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Evaluation of Tilapia Effluent with Ion Supplementation for Marine Shrimp Production in a Recirculating Aquaculture System

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Abstract

Reuse of fish effluent for the culture of marine shrimp, such as Pacific white shrimp, *Litopenaeus vannamei*, could provide an opportunity for the US shrimp farming industry to ease constraints (e.g., environmental concerns and high production costs) that have limited them in the past. In this study under laboratory-scale conditions, the feasibility of culturing *L. vannamei* in effluents derived from a commercial facility raising tilapia in recirculating aquaculture systems (RAS), supplemented with various salt combinations, was compared to the shrimp's survival and growth in well water supplemented with 17.6 (control) and 0.6 (freshwater treatment) g/L synthetic sea salt. Three independent trials were conducted in RAS in which survival and growth in the control, the freshwater treatment, and two effluent treatments were compared. Water quality during this study was within safe levels and no differences ($P < 0.05$) between treatments were observed for dissolved oxygen, nitrite, pH, total ammonia nitrogen, and temperature. However, average nitrate and orthophosphate levels were consistently more than an order of magnitude greater in the effluent treatments compared to the control and the freshwater treatments. Survival and growth of shrimp over 6-wk periods did not vary significantly between the control and the freshwater treatments; however, shrimp tested in the tilapia effluents often exhibited significant effects ($P < 0.05$) depending on the salts added. In the low-salinity waters, correlations ($P < 0.05$) were observed between Ca^{2+}, Mg^{2+}, Ca^{2+} and Mg^{2+}, K^+, Na^+ : K^+ and Ca^{2+} : K^+, and shrimp survival and growth. The results of this study revealed that *L. vannamei* can be raised in tilapia effluent when supplemented with synthetic sea salt (0.6 g/L), CaO (50 mg/L Ca^{2+}), and $MgSO_4$ (30 mg/L Mg^{2+}).

In the USA, shrimp is a high-value food that accounted for a $3.8 billion trade deficit in 2004 (Harvey 2005). The high level of shrimp importation is because of an inability to supply internal demands by national fisheries or aquaculture. Aquaculture of marine shrimp in the USA has been severely restricted because of high production costs and the value of coastal real estate located close to market bases. In addition to the latter constraints, the USA, as in other countries, has severely tightened and enforced its environmental regulations (Boyd 2003). These reasons have limited the development of sustainable shrimp farming in the USA. Nevertheless, the latter constraints can be addressed to varying degrees. For example, labor costs and environmental quality could be managed through automation of production systems and application of water reuse facilities. Real estate costs could be reduced by moving production facilities away from the coast.

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However, as most of the larger markets in the USA are in coastal areas, where by 2025, 75% of all Americans will reside (Culliton 1998), this will necessitate moving away from the marketplace as well as losing access to high-quality marine waters. Providentially, many penaeid shrimp are hyperosmotic in low-salinity waters (Castille and Lawrence 1981).

Because Pacific white shrimp, *Litopenaeus vannamei*, are excellent osmoregulators, research into this species has increased, especially as this relates to the feasibility of rearing these animals in low-salinity waters (Laramore et al. 2001; Atwood et al. 2003; McGraw and Scarpa 2003; Saoud et al. 2003; Sowers et al. 2005). However, low-saline water sources vary greatly in their ionic composition such that shrimp growth and survival may be compromised. Consequently, when contemplating the culture of marine shrimp in low-salinity waters, preliminary laboratory-scale tests must be undertaken upon rearing waters to assess their suitability, irrespective of facility type employed (e.g., ponds, recirculating aquaculture systems [RAS], etc.).

RAS have many advantages over pond or flow-through culture systems including conservation of water and supplemented ions, tighter control of water quality and biosecurity that improves survival and growth, enhanced effluent handling and discharge, and reduction in the risks of introducing disease and pollutants (Skjølstrup et al. 2000; Menasveta 2002; Timmons et al. 2002). Additionally, maximization of spent water use, for example, through polyculture (Tian et al. 2001), or use of RAS effluents for farming marine shrimp, might enhance water conservation, profitability, and ease environmental impacts. Although several studies have examined the combination of RAS and polyculture (e.g., aquaponics), comparatively few (Schneider et al. 2005) have suggested the application of RAS wastewater as a method for supplementing aquaculture income by producing a "catch crop." Accordingly, the present study considered the feasibility of rearing *L. vannamei* in effluents derived from an inland commercial Nile tilapia, *Oreochromis niloticus*, RAS production facility. The tilapia effluent was essentially considered as waste stream but had been heated and contained supplemental salt (NaCl). An ability to raise shrimp in this waste stream would therefore represent increased exploitation not only of the heat energy but also maximize the use of added salt. Research described herein includes evaluations of water ion concentrations and ion supplementation. A zero-exchange RAS was employed for these studies.

