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Evaluating a low-cost salt mixture in brackish water intensive shrimp (*litopenaeus vannamei*) production systems

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Abstract

Inland production of marine shrimp provides high-quality shrimp to consumers. Artificial sea salts are added to local water to provide the essential minerals required by shrimp; however, commercial salts are expensive. An experiment evaluated different combinations of a homemade, least-cost salt mixture (LCS) and a common commercial sea salt (CSS). The LCS formulation was made using six salts: NaCl, $MgSO_4$, $MgCl_2$, $CaCl_2$, KCl, and $NaHCO_3$. The five treatments in this study were as follows: 100% LCS, 75/25% LCS/CSS, 50/50% LCS/CSS, 25/75% LCS/CSS, and 100% CSS; each treatment was randomly assigned to four 1 m^3 tanks. There were some significant differences between treatments in DO, pH, and nitrite concentration, but these differences were subtle. There were no significant differences in mean weight, growth rate, FCR, biomass, or survival of shrimp. The 100% LCS salt formulation was 65% less expensive than the CSS mixture by weight, and the cost per kg of shrimp produced was 57% lower using the LCS. In fact, even the 50/50% treatment had a significantly ($p \le 0.05$) lower cost kg^{-1} shrimp than the 100% CSS treatment. These results indicate that this LCS formulation is suitable for intensive shrimp production, and the cost savings may be substantial.

KEYWORDS
hybrid systems, inland marine aquaculture, intensive production, RAS, salt formulation, shrimp

1 | INTRODUCTION

Inland production of Pacific white shrimp (*L. vannamei*) is becoming more common in the aquaculture industry, as this popular seafood item can be raised year-round using recirculating aquaculture systems (RAS). RAS can be constructed indoors, providing a tightly controlled environment that facilitates enhanced biosecurity, higher animal stocking densities, minimal space, and limited water exchange compared to traditional ponds. Producers can provide a fresh, never-frozen, high-quality, and consistent product to nearby markets, practically anywhere (Timmons & Ebeling, 2010). Developments in RAS technology come as aquaculture supplies approximately half of the world's seafood, and an increase in overall production is needed to meet global demand in the future (FAO, 2018).

Marine shrimp RAS techniques include clear-water (CW), biofloc (BF), and hybrid systems (HY) which have features of both CW and BF (Ray et al., 2017; Tierney & Ray, 2018). CW systems utilize robust external filtration for solids removal and biological filtration (Ebeling & Timmons, 2012). Solids filters like foam fractionators and settling chambers remove settleable and suspended solids, while aerated, external biofilters provide increased surface area for the accumulation of nitrifying bacteria, which convert toxic ammonia and nitrite to less toxic nitrate via nitrification (Boyd & Tucker, 2014; Ray et al., 2010). In contrast to CW, BF systems do not have an external biofilter; rather, microbes suspended directly in the water column perform biological filtration and may provide supplemental nutrition to shrimp (Avnimelech, 2015; Browdy et al., 2012). Hybrid systems utilize external biofilters but allow the accumulation of some solids

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in the water column, which is an attempt to integrate the benefits of both CW and BF (Fleckenstein et al., 2018; Tierney & Ray, 2018).

While inland marine shrimp RAS popularity has increased in the United States, Canada, Europe, and other regions, research is still needed to identify and improve the economic viability (Quagrainie, 2015). RAS facilitate very low water use rates; however, some water exchange does occur and commercial marine salt mixes are typically used to provide the needed minerals for marine shrimp (Ray & Rode, 2019; Tidwell, 2012). Most indoor RAS use brackish water at a salinity of 10 g L^{-1} or higher, as shrimp tend to be more susceptible to toxic ammonia, nitrite, and nitrate at lower salinities (Ray & Lotz, 2017). Artificial marine salt water can be used for about three production cycles using the same water before elevated nitrate levels begin to degrade shrimp growth and survival (Furtado et al., 2015; Kuhn et al., 2010). Complete commercial sea salts are expensive, but homemade mixtures must be carefully formulated as deficiencies in certain minerals can be detrimental to shrimp performance (Boyd & Thunjai, 2003; Davis et al., 2004; Roy et al., 2007, 2010; Sowers et al., 2004; Valenzuela-Madrigal et al., 2017). If a relatively inexpensive, supplemental artificial salt mix can be evaluated in RAS, production costs will decrease, allowing for additional growth in the RAS sector. Further, water exchanges could occur more frequently, which may lead to improved water quality.

