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SURVIVAL OF PACIFIC WHITE SHRIMP, , IN LOW-SALINITY AND MIXED-ION ENVIRONMENTS

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SURVIVAL OF PACIFIC WHITE SHRIMP, *Litopenaeus vannamei,* IN LOW-SALINITY AND MIXED-ION ENVIRONMENTS.

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A Thesis
Presented to
the Graduate School of
Clemson University

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In Partial Fulfillment
of the Requirements for the Degree
Master of Science
Biological Sciences

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by
Kirk J. Parmenter
August 2007

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Accepted by:
Stephen Klaine, Committee Chair
Craig Browdy
John Hains

ABSTRACT

This study was conducted to (1) evaluate the effectiveness of two different salt mixtures, MI-2 ($NaCl$, $MgSO_4$, $MgCl_2$, KCl, $CaCl_2$, and $NaHCO_3$) and MI ($NaCl$, $MgCl_2$, KCl, and $CaCl_2$) as compared to artificial sea salt (SS) to provide a sufficient environment for the survival of *Litopenaeus vannamei* and (2) determine if additions of the MI-2 salt mixture to low concentrations of sea salt would result in comparable survival as that seen in pure SS environments.

Tests conducted at 5 g/L of total dissolved solids showed that the pure SS treatment had a significantly higher survival rate than the MI-2 and MI combinations. The survival for the MI-2 and MI treatments were not significantly different from each other. Tests conducted at 15 g/L did not show significant differences between the SS and MI-2. In the tests comparing survival of shrimp in pure SS treatments and treatments of SS with MI-2 additions there was no significant difference in survival among the different treatments.

The results indicate that additions of the MI-2 mixture to small amounts of SS can create an environment where the shrimp are able to survive as well as they do in the pure SS treatments. MI-2 additions possibly could be used to lower production costs by replacing a portion of the SS which is significantly more expensive than the MI-2 salts. At higher salinities additions of SS may not even be necessary

DEDICATION

I would like to dedicate this to my family whose support has been an integral part in the successful completion of this task. In particular I would like to dedicate this to my mother, Johanna, for without her love and support I never could have made it this far.

ACKNOWLEGEMENTS

I would like to take this opportunity to thank my major professor, Dr. Stephen Klaine for his support and guidance during the writing portions of this endeavor. I would also like to thank Dr. Joseph Tomasso for his support and guidance during the experimental portion and writing portion of this task and also thank him for giving me the opportunity to complete this endeavor. I would also like to thank the other members of my committee, Dr. John Hains, and Dr. Craig Browdy without whom this could not have been possible. A special thanks to my friend and lab partner Joseph Bisesi for all of his help in this entire process. Thanks also to Dr. Shawn Young for his help with all of the questions that I had for him.

Most of all I would like to thank my family, especially my mother and my brother, Earl, for help when I needed it the most. I would also like to thank my wife Laura for all of her love and support.

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LITERATURE REVIEW

Inland Aquaculture

Several obstacles make coastal mariculture in developed countries economically challenging. The ever increasing cost of land and strict environmental regulations (Wirth and Luzar 2000) are two of the main challenges facing the business of coastal mariculture. One promising solution to dealing with the problem of high land cost in coastal areas is the increase of inland mariculture practices. However a major problem presents itself when operations are moved inland. The facilities can no longer gain quick and easy access to seawater. To overcome this problem the mariculture facilities must import seawater, use brackish-water wells, or prepare their own water by the addition of salts. Sea salt or artificial sea salt is an expensive option for inland aquaculture practices. The goal of some recent research has been to find a more economical substitute for artificial sea salt in the form of cheaper mixed salts (Atwood et al. 2003, Sowers et al. 2006).

One key to the success of inland marine aquaculture practices is to determine the minimal-ion concentrations that can support production in these inland systems where seawater is not easily available. This involves finding the lowest level of certain environmental ions at which the shrimp can still perform all physiological functions. In attempts to find an answer to the necessary concentration of certain ions some recent studies have examined the performance of *L. vannamei* in water containing low concentrations of dissolved

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solids (Sowers et al. 2005) and in low-salinity water that has been augmented with mixed salts (Sowers et al. 2006). These studies are attempting to find seawater substitutes that can be used at the lowest total dissolved solid level possible. Knowing the lowest concentrations that are required allows producers to reduce production costs by not adding more salt than is necessary. It is important to note that culturing shrimp at the lowest salinity level possible is not without its risks. At lower salinities problems can arise such as increased stress and increased nitrite toxicity (Sowers et al. 2004).

<div align="center">Litopenaeus vannamei-The shrimp of choice</div>

The "Top Ten Seafoods" summary of the National Fisheries Institute showed that shrimp was the leading seafood consumed in the United States from 2001 to 2005 (Wirth and Davis 2003, Johnson 2005). The demand for seafood far outweighs the production in the United States. About 85 percent of the total seafood consumed in the U.S. in 2001 was imported, primarily from Southeast Asia. There is an inability of domestic shrimp farmers to compete with low costs of imported shrimp due to cheaper land costs and less strict environmental regulations in some Asian and Latin American countries (Briggs et al. 2004). It appears that the best market for U.S. farmers is in fresh shrimp that are a variety of sizes when taken to market (Wirth and Davis 2003).