Materials and Methods

The culture of *L. vannamei*, initiated with $>PL_{25}$ (e.g., PL_{25} = 25-d-old postlarvae) in half-strength seawater (18 g/L salinity), was compared to salinity-challenged conditions over a 42-d period for three independent trials (Trials A–C). Prior to experimental start, preliminary trials were undertaken in order to establish survival of shrimp in tilapia effluents with and without added synthetic sea salt. These studies determined no significant differences (one-way ANOVA) in juvenile shrimp survival between treatments in which synthetic sea salt was added at $\geq$ 0.6 g/L. Subsequently, trials were undertaken to optimize shrimp survival and growth in tilapia effluents with minimum salt additions.

Shrimp Suppliers and Acclimation

Certified, specific pathogen-free shrimp postlarvae (PL) were supplied by commercial and research hatcheries. For the aforementioned preliminary trials, PLs were obtained from a commercial hatchery (Harlingen Shrimp Farms Ltd., Los Fresnos, TX, USA). For the trials presented in this study, PLs were acquired from The Oceanic Institute (Kailua-Kona, HI, USA). PL shrimp were air-freighted overnight. Dissolved oxygen (DO) was above saturation, water temperature between 18 and 21 C, and salinity either 18 (Harlingen Shrimp Farms) or 25 g/L (The Oceanic Institute). After arrival, shrimp were acclimated to well water supplemented with 22 g/L synthetic sea salt (Crystal Sea, Marineland, Baltimore, MD, USA). Once acclimatized, shrimp were transferred to aquaria outfitted with mechanical and biological filtration units. System water quality was DO $>$ 5.75 mg/L, total ammonia nitrogen (TAN) $<$ 0.30 mg/L,

and temperature was 28 ± 1.0 C. Animals were maintained under these holding conditions for a minimum of 72 h and until all the shrimp attained $>PL_{25}$.

The $>PL_{25}$ shrimp were acclimated to lower salinities using freshwater from a local well source. Salinity was adjusted using the following scheme according to Van Wyk et al. (1999): 32–16, 16–8, 8–4, 4–2, and 2–1 g/L with salinity reductions in steps of 2.0, 1.0, 0.5, 0.25, and 0.13 g/L per h, respectively. Once salinity levels in the acclimation tanks matched that of preselected experimental salinities (1.0–18.0 g/L), studies commenced.

Experimental Systems and Stocking Densities

Figure 1 provides an overview of an experimental system used during salinity challenges.

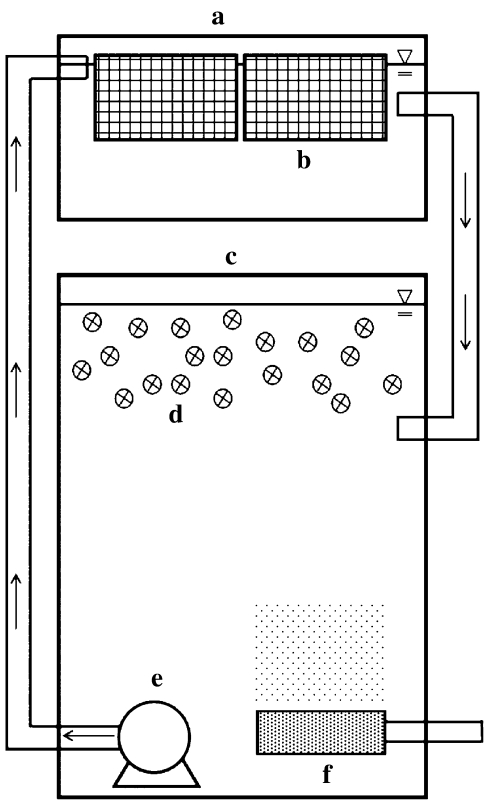

FIGURE 1. *Schematic of a system used to test shrimp performance: (a) three 38-L aquaria, (b) two 1.9-L breeder nets per aquarium, (c) 125-L nitrification reactor, (d) 20 L of Kaldness media, (e) water pump, and (f) 20-cm air diffuser.*

Three 38-L aquaria were used for each treatment, and each aquarium was outfitted with a 50 W Whisper® submersible heater (Tetra, Blacksburg, VA, USA) and two Lee's breeder nets (L. Schultz Inc., San Marcos, CA, USA). Each 1.9-L breeder net was initially stocked with 15 shrimp (8 shrimp/L) totaling 30 shrimp per aquaria. The 125-L nitrification reactors (Fig. 1) contained 20 L of Kaldness media (KMT) (Kaldnes Inc., Providence, RI, USA) and were fluidized using forced air from a 1 horsepower Sweetwater® regenerative blower that supplies forced air to the entire building. Water was pumped with a 40 W Quiet One Pond Pump (Pentair Aquatics™, El Monte, CA, USA) from the nitrification reactors into the three aquaria (at 200 L/h per aquarium) and the return was gravity fed back to the nitrification reactors.