The purpose of this project was to study the effects of various artificial marine salt mixtures on *L. vannamei* production, water quality dynamics, and salt costs in intensive, hybrid-RAS growout production.

2 | MATERIALS AND METHODS

2.1 | Experimental design and operation

An 84-day experiment was conducted in the Sustainable Aquaculture Development Laboratory (SADL), a 174-m^2 insulated, climate controlled (25℃) building located at the Kentucky State University Aquaculture Research Center. Twenty 1.0-m^2 round bottom polyethylene tanks with a water volume of 1.0-m^3 were used for this project. To maintain proper temperature suitable for shrimp growth, each tank contained one-1000W titanium heater, which was set to 28 ℃. Each tank had one 18-L settling chamber used for solids removal, which is similar to the one described by Ray et al. (2010). Each settling chamber had a 5 cm diameter pipe suspended in the center which acted as a baffle to slow the flow of water. Solids then settled at the bottom and relatively clear water flowed out near the top of the chamber. In addition, each tank had an 18-L external moving bed biofilm reactor (MBBR) used for biological filtration, which included 6-L of biomedia (Curler Advance X-1, Aquaculture systems technologies, LLC). To promote the nitrification process, biomedia was taken from an established biofilter connected to a 3.4-m^3 HY nursery raceway, 3-L of which was placed in each of the 20 MBBRs used for this project. All tanks had four 15-cm long ceramic diffusers receiving blown air from two regenerative blowers, with three of the diffusers placed inside the shrimp tank and the fourth inside the biofilter to keep the biomedia aerated and mixed (Boyd & Tucker, 2014). A small pump was placed in all tanks to pass water through the filtration components, and flow rate was set to approximately 3.5 L min^{-1}.

TABLE 1 The formulation of the least cost salt (LCS) mixture at 15 g L^{-1} salinity to make 1.0 m^3 of artificial seawater

Salt	Weight (g)
NaCl	11,310
$MgSO_4$	1,830
$MgCl_2$	855
$CaCl_2$	376
KCl	240
$NaHCO_3$	90

Five treatments were created for this experiment with four randomly assigned replicate tanks each. Depending on treatment, the tanks received their salinity from either a low cost salt mixture (LCS), a commercially-produced complete sea salt mixture (CSS) (Crystal Sea Marine Mix, Marine Enterprises International, Baltimore, MD, USA), or a mixture of the two. The LCS (Table 1) consisted of sodium chloride (NaCl), magnesium sulfate ($MgSO_4$), magnesium chloride ($MgCl_2$), calcium chloride ($CaCl_2$), potassium chloride (KCl), and sodium bicarbonate ($NaHCO_3$). The CSS is a proprietary formula; more information on the product can be found on the company's website (https://www.meisalt.com/Crystal-Sea-Marinemix). The treatments in this study were as follows: 100% LCS (100), 75/25% LCS/CSS (75), 50/50% LCS/CSS (50), 25/75% LCS/CSS (25), and100% CSS (0); treatment abbreviations were indicative of the percentage of LCS used, and the treatments will be referred to as 100 treatment, 75 treatment, 50 treatment, 25 treatment, and 0 treatment.

2.2 | Shrimp husbandry

Post-larvae shrimp were received from a hatchery (American Mariculture, Inc) and placed in a 3.4-m^3 HY nursery raceway for 45 days. Shrimp were provided four Raceway Plus diets (Ziegler Brother, Inc), which differed in crumble sizes but had the same nutritional value (50% protein, 15% lipid, 1.0% fiber, 10% moisture, and 7.5% ash). The shrimp were then slowly transitioned to Ziegler PL Raceway 40-9 1.5-mm diet (40% protein, 9.0% lipid, 3.0% fiber, 10% moisture, and 13% ash) and the Ziegler Hyper-intensive Shrimp 35 2.4-mm diet (35% protein, 7.0% fat, 2.0% fiber, 12% moisture, and 15% ash). Shrimp only received the Ziegler Hyper-intensive Shrimp 35 2.4-mm diet for the last 10 days of the nursery period and for the entirety of the growout-phase experiment.

