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Effects of Potassium, Magnesium and Age on Growth and Survival of *Litopenaeus vannamei* Post-Larvae Reared in Inland Low Salinity Well Waters in West Alabama

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Abstract—The production of *Litopenaeus vannamei* in inland low-salinity well water is a growing industry in several regions of the world. The state of Alabama in the southeastern USA is one such region with a large saline aquifer that could be utilized for shrimp culture. However, some farmers are experiencing problems rearing marine shrimp while others are having considerable success. Previous work has correlated low levels of potassium and/or magnesium to poor shrimp survival. The problem is further complicated by the fact that the age at acclimation may also influence survival. In our present study, we evaluated the effects of potassium, magnesium, and the age of acclimation on growth and survival of PL at two farms. The first experiment was run in a static system utilizing four replicate tanks per treatment. Fifty PL_{17} (0.0066 g) that had been acclimated to 4 ppt seawater were stocked into each tank and the following treatments evaluated: low salinity well water (LSWW) without mineral supplements, LSWW with KCl, LSWW with $MgCl_2$, and LSWW with KCl and $MgCl_2$ added to the water. Shrimp were harvested, counted and weighed after 4 wk. Survival was significantly higher in treatments receiving mineral supplements whereas biomass was only higher in the two treatments with potassium supplements. The second experiment was set up initially as a static system filled with 8.5-ppt reconstituted sea water that was then converted to a flow-through system using LSWW. This experiment evaluated the effect of PL age at acclimation on survival and growth at four different ages (PL_{15}, PL_{19}, PL_{23}, and PL_{27}). All tanks were stocked with 50 PL_{13} *L. vannamei.* Two days after stocking, and then at 4-d intervals, a series of four tanks were converted to flow through (rate of 40 L/hr) using LSWW. After acclimation, water flow was maintained in all tanks until 28 d after stocking when tanks were harvested and surviving shrimp were counted and weighed. Survival and growth increased with PL age when shrimp were acclimated to inland low salinity well water.

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Production of *Litopenaeus vannamei* in inland low-salinity well water (LSWW) is an emerging industry in several states including Alabama (Saoud et al. 2003). Unfortunately, some farmers are experiencing problems rearing this shrimp while others are having considerable success. Similar problems with inland saline ground water have been reported in other countries, such as Australia, while working with penaeid shrimp (personal communication, Adrian Collins) and snapper (Fielder et al. 2001). Researchers at various institutions are working to identify the reasons for the difference in survival and growth among farms and to develop mitigation strategies (Smith and Lawrence 1990; McGraw et al. 2002; Samocha et al. 2002; Saoud et al. 2003). Previous work at our laboratory suggests that lack of potassium and/or magnesium in some well waters could negatively affect survival and growth (McGraw and Scarpa 2003; Saoud et al. 2003). Furthermore, McGraw et al. (2002) and Saoud et al. (2003) reported that post-larvae (PL) age at the time of low salinity acclimation also affects survival and growth. However, these results have not yet been corroborated on farms where they might be affected by natural productivity and varying environments. The present study evaluated the effects of potassium and magnesium supplementation to LSWW on acclimation, survival, and growth of *L. vannamei* PL at two farm sites in west Alabama. Furthermore, because PL age has been found to influence tolerance to low salinity, we also evaluated the effects of PL age on growth and survival of *L. vannamei*.

Materials and Methods

The present research was conducted at two startup commercial shrimp farms in west Alabama. On each farm, a series of 16 circular polyethylene tanks (760 L each) were installed. Each tank was equipped with a central screened drain, water inlet, and two submerged air diffusers. The first experiment was performed in a static system and was used to evaluate the effect of K^+ and Mg^{2+} supplements on PL survival and growth. The second experiment was conducted in a flow-through system using LSWW aged in a surface pond. The flow-through system was used to evaluate the effect of PL age on salinity acclimation, survival, and growth. At both locations, every treatment was assigned four replicate tanks, each stocked with 50 PL. The PL were offered a commercial feed (PL Redi-Reserve, Ziegler Bros., Gardner, Pennsylvania, USA) for 2 wk, and a crumbled shrimp feed (35% protein; Rangen, Buhl, Idaho, USA) during the remaining weeks. Temperature was measured hourly using a submerged data logger. Dissolved oxygen was measured daily using a YSI oxygen meter, and ammonia-N concentration was evalutated twice weekly. These water quality parameters averaged 30 C, 7 mg/L and ≤ 0.1 mg/L, respectively.

In the first experiment, PL_{17} (0.0066 g) that had been acclimated to artificial seawater (Crystal Sea, Marine Enterprises International, Baltimore, Maryland, USA) were stocked into each tank filled with LSWW that had been aged in a pond. The composition of the LSWW used is reported as C-water by Saoud et al. (2003) and had a salinity of 6.1 ppt. The following treatments were evaluated: LSWW with 57.24-g KCl , LSWW with 305.90-g $MgCl_2$, and LSWW with KCl and $MgCl_2$ (57.24 + 305.90 g, respectively) added to the water. Amounts of KCl and $MgCl_2$ added were enough to raise K^+ and Mg^{2+} concentrations up to levels comparable to what is in natural seawater at 4 ppt (50-ppm K and 130-ppm Mg). Shrimp were harvested, counted and weighed after 21 days.

