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Effect of Low Salinity on Growth and Survival of Postlarvae and Juvenile *Litopenaeus vannamei*

SUSAN LARAMORE

Harbor Branch Oceanographic Institution, Inc., Aquaculture Division, 5600 U.S. 1 North, Ft. Pierce, Florida 34946 USA

C. ROLLAND LARAMORE

Aquatic Animal Health Laboratory, 5600 U.S. 1 North, Ft. Pierce, Florida 34946 USA

JOHN SCARPA

Harbor Branch Oceanographic Institution, Inc., Aquaculture Division, 5600 U.S. 1 North, Ft. Pierce, Florida 34946 USA

Abstract

The effect of low salinity on survival and growth of the Pacific white shrimp *Litopenaeus vannamei* was examined in the laboratory due to the interest of raising shrimp inland at low salinities. In three separate experiments, individual *L. vannamei* postlarvae (~ 0.1 g) were cultured at salinities of either 0.5, 1, 1.5, 2, or 3 ppt (N = 5 or 10/treatment) for 18 to 40 d at 30 C in individual 360-mL containers. In each experiment controls of 0 and 30 ppt were run. There was no postlarval survival at salinities < 2 ppt. Survival was significantly different (P < 0.01) at 2 ppt (20%) compared to 30 ppt (80%). Growth was also significantly different (P < 0.01) at 2 and 3 ppt compared to 30 ppt (416%, 475%, and 670%, respectively). A fourth experiment compared juveniles (~ 8 g) and postlarvae (~ 0.05 and 0.35 g). Shrimp were cultured at salinities of 0, 2, 4, and 30 ppt for 40 d at 25 C, in individual 360-mL and 6-L containers (N = 7/treatment). There was no postlarval survival at < 2 ppt. Postlarval survival at 4 ppt (86%) was not significantly different (P > 0.05) from 30 ppt (100%). Juveniles exhibited better survival at lower salinities (100% at 2 ppt) than 0.05 and 0.35 g postlarvae (29% and 14% respectively, at 2 ppt). The effects of salinity on growth varied with size/age. Final growth of 0.05 g postlarvae at 2 ppt (693%) was significantly less (P < 0.01) than at 4 ppt (1085%) and 30 ppt (1064%). Growth of 0.35 g postlarvae was significantly less (P < 0.01) for 4 ppt (175%) than for 30 ppt (264%). There was no growth data for juveniles (8 g). It appears from these experiments that the culture of *L. vannamei* poses risks when performed in salinities less than 2 ppt.

The life cycle of most penaeid species is well known; generally maturing and spawning take place in oceanic water (Williams 1984; Treece and Yates 1993). The larvae migrate into lower salinity estuarine environments where they metamorphose into postlarvae (Gunter et al. 1964). Consequently, penaeid shrimp are subjected to a variety of salinities, which varies at different life stages. Larger postlarvae, in conjunction with their movement into estuarine environments, are attracted to lower salinities (Mair 1980). However, salinity tolerance varies with species. *Litopenaeus vannamei* postlarvae were found to prefer lower salinities (1–8 ppt) than *P. californiensis* (9–26 ppt) or *P. brevirostris* (15–23 ppt) (Mair 1980). *Penaeus setiferus* has been shown to tolerate salinities of 1–40 ppt (Gunter and Hildebrand 1954). The brown shrimp *Penaeus aztecus* is found at salinities ranging from 0.5–13 ppt (Herke et al. 1987).

Although a species may be found at both extremely low and high salinities, this does not mean that the species can achieve maximum growth and survive in such environments. For example, *L. setiferus* tolerates a wide range (1–40 ppt) of salinity (Gunter and Hildebrand 1954), but survival is greatest between 15 and 25 ppt and growth increases with increasing salinity (Rosas et al. 1999). Likewise *P. chinensis* larvae survive at 10 ppt, with best survival occurring at 30–40 ppt and maximum growth at 20 ppt (Chen et al. 1996).

