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# Effects of varying levels of aqueous potassium and magnesium on survival, growth, and respiration of the Pacific white shrimp, *Litopenaeus vannamei*, reared in low salinity waters

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## Abstract

Inland shrimp culture is being practiced in several regions of the United States. In Alabama, the culture of shrimp (*Litopenaeus vannamei*) in inland low salinity well water (approximately 4.0 ppt) faces several challenges. The ionic composition of these waters is deficient in several key minerals, including potassium ( $K^+$ ) and magnesium ( $Mg^{2+}$ ). The objective of the present study was to evaluate the effects of several aqueous  $K^+$  and  $Mg^{2+}$  concentrations on survival, growth, and respiration in juvenile *L. vannamei*. Two experiments, a 14-day trial with postlarvae and a 7-week trial with juvenile ( $\sim 0.2$  g) shrimp were conducted to evaluate effects of  $K^+$  supplementation to culture water. Four different levels of  $K^+$  (5, 10, 20, and 40  $mg\ l^{-1}$ ) were utilized and a treatment of 4 ppt reconstituted seawater was used as a reference for comparison to ideal ionic ratios. Additionally, a 6-week growth trial ( $\sim 1$  g juvenile shrimp) was performed to evaluate the effects of five concentrations of  $Mg^{2+}$  (10, 20, 40, 80, 160  $mg\ l^{-1}$ ). Following completion of growth trials, measurements of basal respirometry rates were conducted to assess stress. Results from the 7-week  $K^+$  growth trial indicated significant differences ( $P < 0.05$ ) in survival and growth among treatments. Individual weight, specific growth rate, and percent weight gain appeared to increase with increasing  $K^+$  concentration (decreasing Na:K ratios). Results from the  $Mg^{2+}$  experiment reveal a significant difference in survival between the lowest  $Mg^{2+}$  treatment (60%) and all other experimental treatments (90–97%). However, no differences in growth were observed. Shrimp respiration in the lowest  $Mg^{2+}$  treatment (10  $mg\ l^{-1}$ ) was significantly higher than in the 80  $mg\ l^{-1}$  treatment. These results suggest a potentially higher energetic cost associated with depressed aqueous  $Mg^{2+}$  concentrations that are common in low salinity environments.

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## 1. Introduction

The inland culture of shrimp, particularly the Pacific white shrimp, *Litopenaeus vannamei*, is becoming more widespread in the Western hemisphere. Depending on

their source, inland waters available for shrimp culture are usually of different salinities and possess different ionic compositions (Boyd and Thunjai, 2003). The ability of *L. vannamei* to tolerate a wide range of salinities (0.5–40 ppt) has made it a popular species for low salinity culture (McGraw et al., 2002; Samocha et al., 1998, 2002). Despite the relative success of some farmers in culturing *L. vannamei* in inland low salinity waters,

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problems still arise from deficiencies in the ionic profiles of pond waters (Saoud et al., 2003; Atwood et al., 2003). The lack of a necessary mix of essential ions, including potassium ( $K^+$ ) and magnesium ( $Mg^{2+}$ ), has been demonstrated to limit growth and survival of shrimp (Saoud et al., 2003; Davis et al., 2005).

Alabama has several saltwater aquifers (Feth, 1970) that are being utilized as sources of low salinity water for aquaculture (Saoud et al., 2003). Farmers in west Alabama have been successful in raising *L. vannamei* in inland low salinity waters by raising the  $K^+$  and  $Mg^{2+}$  levels of their pond waters to correct ionic ratio imbalances (McNevin et al., 2004). McNevin et al. (2004) observed increased shrimp production in Alabama low salinity waters (2–4 ppt) by raising the levels of  $K^+$  ( $6.2 \text{ mg l}^{-1}$ ) and  $Mg^{2+}$  ( $4.6 \text{ mg l}^{-1}$ ) to  $40 \text{ mg l}^{-1}$  and  $20 \text{ mg l}^{-1}$  using muriate of potash and potassium–magnesium sulfate, respectively. Furthermore, various studies have demonstrated a benefit to having appropriate levels or ratios of  $K^+$  and  $Mg^{2+}$  as well as other minerals during postlarval acclimation to low salinity waters (McGraw et al., 2002; McGraw and Scarpa, 2003; Saoud et al., 2003; Davis et al., 2005).

Both  $K^+$  and  $Mg^{2+}$  are ions essential for normal growth, survival, and osmoregulatory function of crustaceans (Mantel and Farmer, 1983; Pequeux, 1995). Potassium is the primary intracellular cation and is also important in the activation of the  $Na^+K^+ATPase$  (Mantel and Farmer, 1983), which is a key component of extracellular volume regulation. The lack of adequate levels of aqueous  $K^+$  could thus be potentially detrimental in terms of the ability to effectively osmoregulate, because enzyme activity can be directly related to  $K^+$  concentration (Burse and Lane, 1971). In penaeid shrimp, hemolymph  $K^+$  is regulated within a narrow range despite decreases in external salinity of the medium (Dall and Smith, 1981). Closely linked to the function of the  $Na^+K^+ATPase$  is adequate availability of  $Mg^{2+}$ , which serves as a cofactor (Mantel and Farmer, 1983; Furriel et al., 2000). The lack of adequate  $Mg^{2+}$  or  $K^+$  can affect  $Na^+K^+ATPase$  activity in crustaceans (Mantel and Farmer, 1983; Pequeux, 1995; Furriel et al., 2000). Magnesium also plays a role in the normal metabolism of lipids, proteins, and carbohydrates serving as a cofactor in a large number of enzymatic and metabolic reactions (Davis and Lawrence, 1997).