The second experiment was designed to evaluate the effect of PL age (PL_{15}, PL_{19}, PL_{23}, and PL_{27}) at acclimation on survival and growth. The composition of the LSWW used in this study is reported by Saoud et al. (2003) as R-water and had a salinity of 4.5 ppt. All tanks were filled with aged LSWW and 1.2 kg of a marine salt mix (Crystal Sea, Marine Enterprises International, Baltimore, Maryland) was added to raise salinity to 8.5 ppt. Fifty PL_{13} *L. vannamei* which were previously acclimated to this salinity were added to each tank. At ages PL_{15}, well water was added to four tanks at a rate of 40 L/hr. Four days later (PL_{19}), water was added to four more tanks at the same rate. The procedure was repeated for PL_{23} and PL_{27}. Water flow was maintained in all tanks until 28 d after stocking when tanks were harvested and surviving shrimp were counted and weighed.

Growth and survival was analyzed using one-way ANOVA to determine significant differences ($P < 0.05$) among treatment means. Student-Neuman-Keuls multiple comparison test was used to determine significant differences between treatment means. All statistical analyses were conducted using the Statistical Analysis System (V8e, SAS Institute Inc., Cary, North Carolina USA).

Results and Discussion

Survival and growth were positively impacted by K^+ and Mg^{2+} supplementation. Survival of shrimp in the control (LSWW without mineral additions) was 58%, compared to 75% or greater in all other treatments (Table 1). Although survivals among treatments were not different from each other, they were all significantly different from the control, indicating that both K^+ and Mg^{2+} supplements improved survival. However, there were no significant differences in growth among treatments. When growth and survival results are combined and expressed as harvested biomass, a significant effect of K^+ is observed (Table 1). These results confirm the findings of short term bioassays which indicated that both K^+

Table 1. *Survival and growth of post larval shrimp reared in low salinity well water. In Exp. 1 (initial PL weight = 0.0066 g) water was supplemented with potassium and magnesium. In Exp. 2 (initial PL weight = 0.0039 g) shrimp were acclimated to well water at various ages.*

Experiment	Treatment	Biomass (g)	Final Weight (g)	Growth (%)	Survival (%)
1	KCl	24.2^{z}	0.65^{z}	$7{,}120^{z}$	76.5^{z}
	$MgCl_2$	15.4^{y}	0.40^{z}	$4{,}414^{z}$	75.0^{z}
	KCl + $MgCl_2$	23.5^{z}	0.54^{z}	$5{,}896^{z}$	89.0^{z}
	Control	11.9^{y}	0.42^{z}	$4{,}579^{z}$	58.0^{y}
	PSE[b]	2.47	0.08	869.8	4.6
2	PL15	14.5^{x}	0.42^{x}	$10{,}685^{x}$	67.0^{y}
	PL19	26.2^{y}	0.68^{y}	$17{,}452^{y}$	76.0^{y}
	PL23	34.8^{y}	0.85^{y}	$21{,}862^{y}$	82.5^{yz}
	PL27	55.2^{z}	1.17^{z}	$30{,}227^{z}$	94.5^{z}
	PSE	3.69	0.08	1995.5	4.75

Values represent the means of four replicates. Numbers within the same column with different superscripts are significantly different ($P < 0.05$).
PSE = Pooled Standard Error.

and Mg^{2+} levels influence survival (Saoud et al. 2003). The supplementation of K^+ appears to enhance both survival and growth, whereas Mg^{2+} appears to primarily influence survival.

Based on initial short term studies, there is a clear influence of PL age on the ability of PL to tolerate low salinity conditions (McGraw et al. 2002; Saoud et al. 2003). Results of the present 28-d growth trial indicate that survival and growth increased with PL age when shrimp were acclimated to inland LSWW (Table 1). Growth, expressed as percent weight gain of shrimp acclimated at PL_{15} (10,685%) was significantly lower from growth of shrimp acclimated to LSWW at age PL_{27} (30,227%). Furthermore, survival of shrimp acclimated as PL_{15} (67%) was significantly different from survival of shrimp acclimated as PL_{27} (94.5%). The differences among treatments might be attributed to better survival of PL acclimated to low salinities at older ages (McGraw et al. 2002; Saoud et al. 2003) or to the fact that shrimp in the treatment PL_{27} had spent additional time in more natural seawater, where they were presumably exposed to less osmotic stress and thus grew larger.

The results of the two experiments described in the present study suggest that when possible farmers should nurse post-larval shrimp in reconstituted seawater prior to LSWW acclimation and release into growout ponds. Moreover, farmers with low salinity well waters that are low in K^+ and Mg^{2+} could supplement K^+ and Mg^{2+} to their ponds to improve survival and possibly growth. It should be noted, that during the shrimp growout season of 2002 and 2003, some farmers in west Alabama supplemented muriated potash (KCl) and Dolomite ($CaMg(CO_3)_2$) to their ponds and reported a strong improvement in survival and growth compared to previous years. Results of this research, as well as on-farm observations, confirm that at least a portion of the low survival of shrimp observed by farmers culturing shrimp in LSWW in Alabama can be attributed to inadequate levels of minerals in the water as well as stocking PL at too early an age.

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