After the nursery phase, the shrimp were stocked into each experimental tank with an average individual weight of 4.3 g at a density of 250 shrimp m^{-3}. All tanks were fed the same amount of feed three times a day at approximately 0800, 1200, and 1600 hours. Feed amounts were adjusted throughout the study based on periodic checks for uneaten feed in the tank and by visually examining the guts of the shrimp from each tank. After 84 days, the shrimp were harvested from all tanks; the shrimp individual weight (g), total tank biomass (kg m^{-3}), survival (%), feed conversion ratio (FCR), growth rate (GR), and the cost of salt per kg of shrimp were all calculated.

2.3 | Water quality

Temperature (°C), dissolved oxygen (DO), pH, and salinity were measured twice daily at approximately 0800 and 1500 hours using a YSI Professional Plus Multi-Meter (YSI Incorporated). To buffer against pH swings, sodium bicarbonate was added to tanks if the pH was below 7.8 in the morning readings. Salinity was maintained at approximately 15 g L^{-1} during the experiment by adding fresh municipal water to all tanks once a week to replace evaporation loss. Total ammonia nitrogen (TAN) and nitrite (NO_2-N) were measured weekly in each tank with Hach methods 8155 and 8507 using a Hach DR6000 spectrophotometer. Likewise, turbidity (Nephelometric turbidity units; NTU) was measured weekly using a Hach 2100Q Portable Turbidimeter (Hach Company).

2.4 | Salt cost

The cost of salt ($USD) at a salinity of 15 g L^{-1} and volume of 1.0 m^3 was calculated for all treatments. The cost per treatment was generated by using the price of the complete sea salt (CSS) mix and ingredients used to create the low cost salt (LCS) mixture, matched with the total amount of each salt needed per treatment to reach the desired salinity level. Shipping costs were not included in these calculations, as such costs likely vary based on manufacturer and location. In order to incorporate the cost of salt ($USD) and the relative shrimp production values as one metric, the cost of salt per kg of shrimp produced was calculated for each treatment.

2.5 | Data management and analysis

Statistical analyses were conducted using Sigmaplot 13.0 (Systat Software, Inc., Chicago, IL, USA) with an α-value of 0.05 used to determine whether significant differences existed between treatments. A repeated measures analysis of variance (RM ANOVA) was used to compare the water quality data; this test can detect subtle differences in data that are consistent over time. A one-way ANOVA was used to analyze final shrimp production data and the salt cost per kg of shrimp between treatments. To ensure normality, a Shapiro-Wilk test was used; a Brown-Forsythe test was used to determine equal variance. All pairwise multiple comparison procedures were conducted using a post hoc Tukey's Honestly Significant Differences Test.

3 | RESULTS

3.1 | Water quality

The 75 treatment had significantly higher DO than the 100, 50, and 25 treatments ($p \leq 0.001$), and the 0 treatment had significantly higher DO than the 25 treatment ($p \leq 0.017$) (Table 2). No other significant differences in DO concentration were detected between treatments. Regarding pH, the 0 treatment was significantly higher than all other treatments, and no other significant differences were found between treatments. No significant differences were found between treatments with TAN and turbidity; however, the 100 treatment had significantly higher NO_2-N concentration than the 25 treatment (Table 2). No other significant differences in NO_2-N concentration were found between treatments. The 0, 100, and 50 treatments all had an average NO_2-N level of 1.2 mg L^{-1}, while the 25 and 75 had an average of 0.8 and 0.9 NO_2-N mg L^{-1}, respectively.

3.2 | Shrimp production

There were no significant differences found between any of the treatments with shrimp production metrics (Table 3). Average weight at the end of the study ranged from 22.2 to 22.9 g, FCR from 1.6 to 2.1:1 and biomass ranged from 3.2 to 4.0 kg m^{-3}. Survival had a wide range on a tank by tank basis: from 41% to 84%.

3.3 | Salt cost

The cost of salt needed to bring 1m^3 of water to 15 g L^{-1} salinity ranged from $8.83 (100 treatment) to $25.08 USD (0 treatment) (Table 4). The 100 and 75 treatments were both significantly lower in the cost of salt per kg of shrimp than the 0, 25, and 50 treatments (Table 4). The 50 treatment also had significantly lower cost per biomass of shrimp than the 0 and 25 treatments. Notably, the cost of salt per kg of shrimp in the 100 and 75 treatments at $3.20 is 52% less than that of the 0 treatment at $6.66.