Shrimp were fed a 35% protein, ground shrimp feed (Melick Aqua Feeds, Catawissa, PA, USA) to excess. Accumulated feed was removed by siphoning at the start of each day (thereby allowing overnight feeding). Aquaria were painted black on the exterior walls to minimize visual stress. A photoperiod of 12 h with an additional 15 min of low-intensity light (used to simulate dawn and dusk) was implemented daily using a 24-h Intermatic model ET100C (Intermatic Inc., Spring Grove, IL, USA) control system.

Water Source and Ionic Supplementation

Table 1 summarizes the water treatments employed during the study for the three independent trials, designated A, B, and C. In each trial, control water always consisted of well water (0.4 g/L salinity) supplemented with synthetic sea salt to 18 g/L salinity, whereas the freshwater treatment always consisted of well water supplemented with 0.6 g/L sea salt. Tilapia effluents, collected from the bottom of settling basins at the local commercial farm (Blue Ridge Aquaculture, Martinsville, VA, USA), were supplemented with various salt combinations (Table 1) including synthetic sea salt, CaO (technical grade calcium oxide, Fisher Scientific, Fairlawn, NJ, USA), $MgSO_4$ (epsom salt, Kroger Co., Cincinnati, OH, USA), and NaCl (noniodized table salt, Morton Salt, Louisville,

TABLE 1. *Tilapia effluent treatments with respective ion supplementation.*

Trial	Treatment	Water source (corresponding salinity [ppt])	Ion supplementation (concentration)
	Effluent 1	Tilapia effluent (1.5)	Sea salt (0.6 g/L)
A	Effluent 2	Tilapia effluent (1.5)	No supplementation
	Effluent 1	Tilapia effluent (1.1)	Sea salt (0.6 g/L), Ca^{2+} (50 mg/L), Mg^{2+} (30 mg/L), SO_4^{2-} (25 mg/L)
B	Effluent 2	Tilapia effluent (1.1)	Ca^{2+} (50 mg/L), Mg^{2+} (30 mg/L), Na^{+} (99 mg/L), SO_4^{2-} (25 mg/L), Cl^{-} (150 mg/L)
	Effluent 1	Tilapia effluent (1.2)	Sea salt (0.6 g/L), Ca^{2+} (50 mg/L), Mg^{2+} (30 mg/L), SO_4^{2-} (25 mg/L)
C	Effluent 2	Tilapia effluent (1.2)	Sea salt (1.0 g/L), Ca^{2+} (50 mg/L), Mg^{2+} (30 mg/L), SO_4^{2-} (25 mg/L)

Each trial (A–C) also included a control (18.0 g/L) and freshwater treatment (1.0 g/L).

KY, USA). Calcium and Mg^{2+} were supplemented to increase the divalent cation concentrations in the tilapia effluent because these proved to be in deficit. The tilapia effluent was treated using nitrification to reduce elevated ammonia (typically 2.0–3.5 mg/L) levels prior to experimentation. The effluent was aerated with cycled KMT media in 166-L drums until TAN was reduced to levels <0.30 mg/L. This reduction in TAN was typically observed within a 24-h period.

Sampling and Monitoring

Water quality was monitored using the methods and frequencies noted in Table 2. All sampling events were conducted at equal intervals

TABLE 2. *Methods and number of sampling events used to determine water quality constituents.*

