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GROWTH AND SURVIVAL OF THE PACIFIC WHITE SHRIMP, Litopenaeus vannamei, IN SEA SALT AND OTHER IONIC ENVIRONMENTS

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GROWTH AND SURVIVAL OF THE PACIFIC WHITE SHRIMP, *Litopenaeus vannamei*, IN SEA SALT AND OTHER IONIC ENVIRONMENTS.

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ABSTRACT

The Pacific white shrimp, *Litopenaeus vannamei*, is a euryhaline and eurythermal shrimp species. The robust nature of this species makes it a popular selection among shrimp aquaculturists. The high cost of coastal property as well as strict environmental regulations in developed countries has caused many shrimp farmers to move their operations further inland. Therefore, many inland farmers must bring in seawater, use brackish well water, or create their own environments using artificial sea salts. The purpose of this research was to determine if a sea salt mixture, ACS, composed of less expensive salts (NaCl, $MgSO_4$, $MgCl_2$, KCl, $CaCl_2$ and $NaHCO_3$), can produce acceptable survival and growth in a large-scale aquaculture system compared to dilute seawater or mixtures of dilute seawater and the less expensive salts. The first trial consisted of 36 tanks with various 5 g/L and 15 g/L total dissolved solids treatments. There was no significant difference in survival or feed conversion ratio during the first trial. There was a significant decrease in final individual weight, and total harvest with increasing ACS salts. Extremely high nitrite levels, 16.2 ± 3.80 mg/L (mean ± SD), may have caused the decreases in these parameters. Trial 2 consisted of 18 tanks with various 15 g/L TDS treatments. There was no significant difference in survival, final individual weight, feed conversion ratio, or total harvest. A cost analysis was conducted and showed that ACS salts may significantly decrease the cost

of shrimp culture when partially or completely substituted for artificial sea salts that many inland farmers are forced to use. It may be possible for inland farmers to use the ACS salt mixture but precautions must be taken to ensure that nitrite levels do not become limiting.

DEDICATION

I dedicate this work to my mother, Judith A. Bisesi, my father, Joseph H. Bisesi, Sr., and my sister, Melanie R. Bisesi. Without their patience, love and devotion none of this would have been possible.

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LITERATURE REVIEW

According to the Top Ten Seafood's summary prepared by the National Fisheries Institute, shrimp was the leading seafood consumed in the United States from 2001 to 2005 (Wirth et al. 2003; Johnson 2005) Therefore, shrimp aquaculture is becoming increasing popular in the United States (U.S.). U.S. farmers are unable to compete with the low cost of imported frozen shrimp; therefore they are interested in marketing their shrimp to fresh seafood dealers. The Pacific white shrimp, *Litopenaeus vannamei*, seems to be the best candidate for U.S. aquaculture because of its robust nature (Wirth et al. 2003). *L. vannamei* is a euryhaline species that is capable of surviving over a large range of salinities and temperatures. Its native range is along the pacific coast of Mexico, Central and South America (Briggs et al. 1991; Rosenberry, 2002).

Pacific white shrimp as an aquaculture species

There are many advantages for the use of *L. vannamei* as an aquaculture species. They are able to survive salinities ranging from 0.5-45 g/L with growth being best between 10-15 g/L (Briggs et al. 1991). *L. vannamei* can tolerate temperatures between 15°C and 33°C with growth being best between 23°C and 30°C (Rosenberry, 2002). Another study showed that *L. vannamei* survive best between 20 and 30°C, but exhibit the best growth between 25 and 35°C (Ponce-Palafox et al. 1997). Pacific white shrimp do not require a diet as rich in protein as other shrimp species (Briggs et al. 1991). *L. vannamei* is an open thelycum species therefore spawning can be induced in captivity. This enables selection for enhanced features such as disease resistance. *L. vannamei* can be cultured successfully at densities as high as 400/m^2 in high density re-circulating systems (Briggs et al. 1991; Browdy et al. 2005).

<u>Growth of Pacific white shrimp in low salinity water</u>

Many studies have been conducted looking at growth and survival of *L. vannamei* in low salinity water. One study showed that *L. vannamei* actually prefers lower salinities over many other penaeid species, such as *P. californiensis*, *P. brevirostris*, and *P. stylirostris* (Mair, 1980). Another study found that *L. vannamei* grew better in 5 and 15 ppt sea water as opposed to higher salinities (Bray et al. 1994). There has been some conflicting research that found that *L. vannamei* did not survive below 2 g/L. Survival did not differ significantly at 2 and 3 g/L but was significantly higher at 30 ppt (Laramore et al. 2001). Another study showed that *L. vannamei* survive and grow better when salinity is over 20 g/L TDS but they did not test below 20 g/L (Ponce-Palafox et al. 1997).

Low salinity does not seem to affect osmotic regulation in the Pacific white shrimp. One group of researchers found that the hemolymph osmolality of *L. vannamei* is isosmotic to seawater at 718 mOsm/kg and is able is able to osmoregulate down to salinities of 5 g/L. Salinities lower then 5 were not tested (Castille et al. 1980). More recent research found that mixed salt and sea salt environments do not effect osmotic regulation in environments as low as 2 g/L TDS (Sowers et al. 2005).

Inland aquaculture of the Pacific white shrimp

The high cost of coastal property has caused many seafood aquaculturists to move their operations further inland. The problem is that once farms are moved inland they are no longer within the reach of seawater. Therefore, farmers must create artificial ionic environments using brackish well water or by adding salts. The high cost of salts has led researchers to investigate growing shrimp at the lowest salinity possible as well as the use of mixed-salt alternatives (Atwood et al. 2003; Sowers et al. 2005; Sowers et al. 2006). Mixed salts are compilations of the major components of artificial sea salts without many of the trace ions. The advantage of these salts is that each component can be purchased in bulk to reduce the cost of creating artificial environments. Another option that farmers may use to reduce operational costs and discharging of excess nutrients is the use of a no-exchange water management strategy. One study has shown that there is no statistical difference in growth and survival of *L. vannamei* when using exchange vs. no-exchange water management strategies (Hopkins et al. 1996). The use of no exchange water management strategies in greenhouse enclosed raceways has been shown to provide acceptable production characteristics in multiple pilot and commercial scale systems (Samocha et al. 2002; Browdy et al. 2005).