Salinity tolerance has also been shown to vary with the size or age of shrimp among a wide variety of species (Mair 1980; Charmantier et al. 1988; Ogle et al. 1992; Kumlu and Jones 1995; Tsuzuki et al. 2000). However, few studies have compared shrimp that varied substantially in age though Tsuzuki et al. (2000) compared *Farfantepenaeus paulensis* postlarvae that ranged from PL10–PL80.

Litopenaeus vannamei is the species of choice in shrimp aquaculture in the Western Hemisphere (Rosenberry 1998). The expense and scarcity of coastal areas in North America has made the establishment of saltwater ponds or flow-through shrimp operations economically risky (Van Wyk et al. 1999). Research has focused on the possibilities of raising these shrimp in low salinity or near-fresh water recirculating systems (Samocha et al. 1998; Van Wyk et al. 1999).

Few studies have focused on growth and survival of *L. vannamei* at very low salinities (Ogle et al. 1992; Bray et al. 1994; Samocha et al. 1998). The aim of this study was to determine if there was a difference in survival and growth of *L. vannamei* at very low salinities (<5 ppt) compared to that of sea water (30 ppt) and to determine whether these differences changed with the size (or age) of shrimp.

Materials and Methods

Experimental Design

Four separate experiments were conducted in the Harbor Branch Oceanographic Institution (HBOI) research laboratory. Controls for each experiment included HBOI fresh well water (chloride concentration of ~ 300 ppm) and HBOI salt well water (28–30 ppt). Fresh water was filtered (35 μm). Salt water was passed through a series of cartridge filters (25–1 μm) prior to UV treatment. HBOI fresh water was added to this salt water to make salinities of 0.5, 1, 1.5, 2, 3, or 4 ppt.

Previous studies had indicated that *L. vannamei* survived as well at 5 ppt as at 30 ppt (Ogle et al. 1992; Bray et al. 1994; Samocha et al. 1998). The range of salinities in this study were chosen to determine at what point below 5 ppt *L. vannamei* survival would begin to decline. Shrimp were acclimated to each salinity over a 5-d period prior to the start of the experiment by exchanging half, or slightly more, of the volume of the original salt water with fresh well water each day until the appropriate salinity was reached for each experimental group.

Pacific white shrimp *Litopenaeus vannamei* used in all trials were obtained from the HBOI shrimp facility. Postlarval shrimp (0.05–0.35 g) were individually housed in 360-mL styrofoam cups with plastic lids to which approximately 200 mL of water was added. Juvenile shrimp (~8 g) were individually housed in 6-L styrofoam containers to which 2 L of water was added. Additional aeration was not supplied. Shrimp were fed once a day with a pelleted diet containing 42% protein formulated by Bonney, Laramore and Hopkins, Inc. (Ft. Pierce, Florida, USA). Feces and uneaten food were removed each day prior to an 80% water exchange. Molting was recorded, as were observations on the health and physiological conditions, for each individual.

Initially and weekly thereafter, shrimp were individually blotted dry and weighed to 0.0001 g on a Mettler AE50 electronic balance (Mettler Instrument Corp., Hightown, New Jersey, USA). Concurrently, containers were cleaned to remove the developing biofilm and 100% of the water was exchanged.

Experiments 1–3

The first set of experiments (1–3) was undertaken to determine the minimum level of salinity in which late stage postlarvae (~PL-30) could survive and grow. Postlarvae averaging 0.106 ± 0.025 g (SD, N =

95) were used. Ten PL's were used in each treatment group in the first two experiments, whereas only five PL's per treatment group were used in the third experiment. All trials were conducted in an incubator at 30 C.

The first experiment compared salinities of 0.5 and 1 ppt to fresh and salt water controls. The second experiment examined the effects of 1.5 ppt compared to the controls. Based on those results, the third experiment compared 1, 2, and 3 ppt to fresh and salt water controls. The first two experiments were run for a period of 18 d and were terminated when shrimp in all treatment groups had died except for the salt water controls. The third experiment was conducted for a period of 40 d.