The domestication of penaeds in general has been slow. The main reasons have been due to the difficulties of closing the life cycle and producing commercial quantities of larvae (Rothlisberg 1998). Despite these problems,

over the past 20-25 years *L. vannamei* has become one of the primary cultured species of shrimp not only in the U.S. but also many Asian countries as well as many Latin American countries.

The physical characteristics of *L. vannamei* are what make it a promising aquaculture species. Researchers have been able to close its life cycle since L*. vannamei* is an open thelycum species. This allows for high production of larval animals for culture. The ability to select for SPF (specific pathogen free) and SPR (specific pathogen resistant) lines to counter some of the recent production losses due to disease outbreaks has been another beneficial quality of this shrimp species (Briggs et al. 2004). High growth rates as well as wide salinity and temperature tolerances make this the species of choice (Briggs et al. 2004). Another aspect that makes this species appealing for production is that consumer preference in the U.S. seems to be for white shrimp such as *L. vannamei*, *L. stylirostris* and *L. setiferus* and it seems that the taste of these species is preferred to the *L. monodon* species (Rosenberry 2002).

<u>*L. vannamei*-Salinity Research</u>

A very promising result for inland aquaculture was found in a survey conducted by the UF/IFAS Food Science and Human Nutrition Department of the University of Florida. This survey found that *L. vannamei* grown in freshwater were actually preferred for their taste over ones that were either grown in brackish or salt water, including even those that had been harvested from the sea (UF/IFAS, 2003). These results have some very promising implications for

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the development of inland aquaculture facilities that grow their shrimp in water containing low total dissolved solids.

The native range of *L. vannamei* stretches from the pacific coast of Mexico as far south as Peru (Rosenberry 2002). Salinity plays a key role in the distribution of many marine animals and *L. vannamei* is no exception. It is known that the salinity preferences vary among the many species of shrimp. It was shown that of four species tested, *L. vannamei*, *L. califoniensis*, *L. brevirostris,* and *L. stylirostris,* it was *L. vannamei* that had the lowest salinity preference of the four species of postlarval shrimp that were used in the experiment (Mair 1980). The preferred salinities for *L. vannamei* ranged from 1-8 ppt for the postlarval shrimp in the experiment. It has been shown that the actual salinity range that *L. vannamei* can tolerate is from 0.5-45 ppt but it does best in a range between 7 and 34 ppt (Briggs et al. 2004). The tolerance of a wide range of salinities is one of the factors that make this species well suited for aquaculture and in particular inland aquaculture at low salinities.

The main limiting factor in raising *L. vannamei* in environments containing low total dissolved solids is the ability of *L. vannamei* to osmoregulate in those conditions. It has long been shown that salinity changes in nature can lead to shifts in the invertebrate fauna that inhabit certain areas due to their inability to survive at certain salinities (Parker 1955). Mature shrimp are not usually found at low salinities in natural settings and it seems that the osmoregulatory capabilities of shrimp change during the maturation process (Castille and Lawrence 1981a).

One study found that osmoregulatory capabilities of *L. japonicus* increased progressively up through the post larval stages (Charmantier et al. 1988). Other studies have found similar results with prawns of *Penaeus plebjus*, *Penaeus esculentus*, *P. merguiensis*, and *Metapenaeus bennettae* that were tested (Dall 1981). The late larval and post larval stages were efficient osmoregulators but this ability was later modified as the post larvae further developed. Further evidence supporting the idea of superior regulation capabilities in the younger post larvae has been found in other studies (Mcgraw et al. 2002). Similar results have been shown for related species such as *L. monodon* (Cawthorne et al. 1983).   Another study found somewhat differing results that showed larger post larvae were attracted to lower salinities but the preference was still for higher salinities in the juvenile shrimp (Mair 1980).

The variability seen in the ion regulatory capability at different stages of the life cycle corresponds well with the location of the members of each life cycle stage in the environment. Adult females release their eggs in offshore waters. The eggs hatch and the animals progress through the larval stages as they move toward the inshore waters and open estuaries where they are very likely to encounter lower salinities. After a certain amount of time the older and larger juveniles head back for open water. The greatest ion regulatory capabilities seem to be in the life cycle stages that are exposed to the greatest salinity variation (Dall 1981, Laramore et al. 2001).

L. vannamei may have the ability to survive in a wide range of salinities but its growth can vary significantly among different salinity conditions. One study found that there was a significant difference in growth for postlarval shrimp that were kept in 2 and 3ppt treatments as compared to 30ppt treatments (Laramore et al. 2001). Not only was growth significantly lower for the shrimp grown in the lower salinities they also showed decreased survival. The shrimp in the 2ppt treatments had a 20% survival while the 30ppt treatment had 80%survival and there was no survival of shrimp in treatments below 2ppt. The researchers also found that size and age factored into the observed effects of salinity on growth and survival. Results from a different study showed higher growth in 5 and 15ppt treatments compared to treatments of 25, 35, and 49ppt (Bray et al. 1994). No significant difference in survival was observed among the different treatments. In yet another study, results showed the best growth at salinities above 20% and temperatures between 20 and 30°C (Ponce-Palafox et al. 1997).