It is also well known that oxygen consumption can be affected by variations in environmental factors such as salinity, diet, activity level, temperature, and body weight (Mantel and Farmer, 1983; Brett, 1987). The impact of salinity on penaeid shrimp physiology has been examined by various authors such as Villareal et al. (1994) and Spanopoulos-Hernández et al. (2005). Less studied, however, is the impact of various ionic profiles of iso-

Table 1

Mean water quality parameters for growth trials with juvenile *L. vannamei* reared in low salinity waters

Parameter	14-day $K^+$ trial	$K^+$ growth trial	$Mg^{2+}$ growth trial
Dissolved $O_2$ ( $\text{mg l}^{-1}$ )	$7.98 \pm 0.04$	$7.24 \pm 0.05$	$7.3 \pm 0.13$
Temperature ( $^{\circ}\text{C}$ )	$25.2 \pm 0.07$	$27.3 \pm 0.1$	$27.0 \pm 0.1$
Salinity (ppt)	$4.2 \pm 0.02$	$4.1 \pm 0.05$	$4.1 \pm 0.06$
pH	$8.1 \pm 0.0$	$8.0 \pm 0.02$	$8.1 \pm 0.03$
TAN <sup>a</sup> ( $\text{mg l}^{-1}$ )	$0.08 \pm 0.01$	$0.09 \pm 0.04$	$0.03 \pm 0.01$
$NO_2$ ( $\text{mg l}^{-1}$ )	$0.54 \pm 0.1$	$0.06 \pm 0.04$	$0.04 \pm 0.01$

Values represent the mean  $\pm$  standard deviation.

<sup>a</sup> Total ammonia nitrogen.

saline inland low salinity well water on shrimp respiration. Consequently, the objective of the present study was to evaluate survival, growth, and respiration of *L. vannamei* maintained in artificial low salinity waters with different concentrations of  $K^+$  and  $Mg^{2+}$ .

## 2. Materials and methods

### 2.1. Culture conditions

The following study was conducted at the North Auburn Fisheries Research Station in Auburn, Alabama. Postlarval *L. vannamei* were obtained from Harlingen Shrimp Farms (Los Fresnos, TX, USA). Postlarvae (PL) were acclimated from 20 ppt to low salinity water (4.0 ppt) over a period of 8 h and maintained in a 220-l polyethylene nursery tank connected to a biological filter. During the first week, PL were maintained on a combination of *Artemia* nauplii (100 nauplii per shrimp) and a daily ration of commercial feed, PL Redi-Reserve (Ziegler Bros. Gardner, Pennsylvania, USA) at 25–50% body weight. Thereafter, shrimp were offered a commercial feed (Rangen 35% protein, Buhl, Idaho) and reared in the nursery system until they were of appropriate size for commencement of growth trials.

The experiment was conducted in twenty, 150-l polyethylene tanks. Each experiment consisted of five treatments with four replicate tanks per treatment. Individual tanks were equipped with an airlift biofilter, submerged air diffuser, and submersible heater to maintain an adequate temperature ( $27 \pm 0.5$   $^{\circ}\text{C}$ ). Shrimp were offered a commercial feed (Rangen 35% protein) four times daily using an automatic feeder. Light control was set at 16 h day and 8 h night. Dissolved oxygen (DO), pH, salinity, and temperature were measured daily whereas ammonia nitrogen and nitrite nitrogen were measured twice weekly according to Solorzano (1969) and Parsons et al. (1985), respectively (Table 1).

## 2.2. Artificial low salinity water

Artificial low salinity water was prepared 2 weeks prior to the commencement of each experiment. Experimental waters for the 14-day PL trial and the  $K^+$  growth trial were prepared using well water from the North Auburn Fisheries Station. Treatment waters were made by filling the tanks with 0.5 ppt reconstituted seawater (Crystal Sea Salt, Baltimore, Maryland, USA) and supplementing with  $40 \text{ mg l}^{-1}$  calcium from  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ , and  $40 \text{ mg l}^{-1}$  magnesium from  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ . Thereafter, KCl was added to four replicate tanks per treatment at levels of 5, 10, 20, and  $40 \text{ mg l}^{-1} K^+$ . Finally, salinity in all treatments was raised to 4.0 ppt using rock salt (NaCl). A 4 ppt reconstituted seawater treatment was also added as a reference. The reconstituted seawater reference had  $K^+$  levels (and thus Na:K ratios) similar to that of the treatment with the highest  $K^+$  supplement ( $40 \text{ mg l}^{-1}$ ).

Artificial low salinity waters for the  $Mg^{2+}$  growth trial were prepared similarly to the waters with potassium. Reconstituted seawater (0.25 ppt) was supplemented with  $40 \text{ mg l}^{-1}$  calcium from  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  and  $40 \text{ mg l}^{-1}$  potassium from KCl. After that, magnesium ( $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ ) was added to provide concentrations of 10, 20, 40, 80, and  $160 \text{ mg l}^{-1}$  per tank to four replicate tanks per treatment, respectively. The waters were then raised to 4.0 ppt using rock salt (NaCl). Experimental waters were analyzed for major ions using ICAP (Inductively coupled argon plasma spectrophotometry) (Table 2) and flame photometry (Cole Parmer digital flame photometer, Model 2655-00, Vernon Hills, Illinois) (Clesceri et al., 1998).