Experiment 4

Based on the results of the first three experiments, a fourth experiment was designed to assess the effects of size/age on growth and survival. Seven shrimp were used for each treatment group. Three sizes of shrimp were examined: postlarvae (~PL-25) averaging 0.048 ± 0.003 g (SD, N = 28), postlarvae (~PL-40) averaging 0.346 ± 0.042 g (SD, N = 28), and juveniles averaging 7.87 ± 0.605 g (SD, N = 28). These shrimp were cultured in HBOI fresh well water, 2 ppt, 4 ppt, or HBOI salt well water. Solutions were prepared as in the first three experiments. Water (150 mL) was added to containers that housed the 0.05-g postlarvae, while 200 mL was added to the containers that housed the 0.35-g postlarvae. Juveniles were maintained in 2,000 mL of water. Procedures were the same as in the first three experiments, except that containers were kept at room temperature (25 C) due to the lack of incubator space needed to accommodate the larger containers.

The experiment was run for 40 d. Temperature and dissolved oxygen (DO) were monitored twice a week using a YSI model 55 dissolved oxygen meter (YSI Inc., Yellow Springs, Ohio, USA). Temperatures ranged from 25.3 C in the large containers to 26.3 C in the small containers. DO readings averaged 6.4 mg/L in the small containers and 5.6 mg/L in the large containers. Salinity, ammonia, nitrite, nitrate and pH were measured once a week using a Hach DR3 spectrophotometer and pH meter model 4180002 (Hach Company, Loveland, Colorado, USA). Except for salinity, all parameters were measured prior to water exchange. Salinities were kept constant among treatment groups.

All treatment groups and container sizes had a similar pH value which ranged from 7.00–7.25. Total ammonia-nitrogen (TAN) varied with size/age. The average TAN was 0.91 mg/L (0.008 un-ionized ammonia or UIA) for the smallest PL's (0.05 g), 1.89 mg/L (0.011 UIA) for the larger PL's (0.35 g), and 2.3 mg/L (0.013 UIA) for the juveniles (8 g). Nitrite-N did not vary between treatments and ranged from 0.17–0.2 mg/L. Average nitrate-N was 1.3 mg/L for the 0.05-g PL's, 1.7 mg/L for the 0.035-g PL's, and 1.25 mg/L for the 8-g juveniles.

Statistics

Mean percentage survival and growth were calculated on a weekly basis. In the first three experiments, data were analyzed using one-way ANOVA (Underwood 1997). Two-way ANOVA was used to test the effects of both size and salinity on growth and survival in the fourth experiment (Underwood 1997). Arc-sin transformations of percentages were performed before survival data were analyzed, whereas square root transformations of percentages were performed before growth data were analyzed (Underwood 1997). Results were considered to be significant if $P < 0.05$. When needed, comparison of means was made using the Student-Newman-Keuls (SNK) multiple range test (Underwood 1997).

Results

Survival

Most shrimp were unable to survive in salinities less than 2 ppt (Tables 1–4). In

TABLE 1. *Mean ± SEM (N = 10) survival of postlarvae (0.1 g) cultured for 18 d at different salinities at 30 C (Exp 1). Means in the same column having different letters are significantly different (P < 0.05).*

Salinity (ppt)	Survival (%)
0	0 b
0.5	0 b
1	0 b
30	90 ± 2.9 a

TABLE 3. *Mean ± SEM (N = 5) survival of postlarvae (0.1 g) cultured at different salinities for 40 d at 30 C (Exp 3). Means in the same column having different letters are significantly different (P < 0.05).*

Salinity (ppt)	Survival (% 18 d)	Survival (% 40 d)
0	0 c	0 b
1	0 c	0 b
2	40 ± 3.3 b	20 ± 2.9 b
3	60 ± 3.3 ab	40 ± 3.3 ab
30	100 a	80 ± 2.9 a

most trials this was evident before the termination point (Tables 3, 4). A size effect was evident (Table 4), as supported by the fact that juveniles exhibited better survival (100% and 14%) at lower salinities (2 ppt and 0 ppt, respectively) compared to postlarvae (2 ppt: 14%, 29%; 0 ppt: 0%) after 40 d. All experiments showed that percent survival decreased significantly when shrimp were maintained in salinities of 2 ppt or less (Tables 1–4).

