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Acclimation of *Litopenaeus vannamei* Postlarvae to Low Salinity: Influence of Age, Salinity Endpoint, and Rate of Salinity Reduction

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Abstract.—Inland culture of *Litopenaeus vannamei* in low salinity well waters is currently conducted on a small scale in a few areas in the U.S. To successfully rear shrimp in low salinity water, postlarvae (PL) must be transferred from high-salinity larval rearing systems to low-salinity growout conditions. To determine effective transfer methods, a series of experiments were conducted under controlled conditions to evaluate the influence of PL age, rate of acclimation, and salinity endpoint on 48 h survival of shrimp. Three age classes of *L. vannamei* PL (10, 15, and 20-d) were acclimated from a salinity of 23 ppt to treatment endpoint salinities of 0, 1, 2, 4, 8, and 12 ppt. Survival of PL_{10} acclimated to 0, 1, or 2 ppt salinity was significantly lower than survival of PL acclimated to salinities of 4, 8, and 12 ppt. Survival of PL_{15} and PL_{20} shrimp was only reduced for the 0 ppt salinity treatment, thus indicating a clear effect of age on salinity tolerance. The same age classes of PL were acclimated from 23 ppt to final salinity endpoints of 1 or 4 ppt at three different rates of salinity reduction: low, 19%/h; medium, 25%/h, and high, 47%/h. Survival was not significantly influenced by the acclimation rates for any of the three PL age classes. As in the fixed rate experiments, survival of the 10-d-old PL was significantly lower for shrimp acclimated to the 1 ppt endpoint compared to the 4 ppt endpoint. Under the reported conditions, age appears to influence PL tolerance to a salinity endpoint. A 10-d-old PL can be acclimated to 4 ppt with good survival, whereas 15- and 20-d-old PL can be acclimated to a salinity of 1 ppt with good survivals.

Inland production of shrimp using water from saline aquifers is providing an alternative to traditional coastal aquaculture and a diversification of agriculture. Currently, shrimp are being farmed in low salinity water pumped from aquifers in states such as Alabama, Arizona, Florida, and Texas. Information from these farms is largely anecdotal but indicates that adequate growth rates and acceptable production of *Litopenaeus vannamei* is possible (Scarpa and Vaughn 1998; Samocha et al. 1999; Rosenberry 1999). Samocha et al. (1998b) observed that *L. vannamei* growth did not differ in culture systems with 2, 4, or 8 ppt saline water.

Experience has demonstrated that marine shrimp culture in low-salinity well water is technically feasible, but early survival is highly dependent on acclimation to the low salinity water. Most recommendations for acclimation procedures have been developed for acclimation to coastal pond waters, (e.g., Villalon 1991) and are often based on practical experience and not stepwise investigations. Additional information is available from stock enhancement and ecology studies that discuss abrupt transfers of shrimp from full strength seawater directly into waters of various salinities (Parado-Estepa et al. 1987; Chen and Lin 1994; Kumlu and Jones 1995; Aquacop 1991; Rosas et al. 1999).

Of the commercial species currently cultured in the Western hemisphere, *L. vannamei* is probably one of the best adapted species for culture in waters of low salinity. Keiser and Aldrich (1976) indicated that native species such as *Litopenaeus setiferus* and *Farfantepenaeus aztecus* do not tolerate low salinity (< 2 ppt) environments. Mair (1980) stated that out of four species endemic to the west coast of Mexico, *L. vannamei* is the most tolerant to low salinity. Although this species does well at low salinity, Aquacop (1991) reported that based on salinity shock experiments, salinity tolerance increased as the shrimp grew from a 2-d-old to 20-d-old postlarvae (PL). Similar

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results were reported by Samocha et al. (1998a) who evaluated a salinity stress test for determining the quality of postlarvae.

In general, the ability of shrimp to tolerate changes in salinity appears to be influenced by age, species, and environmental factors. If commercial culture in low salinity environments is to succeed, we must understand factors that influence survival of the PL when transferred from high salinity hatchery/nursery systems to low salinity culture systems. Consequently, the first step in promoting low salinity culture of *L. vannamei* will be to establish acclimation procedures. The objectives of this research were to evaluate the effects of PL age, final acclimation salinity, and rate of salinity reduction on survival of *L. vannamei* PL over a 48-h holding period.

