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John T. Ogle
Gulf Coast Research Laboratory

Kathy Beaugez
Gulf Coast Research Laboratory

Jeffrey M. Lotz
Gulf Coast Research Laboratory, jeff.lotz@usm.edu

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EFFECTS OF SALINITY ON SURVIVAL AND GROWTH OF POSTLARVAL *PENAEUS VANNAMEI*

JOHN T. OGLE, KATHY BEAUGEZ, AND JEFFREY M. LOTZ
Gulf Coast Research Laboratory, P. O. Box 7000, Ocean Springs, Mississippi 39564

ABSTRACT Eight and 22-day-old *Penaeus vannamei* postlarvae were exposed to several salinities for 24 hours and 120 hours by direct transfer from 32 ppt salinity to lower salinity waters. The challenge study included six experiments conducted on 8-day-old postlarvae (PL-8) and five experiments conducted on 22-day-old postlarvae (PL-22). Each experiment consisted of ten replicates of ten animals each. Shrimp were held in 1 L plastic containers with 500-ml of seawater. Lowered salinity resulted in lower survival for shrimp of both ages. Longer exposure time resulted in lower survival for shrimp of both ages. Younger shrimp exhibited lower survival than older shrimp. Survival of 8-day-old postlarvae after 24-hour exposure to salinities of 32, 16, 8, 4, and 2 ppt was 97.3%, 92.8%, 19.8%, 8.2% and 1.7%, respectively. Survival of 22-day-old postlarvae after 24-hour exposure was 99.2%, 97.8%, 83.8%, 63.4% and 40.2%, respectively. A second series of experiments investigated the effect of salinity upon growth of 22-day-old postlarvae which had been acclimated to four different salinities (16, 8, 4, and 2 ppt). Thirty shrimp were stocked in triplicate into 113 L (30 gal) aquaria and fed a prepared commercial feed. Growth was determined after 30 days at 16°C and 28-30°C. Growth was greatest at higher temperatures, but statistically significant differences due to salinity were not detectable. Nonetheless, best observed growth occurred at the intermediate salinities of 8 and 4 ppt.

Introduction

Shrimp of the genus *Penaeus* encounter a wide variety of salinities throughout their life cycle. Most penaeid species mature and reproduce in high salinity open ocean waters. Subsequently, the larvae migrate into lower salinity estuarine nursery areas where they metamorphose into postlarvae and grow rapidly (Gunter *et al.* 1964). Juveniles of *Penaeus aztecus* have been captured in Gulf of Mexico waters of 0.80 ppt salinity, and juveniles of *P. fluviatilis* (= *P. setiferus*) have been found in salinities as low as 0.42 ppt (Gunter and Shell 1958). At the other extreme, juvenile *P. setiferus* have been captured in water with salinities as high as 43.3 ppt (Gunter 1961) and *P. brasiliensis* have been captured in waters ranging from 40-60 ppt (Chung 1980).

Although young shrimp tolerate a wide variety of salinities, they may show preferences for salinities within narrower ranges. For example, Chung (1980) found that 80% of the postlarval *P. brasiliensis* which he tested preferred salinities from 5-28 ppt, even though the animals had been captured in a high salinity (40-60 ppt) lagoon. On the other hand, Zein-Eldin (1963) demonstrated that wild-caught *P. aztecus*, *P. duorarum*, and *P. setiferus* postlarvae all had high survival rates and exhibited no differences in growth rate in salinities ranging from 2 to 40 ppt. This led Zein-Eldin to surmise that salinity may be of less importance than other factors in postlarval development.

Penaeus vannamei, the most popular aquacultured shrimp in the Western Hemisphere, has been reported to have the greatest tolerance for low salinity water (1-8 ppt) of four penaeids from west Mexico (Mair 1980). In Ecuador, wild-caught *P. vannamei* postlarvae have been reported to grow in ponds with salinities below 2 ppt by Chauvin (1983) and in 0 ppt salinity by Garston (1986). Wulff (1987) reported culturing this species in Arizona utilizing total freshwater. In laboratory experiments, Olin and Fast (1987) determined that transfer of 5 to 12-day-old postlarval *P. vannamei* from 32 ppt salinity water directly into water of 20 or 36 ppt salinity resulted in survivals of 89% and 99%, respectively.