Temperature has also been shown to have some effects on shrimp survival and growth. It was demonstrated that two penaeid species were maintained fairly well in 10-30% seawater but as the temperature was lowered the osmotic regulatory capabilities of the shrimp were also lowered (Williams 1960). One study showed that production of *L. monodon* was possible in the cold-temperate climate of Italy but that it was temperature that was the main limiting factor in the production process (Lumare et al. 1993). It was found that growth rate increased directly with temperature but that the amount of increase

varied with the size of the *L. vannamei* that were being tested (Wyban et al. 1995). The researchers state that the optimal conditions for growth and survival correspond with the natural conditions of the environments from which this species originated (Ponce-Palafox et al. 1997).

The previously mentioned studies examined growth and survival in low-salinity seawater while some other research has focused on shrimp survival in mixed-salt environments with low total dissolved solids as well as other conditions of low total dissolved solids that contain sea salt that has been augmented with mixed salts (addition of ion combinations to artificial sea salt environments). One study found 0% survival in treatments that contained only mixed salts at 2g/L total dissolved solids with no sea salt additions (Atwood et al. 2003). However a significant increase in survival was observed in treatments that had mixed salts and sea salt in the solutions. For the particular mixture of salts that was being used in this research it was necessary to add sea salt to get the shrimp to survive and grow. In a production scale study performed by Sowers et al. (2006) growth was measured and compared for *L. vannamei* raised in 1g/L dilute sea water plus 1g/L mixed salts as opposed to those raised in 2g/L and 20g/L dilute seawater. Survival amongst the 2, 1+1, and 20g/L treatments did not significantly differ. Final shrimp weights also did not differ among the three treatments. Although neither of these two characteristics differed between the treatments there was a significant difference in total harvest weight. The

20g/L treatment had a higher total harvest weight due to the combined effect of slightly higher survival and final individual weights.

Another study conducted by Sowers et al. (2005) showed a decrease in survival rates for shrimp grown in mixed-salt environments as compared to shrimp grown in 2g/L sea-salt environments. In this same study it was also found that the shrimp had similar growth in both the 2 and 20g/L treatments. This experiment showed that salinity did not affect the growth of *L. vannamei* but these results are conflicting with some of the results that I have previously mentioned in which Laramore et al. (2001) had found salinity did affect the growth. On the other hand a different study found no difference in growth for treatments between 2 and 16g/L in which the shrimp were exposed for 4 weeks (Ogle 1992). These conflicting results could be the result of differing exposure times or varying sizes of shrimp used.

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L. vannamei- Production Research
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Due to the growing likelihood of inland aquaculture seeing significant increases in the future, many studies have examined the suitability of inland sites to house aquaculture facilities. Some work was conducted that compared brackish-well waters taken from several southern states to test their suitability for *L. vannamei* culture (Saoud et al. 2003). This study found that some well water was unsuitable for shrimp culture at all post larval stages of the shrimp. The survival of the shrimp was positively correlated with ions such as K, Mg, and SO_4 found in the well water while it was negatively correlated with a high

<div align="center">

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concentration of iron in the water. One group of researchers point out that problems related to shrimp stress and high mortality caused by imbalances of major ions in the water has been much higher in the United States in comparison to inland aquaculture sites in Thailand (Boyd and Thunjai 2003). The most likely reason given by them is that in Thailand pond water is usually prepared by mixing fresh water and brine solution which usually ends up being very similar to seawater in its ionic composition. In the U.S. where often times ground water or surface water is mixed with granular salts the ionic proportions may differ greatly from those in seawater.

Many euryhaline shrimp species require a minimum concentration of certain ions in order to survive. It was discovered that the addition of K^+ significantly increased shrimp survival (Mcgraw & Scarpa 2003). These researchers determined that shrimp grown in "freshwater" needed K^+ in a minimum concentration of 1ppm.   Results from another study showed some variability in shrimp performance at different Na/K ratios (Zhu et al. 2004). One group of researchers conducted a study to see if it was possible to offset some of the problems associated with incorrect ionic proportions by adding certain ions to supplement pond waters (McNevin et al. 2004). Potassium and magnesium concentrations were increased in this study by adding muriate of potash and potassium-magnesium sulfate to experimental ponds. The addition of these ions caused a largely significant increase in production for the ponds. This

demonstrates that it is possible to use ions to supplement well water to make it more suitable for shrimp aquaculture.

Considering production ponds in which the water is kept and recycled can also be very crucial for inland production facilities. At these production sites where seawater is not easily accessible many times water is reused in the next production cycle. This helps reduce costs associated with artificial sea salt and other salts that must be added to well water to create the salt water environment. Results from one study found that no statistical difference in growth and survival was found between production ponds that operated with and without water exchange although a trend of slightly larger size at harvest was observed for ponds operated with water exchange (Hopkins et al., 1996). A major benefit was apparent from the energy differences between the two different exchange practices. The ponds that were operated with water exchange had energy costs that were 31.5% higher than the ponds that had no exchange. No exchange has strong implications for the effectiveness of reducing production costs by using mixed salts. Recycling the water reduces salt use which is one of the major costs in production.

