EVALUATION OF AN ALTERNATIVE SALT MIXTURE AND THE LEVELS OF MAGNESIUM IN LOW SALINITY WATER FOR PACIFIC WHITE SHRIMP *Litopenaeus vannamei*

By

Galkanda-Arachchige Harsha Sameera Chathuranga

A dissertation submitted to the Graduate Faculty of
Auburn University
in partial fulfillment of the requirements for the Degree of
Doctor of Philosophy

Auburn, Alabama
May 01, 2021

Keywords: Pacific white shrimp, low salinity, hemolymph osmolality, growth performance, survival, osmoregulation, ionic composition

Approved by

D. Allen Davis, Chair, Professor of School of Fisheries, Aquaculture, and Aquatic Sciences
Luke A. Roy, Co-Chair, Associate Extension Professor of School of Fisheries, Aquaculture, and Aquatic Sciences
William Daniels, Associate Professor of School of Fisheries, Aquaculture, and Aquatic Sciences
Joseph Tomasso, Professor of School of Fisheries, Aquaculture, and Aquatic Sciences

Abstract

One of the primary challenges faced by inland shrimp farmers today is the higher cost of reconstituted sea salts (RSS) which represents a considerable financial burden considering the volume of salt necessary for the acclimation process or nursery and growth phases in inland shrimp farming. As an alternative to replace expensive RSS, the current study was designed to test the efficacy of a low-cost salt mixture (LCSM) in different production phases of Pacific white shrimp at 3, 6 and 15 g/L salinities under laboratory and farm conditions. The low-cost salt mixture (LCSM) was formulated based on Parmenter *et al.* (2019) to yield Na^+, K^+, Ca^{2+} and Mg^{2+} concentrations closely comparable to those of diluted seawater using agriculture grade sodium chloride, magnesium chloride, magnesium sulfate, muriate of potash (potassium chloride), calcium chloride, and sodium bicarbonate. In addition to the validation of LCSM, the same mixture was modified by decreasing the Mg level to allow for different Mg levels in the culture medium (100, 78, 55, 30, 17, 13 and 12 mg/L) in order to determine the effect of different Mg levels in low salinity (3 g/L) water on growth, survival, hemolymph osmolality, cationic composition in hemolymph, carapace and whole-body of Pacific white shrimp.

Numerous laboratory-based trials were conducted at the E.W. Shell Fisheries Center at Auburn University in Auburn, Alabama and at the Alabama Fish Farming Center (AFFC) in Greensboro, Alabama at 3, 6 and 15 g/L salinities to test the efficacy of LCSM to rear Pacific white shrimp. On-farm evaluation of LCSM along with salinity acclimation of PL was carried out in two tank-based systems installed on levees adjacent to shrimp production ponds at Greene Prairie Aquafarm (GPA), southeastern Greene County, Alabama. Salinity acclimation was done from 32 g/L to 1.5 g/L salinity (PL size = 0.009 ± 0.02g) within 3-days by pumping (flow rate~3.5 L/min) low salinity pond water (1.5 g/L) into each tank. At the conclusion of the on-farm nursery trial, no significant differences existed in either survival (89-94%) or growth of shrimp post-larvae between RSS and LCSM treatments, which was conducted for 21-days following the salinity acclimation. These data were confirmed by the laboratory-based 21-day nursery trials, which had no significant differences in either survival or growth of shrimp post-larvae between RSS and

LCSM treatments at all salinities (2, 6 and 15 g/L). At the conclusion of the 42-day growth trials, no significant differences were observed in survival, growth, osmoregulation and levels of cations in shrimp hemolymph between RSS and LCSM treatments at all salinities (3, 6 and 15 g/L) examined. At the conclusion of Mg^{2+} trial, a subsequent reduction ($P<0.05$) was noted in final weight, weight gain, hemolymph osmolality, osmoregulatory capacity, Mg^{2+} concentration in hemolymph, carapace and whole body of shrimp in respond to the reducing levels of Mg^{2+} in low salinity (3 g/L) water. Reductions in hemolymph osmolality and Mg^{2+} concentration in hemolymph are likely indicative of stress, which is assumed to be due to the dysfunction of osmoregulation in shrimp caused by low levels of Mg^{2+} in culture water. Results reflect the potential use of LCSM to replace RSS which could be an excellent solution to reduce the cost of production for inland low salinity shrimp aquaculture, thereby helping to further stimulate industry growth.