The only experimental data for *P. vannamei* growth in low salinities is that of Huang (1983). He measured the growth of postlarvae *P. vannamei* after 30 days in waters of 5, 15, 25, 35, and 45 ppt and found that fastest growth occurred at 25 ppt, while poorest growth occurred at 45 ppt.

Studies on the survival and growth of postlarval *P. vannamei* in low salinity water are valuable to shrimp farmers along the Northern Gulf of Mexico. Coastal salinities may be as low as 2 ppt in the spring (Ogle 1989), when postlarvae are generally stocked into ponds. Further, shrimp in ponds are often subjected to rapid salinity drops after rainfall.

In this paper, we report the results of investigations concerning the effect of rapid salinity decreases on the survival of postlarval *P. vannamei*. We also attempt to determine the effect of low salinity on growth of *P. vannamei* after a period of acclimation to low salinity.

MATERIALS AND METHODS

All studies were conducted using artificial sea salts (Instant Ocean) dissolved in tap water. For each experiment, waters of various salinities were prepared from a common stock of seawater with a salinity of 32 ppt. At least three days prior to each experiment, the stock seawater was diluted with fresh tap water to achieve salinities of 16, 8, 4, and 2 ppt. Higher salinities (32 and 16 ppt) were measured with a refractometer, while lower salinities (8, 4 and 2 ppt) were measured with a hydrometer. During each experiment, continuous illumination was provided by six 40-watt fluorescent bulbs.

Five-day-old postlarval shrimp were obtained from two Florida-based commercial hatcheries. Shrimp arrived at 16-18°C in 32-34 ppt seawater. Postlarvae from each shipment were held for three days in 32 ppt seawater and fed brine shrimp nauplii (*Artemia* sp.) three times daily, *ad libitum*, before beginning experiments. Each shipment of shrimp was divided into three groups, one for each of three studies.

SALINITY SHOCK

Study 1: 8-day-old postlarvae

The first group of shrimp from each shipment was challenged when they were 8 days old by direct transfer into waters of one of five salinities (32, 16, 8, 4, or 2 ppt). White 1 L plastic bowls containing 500 ml of seawater were used as experimental chambers. Ten chambers for each salinity (32, 16, 8, 4, and 2 ppt) were utilized. Each chamber was stocked with ten postlarvae. Two hours were required to transfer the postlarvae to the experimental chambers. Chambers were loosely fitted with lids to reduce evaporation. Temperatures were maintained by placing chambers in a constant-temperature water bath.

Experiments were conducted on six separate shipments of 8-day-old postlarvae. Animals were fed live *Artemia* sp. nauplii and freeze-dried calanoid copepods (Kordon microplankton) immediately after being stocked and three times daily thereafter. Dead shrimp were removed daily. Survival of animals in each chamber was recorded at 24 hours and 120 hours.

Study 2: 22-day-old postlarvae

The second group of animals from each shipment was held in water of 32 ppt salinity until they were 22-days-old. The shrimp were then challenged by direct transfer to waters with salinities of 32, 16, 8, 4, and 2 ppt. All procedures employed in Study 1 were followed for the five experiments conducted on 22-day-old postlarvae.

SALINITY ACCLIMATION

Study 3: Survival and growth of 22-day-old post-larvae acclimated to lower salinities

The third group of postlarval shrimp was gradually acclimated to the various salinities prior to the start of the experiments. Shrimp were divided among four 113 L aquaria and acclimated separately to the lower salinities of 16, 8, 4, and 2 ppt. Salinity in each aquarium was lowered at a rate of 2 ppt per day by adding aged tap water. The acclimation regime achieved the desired salinities by the time that the experiment was initiated (Day 0). Acclimation of shrimp to 2 ppt began 14 days prior to stocking, to 16 ppt began seven days before stocking and so on. During the acclimation period, shrimp were gradually weaned from a diet of *Artemia* sp. nauplii and copepods to a Rangen-Zeigler postlarval feed.