The fourth experiment examined the effects of both size and salinity on survival. A significant salinity effect was seen at day 18 when shrimp in 2, 4 or 30 ppt, regardless of size, had a significantly higher ($P < 0.05$) survival (100%) than those at 0 ppt (14%, 43%, and 57% respectively). By the end of the experiment a significant lower survival ($P < 0.05\%$) was apparent between 2 ppt and both 4 and 30 ppt (Table 4). A significant interaction between size and salinity was seen by day 40, with fewer postlarvae surviving at 2 ppt (29% and 14%) compared to 100% survival for juveniles (Table 4).

TABLE 2. *Mean ± SEM (N = 10) survival of postlarvae (0.1 g) cultured for 18 d at different salinities at 30 C (Exp 2). Means in the same column having different letters are significantly different (P < 0.05).*

Salinity (ppt)	Survival (%)
0	10 ± 2.9 b
1.5	0 b
30	90 ± 2.9 a

Growth

No difference in growth was seen within the first 18 d in any of the four experiments. Growth differences were seen in the two experiments (3 and 4) that were run for 40 d. Both size and salinity had a significant effect on growth.

Experiment 3 (Fig. 1) showed that by day 21, shrimp at 2 ppt had a significantly ($P < 0.05$) lower growth rate (170%) than at 3 and 30 ppt (233 and 230% respectively). By day 35 shrimp maintained at 2 ppt had a significantly lower growth rate (280%) than at 3 ppt (387%) and both were significantly lower than those held at 30 ppt (490%) ($P < 0.001$). At experimental termination (40 d), growth at both 2 and 3 ppt

TABLE 4. *Mean ± SEM (N = 7) survival of postlarvae and juveniles (0.05, 0.3, 8 g) cultured at different salinities for 40 d at 25 C (Exp 4). Means in the same column having different letters are significantly different (P < 0.05).*

Size (g)	Salinity (ppt)	Survival (% 18 d)	Survival (% 40 d)
0.05		57 ± 2.7	0 c
0.3	0	43 ± 2.7	0 c
8		14 ± 2.9	14 ± 2.9 bc
0.05		100	29 ± 2.6 b
0.3	2	100	14 ± 2.3 bc
8		100	100 a
0.05		100	86 ± 2.3 a
0.3	4	100	86 ± 2.3 a
8		100	86 ± 2.9 a
0.05		100	100 a
0.3	30	100	100 a
8		100	100 a

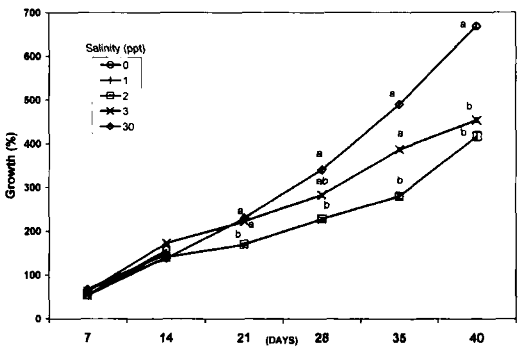

FIGURE 1. *Mean ± SEM* (N = *5) growth of* Litopenaeus vannamei *(0.1 g) at various salinities (Experiment 3). A significant difference was evident at week 3 between 2 and 30 ppt and at week 6 between 3 and 30 ppt* (P < *0.05). Values with separate letters are considered significantly different.*

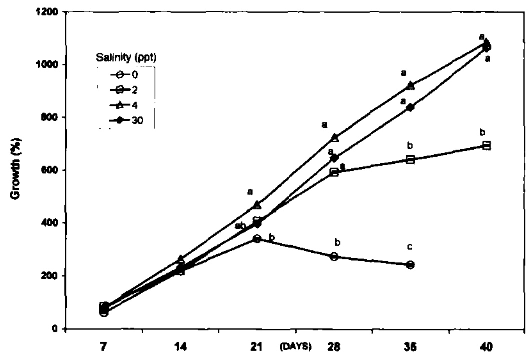

FIGURE 2. *Mean ± SEM* (N = *7) growth of* Litopenaeus vannamei *(0.05 g) at various salinities (Experiment 4). A significant difference in growth was seen in week 5 between 2 ppt compared to 4 and 30 ppt* (P < *0.01). Values with separate letters are considered significantly different.*

remained significantly lower ($P < 0.01$) than at 30 ppt, and growth at 3 ppt was no longer significantly different from growth at 2 ppt.