Parameter	Number of sampling events (*n*) Trial A	Trial B	Trial C	Method
Ammonia-N, total	18	20	19	Nessler method, HACH method 8038[a,b,c]
Alkalinity	6	8	8	Sulfuric acid method, HACH method 8203[a,b]
Ions (Ba^{2+}, Ca^{2+}, Fe, K^{+}, Mg^{2+}, Mn, Na^{+}, Pb, Cl^{-}, So_4^{2-})	2	3	3	Ion Chromatograhy[c,d,e], DIONEX 120, AS 40 autosampler, outfitted with AG 9HC and AS 9HC columns
Chloride	0	11	7	Mercuric thiocyanate method, HACH method 8113[a]
Color, apparent	8	10	7	Platinum–cobalt standard method, HACH method 8025[a]
Dissolved oxygen	20	21	18	YSI model 85 (Yellow Springs, OH, USA)
Hardness, calcium	0	10	7	Titration with EDTA, HACH method 8204[a,b]
Hardness, total	6	8	7	Titration with EDTA, HACH method 8213[a,b]
Nitrite-N	13	12	12	Diazotization method, HACH method 8507[a,c]
Nitrate-N	13	8	10	Cadmium reduction method, HACH method 8039[a,b]
Orthophosphate	6	8	9	Ascorbic acid method, HACH method 8048[a,b,c]
pH	16	15	12	HI 9024 pH meter (HANNA Instrument, Woonsocket, RI, USA)
Salinity	22	24	18	YSI model 85 (Yellow Springs, OH, USA)
Temperature	20	29	21	YSI model 85 (Yellow Springs, OH, USA)
Turbidity	8	8	5	HF Scientific DRT-15 CE turbidimeter (HF Scientific Inc., Fort Myers, FL, USA)

EDTA = Ethylenediaminetetraacetic acid.

[a] Hach Co.

[b] Method developed/adapted from Standard Methods for the Examination of water and Wastewater, APHA (1998).

[c] USEPA approved for wastewater analysis.

[d] Standard Methods for the Examination of Water and Wastewater, APHA (1998).

[e] DIONEX Corp., Sunnyvale, California, USA.

during the trials. HACH samples were analyzed using a HACH DR/2400 spectrophotometer and a HACH digital titrator (Hach Co., Loveland, CO, USA). A modification to the Nessler method (HACH 8038) was used for the high-salinity treatments, which included 10 drops of mineral stabilizer instead of 3 that is typically used for freshwater samples. A standard comparison was conducted for 0.50 mg/L TAN between dionized water and 18 g/L seawater (dionized water with synthetic sea salt). Triplicate measurements for TAN resulted in the following means (95% confidence intervals) for the freshwater and 18 g/L seawater samples, respectively, 0.490 (0.430–0.550) and 0.492 (0.480–0.503) mg/L. Most of the samples (Table 2) were analyzed immediately after sampling events. Samples that were not immediately analyzed (including analysis for anions and cations) were handled and stored in accordance with Standard Methods for the Examination of Water and Wastewater (APHA 1998). As an extra precaution, to ensure that water quality did not degrade over time, apparent color, calcium hardness, chloride (HACH method), salinity, and total hardness also were monitored. However, these parameters were not an issue during this study. Unionized ammonia was determined using the following equilibrium equations (Emerson et al. 1975):

$$\%NH_3 = \frac{1}{(10^{pK_a - pH} + 1)} \quad (1)$$

$$pK_a = 0.09018 + \frac{2729.92}{T} \quad (2)$$

where T = temperature in degrees Kelvin.

Performance Indicators

The impact of varying treatments (Table 1) was monitored by assessing survival, growth, and specific growth rates (SGR). Survival was determined by counting shrimp every 24 h for the first week and thereafter at 48-h periods. On Days 1 and 42, shrimp were patted dry using Kim Tech Wipes (Kimberly-Clark, Roswell, GA, USA) and weighed using an A&D HM-202 analytical balance (A&D Engineering Inc., Milpitas, CA, USA) to the nearest 0.0001 g. SGR (Eqn 3) was determined using the following formula (Ricker 1975):

$$\mathrm{SGR}\left(\frac{\%}{\mathrm{d}}\right) = \frac{100[\log_e \text{shrimp final mass (g)} - \log_e \text{shrimp initial mass(g)}]}{\text{time(d)}} \quad (3)$$

Statistical Analysis

Statistical analysis was performed using SAS version 9.1 for Windows (SAS Institute Inc., Cary, NC, USA). Differences in water quality were considered significant when $P < 0.05$. A one-way ANOVA was employed with a Duncan's Multiple Range Test (where appropriate) to test significant differences ($P < 0.05$) between treatments for 6-wk growth and survival. A two-tailed Pearson's correlation coefficient analysis was utilized to determine correlations between survival and growth with various ions in the low-salinity treatments.

Results

Water quality results are presented in Table 3. There were no differences in DO, nitrite, pH, TAN, unionized ammonia, or temperature between treatments. Unionized NH_3-N concentrations were notably highest during Trial A. In particular, the freshwater treatment and Effluent 1 experienced a 24 h unionized NH_3-N spike of 0.20 mg/L between 4 and 5 d (data not shown). Nitrate and orthophosphate on average were consistently more than an order of magnitude greater in the effluent treatments compared to the control and the freshwater treatments. Alkalinity and turbidity did not differ significantly between treatments. The following unreported water quality parameters that were used to check water quality consistency did not deviate more than 75% (apparent color), 30% (calcium hardness), 19% (chloride, HACH method), 17% (total hardness) when all treatments during the entire study were considered.