These experiments were performed in triplicate in all-glass aquaria containing 113 L of seawater. Each aquarium was stocked with 30 postlarvae. Shrimp were individually weighed to the nearest 0.01 g on an electronic balance. A subsample of 25 postlarvae from each of the salinities was weighed on Day 0 after the acclimation period and prior to the start of each experiment. When the experiments began, postlarvae were less than 0.01 g (wet weight). All surviving shrimp were weighed on Day 30. Total weight gain was used as an indicator of growth because Zein-Eldin (1963) and Raj and Raj (1982), working with postlarval penaeid shrimp, found that weight increased faster than length and was therefore a more sensitive indicator of growth.

Three separate experiments were conducted in this study. The first two experiments were run simultaneously using shrimp from the same shipment. In experiment 1, water temperatures were maintained at 30°C ± 3°C (high temp). In experiment 2, water temperatures were maintained at 16°C ± 2°C (low temp). The third experiment was run at a different time and utilized a different shipment of shrimp than the first two experiments. During experiment 3, water temperatures were maintained at 28°C ± 1°C. Temperatures were controlled by regulating the room temperature or by aquarium heaters. All three experiments were conducted at each of four salinities (16, 8, 4, and 2 ppt).

No substrate was used in the aquaria. Filtration was provided by an external power filter (Dynaflow 600). Previous experiments on 22-day-old postlarvae demonstrated that the postlarvae were too small to handle the currents and would be held against the filter screens during the first week. Death resulted when the shrimp were held against the screens for periods longer than an hour. To prevent loss of postlarvae, the intake pipe of the power filter was screened. Additionally, during the first week of growth the filters were run only an hour a day.

(Use of trade names does not imply endorsement.)

Salinities were checked twice weekly and maintained at the appropriate salinity levels by the addition of freshwater. Salinity fluctuations were less than 1 ppt. Addition of plexiglass lids reduced evaporation and prevented the shrimp from jumping out of the tanks.

DATA ANALYSIS

Survival data were analyzed as percent survival using the Kruskal-Wallis nonparametric analysis of variance. Pairwise comparisons were performed with the Mann-Whitney U-test using Bonferroni's criterion of significance. Differences were considered to be significant if $P < 0.05$.

Growth was determined as final wet weight of shrimp. The analysis of growth as a function of temperature and salinity was analyzed using a two-way nested analysis of variance where the replicated aquaria provided the error term for hypothesis testing. Differences were considered to be significant if $P < 0.05$.

RESULTS

SALINITY SHOCK

Effect of age at exposure on ability to survive a salinity drop

Figure 1 displays the survival of 8-day-old postlarvae (PL-8) and 22-day-old postlarvae (PL-22) of *P. vannamei* at 24 hours and 120 hours after challenge to various lowered salinities. The Kruskal-Wallis test detected significant differences in overall survival (combined 24 hours and 120 hours) between PL-8 and PL-22. Mann-Whitney U-tests at each salinity revealed that overall survival (combined 24 hours and 120 hours) was significantly lower for eight-day-old postlarvae than for 22-day-old postlarvae at 16, 8, 4, and 2 ppt salinity. No differences in survival due to age were detected at the initial salinity of 32 ppt.

Effect of salinity challenge on survival of 8-day-old postlarvae

Survival of 8-day-old postlarvae after 24 hours exposure to salinities of 32, 16, 8, 4, and 2 ppt was 97.3%, 92.8%, 19.8%, 8.2%, and 1.7%, respectively. The Kruskal-Wallis test detected significant differences in overall survival due to salinity. There was no significant difference (Mann-Whitney U-test) in survival percentage between PL-8s transferred to 32 ppt (control) and those transferred to 16 ppt (Figure 2). There was no significant difference in survival between PL-8s exposed to 4 and 2 ppt. Survival was significantly higher for PL-8s transferred to 8 ppt than those transferred to lower salinities. There was a significant reduction in survival between animals exposed to 16 ppt and those exposed to 8 ppt. This trend was obvious in both the 24-hour and 120-hour experiments.

Figure 2 reveals that although the observed survival rates were lower at 120 hours than at 24 hours, the differences were statistically significant by the Mann-Whitney U-test only at the higher salinities (32 ppt and 16 ppt).