Materials and Methods

Three shipments of high health *L. vannamei* were obtained from three different commercial shrimp hatcheries. Shrimp were received as 8-d-old postlarvae (PL_8) and acclimated to a recirculating holding system maintained at 26 C and 23–24 ppt salinity. Seawater was produced using a local freshwater source (20-ppm total hardness) and sea salt (Marine Biomix or Instant Ocean). Newly received PL were held for 48 h before acclimation began. During holding, PL were fed artemia and a commercial shrimp feed.

The test system consisted of a series of 19-L containers and a water distribution system. The distribution system consisted of a reservoir, pump, tubing, and series of drip emitters designed to deliver freshwater to each container. Supplemental aeration was provided, and the system was housed in a temperature-controlled room. All treatments were replicated four times with each replicate receiving 15 PL. Prior to each acclimation experiment, 30 shrimp were towel dried and individually weighed to the nearest 0.1 mg to estimate the mean population weight.

Constant Rate of Salinity Reduction

The constant acclimation rate experiment was conducted twice. During the first trial we used a refractometer to monitor salinity. For all other trials, a temperature compensated, electronic conductivity meter (Yellow Springs Instrument Co., Inc. Yellow Springs, Ohio, USA) was used to measure salinity. Shrimp from three ages (PL_{10}, PL_{15}, and PL_{20}) were acclimated to six salinity endpoints (0, 1, 2, 4, 8, and 12 ppt). During acclimation, drip emitters were adjusted to produce a 4-ppt/h decrease in salinity from a starting salinity of 23–24 ppt to an endpoint of 2 ppt. Thereafter, a rate change of 1 ppt/h was used. Salinity was checked every 15 min in all replicates, and drip rates were adjusted to maintain the required salinity change. Two L of seawater were maintained in each container, with the volume being reduced to 2 L at the end of each h. Treatment levels were reached incrementally. Survival was determined for each treatment at 24 and 48 h after the last endpoint was reached.

Variable Rates of Salinity Reduction

A third trial was conducted to evaluate effects of three acclimation rates and two salinity endpoints, 1 and 4 ppt on PL survival. Salinities were reduced at a constant rate corresponding to low (19.4%), medium (24.6%), and high (46.7%) rates of reduction per h. The six treatments were tested with three age groups (PL_{10}, PL_{15}, and PL_{20}) previously used. Trials were conducted as previously described except the adjustable drip emitters were replaced with constant rate emitters. Each container was stocked with 15 shrimp in 5 L of seawater. Salinity was checked every 0.5 h, and the volume of the tank adjusted back to 5 L every h during the acclimation procedure. Survivals were determined 24 and 48 h from the start of the each acclimation trail.

Statistical Analyses

Survival data were arcsine transformed, and both the original data and transformed

TABLE 1. *Mean survival (%) of 10-, 15- and 20-d-old postlarval* L. vannamei, *24 and 48 h after reaching the target salinity endpoint (Trial 1). Values within a column having a* are significantly different from the control (12 ppt), using Dunnet's* t-test ($P < 0.05$).

	PL age (d)					
	10		15		20	
Time after endpoint (h)	24	48	24	48	24	48
Salinity						
0	8*	0*	77*	7*	42*	8*
1	13*	5*	98	90	87	92
2	97	50*	100	85	100	87
4	100	92	100	95	100	90
8	100	82	100	93	100	85
12	100	80	100	95	100	83
Pooled standard error	3.8	6.0	3.7	4.5	9.9	5.2

data analyzed. Analysis of variance was used to determine significant ($P < 0.05$) differences of the main effects. Dunnet's *T* test was used to compare all treatment salinities against the control salinity (12 ppt). Statistical analyses were conducted using SAS methods (version 6.12, SAS institute Inc. Cary, North Carolina, USA).

Results

Constant Rate Acclimation

The three age classes of PL (PL_{10}, PL_{15}, PL_{20}) used in the first trial had initial mean weights ± SD of 2.0 ± 0.95, 3.6 ± 1.4, and 3.8 ± 2.7 mg, respectively. Postlarvae used in the second trial had initial mean weights of 1.5 ± 0.7, 3.8 ± 2.3, 6.0 ± 5.5 mg, respectively. Results were similar for both trials (Tables 1, 2). Based on a two-way analysis of variance, a significant interaction occurred between age of PL and salinity endpoint for both trials. Hence, data was analyzed for each age class. Survivals after 48 h ranged from 0–50% for PL_{10} acclimated to 2 ppt and below. These values were significantly lower than those observed for PL exposed to 4 ppt and above, which had survival rates of 80–100%. Survivals of PL_{15} and PL_{20} were lower for shrimp exposed to 0 ppt (0–8%) than for those in which the salinity was reduced to 1 ppt and above (82–100%). There were no differences in survivals for shrimp acclimated to the higher salinities.