Many inland wells contain brackish water similar in composition to dilute seawater. Inland farmers have starting using this well water for *L. vannamei* production. The suitability of many inland wells for the production of *L. vannamei* has been examined. Research has shown that some inland wells are able to support good survival depending on the age the post larvae are added to the production tanks (Saoud et al. 2003). The addition of mixed salts to these inland well waters could increase the total dissolved solids which may support better survival without significantly increasing production costs.

Mixed salt Pacific white shrimp aquaculture

There have also been a few studies conducted to test the possibility of using mixed salt alternatives alone or as sea salt additives. Recent research has shown that the addition of K^+ to freshwater (<1 g/L total ion concentration) exhibited increased *L. vannamei* survival of 20 to 43% while the addition of Mg^{2+} and SO_4^{2-} had no effect (McGraw et al. 2003). Other research showed that when K^+ and Mg^{2+} were added to low salinity well water (2.5 g/L) *L. vannamei* survival increased from 19% with a production of 595 kg/ha to 67% with a production of 4,068 kg/ha. Potassium concentrations were near nominal seawater concentrations while magnesium levels were far below those seen in natural sea water (McNevin et al. 2004). Both of these studies show that K^+ is essential in a mixed salt to produce acceptable *L. vannamei* survival.

The use $CaCl_2$ and NaCl alone and as an additive, has produced mixed results. $CaCl_2$ and NaCl alone produced very low survival but when $CaCl_2$ and NaCl were added to tanks with low amounts of sea salt, survival was drastically improved (Atwood et al. 2003). Use of a mixed salt containing the chlorides of Na^+, K^+, Ca^{2+}, and Mg^{2+} has shown promising results. In one experiment the treatments containing Na^+/K^+ ratios approximately the same as sea water produced the highest survival. Survival was not as high in any of the mixed salt treatments as in the artificial sea salt treatments (Sowers et al. 2005). However, one large scale production experiment showed that 20g/L dilute seawater; 2g/L dilute seawater, and 1g/L mixed salts in addition to 1g/L dilute seawater showed similar survival and growth (Sowers et al. 2006).

Nitrite effects on the Pacific white shrimp

One of the main water quality concerns of aquaculturists is the amount of nitrite in the culture systems. The high stocking density of these systems often causes nitrite levels to greatly increase before bacteria can be established to complete the nitrogen cycle and decrease nitrite concentrations (Tomasso et al. 1980). In order for bacteria to convert toxic ammonia, an excretory product of aquatic animals, to nitrate, which is much less toxic, it must first oxidize the ammonia to nitrite. Nitrite is also toxic to aquatic animals and must be oxidized by bacteria to form nitrate. Research on fish physiology has shown that nitrite can be taken up via the gills via the chloride-uptake pathway (Tomasso et al. 1980) and through the gut (Grosell et al. 2000). Once inside fish, nitrite exhibits its effects by oxidizing the iron in the heme group of hemoglobin converting it to methemoglobin. Methemoglobin is unable to bind oxygen and therefore the animal may suffocate even if environmental dissolved oxygen levels are high enough (Tomasso et al. 1979; Huey et al. 1980).

Nitrite may also have other physiological effects such as competing for uptake of chloride which can cause chloride depletion. Nitrite can cause an efflux of potassium from skeletal and muscle and erythrocytes which disturbs intercellular and extracellular K^+ levels. Nitrite can also oxidize other heme proteins and can induce vasodilatation which causes heart rate to increase to re-establish blood pressure. It can also form nitric oxide which can interfere with hormone function and inhibit steroid hormone synthesis (Jensen, 2003).

The toxic pathway of nitrite has not been determined for crustaceans. Some researchers believe that nitrite may exert similar effects on the oxygen carrying molecule of shrimp, hemocyanin (Chen et al. 1995). Although the interaction of nitrite and hemocyanin is not fully understood, nitrite has been shown to have toxic effects on shrimp (Gross et al. 2004). Research has shown that nitrite toxicity in the Pacific white shrimp increases with decreasing salinity. Nitrite uptake also may be lower in environments containing mixed salts (Sowers et al. 2004). A more involved study showed that at 15 g/L the 24, 48, 72, 96, and 144h nitrite-N LC50's for *Litopenaeus vannamei* were 187.9, 142.2, 92.5, 76.5, and 61.1 mg/L respectively. The safe rearing level in water with 15 g/L TDS was estimated at 6.1 mg/L (Lin et al. 2003). Other researchers found that the Nitrite-N 96h LC50 for *L. vannamei* in low salinity (2 g/L) brackish water was approximately 9 mg/L. They concluded that the safe rearing level in water of 2 g/L salinity may be less then 0.45 mg/L. They also found that exposing *L. vannamei* to 4 mg/L nitrite for 2 days followed by a period of freshwater renewal will reduce their growth but not their survival (Gross et al. 2004).

Another study (Cohen et al. 2005) involving the characterization of water quality factors during intensive raceway production of *L. vannamei*, conducted at 30 g/L salinity, showed nitrite levels as high as 26 mg/L. Even at these high nitrite levels survival and growth were acceptable. The 144h nitrite LC_{50} for *L. vannamei* at 25 g/L and 35 g/L is 152.4 mg/L and 257.2 mg/L, respectively (Lin et al. 2003). The 10% safe rearing level at 30 g/L would be in between 15.2 and 25.7 mg/L. Though this was exceeded in this study these high levels were only seen during the last week and therefore did not affect the production characteristics in this study. The authors of this study attribute the slow increase in nitrite to a possible acclimation of the shrimp to high nitrite.