Effect of salinity challenge on survival of 22-day-old postlarvae

Survival of 22-day-old postlarvae after 24 hours exposure to 32, 16, 8, 4, and 2 ppt was 99.2%, 97.8%, 83.8%, 63.4%, and 40.2%, respectively. Figure 3 exhibits the effect of salinity challenge on 22-day-old postlarval survival. The Kruskal-Wallis test detected significant differences in overall survival due to salinity. Significantly lower survival was detected for animals transferred from 32 ppt to 4 and 2 ppt salinity (Mann-Whitney U-test). Survival of animals exposed to 4 ppt was lower than survival of animals exposed to 8 ppt. Survival was also lower for animals exposed to 8 ppt than for animals exposed to 16 ppt. There was no significant difference in survival percentage between PL-22s transferred to 32 ppt (control) and those transferred to 16 ppt.

Survival of postlarvae after 24 hours exposure was generally higher at all salinities than after 120 hours exposure. Statistically significant differences for time of exposure are also indicated on Figure 3.

SALINITY ACCLIMATION

Effect of acclimation to lowered salinity on survival of 22-day-old postlarvae

Figure 4 compares the survival of shrimp acclimated to lower salinities at two temperatures. The Kruskal-Wallis test failed to detect differences in survival due to salinity at a temperature of 16°C. However, differences were detected at the higher temperatures where shrimp displayed a greater sensitivity to salinity (Mann-Whitney U-tests detected lower survival at 2 ppt than at 16 ppt).

Effect of salinity acclimation and temperature on growth rate

Postlarval *P. vannamei* grew fastest at higher temperatures (Figure 5). The two-way nested analysis of variance detected greater final weights for shrimp reared at 30°C than those at 16°C. Animals maintained at 28-30°C were nearly twice as large as those grown at 16°C.

There were no statistically detectable differences in growth among shrimp maintained at the four salinities. However, the greatest final weights were attained in the intermediate salinities (8 and 4 ppt) at both temperatures.

Interestingly, there were large differences between shipments of shrimp in overall performance. Animals in experiment 2 (30°C) reached 1 g in both 8 ppt and 4 ppt

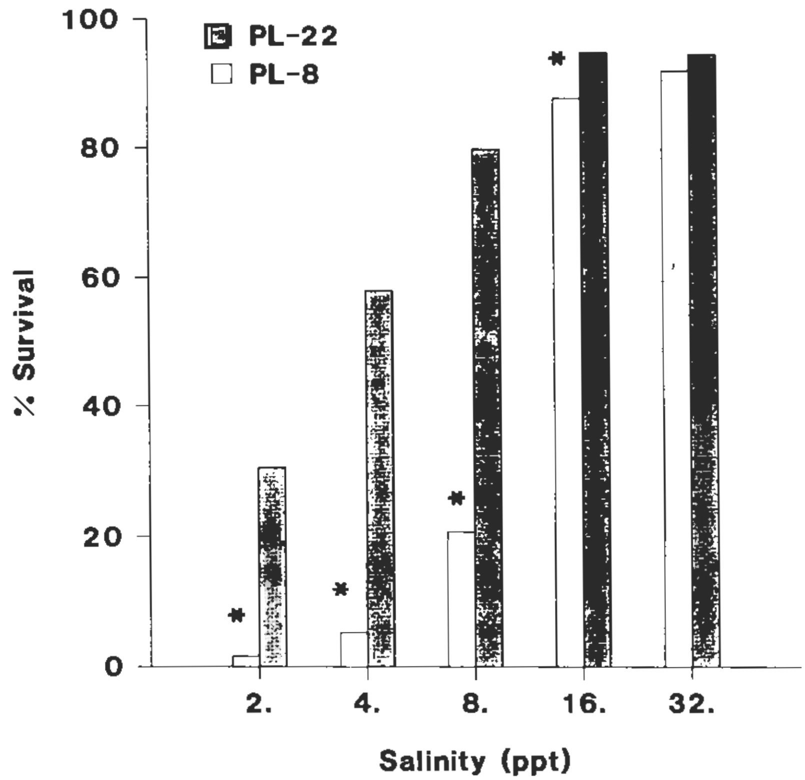

Figure 1. Survival of salinity shock by 8-day-old and 22-day-old postlarval *Penaeus vannamei*. Asterisks denote statistically significant differences in survival between shrimp of the two ages.