Variable Acclimation Rate

The three age classes of PL (PL_{10}, PL_{15}, PL_{20}) used in the third trial had initial mean weights ± SD of 2.2 ± 0.5, 5.3 ± 2.5, and 14.5 ± 6.6 mg, respectively. Salinity endpoint and acclimation rate had no effect on survival of PL_{15} and PL_{20}. Survivals after 48 h ranged from 83% to 98% (Table 3). However, significant differences were found as early as 24 h after acclimation with PL_{10} (Table 3). Acclimation rate did not significantly influence survival, whereas the salinity endpoint did. Survival of PL_{10} acclimated to 4 ppt was significantly higher than that of PL_{10} acclimated to 1 ppt, irrespective of the rate of acclimation.

Discussion

This research evaluated the response of three age classes of *L. vannamei* PL to six salinity endpoints at a constant rate of salinity reduction, as well as three salinity reduction rates at two salinity endpoints. The trials were conducted with postlarval shrimp obtained from three commercial hatcheries to provide variety. Only slight differences in responses were observed between trials, indicating that the observed responses are representative for *L. vannamei.* Differences in 24-h survivals at 2 ppt for

TABLE 2. *Mean survival (%) of 10-, 15- and 20-d-old post larval* L. vannamei, *24 and 48 h after reaching the target salinity endpoint (Trial 2). Values within a column having a * are significantly different from the control (12 ppt), using Dunnet's* t-*test* (P < 0.05).

	PL age (d)					
	10		15		20	
Hours after endpoint (h)	24	48	24	48	24	48
Salinity						
0	50*	0*	0*	0*	0*	0*
1	57*	35*	100	88	97	82
2	70*	48	100	95	100	90
4	100	100	100	93	100	100
8	100	92	100	90	100	93
12	100	100	100	95	98	95
Pooled standard error	6.9	7.0	—	4.7	1.5	4.3

the PL_{10} used in the first two trials (Table 1, 2) were probably due to procedural differences in reading salinity. In Trial 1, a temperature compensated refractometer was used, which measured to the nearest 0.5 ppt. A conductivity meter, which read to the nearest 0.1 ppt, was used in Trials 2 and 3. Consequently, the lower endpoint salinities (0, 1, and 2) during the first trial might have actually been slightly higher (or lower) than the target treatment values. If salinity was higher, this may account for the slower mortality rate observed in the first experiment. However, the observed response could also be due to differences in population. Despite minor differences, the data collected in Trials 1 and 2 confirms an age-specific tolerance to salinity reduction in postlarval *L. vannamei* shrimp.

In the present study, two-way analyses of variance indicated that survival at the tested salinity endpoints was a function of age. This was demonstrated by the low survival of PL_{10} at salinity endpoints of 2 ppt and below; whereas PL_{15} and PL_{20} tolerated salinity as low as 1 ppt. The same age effect was seen in the variable rate trial using 1 and 4 ppt as the final endpoint.

Age of PL has been implicated as an important factor for high survival of various shrimp species. Using *L. vannamei* as the test species, both Aquacop (1991) and Sam-

TABLE 3. *Mean survival (%) of 10-, 15- and 20-d-old postlarval* L. vannamei *24 and 48 h after reaching the two target salinity endpoints using three rates of acclimation. Difference in percent survival due to the main effects and their interactions are indicated at the bottom of the table (nsd = no significant difference).*

		PL age (d)					
		10		15		20	
Time after initiation (h)		24	48	24	48	24	48
Salinity	Rate						
1	High	40	23	100	88	98	87
	Medium	57	42	100	92	100	83
	Low	65	43	100	92	100	88
4	High	97	77	100	93	100	93
	Medium	98	77	100	98	100	88
	Low	98	83	100	88	100	92
P value	Salinity	0.0001	0.0001	nsd	nsd	nsd	nsd
	Rate	0.2080	0.2481	nsd	nsd	nsd	nsd
	Interaction	0.3795	0.4392	nsd	nsd	nsd	nsd