Another benefit that could be related to non exchange is shown by Moss et al. (1992) who demonstrated that *L. vannamei* growth is enhanced by unknown growth factors that are produced within an intensive shrimp production pond. Solids that were greater than 5.0 µm tended to be microalgae and microbial-detrital aggregates and the presence of these solids and solids

generally larger than 0.5 µm significantly increased the growth rates of the experimental shrimp. Increased growth of oysters, brine shrimp, and calanoid copepods has also been correlated with the presence of solids produced authocthonously in the production ponds (Moss et al. 1992). It seems that inland production ponds that recycle their water could have some cost effective benefits from the practice.

Other variables must also be considered and dealt with if an inland aquaculture facility is going to be successful. A study was conducted that examined how different soils may affect the growth of L. *vannamei* in culture ponds (Ritvo et al. 1998). Soil taken from ponds that had shown either "good" or "bad" production history were used to compare growth rates between the two substrate classes. Mean weight gain for postlarval shrimp on the "good" substrate was significantly higher than those raised on the "bad" substrate. This is a substantial finding because it indicates factors other than the water can influence growth and survival of cultured shrimp. These results most likely will have more of an impact on stagnant commercial ponds in comparison to flow through production facilities (Ritvo et al. 1998). A different study found another aspect of the production process other than the water that could influence productivity (Moss & Moss 2004). It was found that the use of artificial substrates significantly increased the final weights of the cultured shrimp. These are just a few of the other factors that must be considered when trying to maximize production output.

It is obvious that there are many issues and obstacles that must be considered if inland shrimp aquaculture facilities are going to be successful. The physiological tolerances of the shrimp are crucial to the successful culture of a particular species. While *L. vannamei* has many characteristics that make it a good candidate for production, and *L. vannamei* has shown many promising physiological characteristics, an interesting point was discovered by Castille et al. (1993). They found that growth rates could vary between different populations of *L. vannamei* and that the correlation of post larval growth to larval survival was a good indicator of the quality of the post larvae of that population. Selection of a certain line could be a factor in the success of production.

Issues do arise with low-salinity production such as whether or not to recycle the water. Other problems such as increased nitrite toxicity at low salinities become very important (Gross et al. 2004, Lin & Chen 2003, Sowers et al. 2004). The large daily pH cycle arising from the low buffer capacity of low salinity culture is another obstacle for inland aquaculture (Allan & Maguire 1992). The negative effects of low salinity production such as those mentioned above are a major obstacle for inland aquaculture. These issues must be considered when determining whether or not supplement ionic mixtures are a viable option for aquaculture production. If a cheaper alternative to sea salt is found then higher salinities could be used which would resolve some of these problems

INTRODUCTION

High land costs as well as strict environmental regulations limit the growth of mariculture in coastal regions of developed countries (Wirth & Luzar 2000). The development of successful mariculture techniques at inland facilities could be the key to making mariculture more economically profitable. Identifying the minimal environmental-ion concentrations necessary for survival is one step in improving the economic potential of inland culture practices. Identifying a possible replacement for artificial sea salt, which is expensive, could also help cut production costs. A number of recent studies have examined the culture potential of Pacific white shrimp, *Litopenaeus vannamei*, in low salinity environments (e.g, Ogle 1992, Bray et al. 1994, Ponce-Palafox et al. 1997, Laramore et al. 2001; McGraw et al. 2002; Atwood et al. 2003). Few studies have been conducted to asses shrimp survival in water that is not solely comprised of dilute seawater (Atwood et al. 2003; McNevin et al. 200; Sowers et al. 2005; Sowers et al. 2006).

The experiments presented here are a continuation of previous work conducted in our laboratory (Atwood et al. 2003, Sowers et al. 2004, Sowers et al. 2005, Sowers et al. 2006) that examined shrimp survival and growth in mixed-ion (MI) environments consisting of a mixture of the chlorides of sodium, potassium, calcium, and magnesium which account for the majority of the ions is sea water. In addition to the original MI solution, a second ion solution (MI-2), consisting of $NaCl$, $MgSO_4$, $MgCl_2$, KCl, $CaCl_2$ and $NaHCO_3$, was also

investigated. The main objective in this set of experiments was to compare the survival of *L. vannamei* in environments of dilute seawater, the mixed-ion solutions, and combinations of dilute seawater and the MI solutions. Equivalent survival in the mixed-ion solutions would allow for complete substitution of the more expensive sea salt for the less expensive mixed salts. If equivalent survival was only seen in the combination treatments it would allow for a partial substitution of the sea salt.