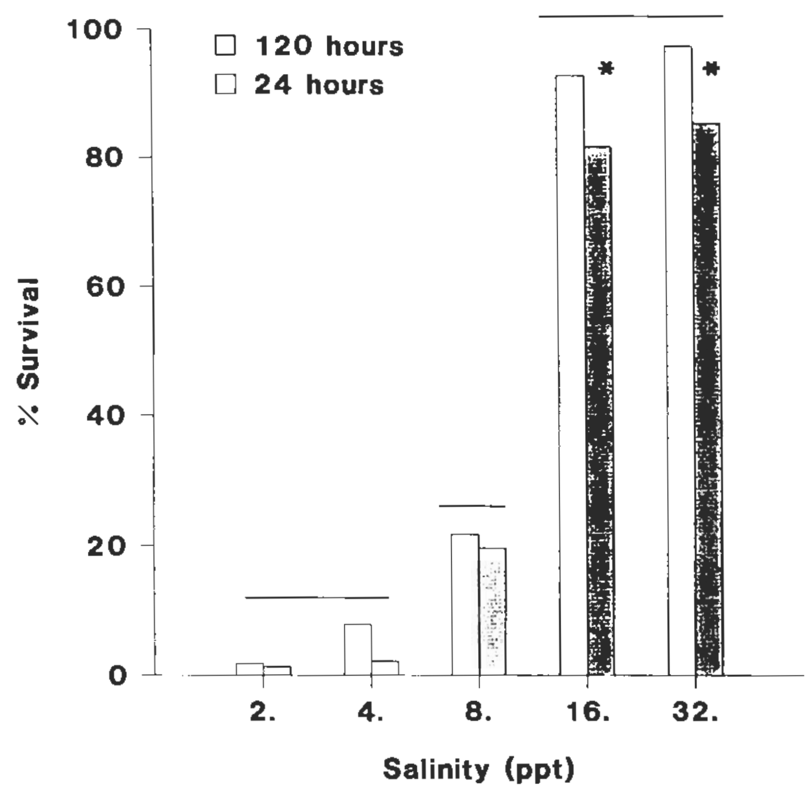

Figure 2. Survival of salinity shock by 8-day-old postlarval *Penaeus vannamei* for 24 and 120 hour exposures. Salinities sharing a line are not statistically different from one another. Asterisks denote statistically significant differences in survival between shrimp exposed for 24 hours or 120 hours at each salinity.

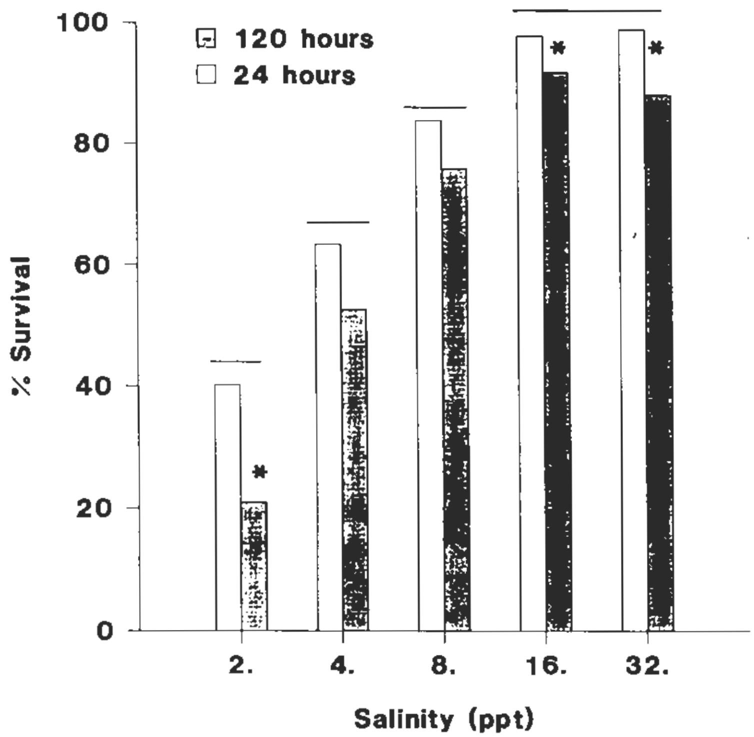

Figure 3. Survival of salinity shock by 22-day-old postlarval *Penaeus vannamei* for 24 hour and 120 hour exposures. Salinities sharing a line are not statistically different from one another. Asterisks denote statistically significant differences in survival between shrimp exposed for 24 hours or 120 hours at each salinity.