Survival and growth over the 6 wk for Trials A–C are noted in Table 4. No differences in

TABLE 3. *Water quality results, mean values with 95% confidence intervals.*

Parameter	Treatment	Trial A	Trial B	Trial C
	Control	0.56 (0–1.6)	0.22 (0–0.49)	0.17 (0.098–0.24)
	Freshwater	0.58 (0–1.8)	0.084 (0–0.16)	0.079 (0–0.17)
	Effluent 1	0.36 (0–0.97)	0.22 (0.10–0.34)	0.17 (0–0.38)
Ammonia-N, total (mg/L)	Effluent 2	0.31 (0–0.24)	0.20 (0.066–0.33)	0.18 (0.061–0.31)
	Control	0.077 (0–0.19)	0.034 (0–0.075)	0.038 (0.026–0.050)
	Freshwater	0.087 (0–0.024)	0.014 (0–0.032)	0.018 (0.002–0.034)
	Effluent 1	0.074 (0–0.25)	0.052 (0.018–0.086)	0.042 (0–0.10)
Ammonia-N, unionized (mg/L)	Effluent 2	0.058 (0–0.12)	0.050 (0.007–0.094)	0.045 (0.017–0.073)
	Control	5.87 (5.13–6.61)	5.64 (5.02–6.27)	5.53 (4.79–6.27)
	Freshwater	6.04 (5.53–6.55)	6.00 (5.63–6.37)	6.23 (5.42–7.03)
	Effluent 1	6.61 (5.99–7.24)	5.99 (5.61–6.37)	6.00 (5.31–6.69)
Dissolved oxygen (mg/L)	Effluent 2	6.69 (6.23–7.15)	6.19 (5.85–6.53)	6.05 (5.27–6.84)
	Control	0.014 (0–0.40)	0.057 (0–0.13)	0.012 (0–0.037)
	Freshwater	0.026 (0–0.052)	0.013 (0–0.035)	0.003 (0–0.006)
	Effluent 1	0.066 (0–0.22)	0.046 (0–0.12)	0.007 (0–0.015)
Nitrite-N (mg/L)	Effluent 2	0.027 (0.008–0.046)	0.033 (0–0.94)	0.010 (0–0.020)
	Control	3.9 (1.7–6.0)	8.1 (0–18)	8.5 (0–17)
	Freshwater	5.7 (3.0–8.3)	18 (0–38)	4.5 (0–9.6)
	Effluent 1	63 (38–87)	100 (29–170)	61 (0–150)
Nitrate-N (mg/L)	Effluent 2	67 (46–87)	94 (48–140)	74 (0–150)
	Control	2.9 (0.17–5.6)	2.8 (0–5.9)	2.7 (2.0–3.5)
	Freshwater	1.9 (0.65–3.1)	1.9 (0.16–3.6)	0.44 (0.19–0.70)
	Effluent 1	7.3 (6.0–8.6)	4.3 (3.2–5.4)	4.7 (0–10)
Orthophosphate (mg/L)	Effluent 2	6.9 (5.3–8.5)	4.2 (3.3–5.1)	5.0 (2.0–8.0)
	Control	8.42 (8.20–8.65)	8.41 (8.17–8.66)	8.57 (8.39–8.67)
	Freshwater	8.48 (8.18–8.77)	8.44 (8.24–8.64)	8.55 (8.16–8.94)
	Effluent 1	7.3 (6.0–8.6)	4.3 (3.2–5.4)	4.7 (0–10)
pH	Effluent 2	6.9 (5.3–8.5)	4.2 (3.3–5.1)	5.0 (2.0–8.0)
	Control	18.5 (17.0–20.0)	18.2 (16.1–20.4)	18.0 (15.7–20.4)
	Freshwater	1.06 (0.91–1.21)	1.03 (0.94–1.12)	1.03 (0.80–1.27)
	Effluent 1	2.11 (2.02–2.02)	2.10 (2.03–2.18)	1.59 (1.3–1.9)
Salinity (g/L)	Effluent 2	1.59 (1.41–1.76)	1.52 (1.38–1.66)	2.26 (2.10–2.41)
	Control	25.6 (24.1–27.1)	28.0 (26.8–29.2)	29.3 (27.3–31.3)
	Freshwater	25.9 (24.4–27.5)	28.4 (27.8–29.0)	28.3 (26.9–31.2)
	Effluent 1	25.8 (25.0–26.6)	28.7 (27.6–29.8)	29.0 (26.9–31.2)
Temperature (C)	Effluent 2	25.7 (24.7–26.7)	28.2 (27.1–29.3)	28.7 (26.9–30.5)

growth or survival were observed between the control and the freshwater treatment during any of the trials. However, growth and survival in the tilapia effluent (Effluents 1 and 2) did vary significantly ($P < 0.05$) depending on the ion supplementation.