Acknowledgment

I would like to dedicate this dissertation to my major supervisors Dr. Donald Allen Davis and Dr Luke A. Roy, for their enthusiastic guidance, encouragement, and continuous support during the research study. With their continuous support, I never lost my eagerness to progress and it was a great honour for me to study under the supervision of both. I am grateful to Dr William Daniels and Dr. Joe Tomasso for being on my PhD committee and providing input and review of the research. In addition, special thanks go to Dr. Ash Abebe for accepting the invitation to be the outside reader for my dissertation.

My heartfelt thanks are for the all the professors, colleagues, students and staff of the School of Fisheries, Aquaculture and Aquatic Sciences of Auburn University for their precious advice and assistance throughout the research work. Assistance provided by Auburn Laboratory crew including Dr Guillaume Salze, and the crew from Alabama Fish Farming Centre (AFFC) including Dr. Anita M. Kelly, Sunni Dahl and Jesse James, are greatly appreciated. I would also like to acknowledge Greene Prairie Aquafarm for allowing me to carry out the on-farm trial and Greene Prairie Aquafarm and Sumter County Shrimp for supplying pond water for the trials conducted at the Alabama Fish Farming Centre. Furthermore, my special thanks go to Wayamba University of Sri Lanka for granting study leave to me to undergo this invaluable programme at Auburn University. Many thanks to my friends from Auburn: Jan Macguire Miles and Carter Miles, for their support and inspirations to manage and succeed in all work.

Finally, I would like to express my profound gratitude and love to my wife Darshika and my loving angels, Thini and Pini, for their love during the study. Real understanding and continued encouragement of my wife was a great push towards the success of this effort, and she was always besides me sharing the problems and challenges during the study and research. Last but not least, great thanks to my parents & my sisters who always supported me throughout the whole programme. I know that words are not enough to express my gratitude to them.

Table of Contents

List of Tables

CHAPTER II

List of Figures

CHAPTER IV

CHAPTER 1

GENERAL INTRODUCTION

Pacific white shrimp, *Litopenaeus vannamei* is one of the most popular cultured shrimp species (more than 90%) in Americas (Cuzon *et al., 2004)* due to its rapid growth rate, good survival across densities, disease resistance (Cuzon *et al.*, 2004), relatively low dietary protein requirements, and adaptability to wide ranges of salinity and temperature (Moss *et al.*, 2007; Lightner *et al.*, 2009; Rocha *et al.*, 2010). Outside its native range (Eastern Pacific coast from Gulf of California, Mexico to Tumbes, north of Peru), Pacific white shrimp continue to be an important species for world aquaculture, accounting for 85% of total shrimp production in China (Li and Xiang, 2013) and 80% of the farmed shrimp production in the world (Panini *et al.*, 2017).

Until recently, shrimp farming has been largely a coastal phenomenon owing to the need for large volumes of salt water during the grow-out period. Coastal shrimp farms maintain pond salinity levels between 10 and 30 g/L, and commonly exchange 30 to 40% of the pond water volume each day to offset seepage or evaporation losses and maintain environmental conditions (Flaherty *et al.*, 2000). However, due to the remarkable ability to tolerate a wide range of salinities (Castille Jr and Lawrence, 1981; Lester and Pante, 1992; Roy *et al.*, 2010), there is considerable interest in the culture of Pacific white shrimp far from coastal areas in inland ponds filled with low-salinity ground water (1–6 g/L) or in indoor tank systems (~10-20 g/L) (Flaherty *et al.*, 2000; Atwood *et al.*, 2003; Green, 2008). Inland production of shrimp has attracted substantial attention globally due to the criticism concerning coastal culture of shrimp, such as degradation of coastal waters, pond abandonment and destruction of mangrove forests (Flaherty and Karnjanakesorn, 1995; Stevenson, 1996). In addition, several advantages of inland aquaculture such as year-round production, increased biosecurity, more diverse locations and the ability to locate close to major markets or shipping routes, and ability to provide fresh products to consumers, all play a vital role in promoting this culture practice (Ray, 2015).