## 2.3. Effect of $K^+$ on postlarvae

Twenty PL<sub>39</sub> (Mean initial weight, 30 mg) were stocked into each of the 20 experimental tanks with four replicate tanks per treatment (5, 10, 20, and  $40 \text{ mg l}^{-1} K^+$ ). Shrimp were fed ad libitum 4 times daily for 14 days. At the end of the 14-day trial, shrimp were harvested and survival and growth were assessed.

## 2.4. Effect of $K^+$ on juveniles

The same artificial low salinity waters used in the first experiment were utilized for the trial with juveniles. Fifteen juvenile shrimp (mean individual weight: 280 mg) were stocked per replicate tank. Shrimp in each tank were counted weekly and ration was calculated assuming a 1.75 feed conversion ratio and a doubling in size until individual shrimp weighed 1 g (approximately the first 2 weeks). Thereafter, a growth rate of 1 g per week was

Table 2

Ionic composition ( $\text{mg l}^{-1}$ ) of artificial low salinity waters (4.0 ppt) used to culture *L. vannamei* at North Auburn Research Unit compared to seawater

$K^+$ trial	5	10	20	40	Reference	Dilute SW 4.0 ppt
K	9.1	16.8	22.4	39	40.1	47.3
Mg	156.2	170.3	155	169.9	154.1	132.3
Ca	80.1	86.1	81.6	86.2	82.5	42.4
Na	1085	1137	1068	1114	1214	1316.3
Na:K ratio	119:1	68:1	48:1	29:1	30:1	28:1
Mg:Ca ratio	1.95:1	1.98:1	1.90:1	1.97:1	1.87:1	3.1:1
$Mg^{2+}$ trial	10	20	40	80	160	Dilute SW 4.0 ppt
K	37.4	36.3	35.9	38.9	33.2	47.3
Mg	16.8	26.5	46.3	92.4	162.6	132.3
Ca	69.6	71.1	69.5	76.3	66.6	42.4
Na	1397	1331	1308	1334	1069	1316.3
Na:K ratio	37:1	37:1	36:1	34:1	32:1	28:1
Mg:Ca ratio	0.24:1	0.37:1	0.67:1	1.21:1	2.44:1	3.1:1

assumed. At the end of a 49-day growth period, shrimp were harvested, counted and group weighed. Hemolymph was collected using a  $1 \text{ cm}^3$  syringe, and all samples from each tank were pooled in a 1.5-ml Eppendorf microfuge tube. Hemolymph osmolality, chloride,  $\text{Na}^+$ , and  $\text{K}^+$  levels were measured and the remainder of each sample stored at  $-70^\circ\text{C}$  pending further analysis.

## 2.5. Effect of $Mg^{2+}$ on juveniles

Fifteen juvenile shrimp (mean individual weight 1.2 g) were stocked into 4 replicate tanks per treatment (10, 20, 40, 80, and  $160 \text{ mg l}^{-1} Mg^{2+}$ ). Shrimp were counted weekly and were fed as described in the  $K^+$  growth trial. At the end of a 42-day growth period, shrimp were harvested, counted and group weighed. Hemolymph was extracted as previously described and samples from each tank were pooled into a 1.5-ml Eppendorf microfuge tube. Hemolymph osmolality, chloride,  $\text{Na}^+$ , and  $\text{K}^+$  levels were determined and the remainder of each sample stored at  $-70^\circ\text{C}$  pending further analysis.

## 2.6. Hemolymph osmotic and ionic concentrations

In order to determine hemolymph osmotic and ionic concentrations, stored samples were thawed on ice and then sonicated (25 W, 30 s, Heat Systems Microson ultrasonic cell disruptor, Farmingdale, New York) to disrupt the clot (Henry et al., 2003). The samples were then centrifuged ( $10,000 \times g$  for 60 s, Fisher 235B microfuge, Pittsburgh, Pennsylvania) to separate the clot from serum. Total osmolality was measured using a Wescor 5100C vapor

pressure osmometer. Hemolymph chloride ion concentration was determined by Ag titration (LabconCo chloridometer, Petaluma, California). Finally, hemolymph  $\text{Na}^+$  and  $\text{K}^+$  concentrations were measured by flame photometry (Cole Parmer digital flame photometer, Model 2655-00, Vernon Hills, Illinois).

### 2.7. Hepatopancreas mineralization

Whole shrimp were thawed and the hepatopancreas of five intermolt (Robertson et al., 1987) shrimp from each tank removed. Hepatopancreas samples were pooled per tank, then oven dried and wet ashed according to established methods (Association of Official Analytical Chemists, 1984). Both  $\text{K}^+$  and  $\text{Mg}^{2+}$  levels were sent to the Auburn University Soils Laboratory and measured using atomic absorption spectrophotometry.

### 2.8. Respirometry

A Strathkelvin respirometer (Model 928, Strathkelvin Instruments, Glasgow, Scotland) was utilized to determine oxygen uptake by shrimp. Following the completion of growth trials, respiration was measured daily in 6–8 shrimp of similar size (~4–5 g) using flow through respirometry. Oxygen consumption of shrimp from each treatment was performed in water identical in ionic makeup to the one in which the shrimp were reared. Four experimental chambers were constructed using 2" diameter transparent plexiglass pipe cut into 6" sections, capped with quick-disconnect PVC caps and fitted with a small mesh false bottom. The two ends of the chamber connected to tygon tubing for a water inlet and outlet. The volume of each chamber was 200 ml. Each chamber was set up on a magnetic stir plate, and a micro stir-bar was placed underneath the false bottom in order to assure adequate mixing. An oxygen probe was fitted through the top of each chamber. Oxygen saturated water was gravity fed into each chamber from a 40-l tank of previously prepared 4.0 ppt low salinity water. In experiments where aqueous concentrations of  $\text{K}^+$  and  $\text{Mg}^{2+}$  were evaluated, a separate water source containing the appropriate concentration of the ions examined was utilized for each treatment. Temperature was maintained at 27.8 °C throughout the experiment using a heater submerged in the supply tank. Water exiting the chamber was controlled by a flow-restrictor attached to the respirometry chamber and flow rate was set at 5 ml  $\text{min}^{-1}$ . As such, water was exchanged within the chamber every 40 min.