In experiment 4 (Figs. 2, 3) no growth occurred throughout the course of the experiment in any of the juvenile shrimp (8 g) regardless of salinity. Therefore, growth rates were calculated only for 0.05-g and 0.35-g postlarvae. Both size and salinity, as well as the interaction between the two, had a significant effect ($P < 0.001$) from day 21 to day 40. Smaller postlarvae (Fig. 2) exhibited greater growth than larger postlarvae (Fig. 3). At day 28, growth was significantly lower ($P<0.05$) in 0 ppt than in 2, 4, or 30 ppt for all postlarvae. By day 28 (0.05 g) and by day 35 (0.35 g) shrimp in 0 and 2 ppt had a significantly ($P < 0.05$) lower growth rate than 4 or 30 ppt. At experimental termination (day 40), 0.05-g postlarvae in 2 ppt were significantly ($P < 0.01$) smaller than shrimp in 4 and 30 ppt (Fig. 2). All but one 0.35 g postlarvae in 2 ppt had died (growth data not included), and those in 4 ppt were significantly smaller ($P < 0.01$) than in 30 ppt (Fig. 3).

Discussion

Although this study uses size (0.05, 0.1, 0.35, and 8 g) rather than age to assess the effect of salinity on both survival and growth, the approximate ages of the PL's were known (PL 25, 30, and 45). It was determined that comparisons could be made between this study and other studies that focus on either size (Ogle et al. 1992) or age (Bray et al. 1994; Samocha et al. 1998). In aquaculture, where environmental conditions are kept more constant than in nature, age and size are correlated to a greater degree and are often used interchangeably.

Few studies have focused on growth and survival of *L. vannamei* at low salinities.

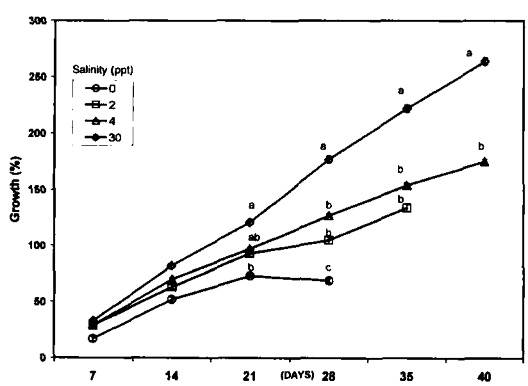

FIGURE 3. *Mean ± SEM* (N = *7) growth of* Litopenaeus vannamei *(0.35 g) at various salinities (Experiment 4). A significant decrease in growth was seen by week 5 between 2 ppt compared to 4 and 30 ppt* (P < *0.01). Values with separate letters are considered significantly different.*

Bray et al. (1994) found no difference in survival rates of 2-g juveniles between 5 and 40 ppt. Samocha et al. (1998) found no difference in growth or survival of 2-g juveniles between 2 and 8 ppt. Ogle et al. (1992) found no difference in growth of 22-d-old postlarvae between 2 and 16 ppt, but did see a decrease in survival. None of these studies directly compared postlarvae with juvenile shrimp.

This study showed that *Litopenaeus vannamei* postlarvae and juveniles can be successfully cultured at 4 ppt. No differences in growth and survival were seen between 4 and 30 ppt. Salinities below 4 ppt negatively affected both postlarval survival and growth. In contrast, juveniles were able to survive as well at 2 ppt as at 30 ppt. Previously published studies confirm these results.

Ogle et al. (1992) assessed the effects of salinity on survival of 22-d-old *L. vannamei* postlarvae over 30 d. No significant differences were found in survival between 4 and 16 ppt, although a significant difference was seen between 2 and 16 ppt. In the first three experiments of the present study, postlarvae in both 2 and 3 ppt exhibited significantly lower survival than at 30 ppt after 18 d. In the fourth experiment similar differences were noted between 2 and 30 ppt, but not between 4 and 30 ppt after 40 d.