MATERIALS AND METHODS

Shrimp were produced by the Oceanic Institute Center for Applied Aquaculture and Marine Biotechnology in Kona, Hawaii, and were air shipped to the Aquatic Animal Research Laboratory at Clemson University. The animals were housed in a 1,500-L recirculating system prior to use in the experiments. During the holding period, the shrimp were fed a commercial shrimp feed (Zeigler Bros., Inc., Gardners, PA, USA; protein ≥ 40%, fat ≥ 9%, fiber ≥ 4%) at least once a day. Water quality tests for ammonia-nitrogen, nitrite-nitrogen, and pH were conducted at least once a week according to APHA et al. (1989). Temperature, salinity, and dissolved oxygen were monitored daily using a meter (Model 85/10 FT, Yellow Springs Instrument Company, Yellow Springs, Ohio, USA). Artificial sea salt (Instant Ocean, Aquarium Systems, Mentor, Ohio, USA) was used in the holding tank and in the experiments where sea salt (SS) is indicated.

The cations in both the MI and MI-2 solutions were present in the same ratios found in SS (Langmuir 1997) with the exception of Ca which is slightly higher in the MI-2 mixture. The proportions of these different salts for a 5 g/L solution can be seen in Table 1.

Table 1. Ion proportions of the three salt combinations for an overall salinity of 5g/L.

Treatment	Sea salt	NaCl	KCl	CaCl$_2$	MgCl$_2$	MgSO$_4$	NaHCO$_3$	TDS
SS	5000	0	0	0	0	0	0	5000
MI-2	0	3778	90	237	293	566	36	5000
MI	0	3970	106	160	764	0	0	5000

Experiments were conducted in glass aquaria each containing 30 L of test solution and one animal. Aquaria were placed in Living Stream systems (Frigid Units, Inc., Toledo, Ohio, USA) that functioned as water baths to maintain the water temperature. Each tank was aerated by its own air stone that ran continuously. Water lost through evaporation was replaced every 48-72 hours. Temperature and salinity were measured before shrimp were placed in the experimental tanks, and full water quality analysis was performed for each tank at the completion of the experiment in the same manner as was previously described for the holding tank. Water quality data for the combined experiments are included in the appropriate figure caption.

Prior to being placed in the experimental aquaria, shrimp were removed from the holding tank and placed into an 80-L aquarium. The salinity was slowly lowered in the aquarium over several days by adding filtered tap water until the experimental salinity was reached. Only after the shrimp were acclimated to the lower salinity were they placed in the experimental tanks. During the experiments dead shrimp were removed from the tank then blotted dry and weighed. At the end of the exposure time all of the surviving shrimp were blotted and weighed.

Thirteen experiments were conducted in total (Table 2). All thirteen experiments were ran as seven day exposure tests. Experiments one through four were designed to determine if the MI and MI-2 solutions would support comparable survival to the SS solution at a salinity of 5 g/L. Experiments five

through eight examined the survival in mixtures of MI-2 and SS solutions in comparison to survival in only SS solutions. Experiment nine was designed to determine if shrimp could survive at salinities as low as 0.25 g/L and to test if addition of MI-2 solution to the SS solution increased survival. Experiments 10 and 11 examined the suitability of MI-2 and SS mixtures at a salinity of 5 g/L while experiments 12 and 13 looked at those mixtures at a salinity of 15 g/L.

A chi square analysis was used to examine the data to determine the effect of the environmental treatment on survival. A chi square test was also used to test for outliers. Experiments 2-4 were analyzed together and the results can be seen in figure 1. Experiments 5-8 were analyzed together and can be seen in figure 2. Experiment 9 was analyzed by itself and the results are in figure 3. Experiments 10 and 11 were analyzed together with results shown in figure 4 while experiments 12 and 13 made were combined and the results are in figure 5. Table 2 shows the treatment groups for each experiment. A p value of 0.05 was considered significant.

Table 2. Treatment groups for experiments 1-13. The figure row shows how experiments were grouped for chi-square analysis and in what figure the results appear. In experiments 5-13, treatment indicates the g L^{-1} of SS : g L^{-1} of MI-2. SS represents artificial sea salt. MI-2 represents total dissolved solids of the MI-2 mixture. MI represents total dissolved solids of the MI mixture.

Experiment	1	2	3	4	5	6	7	8	9	10	11	12	13
Treatment	MI-2 5g/L	MI-2 5g/L	MI-2 5g/L	MI-2 5g/L	2:0	2:0	2:0	2:0	0.25:0	5:0	5:0	15:0	15:0
	MI 5g/L	MI 5g/L	MI 5g/L	SS 5g/L	1.5:0.5	1.5:0.5	1:0	1:0	0.25:1.75	3.34:1.66	3.34:1.66	10:5	10:5
	SS 5g/L	SS 5g/L	SS 5g/L		1:1	1:1	1:1	1:1		1.66:3.34	1.66:3.34	5:10	5:10
					0.5:1.5	0.5:1.5	0.5:0	0.5:0		1:4	1:4	1:14	1:14
							0.5:1.5	0.5:1.5		0:5	0:5	0:15	0:15
Figure	-		1				2		3		4		5

RESULTS AND DISCUSSION

After 7 days of exposure to 5 g/L of MI, MI-2 or SS (Experiments 2-4, Figure 1) the survival rates ranged from 100 to 81%. There was a significant difference (P=0.0106) in survival between the SS treatment and the other two but no significant difference between the MI treatment and the MI-2 treatments. Experiment 1 was excluded from the analysis based on the chi square test (P=0.003). The continued testing of the MI solution was abandoned at this point due to results similar to those obtained from previous experiments (Sowers et al. 2005, Sowers et al. 2006). Survival in the MI-2 solution, while better than in the MI solution, was still significantly different than in the SS solution.