Oxygen sensors were calibrated using an oxygen saturated sample of the experimental water. Following

calibration, intermolt shrimp (Robertson et al., 1987) were blotted dry, weighed, and one shrimp stocked per respirometry chamber. Four replicate respirometry estimations were performed simultaneously. An oxygen probe was placed in each of the chambers, flow rate was measured and shrimp were allowed to acclimate 5–7 h or until respiration rate remained stable for at least 1 h. Oxygen concentration following the acclimation period was measured in  $\text{mg l}^{-1}$ . Respiration rate (R) in  $\text{mg O}_2 \text{ g shrimp}^{-1} \text{ h}^{-1}$  was calculated using the following equation:

$$R = Q \times ([\text{O}_2\text{initial}] - [\text{O}_2\text{final}]) / W$$

In this equation  $Q$  is flow rate ( $\text{l h}^{-1}$ ) and  $W$  is the weight of the shrimp in grams. The oxygen electrode was placed inside the actual chamber where the shrimp was resting because of constraints pertaining to the availability of supplies for the chamber. The placement of the electrode inside the chamber created a scenario in which fresh oxygenated saturated water was mixed with water inside the chamber. However, the flow rate was low (complete exchange in 40 min) and the chamber was adequately stirred to maintain fairly stable oxygen concentration. As such, the respiration values obtained in this experiment could potentially be underestimates of true basal metabolic respiration rates, but do allow for a comparison of respiration rates of shrimp reared in waters containing different ionic profiles.

### 2.9. Statistical analysis

Statistical analyses were performed using SAS (version 8.2, SAS Institute, Cary, North Carolina). Data from both experiments were analyzed using one-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.

## 3. Results

### 3.1. Effect of $\text{K}^+$ on postlarvae

Results from the preliminary  $\text{K}^+$  trial revealed significant differences in survival and mean individual weights after 14 days of culture (Fig. 1). Survivals were low across all treatments with the exception of the reference (78%) which contained ionic profiles most similar to full strength seawater. The lowest  $\text{K}^+$  treatment (5  $\text{mg l}^{-1}$ ) yielded the lowest survival (21.25%)

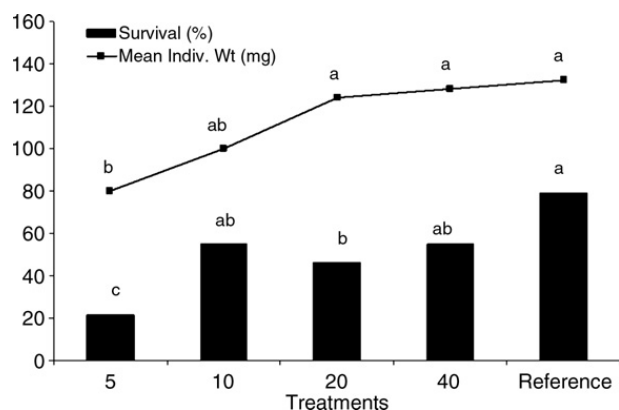


Fig. 1. Survival (%) and mean final weight (mg) of postlarval *L. vannamei* stocked in the 14-day preliminary growth trial with  $K^+$ .

and was significantly different from all other treatments ( $P < 0.05$ ). Survivals in the 10, 20, and 40  $mg\ l^{-1}$   $K^+$  treatments ranged from 46.3–55.0%. The 10  $mg\ l^{-1}$  treatment (46.3%) was significantly different from the control. Individual final weight was highest in the reference (0.132 g) and was significantly greater than weights in either the 5  $mg\ l^{-1}$  (0.80 g) or 10  $mg\ l^{-1}$  (0.99 g) treatments. Individual weights of the 20  $mg\ l^{-1}$  and 40  $mg\ l^{-1}$   $K^+$  treatments were 0.124 g and 0.128 g, respectively, and were also significantly different from the lowest treatment. Water quality parameters during the experiment remained within acceptable limits for the species.

### 3.2. Effect of $K^+$ on juveniles

Significant differences in final weight, survival, and percentage weight gain were observed in the 49-day growth trial in waters with various levels of  $K^+$  (Table 3). Shrimp reared in the 4 ppt reference yielded the highest final weight (4.90 g) and weight gain (1854.6%), significantly different from all other treatments. The final weights and percentage weight gain of shrimp reared in the 20 and 40  $ppm\ K^+$  amended waters were significantly different from the 5  $mg\ l^{-1}$   $K^+$  amended treatment. In general, as  $K^+$  concentration increased, so did mean individual weight and percent weight gain. Survival in the 10, 20, 40  $mg\ l^{-1}$  and reference ranged between 93.3 and 96.7%, and was significantly higher than survival in the 5  $mg\ l^{-1}$   $K^+$  treatment (23.3%).