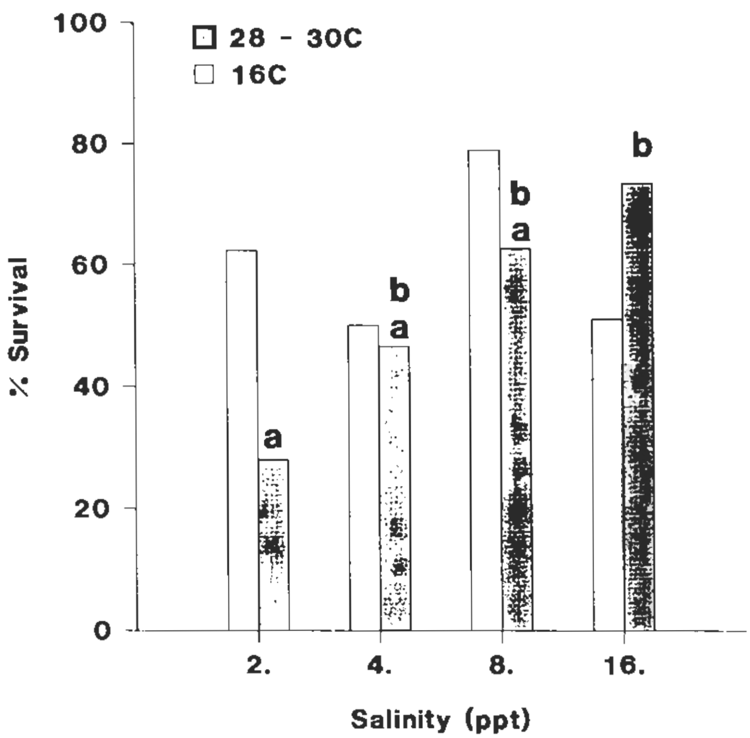

Figure 4. Effects of temperature on survival of 22-day-old postlarval *Penaeus vannamei* acclimated to low salinity. There are no statistically significant differences among salinities for shrimp maintained at 16°C. For shrimp maintained at 28°C and 30°C, salinities sharing a letter are not statistically different from each other.