Bray et al. (1994) compared survival in *L. vannamei* juveniles (2 g) and found no difference in survival between 5 and 35 ppt, over 35 d. Samocha et al. (1998) found no difference in survival between 2, 4, and 8 ppt over 70 d with *L. vannamei* juveniles (2 g). Results of the present study, in which no difference in survival was seen between 2, 4, and 30 ppt for juveniles (8 g) over 40 d, are consistent with these studies. It would appear then that at some point between the postlarval and juvenile stages (0.3–2 g), *L. vannamei* gain the ability to tolerate lower salinities, probably due to enhanced osmoregulatory capabilities as has been reported for other decapod crustaceans (Castille and Lawrence 1981; Charmantier et al. 1988).

Growth was size/age, as well as salinity, dependent. Smaller (0.05 g) postlarvae (PL-25) had a significantly faster rate of growth than larger (0.35 g) postlarvae (PL-40). Postlarvae often increase their rate of growth by 100% each week under laboratory conditions and even higher in ponds (Laramore, unpublished data). That no growth was observed in 8-g animals at any salinity tested points to a flaw in the experimental system. Apparently the containers used were too small to permit growth of these juveniles. Later experiments confirmed that shrimp larger than 4 g do not exhibit good growth in these containers (data not shown).

In contrast to the present study, Ogle et al. (1992) did not find any significant difference in growth of postlarvae between 2 and 16 ppt. However the experiment lasted only 4 wk. In this study, a difference in growth was not observed between 2 and 30 ppt until the third or fourth week. Samocha et al. (1998) also found no difference in growth rates between 2, 4, or 8 ppt, but worked with 2-g juveniles rather than postlarvae. Growth data was not obtained from juveniles in this study, therefore no comparisons can be made. Growth at low salinities was not compared to growth in salt water in either of the previous studies. Bray et al. (1994) found significantly better growth in juveniles at 5 and 15 ppt than at 25 and 35 ppt, but did not test lower salinities. Results of the present study showed significantly lower growth in 2 and 3 ppt than in 30 ppt. Greater growth was also seen at 4 compared to 2 ppt. These results were obtained for all postlarvae (0.05, 0.1, and 0.35 g) tested.

Experiments cited in this discussion differ in design including temperature, salinity, size/age, and duration. Previous experiments used either tanks (Bray et al. 1994; Samocha et al. 1998) or aquaria (Ogle et al. 1992) maintained at 27–30 C. Although experiments 1–3 in the present study were run at 30 C, experiment 4 was performed at room temperature (25–26 C). Temperature, rather than salinity, has been shown to have a significantly greater effect on growth in penaeid shrimp (Herke et al. 1987; Staples and Heales 1991; O'Brien 1994; Tsuzuki et al. 2000) and may partly account for the differences seen between experiments. Other factors, such as size/age, housing and experimental duration may also explain these differences.

It should be noted that shrimp have been successfully cultivated at lower salinities in commercial operations. Ponds in Central and South America are often subjected to heavy rainfall and fresh water influx that decrease the salinity to less than 2 ppt (Teichert-Coddington et al. 1995). Shrimp have been cultivated in commercial operations (A. Duda and Sons, Inc. Cocoa, Florida, USA; Indian River Aquaculture, Vero Beach, Florida, USA) and at HBOI (Van Wyk et al. 1999) at salinities as low as 0.5 ppt.

In conclusion, *L. vannamei* postlarvae grow and survive as well in 4 ppt as in 30 ppt. Juvenile shrimp survive in lower salinities (2 ppt) and can conceivably grow at that salinity, although no supporting data were obtained in the present study. It also appears that some shrimp, no matter what age, are simply more tolerant of low salinities. In the second experiment, one postlarva was still surviving in 0 ppt when the experiment was terminated. In both the third and fourth experiments, one postlarva survived in 0 ppt through week 5. In experiment 4, one juvenile survived in fresh water until experimental termination. It is possible that the variation seen may have a genetic basis. This has implications for the selection of low salinity tolerant shrimp.

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