### 3.3. Effect of $Mg^{2+}$ on juveniles

There were no significant differences in final weights or weight gain (%) of shrimp reared in low salinity waters containing various levels of  $Mg^{2+}$  (Table 3). Final weights ranged from 3.25 to 3.67 g, while weight gain ranged from 144.0 to 181.4% across treatments. Nevertheless, the trend for the treatment with the highest concentration of  $Mg^{2+}$  (160  $mg\ l^{-1}$ ) to yield the largest weight gain, while the treatment with the lowest concentration of  $Mg^{2+}$  (10  $mg\ l^{-1}$ ) to yield the lowest weight gain was worth noting. Survival in the lowest  $Mg^{2+}$  treatment (10  $mg\ l^{-1}$ )

Table 3

Final weight (g), Survival (%), Weight Gain (%), hemolymph osmolality ( $mmol\ kg^{-1}$ ), chloride ( $mEq\ l^{-1}$ ),  $K^+$  ( $mEq\ l^{-1}$ ), and  $Na^+$  ( $mEq\ l^{-1}$ ) for *L. vannamei* reared in low salinity water containing varying levels of  $K^+$  and  $Mg^{2+}$

	Indiv. Wt. (g)	Weight Gain (%)	Survival (%)	Osmolality ( $mmol\ kg^{-1}$ )	Cl ( $mEq\ l^{-1}$ )	$K^+$ ( $mEq\ l^{-1}$ )	$Na^+$ ( $mEq\ l^{-1}$ )
<i>K<sup>+</sup> trial</i>							
5	2.40 <sup>c</sup>	852.8 <sup>c</sup>	23.3 <sup>b</sup>	629.8 <sup>a</sup>	261.4 <sup>a</sup>	7.4 <sup>a</sup>	283.8 <sup>a</sup>
10	2.79 <sup>b,c</sup>	1064.8 <sup>b,c</sup>	95.0 <sup>a</sup>	647.0 <sup>a</sup>	260.8 <sup>a</sup>	7.7 <sup>a</sup>	282.2 <sup>a</sup>
20	3.18 <sup>b</sup>	1145.5 <sup>b</sup>	96.7 <sup>a</sup>	629.3 <sup>a</sup>	267.5 <sup>a</sup>	7.3 <sup>a</sup>	289.2 <sup>a</sup>
40	3.30 <sup>b</sup>	1208.9 <sup>b</sup>	93.3 <sup>a</sup>	609.8 <sup>a</sup>	269.6 <sup>a</sup>	8.0 <sup>a</sup>	260.5 <sup>a</sup>
Reference	4.90 <sup>a</sup>	1854.6 <sup>a</sup>	93.3 <sup>a</sup>	631.3 <sup>a</sup>	264.8	7.6 <sup>a</sup>	275.7 <sup>a</sup>
PSE <sup>1</sup>	0.211	63.4	4.1	12.2	9.32	0.45	11.21
P value	<0.0001	<0.0001	<0.0001	0.37	0.96	0.39	0.77
<i>Mg<sup>2+</sup> trial</i>							
10	3.25 <sup>a</sup>	144.0 <sup>a</sup>	60.2 <sup>b</sup>	616.6 <sup>a</sup>	254.6 <sup>a</sup>	<sup>2</sup>	269.7 <sup>a</sup>
20	3.41 <sup>a</sup>	151.5 <sup>a</sup>	90.0 <sup>a</sup>	655.3 <sup>a</sup>	249.0 <sup>a</sup>	7.7 <sup>a</sup>	272.2 <sup>a</sup>
40	3.33 <sup>a</sup>	156.2 <sup>a</sup>	96.5 <sup>a</sup>	673.1 <sup>a</sup>	246.7 <sup>a</sup>	8.1 <sup>a</sup>	268.9 <sup>a</sup>
80	3.37 <sup>a</sup>	156.9 <sup>a</sup>	93.2 <sup>a</sup>	634.8 <sup>a</sup>	246.4 <sup>a</sup>	7.6 <sup>a</sup>	249.9 <sup>a</sup>
160	3.67 <sup>a</sup>	181.4 <sup>a</sup>	95.0 <sup>a</sup>	658.6 <sup>a</sup>	233.5 <sup>a</sup>	7.7 <sup>a</sup>	263.5 <sup>a</sup>
PSE <sup>1</sup>	0.167	11.87	4.1	22.1	7.31	0.42	14.23
P value	0.49	0.28	<0.0001	0.44	0.6	0.84	0.8

<sup>1</sup>Pooled Standard Error.

<sup>2</sup>Not enough hemolymph to run samples.

Values represent the mean of 4 replicates. Letters that are different are significantly different ( $P < 0.05$ ).

was 60.2%, significantly lower than all other treatments which ranged from 90.0 to 96.5%.

### 3.4. Hemolymph osmotic and ionic concentrations

There were no significant differences in hemolymph osmolality,  $\text{Cl}^-$ ,  $\text{Na}^+$ , or  $\text{K}^+$  in either the  $\text{K}^+$  or  $\text{Mg}^{2+}$  supplementation experiments (Table 3). In the  $\text{K}^+$  trial, mean hemolymph osmolalities were  $629 \text{ mmol kg}^{-1}$ , while hemolymph chloride was  $264.8 \text{ mEq l}^{-1}$ . Water osmolality in this experiment was  $149.0 \text{ mmol kg}^{-1}$ . In the  $\text{Mg}^{2+}$  growth trial, mean hemolymph osmolality was  $647.7 \text{ mmol kg}^{-1}$ , whereas mean hemolymph chloride was  $246 \text{ mEq l}^{-1}$ . Water osmolality was  $130.0 \text{ mmol kg}^{-1}$ , slightly lower in the  $\text{K}^+$  trial. In the  $\text{K}^+$  trial, mean hemolymph  $\text{K}^+$  and  $\text{Na}^+$  was  $7.6 \text{ mEq l}^{-1}$  and  $278.3 \text{ mEq l}^{-1}$ , respectively. In the  $\text{Mg}^{2+}$  trial, mean hemolymph  $\text{K}^+$  and  $\text{Na}^+$  were also similar to those in the  $\text{K}^+$  trial,  $7.8 \text{ mEq l}^{-1}$  and  $264.8 \text{ mEq l}^{-1}$ , respectively.