INTRODUCTION

The Pacific white shrimp, *Litopenaeus vannamei*, is a euryhaline species capable of surviving and growing in a large range of salinities. Because of its robust nature, good taste, and marketability the Pacific white shrimp is often selected by shrimp farmers as the best species for aquaculture (Wyban et. al. 1991; Rosenberry, 2002).

Inland aquaculture of this species is becoming very popular because of the high cost of coastal land as well as strict environmental regulations (Samocha et al. 2002; Atwood et al. 2003; Sowers et al. 2005; Sowers et al. 2006). Many farmers have been able to find inland wells that have high enough salt levels to support growth of these shrimp (Samocha et al. 2002; Saoud et al. 2003). In areas like South Carolina, where these wells are not available, farmers are forced to create their own salt water using artificial sea salt. The high costs of these salts has led researchers to examine the possibility of using mixed salts as substitutes for artificial sea salt (Atwood et al. 2003; McGraw et al. 2003, McNevin et al. 2004; Sowers et al. 2005; Sowers et al. 2006; Parmenter et al. unpublished research). Mixed salts are typically composed of the same major ions as artificial sea salts, in ratios found in seawater, but lack some of the trace ions. They are usually made of cheaper bulk salts such as NaCl and $MgCl_2$. Researchers have found that these salts may be able to support growth by themselves but shrimp grow much better when they are combined with small amounts of artificial sea salt.

The goal of this research was to test the survival and growth of *L. vannamei* in various mixed-salt and sea-salt environments, using a new salt mixture (American Chemical Society, ACS, Sowers et al. 2006). One gram of ACS is comprised of 754 mg NaCl, 57 mg $MgCl_2$, 122 mg $MgSO_4$, 16.4 mg KCl, 45.8 mg $CaCl_2$, and 5.6 mg $NaHCO_3$. The goal was to determine if these mixed salts will support the same growth and survival as sea salt. Lab experiments were conducted by Kirk Parmenter (Clemson University, unpublished) to determine what sea-salt/mixed-salt ratios produced the best survival. Large scale tank studies were then conducted to compare the effects of sea salt, ACS and combinations of sea salt and ACS on production characteristics of *L. vannamei.*

MATERIALS AND METHODS

Post larval Shrimp for the experiments were obtained from Harlingen Shrimp Farms, Ltd. (Los Fresnos, TX, USA). They were held in raceway systems at Waddell Mariculture Center (South Carolina Department of Natural Resources, Bluffton, SC, USA) until stocking into experimental systems.

In the first experiment, thirty-six 3.35 m diameter tanks were used and filled to 71.12 cm. The total volume of each tank was ~6,279L. Tanks were constantly aerated, but no biofiltration or water exchange was used. The experimental treatments for the first experiment are outlined in Table 1. Full strength sea water from the Colleton River, Bluffton, SC, USA, was used to provide the sea salt. This water was diluted with filtered tap water from the Waddell Mariculture Center (290 mg/L total dissolved solids, 34 mg/L calcium, 13 mg/L magnesium, 3 mg/L potassium, 19 mg/L sodium, 34 mg/L chloride)..ACS salts were added after the sea salt concentration of the mixture had been reached. ACS salts were added as agricultural grade or purer chemicals. One month prior to stocking shrimp, tanks were fertilized with 100g of alfalfa to aid in establishing nitrifying bacteria.

The second experiment consisted of 18 tanks and included all the 15 g/L TDS treatments (Table 1). Water from the first experiment was reused for the second experiment in order to keep the nitrifying bacteria that were already established. Nitrite levels were allowed to drop below 1 ppm before tanks were stocked again.

All shrimp to be added to the 5 g/L TDS tanks were moved from the raceway (salinity = 25.1 ± 2.25 g/L (mean ± SD), pH = 7.5 ± 0.46, dissolved oxygen = 5.8 ± 1.35 mg/L, temperature = 29± 1.1 °C, alkalinity (as mg/L CaCO3) = 158 ± 24.9, total ammonia-nitrogen = 1.1 ± 1.79 mg/L, nitrite-nitrogen = 1.4 ± 1.10 mg/L, and nitrate-nitrogen = 6.5 ± 0.71 mg/L.) to a tank containing approximately 20 g/L dilute seawater. The salinity was decreased by ~5 g/L/hour by adding freshwater to the tank until the salinity reached approximately 5 g/L. Shrimp were then moved from the acclimation tank to the experimental tanks. Animals for the 15 g/L TDS treatments were moved directly from the raceway to the experimental tanks. All experimental tanks were stocked at 100 animals per square meter. Shrimp in trials 1 and 2 weighed 1.2 ± 0.10 g and 7.1 ± 0.26 g (mean ± SD) at stocking, respectively

Shrimp were fed a commercial shrimp diet (Ziegler Brothers, Inc., Gardners, PA, USA) on feeding trays twice daily. During each feeding event feeding trays were examined before applying feed. If feed remained on feeding trays animals were not fed until the next scheduled feeding time. Feed conversion ratio (FCR) was calculated by dividing the total amount of feed added by the total biomass taken out minus the total biomass put in.

Dissolved oxygen, salinity, pH and temperature were measured each morning and afternoon using a Yellow Springs Instrument Co. model 556 multiprobe (Yellow Springs, OH, USA). Nitrate, nitrite, ammonia, and alkalinity were measured weekly by means of Hach Reagent kits and a Hach DR4000 spectrophotometer (Hach Chemical Company, Loveland, CO, USA). Turbidity was measured weekly using an HF Scientific Micro 100 Turbidimeter (Fort Myers, FL, USA). Tables 2 and 3 outline water quality characteristics for trials 1 and 2, respectively. When alkalinity dropped below 100 ppm, 500 g $NaHCO_3$ was added to ensure proper buffering capacity. At the end of trial 2, water was analyzed for ion concentrations (Table 4) using inductively coupled plasma spectrometry (Clemson University Agricultural Services Laboratory, Clemson, SC, USA). The samples were not filtered but they were diluted to ensure the ion concentrations were within range of the standards for each ion.