More specifically, during Trial A, Effluent 1 exhibited lower ($P < 0.05$) growth and survival rates compared to the control and the freshwater treatment, whereas in Effluent 2, survival was 0% (by Day 37). In Trial B, addition of sea salt, CaO, and $MgSO_4$ (Effluent 1) or NaCl, CaO, and $MgSO_4$ (Effluent 2) to tilapia effluent resulted in differences ($P < 0.05$) in survival and growth when compared to the control and the freshwater treatment (Table 4). With respect to growth, the following order was obtained, freshwater treatment = control > Effluent 1 > Effluent 2. Consideration of survival illustrated the following: control = freshwater treatment = Effluent 1 > Effluent 2. In Trial C, addition of sea salt, CaO, and $MgSO_4$ (levels noted in Table 1) to the tilapia effluents resulted in similar levels of survival to those observed in the control and the freshwater treatment. In terms of growth, no differences were observed between the control, the freshwater treatment, and Effluent 2. However, differences ($P < 0.05$) were recorded in growth

TABLE 4. *Six-week survival and growth results for Trials A–C.*

Trial	Treatment	Initial mass (g)	Final mass (g)	Specific growth rate	Survival (%)
	Control	0.03442	0.2978[a]	5.14	67[a]
	Freshwater	0.03442	0.2694[a]	4.90	73[a]
	Effluent 1	0.03442	0.1103[b]	2.77	33[b]
	Effluent 2	0.03442	*	*	0[c]
	Pooled error		0.01641		5.199
A	$P > F$		<0.0001		<0.0001
	Control	0.07422	0.6943[a]	5.32	50[a]
	Freshwater	0.07422	0.6995[a]	5.34	46[a]
	Effluent 1	0.07422	0.4977[b]	4.53	46[a]
	Effluent 2	0.07422	0.2750[c]	3.12	27[b]
	Pooled error		0.03905		4.517
B	$P > F$		<0.0001		0.0243
	Control	0.07802	0.4945[b]	4.40	69[a]
	Freshwater	0.07802	0.4649[b]	4.25	50[a]
	Effluent 1	0.07802	0.6256[a]	4.96	61[a]
	Effluent 2	0.07802	0.5271[b]	4.55	74[a]
	Pooled error		0.03564		9.046
C	$P > F$		0.0092		0.3261

Superscript letters denote significant differences between treatments within the respective trial.
* Growth at 6 wk not measured because of 100% mortality by Day 37.

for Effluent 1 animals, which were larger than other treatments in this trial (Table 4).

Recorded mean ion levels (with range) observed for the different low-salinity treatments are presented in Table 5. As might be anticipated, variations in water ion content occurred following additions of the various salts. Because growth and survival of shrimp maintained in the control and the freshwater treatment were similar (Table 4, Trials A–C), the ion data sets indicate that appropriate ion levels were present in the freshwater treatments (Table 5, Trials A–C). However, the ion composition in the effluent treatments (Table 5) demonstrated that the ion levels did not always support shrimp survival and growth (Table 4). Correlations between survival and growth with various ions are presented in Table 6. Calcium, Mg^{2+}, and $Ca^{2+} + Mg^{2+}$ ($P < 0.01$) were significantly correlated with survival in the low-salinity waters. Growth was significantly correlated with Mg^{2+} ($P < 0.05$), and an even stronger correlation was observed with Ca^{2+}, $Ca^{2+} + Mg^{2+}$, $Na^+ : K^+$, and $Ca^{2+} : K^+$ ($P < 0.01$). A negative correlation ($P < 0.05$) was observed between growth and K^+.

Discussion

Water quality (Table 3), except for ion composition (Table 5), was not considered a cause of poor shrimp performance observed in many of the effluent treatments (Table 4). The short-term unionized ammonia spikes of 0.20 mg/L observed during this study were <7% of the reported 24-h 50% lethal concentration (LC_{50}) of 2.95 mg/L (Lin and Chen 2001). Nitrite concentrations during the entire study (<0.38 mg/L) also were considered to be within acceptable levels, for example, Lin and Chen (2003) determined acceptable levels of nitrite to be 6.1 mg/L at 15 g/L salinity. Even though nitrate levels were consistently higher in the effluent treatments when compared to controls and freshwater treatments, the highest concentrations never exceeded 5% of the 48-h LC_{50} (3400 mg/L) for shrimp as determined by Wickins (1976). Furthermore, temperatures were maintained within the recommended range (23–30 C) for favorable growth and survival of *L. vannamei* (Wyban et al. 1995).