### 3.5. Hepatopancreas mineralization

In the  $\text{K}^+$  trial, there were no significant differences observed among treatments in storage of  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Mg}^{2+}$ , or  $\text{Ca}^{2+}$  (Table 4). However, in the  $\text{Mg}^{2+}$  trial there were significant differences in the storage of  $\text{Mg}^{2+}$ . The  $160 \text{ mg l}^{-1}$  treatment had significantly higher hepatopancreatic storage of  $\text{Mg}^{2+}$  ( $0.85 \text{ mg g}^{-1}$ ) when compared to the  $10$  ( $0.62 \text{ mg g}^{-1}$ ),  $20$  ( $0.61 \text{ mg g}^{-1}$ ),

Table 4  
Selected mineral content ( $\text{mg g}^{-1}$ ) of the hepatopancreas for *Litopenaeus vannamei* in growth trials

	Minerals			
	$\text{Na}^+$	$\text{K}^+$	$\text{Mg}^{2+}$	$\text{Ca}^{2+}$
<i>K<sup>+</sup> trial</i>				
5	9.70 <sup>a</sup>	3.74 <sup>a</sup>	1.09 <sup>a</sup>	3.74 <sup>a</sup>
10	11.18 <sup>a</sup>	4.10 <sup>a</sup>	1.02 <sup>a</sup>	4.55 <sup>a</sup>
20	8.95 <sup>a</sup>	3.29 <sup>a</sup>	0.93 <sup>a</sup>	5.23 <sup>a</sup>
40	10.65 <sup>a</sup>	4.06 <sup>a</sup>	1.03 <sup>a</sup>	4.32 <sup>a</sup>
Reference	11.90 <sup>a</sup>	4.11 <sup>a</sup>	0.98 <sup>a</sup>	4.09 <sup>a</sup>
PSE *	1.0	0.47	0.1	0.62
P value	0.394	0.7	0.84	0.54
<i>Mg<sup>2+</sup> trial</i>				
10	13.44 <sup>a</sup>	4.96 <sup>a</sup>	0.62 <sup>b</sup>	3.09 <sup>a</sup>
20	11.48 <sup>a</sup>	4.08 <sup>a</sup>	0.61 <sup>b</sup>	3.09 <sup>a</sup>
40	13.28 <sup>a</sup>	4.75 <sup>a</sup>	0.67 <sup>b</sup>	3.26 <sup>a</sup>
80	11.19 <sup>a</sup>	4.26 <sup>a</sup>	0.74 <sup>a,b</sup>	3.18 <sup>a</sup>
160	11.43 <sup>a</sup>	4.28 <sup>a</sup>	0.85 <sup>a</sup>	3.10 <sup>a</sup>
PSE *	1.03	0.14	0.048	0.046
P value	0.38	0.46	0.019	0.97

Values represent the mean of 4 replicates.

\* Pooled standard error.

Table 5

Respiration rate ( $\text{mg O}_2 \text{ g}^{-1} \text{ shrimp h}^{-1}$ ) of *L. vannamei* cultured in 4.0 ppt low salinity waters with different concentrations of  $\text{K}^+$  and  $\text{Mg}^{2+}$

Treatment	n	Weight (g)	Respiration rate ( $\text{mg O}_2/\text{g shrimp/h}$ )
<i>K<sup>+</sup> trial</i>			
5	7	3.91	0.375 <sup>a</sup>
10	7	4.11	0.415 <sup>a</sup>
20	6	5.45	0.356 <sup>a</sup>
40	6	5.46	0.336 <sup>a</sup>
Reference	6	4.91	0.358 <sup>a</sup>
<i>Mg<sup>2+</sup> trial</i>			
10	8	3.96	0.494 <sup>a</sup>
20	6	4.39	0.434 <sup>a,b</sup>
40	6	4.42	0.344 <sup>a,b</sup>
80	6	5.23	0.299 <sup>b</sup>
160	6	4.17	0.317 <sup>a,b</sup>

and  $40$  ( $0.67 \text{ mg g}^{-1}$ )  $\text{mg l}^{-1} \text{ Mg}^{2+}$  treatments. The  $160 \text{ mg l}^{-1}$  was not significantly different from the  $80 \text{ mg l}^{-1}$  ( $0.74 \text{ mg g}^{-1}$ ) treatment. In the  $\text{Mg}^{2+}$  trial there were no significant differences in hepatopancreas storage of  $\text{Na}^+$ ,  $\text{K}^+$ , or  $\text{Ca}^{2+}$ .