Samples were taken weekly to adjust feed rates and examine the overall health of the shrimp. Three groups of 25 individual shrimp were weighed in order to calculate average weights of the shrimp each week. At the end of the first experiment (36 days), the total harvest from each tank was weighed. Three groups of 25 individuals were weighed per tank, to estimate the average weight of the shrimp in each tank. The total harvest weight was divided by the average individual shrimp weight in order to estimate the number of surviving shrimp in each tank.

At the end of the second experiment (42 days), the total harvest from each tank was weighed. Three groups of 25 individuals were weighed in order to estimate the average weight of the shrimp in each tank. Surviving shrimp in each tank were individually counted to confirm the number of surviving shrimp.

ANOVA, followed by the Tukey-Kramer multiple comparison test, was used to compare weights, percent survival, total harvest, feed conversion ratio and all water quality parameters. Weights and water quality parameters were entered into analyses as tank means. A $P \leq 0.05$ was considered significant.

RESULTS

Trial 1

The first trial had to be terminated after 36 days because of complete mortality in all of the 5 g/L total dissolved solids (TDS) treatments. Mortality was attributed to high environmental nitrite concentrations. Therefore, survival, final weights, FCR and total harvest were only examined in the 15 g/L TDS.

Percent survival for the 15/0, 10/5, 5/10, 0/15 ((g/L Seawater) / (g/L ACS salts)) treatments averaged 47 ± 24 % (mean ± SD) after 36 days and did not differ significantly (Figure 1). Mean final weight averaged 5.6 ± 0.50 g and showed a significant treatment effect with shrimp in the higher seawater treatments being heavier. FCR averaged 4.4 ± 3.23 and did not differ significantly. The total harvest averaged 0.32 ± 0.17 kg/m^2 and was higher in the treatments with higher seawater concentrations. Growth per week for trial 1 averaged 1.01 ± 0.14 g/wk.

Trial 2

Percent survival averaged 84 ± 10% and was not significantly affected by treatment (Figure 1). Final weight averaged 13.1 ± 0.83g for the four treatments and also was not affected by treatment. The FCR averaged 2.69 ± 0.36 and did not differ significantly between treatments. Total harvest averaged 1.03 ± 0.12 kg/m^2 and was not significantly affected by treatments. Growth per week for trial 2 averaged 1.04 ± 0.11 g/wk.

DISCUSSION

Little research has looked at the effects of mixed salts on survival and growth of *L. vannamei*. One lab study (Atwood et al. 2003) showed that when exposing *L. vannamei* to a mixed salt environment consisting of only NaCl, and $CaCl_2$ (2 g/L TDS) survival was less then 10%. A much better survival was obtained after adding 0.25 g/L of artificial sea salt. This might indicate a trace amount of something was missing in this solution in order for *L. vannamei* to survive. Another lab study (Sowers et al. 2005) found that a mixed salt environment of NaCl, $MgCl_2$, $CaCl_2$, and KCl (2 g/L TDS) did not interfere with ion regulation in *L. vannamei* but also did not provide the ions or the correct ratios of these ions needed for normal growth and survival. Survival was significantly higher in the artificial sea salt treatments compared to the mixed salt treatments in different ratios.

A tank grow-out study found that survival, and growth of *L. vannamei* did not differ significantly between shrimp grown in 20 g/L sea water, shrimp grown in 2 g/L sea water, and shrimp grown in 1 g/L sea water with 1 g/L of mixed salts (Sowers et al. 2006). The 20 g/L sea salt treatment of this experiment did show a significantly higher total harvest indicating that the lower TDS may have had an effect on total harvest. Unpublished research by Kirk Parmenter (Clemson University) has shown that the same mixed-ion environment used in the current experiment is not capable of providing normal survival at 5 g/L. When as little as 0.25 g/L of artificial sea salt is added, *L. vannamei* survival increases from 80% to 90% at 2 g/L TDS. This indicates that only a slight amount of sea salt is necessary to provide better survival.

The two trials conducted during the summer of 2006 provide conflicting results for the ability of the ACS mixed salt environment to provide normal survival and growth of *L. vannamei* on a large scale. There was 100% mortality in all of the 5 g/L tanks during the first trial. On the last day of sampling, Nitrite levels in the 5 g/L tanks reached an average maximum of 11.1 ± 3.73 mg/L. The 96-h nitrite LC50 for *L. vannamei* is approximately 14.1 mg/L in water with 5 g/L TDS (Sowers et al. 2004). Therefore, mortality was attributed to the toxic amount of nitrite in these tanks.

Though there was no treatment effect on survival during trial 1 in the 15 g/L tanks, there was an obvious trend between the treatments as seen in Figure 1. There was a significant decrease in final weight, and total harvest weight when substituting all of the sea salt for ACS salts. The 15/0 and 10/5 g/L treatments seem to work much better than the 5/10 and 0/15.

Trail 2 showed no significant differences in survival, final weight, FCR or total harvest weight (Figure 1). This would indicate that substitution of sea salt for ACS salts can provide normal survival and growth even when completely substituting for 15 g/L of ACS salts.

There are two possible explanations for the differences seen in survival and growth between the first and second trials. The first possibility is the difference in initial weight. The average stocking weight from the first and second trials were 1.2 ± 0.10 g and 7.1 ± 0.26 g (mean ± SD), respectively. The smaller shrimp in the first experiment may have had a lower resistance to nitrite or the effects of the ACS salts. Research has shown that after *L. vannamei* post larvae are older then 15 days their gills are fully developed and can be acclimated down to salinities as low as 2 g/L with acceptable survival (McGraw et al. 2002). Since the shrimp used for both trials were much older than 15 days it is unlikely that the differences seen between trials 1 and 2 are due to differences in initial weight.