Even though the initial mass of shrimp used in Trial A were significantly smaller than shrimp used in Trials B and C, these shrimp were $>PL_{30}$ by the time the experiment commenced.

McGraw et al. (2002) noted that PL_{15} can be acclimated to low salinities (1.0 g/L), and PL at this age have developed proper osmoregulation ability because of extensive filament branching of the gills (Palacios et al. 2004).

Treatments varied in ion composition (Table 5), and it was these variations that affected shrimp performance (Table 6). Shrimp in both effluent treatments (Effluent 1 and 2) during Trial A demonstrated poor survival and growth. During this trial, Ca^{2+} levels were consistently lower (<32%) in the effluent treatments than that measured in freshwater treatments. Magnesium was in deficit as well, especially in Effluent 2. Magnesium levels were <44% of those measured in freshwater treatments. This warranted supplementation of Ca^{2+} and Mg^{2+} in the remaining trials (Trials B and C), which resulted in improved shrimp performance (Table 4). Calcium and Mg^{2+} are vital to shrimp because they represent major components of the exoskeleton (Ca^{2+} = 15.95%, Mg^{2+} = 1.19% in Vijayan and Diwan 1996) and are important for normal physiological processes. For example, Ca^{2+} is essential for binding many proteins (Endo et al. 2002), whereas both Ca^{2+} and Mg^{2+} are engaged in enzymatic reactions (Xie et al. 2004). The demand for calcium in crustaceans is highest during ecdysis (Greenaway 1985), and Vijayan and Diwan (1996) suggested that the Ca^{2+} needed for cuticular mineralization is directly absorbed from ambient water.

The results from this study are consistent with Atwood et al. (2003) and Sowers et al. (2005), in that *L. vannamei* were deemed to require sea salt for survival and growth when compared to mixed salt environments at salinities ≤ 2.0 g/L. This is likely because of the essential trace minerals that sea salt contains. Even though the ionic composition in the hemolymph of shrimp is predominately Na^+ and Cl^- (Chen and Chen 1996), penaeid shrimp require additional ions to Na^+ and Cl^- in the culture medium for adequate survival and growth (Cawthorne et al. 1983). Furthermore, Atwood et al. (2003) challenged *L. vannamei* in waters supplemented with 0, 0.25, 1.0 g/L sea salt with and without CaCl and NaCl. These authors found that

TABLE 5. *Mean ion concentration (range) observed in low-salinity treatments.*

		Constituent									
Trial	Treatment	Ba^{2+}(mg/L)	Ca^{2+}(mg/L)	Fe (mg/L)	K^+ (mg/L)	Mg^{2+} (mg/L)	Mn (mg/L)	Na^+ (mg/L)	Pb (mg/L)	Cl^- (mg/L)	So_4^{2-} (mg/L)	Na : K (mg/L)
A	Freshwater	<0.05	89 (58–121)	<0.01	14 (13–14)	74 (70–78)	<0.01	268 (256–280)	<0.01	854 (830–878)	281 (275–286)	35.3
	Effluent 1	<0.05	28 (14–41)	<0.01	78 (73–83)	59 (56–63)	<0.01	558 (438–677)	<0.01	1420 (1370–1470)	369 (350–388)	12.2
	Effluent 2	<0.05	21 (11–32)	<0.01	76 (67–84)	32 (20–43)	<0.01	494 (473–515)	<0.01	668 (655–681)	241 (207–274)	11.0
B	Freshwater	<0.05	97 (57–137)	0.09	16 (11–19)	56 (49–60)	<0.01	242 (227–257)	<0.01	517 (488–569)	133 (120–151)	25.5
	Effluent 1	<0.05	80 (66–98)	0.10	76 (73–80)	58 (53–64)	<0.01	594 (579–606)	<0.01	703 (694–711)	279 (270–285)	13.2
	Effluent 2	<0.05	63 (59–67)	0.09	69 (65–74)	54 (48–59)	<0.01	434 (419–450)	<0.01	389 (353–440)	234 (215–263)	11.0
C	Freshwater	<0.05	63 (63–65)	<0.01	14 (11–17)	54 (44–67)	<0.01	227 (224–229)	<0.01	500 (492–509)	141 (133–148)	27.0
	Effluent 1	<0.05	91 (89–97)	<0.01	98 (97–99)	54 (53–57)	<0.01	576 (525–602)	<0.01	720 (714–723)	372 (344–388)	10.1
	Effluent 2	<0.05	96 (91–101)	<0.01	85 (82–89)	65 (62–69)	<0.01	642 (618–690)	<0.01	1000 (991–1020)	360 (342–373)	12.7

Refer to Table 2 for number of sampling events.

