities. Previous observations (Davis and Rouse, unpublished data), suggest that weight differences of same-age nursed juveniles have minimal influence on the harvest weight of shrimp after growout in ponds. Hence, when considering commercial applications and the cost associated with a nursery system, stocking at moderate or high densities to maximize space utilization may be more appropriate than stocking a low density to optimize growth. Growth rates of PLs in nurseries are good indicators of suitability of culture techniques of the nursery. However, before optimal nursery densities are recommended, the response of the shrimp during the final grow-out stage should also be evaluated.

Another possible explanation for low survival and growth in Experiments 1 and 3 could be due to effects of water temperatures. Zein-Eldin and Aldrich (1965) and Zein-Eldin and Griffith (1966) suggest that high water temperature affected growth and survival of *F. aztecus* more than other environmental factors. They report that production in the laboratory is good at temperatures of 22.5 to 30°C. Shrimp in the experiments we conducted were therefore exposed to temperatures at the upper end of their tolerance levels.

Results of Experiment 3 suggest that eight-day old Gulf brown-shrimp PL growth is greater at a salinity of 32 ppt than at 16 ppt after 14 days of stocking. However, as stated earlier, shrimp size at stocking does not seem to affect harvest size in pond aquaculture. Accordingly, bait farms can be constructed at sites with marine water ranging from high brackish to full strength sea water. However, salinity acclimation appears to have caused a reduction in growth rate. Many species of penaeids are tolerant of salinity shifts at specific ages, generally between PL 12 and PL 40; see Saoud and Davis (2003) and Saoud et al. (2003). The reduction in growth observed in the present experiment might have been due to this reduction in euryhalinity or stress associated to salinity acclimation.

While shrimp nurseries have several advantageous attributes, there are also disadvantages associated with the construction and operation of an intensive nursery system. The construction and operating costs of a nursery are higher than those of pond production (Samocha and Lawrence 1992). Two production cycles per year may not increase farm profitability (Juan et al. 1988). Moreover, the operation of the facility would require more technical expertise (Hirono 1983; Samocha and Lawrence 1992). Finally, the use of a nursery system involves an increased amount of handling of PL, which may have negative effects on PL health and survival. Nonetheless, various researchers reported that it is reasonable to expect positive production results and good survival (> 80%) from nursery systems for shrimp (Davis and Arnold 1998; Samocha et al. 1999; Samocha et al. 2002). As the research progresses and new technology is developed, better nurseries and nursery management techniques will be developed and bait shrimp production is bound to develop into a major industry in the southern coastal states of the US.

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