The other explanation could be the extremely high nitrite levels in the first trial. Although the interaction between nitrite and hemocyanin, the oxygen transporter for crustaceans, has not been determined, nitrite has been shown to be extremely toxic to the Pacific white shrimp in marine environments (Lin et al. 2003; Gross et al. 2004; Sowers et al. 2004). The average maximum amount of nitrite found in the 15 g/L treatments during the first trial was 16.2 ± 3.80 mg/L (mean ± SD). One study showed that the 96-h nitrite LC50 for *L. vannamei* in a 15 g/L TDS environment to be approximately 53.9 mg/L (Sowers et al. 2004). This means that the safe rearing level would be around 5.39 mg/L. Another study (Lin et al. 2003) estimated the 144-h nitrite LC50 for *L. vannamei* in a 15 g/L TDS environment to be 61.1 mg/L. They estimated the safe rearing level to be 6.1 mg/L. The maximum nitrite level in trial 1 exceeded both of the safe rearing levels estimated from these two studies.

Researchers have found that mixed salts and low TDS do not have effects on osmoregulation in *L. vannamei* (Castille et al. 1980; Sowers et al. 2005). Studies have shown that the uptake mechanism of nitrite is through the chloride cells at the gill. In water with low TDS, high nitrite may out-compete chloride for uptake (Tomasso et al. 1980). Since mixed salts do not seem to interrupt osmoregulation is it possible that high levels of nitrite were taken in by the shrimp in these experiments. Even though the nitrite levels were far below the LC_{50} at 15 g/L TDS it may have caused increased stress. As more ACS salts were added, the final weight, and total harvest decreased indicating that the ACS salts may not be providing a needed ion. The compounding effects of ion regulation and elevated nitrite levels may have resulted in the lower final weight, and total harvest in the tanks containing more ACS salts.

During trial 2 the maximum amount of nitrite in all of the tanks averaged 3.76 ± 3.79 mg/L. The decreased nitrite levels in these tanks yielded survival, final weights, and total harvest weights that were not statistically significant from each other and showed no obvious trends.

The high average FCR (4.4 ± 3.23) in the first trial is to be expected due to the high mortality and low growth in the 5/10 and 0/15 tanks. The amount of feed given was determined by estimating a percentage of body weight based on growth and an estimated survival. Since survival was much lower in the 5/10 and 0/15 tanks the extra feed was not converted and therefore increased the FCR. Though the average FCR for trial 2 was much better (2.69 ± 0.36) it was still high for industry standards. It is possible that there was a miscalculation of feed needed for acceptable growth or that feed may have been misweighed during this trial.

There were some statistical differences seen in the AM salinity, PM salinity and AM dissolved oxygen during trial 1(Table 2). Many of the tanks had warped bottoms and sides which could have caused differences in tank volumes. Since all the tanks for each treatment got the same amount of salt this may have caused some of the differences seen in the salinities. Because the salinity differences were compounded by the addition of more ACS salts it may be possible that miscalculation and misweighing also played a role in the higher salinities in these tanks.

The difference in dissolved oxygen was probably just due to variability in the meter. There were no statistical differences seen in the water quality parameters for trial 2 (Table 3). All water quality characteristics, with the exception of nitrite, appeared to be within acceptable ranges for *L. vannamei*.

The analysis of water for specific ions is outlined in table 4. Na^+, Mg^{2+}, K^+, and Cl^- were all very close to their respective nominal values in sea water. Ca^{2+} in the different environments was much higher then nominal values across the board. This may be a result of calcium that was released from feed. To confirm this theory a sample calculation was done, using tank 1 was an example. The total amount of feed added to tank 1 during trails 1 and 2 was 32,625g. The percent Ca^{2+} inclusion of the feed used for this study was 2%. Therefore, 2% of the feed was 654g. In 6,279 liters of water this means an extra 104 mg/L of calcium was added to the water. The concentration of calcium found in tank 1 was 261 mg/L which was 162% of the nominal value of calcium at the salinity in this tank. If you subtract the 104 mg/L of Ca^{2+} that was added via the feed the concentration of calcium in the water becomes 157 mg/L which is 97% of the nominal value at this salinity.

Sulfate was a little less then half of its concentration in typical seawater. Because this condition was found across the board, including the seawater only treatments, the lower sulfate levels may be attributed to complexation with iron and organic material (Langmuir, 1997).

In summary, it seems that the ACS salt mixture, when used by itself or in conjunction with some sea salt, can yield normal production characteristics in some cases. If this mixture were to be used, the pond or raceway would have to be well cycled or have a filtration system that was able to remove nitrite efficiently. High nitrite can have effects on survival as well as growth in the Pacific white shrimp. If nitrite becomes an issue the lower cost of the ACS salt mixture may be offset by lower survival in environments containing these salts. As long as nitrite is monitored closely the ACS salt mixture could be a viable option for inland shrimp farmers.

Before the ACS mixed ion solution can be used more research must be conducted to ensure its ability to produce production characteristics. The experiment must be repeated using external bead filters that may aid in nitrite removal. To ensure that ACS does not effect smaller shrimp the experiment should be repeated using shrimp with a lower initial weight. Finally the same experiment should be repeated at a lower salinity, which could reduce the cost of inland farming even further.

ECONOMIC ANALYSIS OF ACS SALTS

In order for inland aquaculturists to use the ACS mixed salt solution, it must save them money while still exhibiting the same production characteristics as artificial sea salt. A cost analysis was performed using prices obtained from a few chemical companies. Time constraints limited the number of companies that could be contacted for salt prices. More research must be conducted to ensure the best price for each salt was obtained. From the companies contacted, the cheapest price for each salt was used in the analysis. All prices include delivery to the Waddell Mariculture Center, Bluffton, SC, USA. Prices were based on purchasing at least 1 ton of each salt individually.