Although Pacific white shrimp can tolerate a wide range of salinities, it does not mean that this species can achieve maximum growth and survival throughout the entire salinity spectrum. Numerous researchers have evaluated the effects of salinity for Pacific white shrimp post-larvae and juveniles (Mair, 1980; Boyd, 1989; Ogle *et al.*, 1992; Bray *et al.*, 1994; Samocha *et al.*, 1998; Tsuzum *et al.*, 2000; Laramore *et al.*, 2001; Atwood *et al.*, 2003; Sowers *et al.*, 2005). Ogle et al. (1992) found no differences in 22-day old post-larvae in terms of growth and survival between 2 and 16 g/L (4-wk exposures). This was confirmed by Atwood *et al.* (2003) by exposing 0.22 g post-larvae (initial weight of 0.218 g) to 1, 2, 5, 20 g/L salinity solutions in a 3-week nursery trial using artificial sea salt (Atwood *et al.*, 2003). However, Laramore *et al.* (2001) observed a treatment effect of salinity after culturing 0.05 and 0.3 g post larvae for 40 days in 0, 2, 4 and 30 g/L dilute seawater. In both post-larval stages, 0 and 2 g/L salinity yielded significantly lower survival (<29%) compared to 4 and 30 g/L (>86%). Though the argument is still there on suitability of ≤ 2 g/L salinity for post larvae (smaller than PL_{15}), it is clear that salinities higher than 2 g/L have no detrimental effect on survival and growth of Pacific white shrimp post-larvae, once they reach the PL15 stage (McGraw *et al.*, 2002).

The data pertaining to the effects of salinity on larger shrimp is fairly inconsistent and could be due to the between-study variance of experimental design, water quality parameters (temperature, TAN, nitrite, etc.), initial size of animals, experimental duration, handling and acclimation procedures, ionic composition of the culture medium, or other factors. The salinity range of 15-25 g/L was considered ideal to culture Pacific white leg shrimp by Boyd (1989), while Bray et al. (1994) observed superior growth of shrimp at 5 and 15 g/L salinity compared to 25, 35, and 49 g/L salinities, in an experiment conducted in an outdoor tank system with natural productivity (35-day growth trial; 1.6 g initial size).

The remarkable euryhaline nature of the Pacific white shrimp has been found to be due to their exceptional ability to make a new steady state equilibrium with a new medium (comprised of different salinity) by rapidly changing its osmotic concentration in the hemolymph (Castille Jr and Lawrence, 1981; Roy *et al.*, 2007). Shrimp hemolymph functions hyper-osmotic at low salinities to avoid internal dilution and is hypo-osmotic at high salinities to avoid concentration of body fluids (Castille Jr and Lawrence, 1981). The Pacific white shrimp has been found to be one of the best hyperosmotic regulators among its family (Castille Jr and Lawrence, 1981). Osmoregulatory abilities of *L. vannamei* at low salinities has been found to decline naturally when they reach

subadult or adult stages and the smaller shrimp are considered to be the best at osmoregulation (Vargas-Albores and Ochoa, 1992; Gong *et al.*, 2004).

It is well established that, Na^+–K^+-ATPase, V-ATPase, HCO_3 ATPase, carbonic anhydrase (CA) and many other ion-transport enzymes in the gill epithelium membrane are responsible for the osmoregulation in decapod crustaceans (Towle, 1984; Morris, 2001). Among the rest, Na^+–K^+-ATPase plays the major role in transporting Na^+ from the cell into the hemolymph, with K^+ or NH_4^+ serve as counterions (Towle, 1981; Towle, 1984). Investigations have demonstrated that the gill Na^+–K^+-ATPase activity of euryhaline crustaceans could be impacted by the salinity and ionic composition of the culture medium. Significant increases in Na^+–K^+-ATPase activity was noted in euryhaline crabs adapted to diluted seawater, while the activity of Na+–K+-ATPase was reduced during the acclimation to high salinity (Towle *et al.*, 1976; Neufeld *et al.*, 1980; Lima *et al.*, 1997; Castilho *et al.*, 2001; Towle and Weihrauch, 2001; Henry *et al.*, 2002). Bouaricha *et al*. (1991) documented variations in the activity of Na^+–K^+-ATPase in *M. japonicus* in successive development stages, and the Na^+–K^+-ATPase activity increased significantly in subsequent stages after nauplii.

The energy requirement for the osmoregulatory process (maintaining hemolymph concentrations) can be a considerable proportion of total energy expenditure (Hagerman and Uglow, 1982; Lucu, 1990), which could lead to molt-associated mortality problems partly due to the shortage of available energy under the circumstances of low salinity. Therefore, changes in salinity and ionic concentrations in the rearing medium not only induce modifications in the activity of processes directly related to ion transport mechanisms, but also in the processes related to energy consumption of shrimp (Pequeux, 1995; Gong *et al.*, 2000). Based on this phenomenon, once the change in salinity or ionic composition of the medium is greater than that of the osmoregulation range of shrimp, effects on growth and survival of shrimp are expected to occur accordingly (Gao *et al.*, 2016).