4 | DISCUSSION

The temperature, DO, pH, and salinity in this experiment for all treatments fell within the appropriate range for shrimp growth provided by Van Wyk and Scarpa (1999). There were subtle but consistent DO and pH differences over time between treatments that were detected using the RM ANOVA. Using only one decimal place for the data in Table 2 makes it difficult to see these differences (Table 1); however, due to the resolution of the measurements, these data contain the appropriate number of significant figures. All tanks appeared to benefit from using 3-L of seeded biomedia, as the relatively low TAN and NO_2-N levels suggest that nitrification was occurring in all systems (Boyd & Tucker, 2014). Although

TABLE 2 Water quality data over the 84 day long project, analyzed using a repeated measures ANOVA, which can detect subtle but consistent differences over time

	Treatment				
	0	25	50	75	100
Temperature °C	27.72 ± 0.05 (25.15-28.70)	27.73 ± 0.06 (25.06-28.58)	27.86 ± 0.05 (25.26-28.66)	27.74 ± 0.05 (25.21-28.64)	27.69 ± 0.05 (25.21-28.68)
Dissolved Oxygen (mg L^{-1})	6.19 ± 0.04 (5.42-6.97)^{ab}	6.16 ± 0.03 (5.42-6.92)^{c}	6.16 ± 0.04 (5.44-6.83)^{bc}	6.20 ± 0.04 (5.48-6.80)^{a}	6.16 ± 0.03 (5.52-6.88)^{bc}
pH	7.91 ± 0.01 (7.73-8.33)^{a}	7.89 ± 0.01 (7.70-8.26)^{b}	7.89 ± 0.04 (7.69-8.26)^{b}	7.89 ± 0.01 (7.70-8.28)^{b}	7.89 ± 0.01 (7.70-8.28)^{b}
Salinity (g L^{-1})	15.10 ± 0.03 (14.60-15.65)	15.21 ± 0.03 (14.54-15.73)	15.25 ± 0.03 (14.42-15.78)	15.16 ± 0.03 (14.32-15.69)	15.22 ± 0.02 (14.75-15.69)
Ammonia (mg TAN L^{-1})	0.23 ± 0.13 (0.01-1.46)	0.22 ± 0.08 (0.01-1.01)	0.31 ± 0.12 (0.01-1.44)	0.23 ± 0.09 (0.01-1.03)	0.31 ± 0.10 (0.01-1.22)
Nitrite (mg NO_2-N L^{-1})	1.22 ± 0.39 (0.35-3.89)^{ab}	0.80 ± 0.15 (0.38-1.59)^{a}	1.22 ± 0.32 (0.39-3.62)^{ab}	0.91 ± 0.16 (0.43-2.02)^{ab}	1.18 ± 0.19 (0.50-2.26)^{b}
Turbidity (NTU)	32.07 ± 2.84 (18.65-48.80)	33.77 ± 3.21 (16.58-46.90)	30.06 ± 2.84 (15.04-40.70)	28.22 ± 2.16 (19.35-39.30)	32.56 ± 2.56 (19.38-42.40)

Note: Different superscript letters in a row indicate significant differences ($P < 0.05$) between treatments. Data are presented as mean ± SEM (range). The treatments were 100% LCS (100), 75/25% LCS/CSS (75), 50/50% LCS/CSS (50), 25/75% LCS/CSS (25), and100% CSS (0), where LCS is least cost salt and CSS is Crystal Sea Salt mix.

the 100 treatment had significantly higher NO_2-N levels over the 25 treatment, the difference is small and not believed to have had much impact on overall shrimp production between the two treatments. While NO_2-N levels over 1.0 mg L^{-1} for prolonged periods of time can degrade shrimp health and lead to poor survival (Len and Chin, 2003; Ray & Rode, 2019), mean survival rates were not significantly different among treatments.

The mean individual shrimp weights were similar between treatments and similar to those seen in previous indoor shrimp production studies (Fleckenstein et al., 2018; Ray & Lotz, 2017). Shrimp from all treatments were within the desired size range suggested by Quagrainie (2015) that inland famers need to obtain a profit. While the FCRs in this study resembled the 1.8:1 standard seen in most commercial U.S. shrimp farms (Treece, 2014), the 75 treatment was slightly better than the other treatments. Regarding total harvest output, Ray and Rode (2019) suggest that producers aim for 4-5 kg shrimp m^{-3} at the end of a production cycle, which was only accomplished in the 75 treatment (Table 4). However, considering the relatively small size of the tanks used here and the experimental nature of the project, these results seem reasonable. The authors note that some of the tank mortalities occurred overnight when the shrimp would jump and escape past the tank cover and land on the floor. Future studies will include tighter fitting nets over the tops of the tanks. Producers are advised to maintain a 70% survival rate or higher in order to net a return on their initial investment (Van Wyk, 1999). The 75 treatment had the best mean survival in the study; although there were no significant differences, this is an indication that the LCS mixture is suitable for shrimp culture. Future experiments will examine the effects of higher LCS inclusion levels between the 75% and 100% range to more completely assess that range and make sure farmers can safely use a 100% LCS mixture. Future work should also examine the long-term implications of using this salt mixture. Issues such as mineral depletion or accumulation, interactions with accumulating nitrate levels, or other potential contaminants should be examined.