The per-gram cost of each salt was calculated and the total cost to make 1 g of the ACS salt mixture was determined. From this value, cost can be calculated for any salinity and any volume. The sea salt portion of this analysis was calculated using Red Sea© salt (Houston, TX, USA), which is a commercially available artificial sea salt. For this analysis two hypothetical aquaculture systems were used. The first was a typical 1.5 ha pond with a volume of 15,000 m^3 or 15,000,000 L. The second system was a typical super-intensive raceway system with a volume of 500 m^3 or 500,000 L. The calculation of the cost to make 1 g of the ACS salt mixture is given in table 5. The comparison of the cost of using the different treatments in the two hypothetical aquaculture systems is given in table 6.

Depending on which treatment shrimp culturists go with the use of ACS salts can save a lot of money. If a farmer decided to completely substitute for the ACS mixed salt, they could see a savings of ~$70,000 for each pond they stock.

The cost of each treatment from the first part of the analysis was used to calculate the cost per kg of shrimp for these hypothetical systems after using the various treatments for one year. The following factors were assumed for the hypothetical 1.5 ha pond, stocking density: 100 animals per m^2, Crops per year: 1, water exchange: none, final weight: 18g, survival 80%. The following factors were assumed for the hypothetical 500m^3 raceway, stocking density: 300 animals per m^2, crops per year: 3, water exchange: 5% water replacement after each harvest, final weight: 18g, survival: 80%. Results of this analysis can be found in table 7. From the raw cost numbers it looks as if ACS would be more beneficial to pond farmers as opposed to raceway farmers. After considering the cost per kg of shrimp it is obvious that the ACS salt mixture can reduce the operational cost of salt for raceway farmer by $0.32 per kg of shrimp when completely substituting for ACS. Complete substitution for the ACS salts can save pond farmers $3.22 per kg of shrimp but overall it is less expensive to use ACS in a raceway system.

The experiments conducted at the Waddell Mariculture Center, Bluffton, SC, USA in the summer of 2006 show that as long as nitrite is monitored closely, the use of the ACS salt mixture as a partial or complete substitution is possible. The lower cost of the ACS salt mixture may even offset the lower growth and survival if nitrite does become an issue.

Table 1. Description of experimental treatments for trials 1 & 2

	SS (g/L)	ACS (g/L)	TDS (g/L)	# of Reps
Trial 1	5	0	5	4
	3.34	1.66	5	5
	1.66	3.34	5	5
	0	5	5	4
	15	0	15	4
	10	5	15	5
	5	10	15	5
	0	15	15	4
Trial 2	15	0	15	4
	10	5	15	5
	5	10	15	5
	0	15	15	4

SS=amount of dilute seawater, ACS=amount of experimental salts, TDS=total dissolved solids, ACS consists of $NaCl$, $MgCl_2$, $MgSO_4$, $CaCl_2$, KCl, $NaHCO_3$

Table 2. Water quality for the experimental environments in trial 1 (mean ± SD)

Treatment	15/0	10/5	5/10	0/15
AM Salinity (g/L)	13.9 ± 1.51a,b	14.4 ± 0.90b,c	15.1 ± 1.06c	15.4 ± 1.12a
PM Salinity (g/L)	14.0 ± 0.88a	14.4 ± 0.98b	15.1 ± 0.99b	15.5 ± 0.96a
AM Temperature (°C)	25.8 ± 2.58a	26.0 ± 1.29a	26.1 ± 1.28a	25.9 ± 1.31a
PM Temperature (°C)	27.2 ± 1.38a	27.1 ± 1.32a	27.2 ± 1.31a	27.1 ± 1.35a
AM pH	7.7 ± 0.31a	7.7 ± 0.32a	7.6 ± 0.31a	7.6 ± 0.34a
PM pH	7.9 ± 0.32a	7.9 ± 0.34a	7.8 ± 0.33a	7.8 ± 0.37a
AM Dissolved Oxygen (mg/L)	6.6 ± 0.54a	6.6 ± 0.57a,b	6.4 ± 0.58b	6.3 ± 0.67a
PM Dissolved Oxygen (mg/L)	6.8 ± 0.55a	6.7 ± 0.58a	6.6 ± 0.60a	6.7 ± 0.77a
Turbidity (NTU)	125.0 ± 20.77a	131.6 ± 29.77a	148.3 ± 29.59a	151.1 ± 35.02a
Alkalinity (mg/L as $CaCO_3$)	135.1 ± 3.17a	136.4 ± 4.58a	136.0 ± 3.47a	126.3 ± 5.77a
Nitrate-N (mg/L)	8.8 ± 3.22a	11.1 ± 2.58a	11.8 ± 1.28a	9.7 ± 0.68a
Nitrite-N (mg/L)	4.6 ± 1.85a	4.6 ± 1.40a	5.8 ± 1.20a	5.4 ± 0.33a
Total ammonia-N (mg/L)	0.6 ± 0.39a	0.4 ± 0.14a	0.4 ± 0.17a	0.7 ± 0.67a

Nominal values: 0/15 = 15 g/L experimental salt mixture, 15/0 = 15 g/L dilute seawater, 10/5 = 10 g/L dilute seawater + 5 g/L experimental salt mixture, 5/10 = 5 g/L dilute seawater + 10 g/L experimental salt mixture. Water quality characteristics with the same letter do not differ significantly between treatments. Morning values (AM) were taken 1-2 h after sunrise. Afternoon values (PM) were taken 4-6 h prior to sunset.

Table 3. Water quality for the experimental environments in trial 2 (mean ± SD).