The next set of experiments was designed to see if MI-2 salts could be mixed with small amounts of sea salt to increase the survival. Experiments five through eight tested different SS : MI-2 ratios. Survival ranged from 95 to 83.3% (Figure 2, P=.5828). Low SS treatments that were augmented with MI-2 salts had survival percentages that were not significantly different from treatments that only had SS at the same overall salinity. This indicates that MI-2 can partially substitute for SS.

Experiment 9 was designed to determine if MI-2 contributed to survival when a low concentration of SS was present. Survival was significantly higher (P = 0.0081) in the 0.25 g/L SS:1.75 g/L MI-2 than the 0.25 g/L SS treatment (Figure 3), indicating that addition of MI-2 salts are contributing to the increased survival of shrimp.

Combined experiments 10 and 11 as well as combined experiments 12 and 13 were designed to determine if the MI-2 and SS mixtures would have comparable survival rates at salinities that would be more likely to be used in an aquaculture production facility. Combined experiments 10 and 11 used combinations of SS and MI-2 to have an overall total dissolved solids of 5 g/L while combined experiments 12 and 13 had combinations that had overall total dissolved solids of 15 g/L. In 5 g/L total dissolved solids, survival ranged from 100 to 89.5% but was not significantly affected by treatment (P= 0.5226, Figure 4). All treatments in the 15 g/L total dissolved solids had 100% survival (Figure 5). When all experiments are considered together, it appears that MI-2 solutions are more effective at supporting shrimp survival at higher concentrations.

The first set of combined experiments (2-4) showed the survival rates in the three environments (SS, MI, and MI-2), the MI mixture showed similar results as in previous experiments and was not further tested (Figure 1). The second set of combined experiments tested whether or not MI-2 salts could be added to small amounts of sea salt to improve survival. The results of these experiments indicate that low levels of sea salt could be augmented with MI-2 salts and have survival rates that are not significantly different from SS treatments of the same overall salinity. One important use of the MI-2 salts that could be supported by this data is that it would be possible to add MI-2 salts to low levels of SS to bring the overall salinity of production tanks up. The added MI-2 salts would allow for acceptable survival and increase the overall salinity which could offset some

production problems such as high nitrite levels that can be toxic at low salinities (Sowers et al. 2004). MI-2 salts could replace some of the more expensive SS which could help cut production costs. Experiment 9 which was analyzed by itself indicated that the addition of MI-2 did actually improve shrimp survival at low levels of sea salt.

The results of combined experiments 10 and 11 and combined experiments 12 and 13 are promising for the use of alternative salts in the production of *L. vannamei*. Comparable survival rates were seen for SS treatments that had been augmented with MI-2 salts and even the treatments only with MI-2 salts did well.

Previous experiments have shown some promising results using low salinity water that had been augmented with mixed salts (Sowers et al. 2006). Similar production characteristics were seen for *L. vannamei* in a 2 g/L sea salt environment and an environment containing 1 g/L sea salt + 1 g/L mixed salts (Sowers et al. 2006). In our second set of combined experiments similar survival rates were seen in environments containing low levels of sea salt that had been augmented with the MI-2 salts in comparison to the SS environments.

Other experiments have not had such promising results using mixed salt environments for shrimp culture. One study found that it appeared that the addition of sea salt was required for the survival and growth of *L. vannamei.* The same study also found that high levels of sodium appeared to be detrimental to shrimp health (Atwood et al. 2003). That study was not able to determine the

benefits of using mixed salts to replace sea salt but our study showed similar survival rates for environments augmented with mixed salts compared to the SS environments, which supports the benefits of using mixed salts to replace at least part of the sea salt in the treatments.