The cost difference between CSS and LCS mixtures is quite substantial. In the long term, water will be reused for multiple crops of shrimp, typically three, so the costs of salt will be reduced further. However, Maier (2020) developed an economic model for indoor shrimp farming in the U.S. that predicted that in an eight tank farm, the adoption of the 100% LCS mixture reduced the probability of a net loss of income from 17.4% to 7.4%. The economic model predicted no chance of a net loss using a 24 tank facility, and the net present value was $85,400 USD using CSS versus $108,600 USD when the LCS was used. These bottom-line differences indicate that an inexpensive salt mixture can make a large impact on the business of shrimp farming. Also worth noting is that relatively small quantities of salts were purchased for this project. Bags of salts typically weigh about 23 kg; however, in a commercial operation larger quantities of salt would likely be purchased. Buying such items in bulk quantities typically brings down the cost per unit of weight, so salt costs may be reduced further.

TABLE 3 Final shrimp production data from the five treatments presented as mean ± SEM. The treatments were 100% LCS (100), 75/25% LCS/CSS (75), 50/50% LCS/CSS (50), 25/75% LCS/CSS (25), and100% CSS (0), where LCS is least cost salt and CSS is Crystal Sea Salt mix

	Treatment				
	0	25	50	75	100
Average Weight (g)	22.9 ± 0.8	22.2 ± 0.5	22.4 ± 0.2	22.5 ± 0.6	22.5 ± 0.4
Growth rate (g week^{-1})	1.6 ± 0.1	1.5 ± 0.0	1.5 ± 0.0	1.5 ± 0.1	1.5 ± 0.0
FCR	1.8 ± 0.1	1.7 ± 0.1	1.8 ± 0.1	1.6 ± 0.1	2.1 ± 0.2
kg m^{-3}	3.8 ± 0.2	3.9 ± 0.2	3.8 ± 0.3	4.0 ± 0.2	3.2 ± 0.3
Survival (%)	67.2 ± 3.8	70.3 ± 3.9	68.3 ± 5.2	73.1 ± 4.2	57.2 ± 6.2

TABLE 4 The total cost of salt per treatment formulation (1.0 m^3 at 15 g L^{-1} salinity) and the mean cost of salt per kg of shrimp. Different superscripts in a column denote significant differences ($p < 0.05$) between treatments. The treatments were 100% LCS (100), 75/25% LCS/CSS (75), 50/50% LCS/CSS (50), 25/75% LCS/CSS (25), and100% CSS (0), where LCS is least cost salt and CSS is Crystal Sea Salt mix

Total Salt Cost per Formulation		Cost of Salt per kg Shrimp	
Treatment	$USD per 1m^3	Treatment	$USD
0	$25.08	0	$6.66^a
25	$21.02	25	$5.50^a
50	$16.96	50	$4.57^b
75	$12.89	75	$3.20^c
100	$8.83	100	$2.87^c

5 | CONCLUSIONS

In this study, there were minor differences between treatments with DO, pH, and NO_2-N; however, water quality does not seem to have been affected much by salt formulation. Production metrics were similar for all treatments as well, with no significant differences in final weight, growth rate, FCR, tank biomass, or survival. The LCS salt formulation was 65% less expensive than the CSS mixture, and the cost per kg of shrimp produced was 57% lower using the LCS. Overall, this project indicates that a home-made, low-cost salt formulation can be used in intensive shrimp production systems and that, by using this, farmers may be able to substantially reduce production costs.

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ETHICS STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received. The US National Research Council's guidelines for the Care and Use of Laboratory Animals were followed.

DATA AVAILABILITY STATEMENT

The data reported in this manuscript are not currently shared. The data that support the findings of this study are available from the corresponding author upon reasonable request.

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