ocha et al. (1998a) evaluated the response of early PL to salinity shock and also reported an age specific response. The osmoregulatory ability of postlarval *Fenneropenaeus indicus* has also been found to increase with age (Kumlu and Jones 1995). It was reported that hatchery reared shrimp (PL_{7-22}) survived an abrupt salinity change of up to 10 ppt. However, transferring younger shrimp from 30 ppt to 5 and 10 ppt resulted in mass mortalities. Ten-d-old *Farfantepaenaeus paulensis* PL transferred from 30 ppt to salinities of 2 and 5 ppt had 96 and 77% mortality rates, respectively (Tsuzuki et al. 2000). An extended acclimation period of 5 d only slightly decreased mortality of PL_{10} when transferred from 30 ppt salinity to 2 and 5 ppt, while PL_{15-30} acclimated from 30 to 2 ppt had less than 5% mortality. Consequently, the ability of *F. paulensis* to tolerate salinity changes was age dependent with older PL having higher survival or lower mortalities.

In the rate study (Table 3), 10-d-old *L. vannamei* PL were less tolerant of acclimation to low salinity (1 ppt) when compared to older PL (15- and 20-d-old), once again demonstrating an age effect of the PL ability to tolerate salinity changes as measured by 48-h survival. Quite often, the rate of salinity change is implicated in the shrimp's ability to adjust to a new salinity. However, based on the results of this study, survival of PL_{10} (or older PL) was not influenced by the rate of salinity reduction (high, medium, or low). These reductions in salinity took anywhere from about 5 h (high rate to 1 ppt) to 15 h (low rate to 1 ppt). In the present study, PL_{15} and PL_{20} *L. vannamei* were capable of tolerating a high rate of salinity reduction going from 24 ppt to 1 ppt in about 5 h. We were unable to acclimate the PL to 0 ppt during the constant rate acclimation experiment (which took about 8 h). This would indicate that either *L. vannamei* are incapable of tolerating such a low salinity water source, or that a longer period would be required to acclimate PL to 0 ppt.

Pantastico and Oliveros (1980) also found that age and length of the acclimation period influenced the ability of *P. monodon* to survive in low salinities. Van Wyk et al. (1999) reported that 48 h were necessary to acclimate *L. vannamei* (PL_{12}) down to "freshwater" (0.5 ppt). Their recommended acclimation rate decreased from 2 ppt/h for the first 8 h down to 0.063 ppt/h for the last 8 h. They suggested checking PL under a microscope to see if shrimp gills had developed and branched before acclimating to low salinity waters. Gills, particularly the posterior filaments, are known to be important in osmoregulation in penaeid shrimp (Lucu 1990; Pequeux 1995). In this study, acclimation between 5 (high rate) and 15 h (slow rate) made no difference in survival when using PL_{15-20} and acclimating down to a salinity of 1 ppt.

In a more practical acclimation setting such as tanks located on farms, other factors, such as dissolved oxygen, temperature, salinity, and ammonia, may also influence the osmoregulatory ability of shrimp. Tsuzuki et al. (2000) observed an increase in mortality of *F. paulensis* due to the synergistic effects in changes of temperature, salinity, and PL stage. Lower survival rates of PL were observed in response to a change in salinity when the temperature was lower or higher than the optimal temperature for this shrimp. Younger PL stages (10–30 d) were more sensitive to temperature change than older PL (> 30 d). *L. vannamei* (10 g) exposed to low oxygen tension (4–8 kPa) experienced decreased osmoregulatory ability at low salinity and full strength seawater. *L. chinensis* showed a decrease in hemolymph osmolality when exposed to 1.476 mmol/L ammonia-N due to a decline in Ca, Mg, K, Na and Cl ions. Young juvenile *M. japonicus* exposed to a turbidity of 35 nephelometric turbidity units experienced a decrease in osmoregulation ability resulting from impairment of Na and Cl regulation (Lin et al. 1992). Dalla Via (1985) demonstrated how *M. japonicus* juveniles temporarily increased oxygen consumption by 300% after a change in environmental salinity from 37 ppt to 10 ppt.

Summary

Based on the results of this experiment, it is clear that there is an age specific response of postlarvae to salinity acclimation. This is exemplified by the three ages of PL ability to acclimate to 4 ppt irrespective of acclimation rate. Whereas survival was significantly reduced when 10-d-old PL were acclimated to lower salinity levels, acclimating to 1 ppt, even at the slowest rate of salinity reduction tested, resulted in reduced survival. Based on these results PL younger than 15-d-old should not be acclimated to salinities below 4 ppt. However, older PL can be acclimated to 1 ppt with good 48-h survival.

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