### 3.6. Respirometry

Respirometry trials conducted with shrimp reared in low salinity waters containing various levels of  $\text{K}^+$  ions revealed no significant differences in respiration rates (Table 5). Respiration rates ranged between  $0.336$  and  $0.415 \text{ mg O}_2 \text{ g shrimp}^{-1} \text{ h}^{-1}$  for all treatments. However, significant differences in respiration ( $P < 0.05$ ) occurred among respirometry trials containing various levels of  $\text{Mg}^{2+}$  ions (Table 5). Respiration rates ranged between  $0.299$  and  $0.494 \text{ mg O}_2 \text{ g shrimp}^{-1} \text{ h}^{-1}$ . The  $10 \text{ mg l}^{-1} \text{ Mg}^{2+}$  treatment yielded the highest respiration rate ( $0.494 \text{ mg O}_2 \text{ g shrimp}^{-1} \text{ h}^{-1}$ ) and was significantly different from the  $80 \text{ mg l}^{-1}$  treatment ( $0.299 \text{ mg O}_2 \text{ g shrimp}^{-1} \text{ h}^{-1}$ ). The  $160 \text{ mg l}^{-1} \text{ Mg}^{2+}$  treatment produced the second lowest respiration rate ( $0.317 \text{ mg O}_2 \text{ g shrimp}^{-1} \text{ h}^{-1}$ ), however, this was not significantly different from the  $10 \text{ mg l}^{-1} \text{ Mg}^{2+}$  treatment.

## 4. Discussion

Various strategies have been proposed to improve the growth and survival of shrimp reared in inland low salinity well waters. These strategies were devised for specific species reared in inland low salinity waters. However, inland low salinity waters differ from each other, and variations in ionic profiles occur even in waters derived from the same saline aquifer (Saoud et al., 2003). Thus, it is

common for saline well waters to have ionic profiles unsatisfactory for shrimp culture. Mineral supplements, in the form of fertilizers rich in  $K^+$  and  $Mg^{2+}$ , have been suggested as remediation methods whereby the osmoregulatory capacity and thus growth and survival of shrimp cultured in low salinity waters might be ameliorated (Saoud et al., 2003; Gong et al., 2004; McNevin et al., 2004). Results of the present study demonstrate an effect of  $K^+$  and  $Mg^{2+}$  on shrimp physiology.

#### 4.1. $K^+$ trials

Results from the 14-day trial with postlarvae indicate a response to  $K^+$  concentration in the rearing medium. The fact that better growth was observed in the reference treatment was probably because ion ratios were similar to those of natural oceanic water. In experimental treatments, we observed an increase in PL survival and mean individual weight concurrent with an increase in the amount of  $K^+$  (resulting in a decrease in Na:K ratio). Similar results were reported by McGraw et al. (2002) and McGraw and Scarpa (2004), who found that PL age, salinity endpoint, and rate of salinity reduction influence PL survival following acclimation to low salinity rearing environments. In a study with PL<sub>18</sub> and PL<sub>28</sub> (McGraw and Scarpa, 2003) determined that a minimum concentration of  $1 \text{ mg l}^{-1} K^+$  was necessary for adequate survival following a 48-h acclimation period in freshwater (0.7 ppt). Saoud et al. (2003) and Davis et al. (2005) also observed a positive correlation between PL survival following acclimation to low salinity water and levels of  $K^+$ . However, the low survival observed in the preliminary trial suggests that the effects of low salinity acclimation can extend beyond the 24- to 48-h period assayed by Saoud et al. (2003) suggesting that although short term bioassays are a good way to quickly screen waters, longer term studies should also be conducted.

In the present experiment, shrimp reared in 4 ppt reconstituted seawater had significantly higher weight gain when compared to all other treatments. Because the reference and the  $40 \text{ mg l}^{-1} K^+$  treatment had nearly identical Na:K ratios (29:1 vs. 30:1), other ions or ion ratios must have influenced growth and well being. Furthermore, results of the present work indicate that the closer the Na:K ratio is to 28:1 (which is the ratio found in full strength seawater) the better the growth of the animals. The poor survivals and slower growth in the treatment with the lowest concentration of  $K^+$  had the highest Na:K ratio (119:1). In a series of farm trials conducted in west Alabama where shrimp were reared on two separate farms containing different ionic profiles, better growth and survival were obtained in the farm with the Na:K ratio

most similar to full strength seawater (Roy et al., 2006). Our results are similar to those obtained by Zhu et al. (2004) who reported that a molar Na:K ratio of 40–43:1 was adequate for *L. vannamei* reared at 30 ppt. In the study by Zhu et al. (2004), the lack of an adequate Na:K ratio resulted in low activity and death of juvenile shrimp at a salinity of 30 ppt. Fielder et al. (2001) also reported an influence of Na:K ratio on growth of Australian snapper, *Pagrus auratus*, cultured in saline groundwater deficient in  $K^+$ .