The results of these experiments show that MI-2 salts could be used in the production process to lower the costs of shrimp farming that are associated with artificial sea salt prices. An experiment testing the use of the MI-2 salts in the production facility were performed by another graduate student in our lab (Bisesi unpublished results). The results of that study as well as this study showed promising results for using MI-2 as one possible mechanism to reduce production costs.
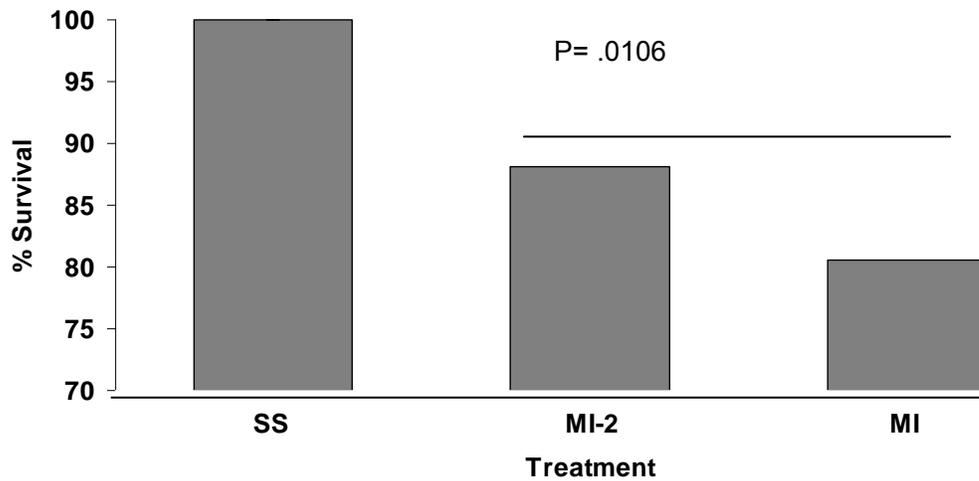
Figure 1. Percent survival for each experimental treatment after 7 days (N=147, final animal weight = 0.55 ± 0.43 g, mean ± SD) in 5 g/L SS, MI, or MI-2 (defined in text). Water quality at the end of the experiments was: nitrite-N = 0.04 ± 0.05 mg L^{-1}, temperature = 26.8 ± 0.3 °C, pH = 7.7 ± 0.14, total ammonia-N = 0.14 ± 0.21 mg L^{-1}. Line indicates that MI and MI-2 are not significantly different from each other. Total dissolved solids 5 g/L.
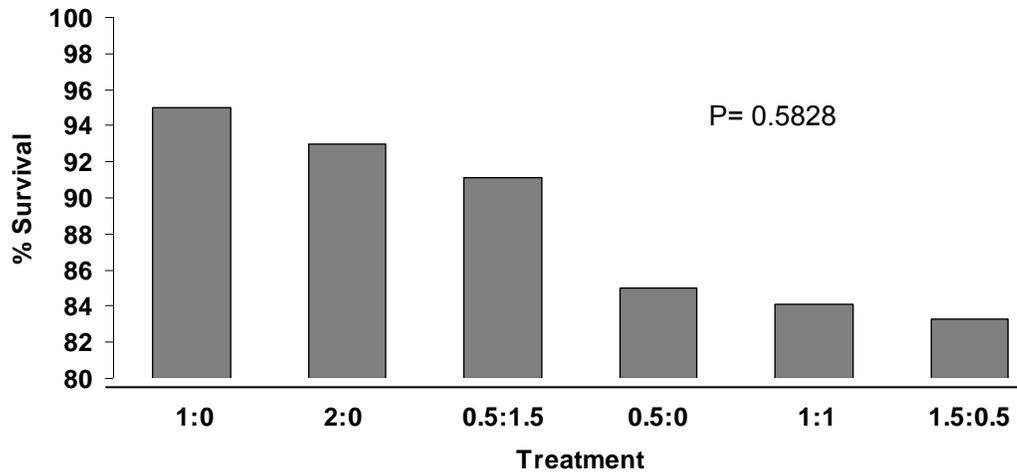
Figure 2. Percent survival for each treatment after 7 days (N=196, final animal weight = 0.32 ± 0.36 g, mean ± SD). The treatment indicates the g L^{-1} of SS : g L^{-1} of MI-2 (SS and MI-2 defined in text). Water quality at the end experiments was: nitrite-N = 0.02 ± 0.03 mg L^{-1}, temperature = 26.5 ± 0.3 °C, pH = 7.46 ± 0.10, total ammonia-N = 0.26 ± 0.33 mg L^{-1}. Total dissolved solids (0.5-2 g/L).