Treatment	15/0	10/5	5/10	0/15
AM Salinity (g/L)	14.6 ± 0.34a	14.5 ± 0.09a	14.1 ± 0.25a	14.2 ± 0.54a
PM Salinity (g/L)	14.6 ± 0.35a	14.5 ± 0.09a	14.1 ± 0.25a	14.2 ± 0.55a
AM Temperature (°C)	27.7 ± 0.10a	27.8 ± 0.11a	27.7 ± 0.09a	27.8 ± 0.09a
PM Temperature (°C)	28.8 ± 0.11a	28.9 ± 0.11a	28.8 ± 0.11a	28.8 ± 0.11a
AM pH	7.0 ± 0.05a	7.0 ± 0.19a	7.1 ± 0.25a	7.2 ± 0.30a
PM pH	7.1 ± 0.06a	7.1 ± 0.19a	7.1 ± 0.24a	7.3 ± 0.32a
AM Dissolved Oxygen (mg/L)	5.6 ± 0.14a	5.5 ± 0.13a	5.4 ± 0.24a	5.2 ± 0.26a
PM Dissolved Oxygen (mg/L)	5.7 ± 0.14a	5.6 ± 0.19a	5.5 ± 0.13a	5.5 ± 0.20a
Turbidity (NTU)	184.9 ± 44.37a	236.4 ± 122.93a	205.8 ± 82.21a	235.0 ± 57.15a
Alkalinity (mg/L as $CaCO_3$)	98.4 ± 7.07a	99.5 ± 16.51a	104.3 ± 26.23a	115.9 ± 39.32a
Nitrate-N (mg/L)	24.4 ± 0.75a	26.3 ± 4.16a	24.6 ± 5.40a	21.0 ± 5.40a
Nitrite-N (mg/L)	1.0 ± 0.61a	1.1 ± 1.12a	0.4 ± 0.98a	0.9 ± 0.46a
Total ammonia-N (mg/L)	0.2 ± 0.04a	0.3 ± 0.24a	0.2 ± 0.05a	0.2 ± 0.04a

Nominal values: 0/15 = 15 g/L experimental salt mixture, 15/0 = 15 g/L dilute seawater, 10/5 = 10 g/L dilute seawater + 5 g/L experimental salt mixture, 5/10 = 5 g/L dilute seawater + 10 g/L experimental salt mixture. Water quality characteristics with the same letter do not differ significantly among treatments. Morning values (AM) were taken 1-2 h after sunrise. Afternoon values (PM) were taken 4-6 h prior to sunset.

Table 4. Sodium, calcium, magnesium, potassium, chloride, and sulfate concentrations of the experimental environments (mean ± SD)

		0/15	15/0	10/5	5/10
Na^+	Nominal	4,136	4,230	4,206	4,091
	Actual	3,751 ± 315	4,095 ± 122	4,002 ± 95	3,889 ± 82
	% Nominal	91	97	95	95
Ca^{++}	Nominal	162	165	164	160
	Actual	297 ± 39	245 ± 28	291 ± 30	302 ± 36
	% Nominal	184	149	178	189
Mg^{++}	Nominal	532	544	541	526
	Actual	501 ± 43	511 ± 15	515 ± 12	517 ± 15
	% Nominal	94	94	95	98
K^+	Nominal	154	157	156	152
	Actual	157 ± 15	171 ± 11	164 ± 7	161 ± 2
	% Nominal	102	109	105	106
Cl^-	Nominal	7,558	7,519	7,525	7,495
	Actual	6,526 ± 562	7,126 ± 159	7,000 ± 87	6,812 ± 173
	% Nominal	86	95	93	91
SO_4^{2-}	Nominal	1,048	1,078	1,092	1,070
	Actual	431 ± 31	361 ± 45	408 ± 44	403 ± 34
	% Nominal	41	34	37	38

Nominal and Actual column units are in mg/L. Nominal values: 0/15 = 15 g/L experimental salt mixture, 15/0 = 15 g/L dilute seawater, 10/5 = 10 g/L dilute seawater + 5 g/L experimental salt mixture, 5/10 = 5 g/L dilute seawater + 10 g/L experimental salt mixture. Nominal values were calculated by converting typical full strength sea water ion concentrations (Langmuir, 1997) to the salinity of the experimental water the day the samples were taken. Sulfate was assumed to be the major form of sulfur in the water. Samples were taken on the final day of the experiment. Ions were analyzed by inductively coupled plasma spectrometry (Clemson University agricultural services lab, Clemson, SC, USA).

Table 5. Calculation of cost to make ACS mixed salt

	NaCl	$MgCl_2$	$MgSO_4$	$CaCl_2$	KCl	$NaHCO_3$
Bulk Individual Salt Cost	$330.00a	$3760.00a	$841.00a	$1068.00b	$647.00a	$640.00a
Individual Salt Cost per Gram	$0.000364	$0.004145	$0.000927	$0.001177	$0.000713	$0.000705
Grams needed for 1 g ACS	0.754000	0.057000	0.122000	0.045800	0.016400	0.005600
Individual Cost to make 1 g ACS	$0.000274	$0.000236	$0.000113	$0.000054	$0.000012	$0.000004
Total Coast to make 1 g ACS	$0.000693					

Prices marked with an "a" were quoted from the Carolina Chemical Equipment Co., North Charleston, SC, USA. Prices marked with a "b" were quoted from Aquacenter, Inc, Lake Village, AR, USA. All bulk individual salt costs are for 1 ton of each salt. Prices from the Carolina Chemical Company were based on purchasing 40,000-45,000 lbs of each salt. Prices from Aquacenter, Inc were based on purchasing three 2800lb pallets. All prices include delivery to the Waddell Mariculture Center, Bluffton, SC, USA

Table 6. Comparison of the cost of using the experimental treatments from the WMC experiment.