Respiration rates have been utilized in other studies with crustaceans to examine the effect of salinity acclimation (Rosas et al., 1997, 1999, 2001; Pillai and Diwan, 2002). To our knowledge, however, no studies have yet addressed the effect of specific ions ( $K^+$ ,  $Mg^{2+}$ ) essential for osmoregulation in low salinity environments on respiration rates in shrimp. In our study, there were no differences in respiration rates among shrimp in the different treatments of the  $K^+$  Trial. The respiration rates for the two lowest  $K^+$  treatments were numerically higher ( $0.375\text{--}0.415 \text{ mg O}_2 \text{ g shrimp}^{-1} \text{ h}^{-1}$ ) than the other three treatments ( $0.336\text{--}0.358 \text{ mg O}_2 \text{ g shrimp}^{-1} \text{ h}^{-1}$ ), but were not significantly different. Respiration rates obtained in our experiments are slightly lower than those observed by Rosas et al. (2001) who reported the effect of salinity acclimation on respiration rates in juvenile *Litopenaeus vannamei* of the approximate same size as animals utilized in our experiments. However, Rosas et al. (2001) examined the effect of rapid salinity reduction to a variety of salinities in shrimp acclimated for a 4-day period, whereas our experiment was conducted with animals that had been residing in experimental waters for several weeks and were fully acclimated, thus resulting in much lower respiration rates. In addition, Rosas et al. (2001) reported their respiration rates as  $\text{mg O}_2 \text{ h}^{-1} \text{ g}^{-1}$  dry weight of shrimp, while in our experiments the weight of the shrimp was expressed as wet weight. While most studies have addressed the effect of acute salinity stress on *L. vannamei* our study examines a more chronic stress event associated with depressed levels of either  $K^+$  or  $Mg^{2+}$ . It is also worth noting that our study was conducted in artificial low salinity water made from reconstituted seawater and supplements of other select ions. These ionic ratios and concentrations would be much different than those of other studies conducted with low salinity waters containing profiles more similar to full strength seawater. The fact that differences in survival and growth were not reflected in respirometry measurements suggests that respirometry studies are not always adequate to evaluate stress in penaeid shrimp. Saoud and Anderson (2004) suggest that scope for growth studies that evaluate total energy assimilation and expenditure are better indicators of shrimp

well being. In the present study,  $K^+$  levels might have affected feed uptake or assimilation thus affecting growth.

#### 4.2. $Mg^{2+}$ trials

Results from the growth trial using various levels of  $Mg^{2+}$  indicate that shrimp growth was not influenced by depressed  $Mg^{2+}$  concentrations. There was a significant effect of aqueous  $Mg^{2+}$  concentrations on hepatopancreas storage of  $Mg^{2+}$ . In a previous study examining dietary supplementation of  $K^+$ ,  $Na^+$ ,  $Mg^{2+}$  for shrimp reared in low salinity water, no differences in hepatopancreas storage were observed (Roy et al., in press). In the present study, increased levels in the water resulted in increased hepatopancreas storage of  $Mg^{2+}$  but not  $K^+$ . Aquatic animals can obtain part or all of their physiological requirements for minerals from the water and mineral stores in tissues are quite often a good indicator of the mineral status of the animal (Davis and Gatlin, 1996). Consequently, the degree of hepatopancreas mineralization can be a useful indicator of physiological reserves for some minerals. The depressed levels of  $Mg^{2+}$  in the hepatopancreas would indicate that mineral status of the shrimp was impaired possibly by being inadequate for physiological demands. This is confirmed by the depressed survival in the treatment with the lowest  $Mg^{2+}$  concentration ( $10 \text{ mg l}^{-1}$ ), indicating a stressful environment due to depressed aqueous  $Mg^{2+}$  levels. Low aqueous  $Mg^{2+}$  concentrations were also associated with an increase in respiration rate in the shrimp (Table 5). The higher respiration rate of shrimp reared in low salinity water containing the lowest  $Mg^{2+}$  levels indicates that the shrimp were stressed and corroborates results of the survival experiment in which shrimp reared in high  $Mg^{2+}$  waters survived better than shrimp in the lowest  $Mg^{2+}$  waters. Results from this experiment are also in agreement with previous studies evaluating the impact of  $Mg^{2+}$  and other ions on survival of postlarvae acclimated short-term and long-term to low salinity water from various west Alabama farms (Saoud et al., 2003; Davis et al., 2005). It appears that similar effects of depressed  $Mg^{2+}$  in postlarvae were also observed in juvenile shrimp.

The present study demonstrated an effect of  $K^+$  on survival and growth but not on respiration and osmoregulation. Such results suggest a possible effect of  $K^+$  on ingestion or assimilation or other functions that do not strongly affect metabolism. Conversely, magnesium levels affected survival and metabolism but not osmoregulation. Hepatopancreas levels of  $Mg^{2+}$  in various treatments suggest an active regulation of magnesium levels, which probably explain differences in respiratory rates. The green gland probably had to work much more in shrimp

maintained in waters with  $10 \text{ mg l}^{-1} \text{ Mg}^{2+}$  for hepatopancreatic levels of magnesium to be similar to those of shrimp at  $80 \text{ mg l}^{-1} \text{ Mg}^{2+}$  (Mantel and Farmer, 1983).

#### 5. Conclusion

Farmers growing shrimp in inland LSWW with depressed levels of  $K^+$  and  $Mg^{2+}$  should continue to supplement  $K^+$  and  $Mg^{2+}$  directly to ponds using fertilizers. Potassium and magnesium should be maintained at adequate concentrations to ensure optimum growth and survival. Ratios of Na:K should approximate the ratio of these two ions found in full strength seawater (28:1). Magnesium levels are also important for shrimp well-being, and can be maintained by regulating ratios of divalent cations in the water. Thus, we suggest that ratios of Mg:Ca should also approximate those found in natural seawater (3.1:1) to ensure adequate survival of *L. vannamei* reared under low salinity conditions. Before stocking postlarvae, farmers should check  $Na^+$ ,  $K^+$ , and  $Mg^{2+}$  concentrations to ensure that ratios and concentrations of these specific ions are not deficient. Further research on rapid assays of stressful environments is also needed. The present experiment demonstrates that respirometry studies are adequate in some cases but not always. Further respirometry studies examining the effects of different ions (specifically  $K^+$  and  $Mg^{2+}$ ) on postlarvae acclimation to low salinity waters may help answer some of the questions associated with the high pond to pond variability in survival during the first several weeks following stocking.

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