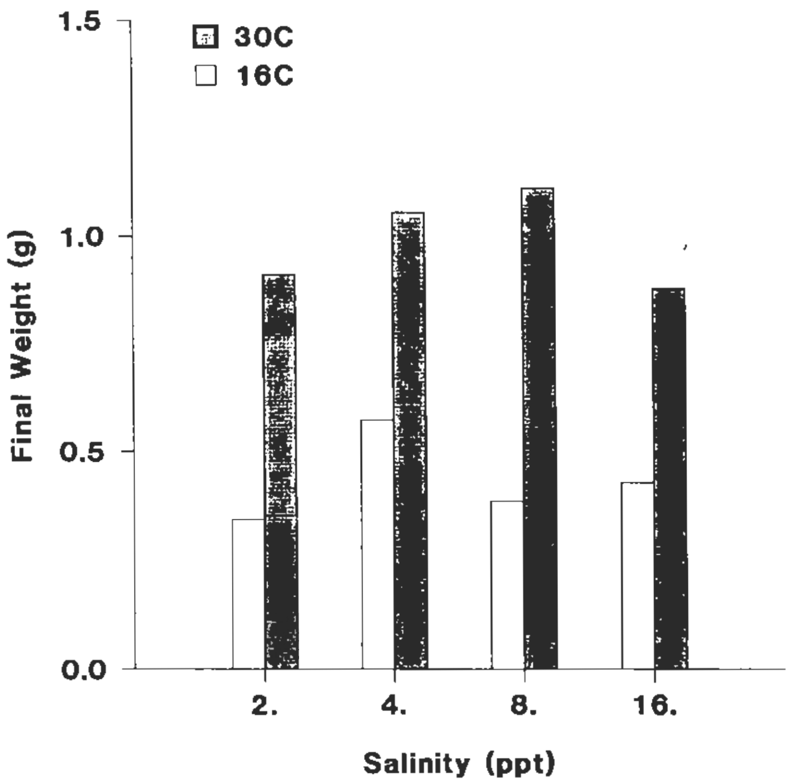

Figure 5. Final weights of *Penaeus vannamei* grown for 30 days at two temperatures. There is a statistically significant difference between the temperatures, but there are no significant differences due to salinity at either temperature.

seawater. Animals grown in both 16 ppt and 2 ppt seawater at 30°C failed to attain a size of 1 g. Animals from a different shipment of shrimp grown at 28°C demonstrated negligible growth at any salinity and therefore were excluded from the growth rate analysis.

DISCUSSION

Our results on the tolerance of postlarval *P. vannamei* to rapidly-lowered salinities are consistent with other studies of penaeids. Zein-Eldin and Aldrich (1965) report lower survival of *P. aztecus* when exposed to reduced salinities for more than 24 hours. Biesiot (1975) noted that 22-day-old *P. aztecus* were more tolerant to challenge by low salinity than 10-day-old postlarvae. In studies on four penaeid species, Mair (1980) found that as shrimp became older and increased in size, their salinity preferences altered and they were apparently able to adjust more readily to lower salinities.

In our studies, postlarvae of *P. vannamei* had greater observed survival at 24 hours than at 120 hours. The differences in survival at a particular age which we noted between 24 hours and 120 hours are due to time of exposure. The survival rate (deaths per hour) is no different at 24 hours than at 120 hours, even though the total number of deaths was greater at 120 hours for each salinity and age.

There was a great deal of variability in survival among the different shipments of postlarvae tested. Ability of postlarvae to withstand stresses is known to vary considerably and is apparently correlated with overall performance in aquaculture settings. In fact, postlarvae collected from full seawater in the wild in Ecuador which are destined for shrimp culture ponds are subjected to a three-minute brackish water stress test bath (15 ppt) to assess their vigor (Maugle, personal communication). A simple salinity stress test was used by Tackaert et al. (1989) to evaluate nutritional differences for three species of penaeids including *P. vannamei*.

Although we were unable to detect statistically significant differences in growth rate attributable to salinity, the greatest observed growth rates occurred at 8 and 4 ppt salinities. In a commercial setting, *P. vannamei* stocked into nursery ponds are expected to achieve a size of 1 g in 30 to 50 days (Shleser and Follett 1984). The growth of shrimp at 30°C in this study was acceptable by this criterion at salinities of 8 and 4 ppt. Growth at 16°C was below this criterion at all salinities. Zein-Eldin and Griffith (1969) have shown that *P. aztecus* and *P. setiferus* postlarvae were adversely affected by low salinities when held at low temperatures. Sturmer and Lawrence (1989) found that a combination of high salinity and high temperature had an adverse effect on production of *P. vannamei* postlarvae.

As with survival, growth of *P. vannamei* under laboratory conditions is highly variable and often unpredictable (Ogle 1992). The lack of growth in the group of shrimp held at 28°C was probably not due to temperature, but indicative of the variability of the animals. Olin and Fast (1989) tested *P. vannamei* and *P. monodon* postlarvae in acclimation studies. When different groups of postlarvae were tested under the same conditions, there appeared to be significant differences in growth among the groups. This points out the critical need for using as many replicates as possible when designing and performing experiments on shrimp growth and survival.

Even though greatest growth was found in waters with salinities of 8 and 4 ppt, percentage survival was better at 16 ppt. We feel that postlarval shrimp would have a higher survival rate if stocked into lower salinity ponds (between 16 and 8 ppt) as PL-22s rather than PL-8s. We do not know if the additional cost of holding the shrimp for the added 12 days would offset the cost of additional postlarvae.

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