TABLE 6. *Pearson's correlation coefficient* (P *value*) *for 42-d survival and growth (data pooled from low-salinity treatments).*

	Ca^{2+}	K^+	Mg^{2+}	Na^+	Cl^-	So_4^{2-}	$Na^+ + K^+$	$Ca^{2+} + Mg^{2+}$	$Cl^- + So_4^{2-}$	$Na^+ : K^+$	$Ca^{2+} : K^+$	$Ca^{2+} : Cl^-$
Survival	0.521**	−0.162	0.474**	−0.043	−0.036	−0.002	−0.063	0.596**	−0.029	0.288	0.274	0.068
	(0.002)	(0.367)	(0.005)	(0.814)	(0.844)	(0.992)	(0.727)	(<0.001)	(0.875)	(0.105)	(0.123)	(0.705)
Growth	0.818**	−0.407*	0.406*	−0.337	−0.178	−0.179	−0.351	0.839**	−0.187	0.558**	0.679**	0.207
	(<0.001)	(0.049)	(0.049)	(0.107)	(0.405)	(0.403)	(0.092)	(<0.001)	(0.381)	(0.005)	(<0.001)	(0.332)

* Correlation is significant at the 0.05 level (two tailed).
** Correlation is significant at the 0.01 level (two tailed).

0.25 g/L sea salt, even with CaCl and NaCl supplementation, was insufficient for adequate survival and growth, whereas 1.0 g/L sea salt supplementation supported growth. The present study demonstrates that 0.6 g/L sea salt supplementation is feasible for shrimp culture, which could potentially save upward of 40% of the costs associated with the addition of synthetic sea salt.

Potassium is a principle intracellular ion (Shiau and Hshieh 2001) and often can be a limiting ion for *L. vannamei* performance under salinity-challenged conditions. Levels of K^+ in this study were more than an order of magnitude greater than the recommended minimum concentration of 1 mg/L required for the culture of *L. vannamei* at low salinities (McGraw and Scarpa 2003). Saoud et al. (2003) observed a positive correlation between K^+ and shrimp survival, while results from this study demonstrated the opposite effect for growth (Table 6). This negative correlation was partly because of the poor performance observed in the tilapia effluent, which has relatively high levels of K^+ (between 49 and 78 mg/L) without the necessary synthetic sea salt supplementation (Effluent 2 in Trials A and B). Zhu et al. (2004) reported that *L. vannamei* weight gain, SGR, and food conversion efficiencies improved as the $Na^+ : K^+$ ratio decreased over the range of 187.4–34.1 mmol/mmol. The $Na^+ : K^+$ ratios in this study were equal to and below this range in the low-salinity treatments and except for the positive correlation observed in Table 6 (in terms of growth), ratios lower than 34.1 mmol/mmol did not impact shrimp performance.

Conclusions

Even though there are conflicting reports regarding the effects of low-salinity waters on survival and growth of *L. vannamei* (Ogle 1992; Bray et al. 1994; Laramore et al. 2001; Atwood et al. 2003; Sowers et al. 2005), this study demonstrated that there were no differences between the high-salinity treatments (18 g/L) and the lowest salinity treatments (1.0 g/L). Moreover, correlations between Ca^{2+}, Mg^{2+}, $Ca^{2+} + Mg^{2+}$, K^+, $Na^+ : K^+$ and $Ca^{2+} : K^+$, and shrimp performance were observed in the

low-salinity waters. This study also demonstrated that *L. vannamei* can be reared in effluents produced by an inland commercial tilapia RAS when the water is supplemented with synthetic sea salt (0.6 g/L), CaO (50 mg/L Ca^{2+}), and $MgSO_4$ (30 mg/L Mg^{2+}).

Fish effluent can be responsible for negative cash flows for producers, but using shrimp as a ''catch crop'' or ''cash crop'' in the effluent is a viable solution that can provide for a sustainable and profitable operation. Reuse of fish effluents for the culture of marine shrimp in RAS systems could provide the US shrimp farming industry an opportunity to overcome limitations that have previously prevented sustainability and economic success in the past.

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