Treatment	1.5 ha Pond	Cost difference	500 m^3 Raceway	Cost difference
0/15	$155,925	$69,548	$5,198	$2,318
15/0	$225,473	$0	$7,516	$0
10/5	$202,290	$23,183	$6,743	$773
5/10	$179,108	$46,365	$5,970	$1,546

All treatments are ratios of artificial sea salt to ACS mixed salts. The artificial sea salt portion of the treatments was calculated using a price from the Red Sea Co. for their artificial sea salt. The proce was based on buying 2200lbs of salt and having it delivered to Clemson University, Clemson, SC, USA. Cost per gram of the Red Sea Co. artificial sea salt was $0.001002101. Cost difference was calculated by subtracting the cost of each volume using the treatment minus the cost using the Red Sea Co. artificial sea salt the make a salinity of 15 g/L for the same volume. The volume of the 1.5 ha pond is ~15,000,000 L. The Volume of the 500 m^3 is ~500,000 L.

Table 7. Salt cost per kg of Shrimp in two hypothetical aquaculture systems using the ACS salt mixture for one year.

1.5 ha Pond		500 m^3 raceway	
Treatment	Cost per kg of Shrimp	Treatment	Cost per kg of Shrimp
15/0	$10.44	15/0	$1.04
10/5	$9.37	10/5	$0.94
5/10	$8.29	5/10	$0.83
0/15	$7.22	0/15	$0.72

Nominal values: 0/15 = 15 g/L experimental salt mixture, 15/0 = 15 g/L dilute seawater, 10/5 = 10 g/L dilute seawater + 5 g/L experimental salt mixture, 5/10 = 5 g/L dilute seawater + 10 g/L experimental salt mixture. 1.5 ha pond assumptions: Bottom area = 15,000m^2, stocking density = 100 animals/m^2 bottom area, final weight = 18g, survival = 80%, water exchange = none, crops per year = 1. 500 m^3 raceway assumptions: Bottom area = 500m^2, stocking density = 300 animals/m^2 bottom area, final weight 18g, survival = 80%, water exchange = 5% water replacement after each harvest, crops per year = 3. Cost of each treatment from table 6.

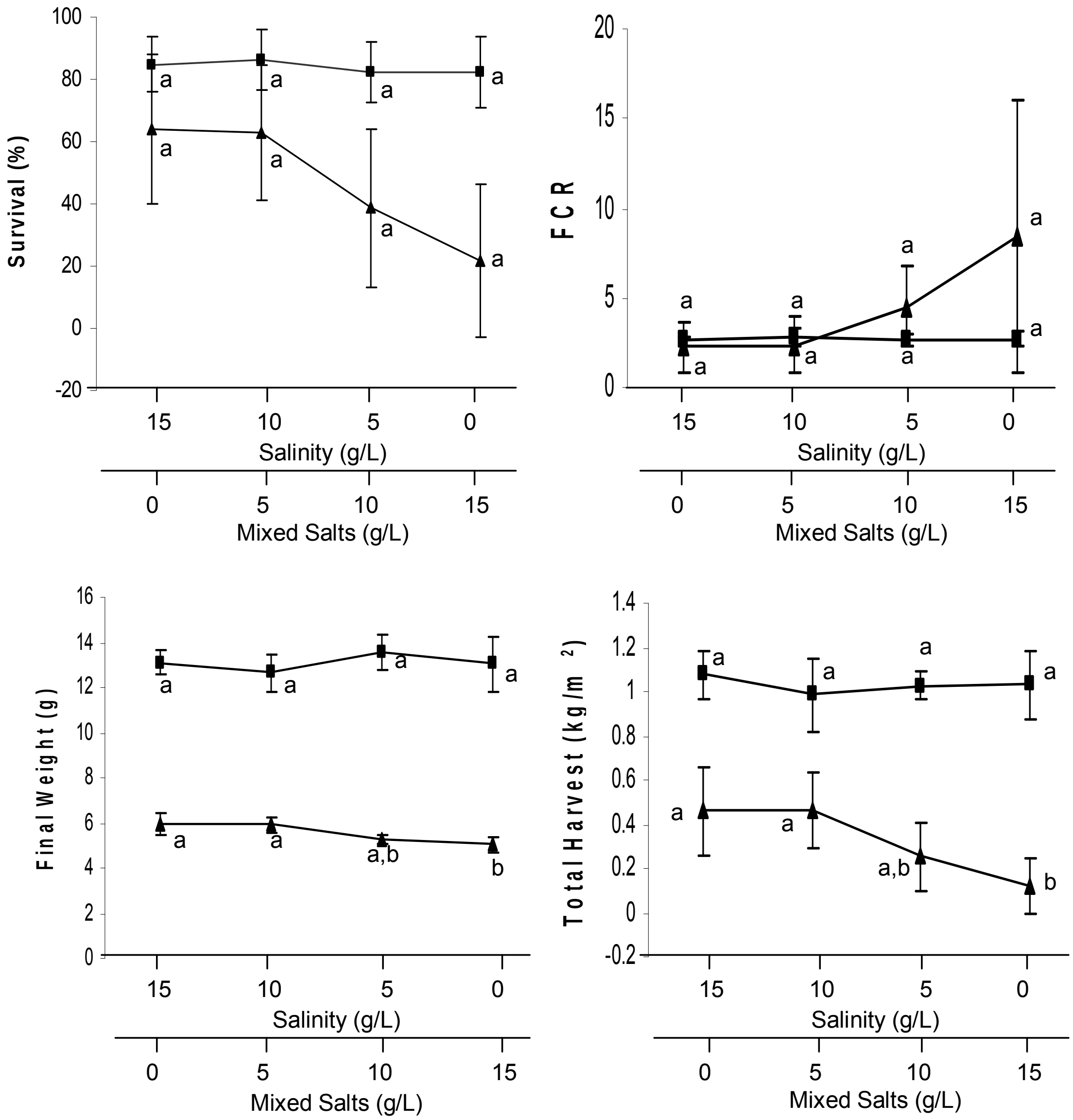

Figure 1. Survival, FCR, final weight and total harvest (mean ± SD) of *Litopenaeus vannamei* for trial1 (Triangles) and 2 (Boxes). Error bars represent ± 1 SD. Treatments with the same letter within each trial do not differ significantly.

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