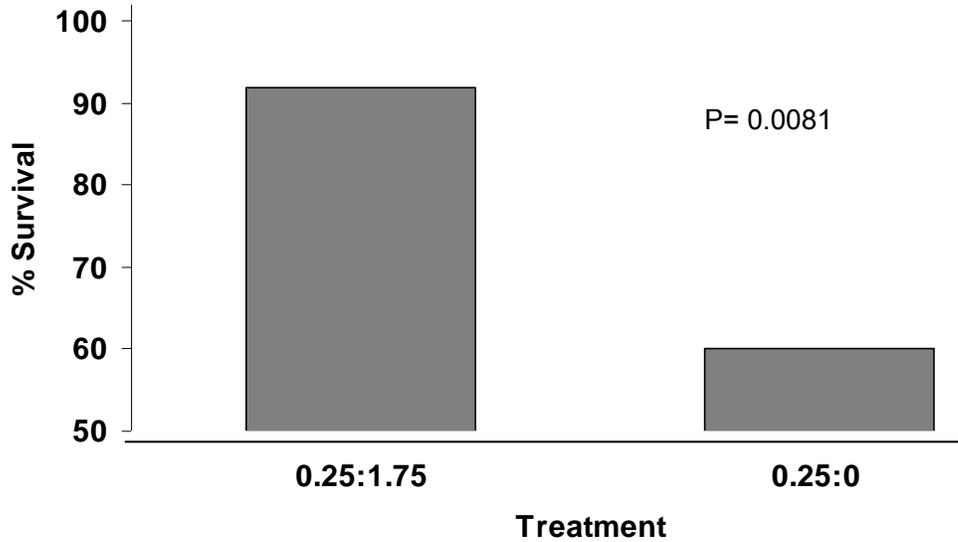
Figure 3. Percent survival for each treatment after 7 days (N=50, final animal weight = 0.24 ± 0.17 g, mean ± SD). The treatment indicates the g L $^{-1}$ of SS **:** g L $^{-1}$ of MI-2 (SS and MI-2 defined in text). Water quality at the end of the experiments was: nitrite-N = 0.03 ± 0.05 mg L $^{-1}$, temperature = 26.4 ± 0.4 °C, pH = 7.33 ± 0.95, total ammonia-N = 0.20 ± 0.31 mg L $^{-1}$. Total dissolved solids 0.25 g/L and 2 g/L.
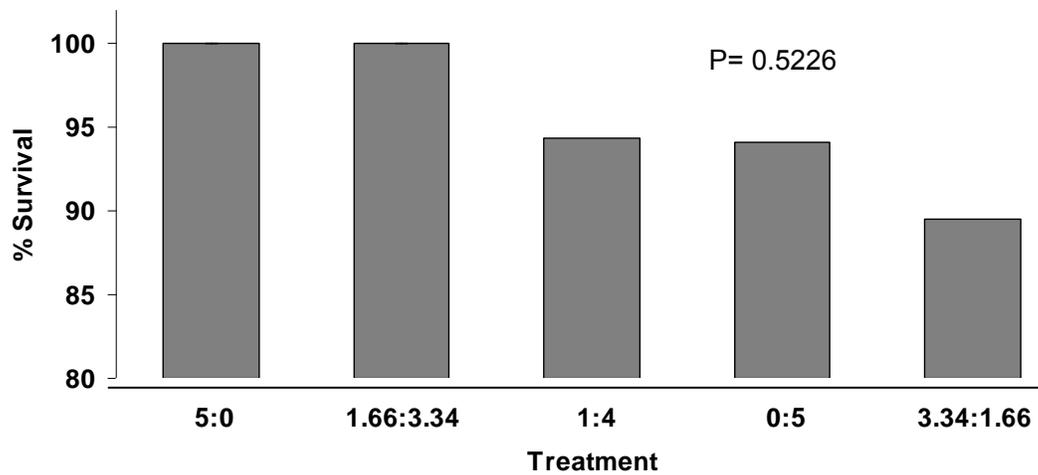
Figure 4. Percent survival for each treatment after 7 days (N=87, final animal weight = 0.97 ± 1.76 g, mean ± SD). The treatment indicates the g L^{-1} of SS **:** g L^{-1} of MI-2 (SS and MI-2 defined in text). Water quality at the end of the experiment was: nitrite-N = 0.03 ± 0.05 mg L^{-1}, temperature = 26.7 ± 0.4 °C, pH = 7.74 ± 0.04, total ammonia-N = 0.16 ± 0.39 mg L -1. Total dissolved solids 5 g/L.
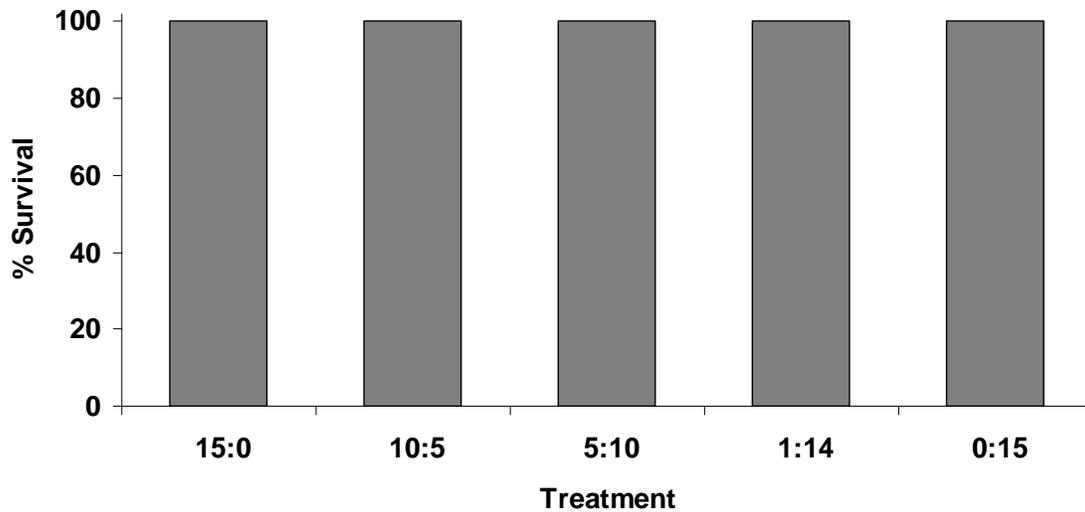
Figure 5. Percent survival for each treatment after 7 days (N=94, final animal weight = 2.48 ± 2.54 g, mean ± SD). The treatment indicates the g L $^{-1}$ of SS**:** g L $^{-1}$ of MI-2 (SS and MI-2 defined in text). Water quality at the end of the experiment was: nitrite-N = 0.24 ± 0.35 mg L $^{-1}$, temperature = 26.4 ± 1.2 °C, pH = 7.96 ± 0.13, total ammonia-N = 0.09 ± 0.29 mg L -1. Total dissolved solids 15 g/L.

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