The ionic composition of culture water may be a more important limiting factor for shrimp growth and survival than the salinity itself. This was highlighted by Saoud *et al.* (2003), Davis *et al.* (2005) and Hou *et al.* (2012), who reported reduced growth and survival of shrimp as a result of deficiencies in certain ions and ionic ratios of sodium (Na^+), potassium (K^+), calcium (Ca^{2+}) and magnesium (Mg^{2+}). However, minimum requirements of those ions and ionic ratios to sustain

physiological functions in Penaeid shrimp are yet to be defined (Boyd, 2018). Due to uncertainty of ionic requirements, inland shrimp farmers often choose to use reconstituted sea salts (RSS), since ionic levels and ratios similar to that of seawater are the safest option for optimal survival and growth of *L. vannamei.*

One of the primary challenges faced by U.S. inland shrimp farmers today is the higher cost of RSS which are scientifically formulated to contain the major, minor, and trace elements to support delicate marine life including fish, corals and invertebrates. Due to higher costs of production in the U.S., profit margins are much less robust than in other regions worldwide. Some of the commercially available RSS contain dechlorinating agents to ensure instant removal of chlorine from tap water. Therefore, there is no doubt about their efficacy for use in highly profitable ventures such as public aquariums, aquaculture, university research, environmental studies, ornamental fish exhibits, and reef aquaria. Currently RSS is used in inland shrimp production systems as well, assuming that ionic levels and ratios similar to that of seawater are best for optimum survival and growth of *L. vannamei* (Atwood *et al.*, 2003; Roy and Davis, 2010). Farmers using semi-intensive ponds for low salinity culture use RSS during the nursery phase to acclimate shrimp from full strength seawater down to the salinity of their ponds prior to stocking. Commercial producers utilizing indoor RAS and bio-floc use culture water formulated with RSS throughout their entire production cycle. Unfortunately, the high price of RSS represents a considerable financial burden to commercial producers considering the volume of salt necessary for the acclimation process in outdoor pond culture or for use in indoor production systems (Quagrainie, 2015). As a result, indoor shrimp producers are forced to re-use brackish water prepared with RSS for as many growing cycles as possible to minimize cost. However, over time nitrate accumulates in culture water, and after 3-4 shrimp crops, can become high enough to suppress shrimp growth or even cause mortality (Kuhn *et al.*, 2010). If less expensive salts options were available, indoor shrimp producers could justify exchanging more water to help dilute toxic nitrate levels.

Several studies have investigated the ability of combinations of chlorides of sodium, potassium, calcium, and magnesium prepared in the same ionic ratios as found in dilute seawater to support survival and growth of Pacific white shrimp (Atwood *et al.*, 2003; Sowers *et al.*, 2005, 2006) and found them to be unsatisfactory unless mixed with RSS (Atwood *et al.*, 2003; Sowers *et al.*, 2005; Sowers *et al.*, 2006). However, a mixed ion solution formulated to yield similar ionic

concentrations of Na^+, K^+, Ca^{2+} and Mg^{2+} as in seawater using sodium chloride, magnesium chloride, magnesium sulfate, potassium chloride, calcium chloride and sodium bicarbonate, successfully replaced RSS, without compromising the growth, survival and food conversion ratio (FCR) of juvenile Pacific white shrimp with mean initial weight of 7.1 ± 0.26 g in a laboratory study (Parmenter *et al.*, 2009). Following the success of this mixed ion solution, the current study was conducted with three major objectives.

1. To evaluate the efficacy of low-cost salt mixture (LCSM) to replace RSS in the salinity acclimation and nursery phase of Pacific white shrimp cultured at different salinities (2, 6 and 15 g/L) under both laboratory and on-farm production conditions.
2. To evaluate the efficacy of LCSM to replace RSS in the growth phase of Pacific white shrimp at different salinities (3, 6 and 15 g/L) under laboratory conditions.
3. To determine the effect of different Mg levels on growth, survival, hemolymph osmolality, cationic composition of hemolymph, carapace and whole-body mineral levels of Pacific white shrimp reared in low salinity water (3 g/L).

References

Atwood, H., Young, S., Tomasso, J. & Browdy, C. (2003) 2283981. Survival and growth of Pacific white shrimp *Litopenaeus vannamei* postlarvae in low-salinity and mixed-salt environments. *Journal of the World Aquaculture Society,* 34**,** 518-523.

Bouaricha, N., Thuet, P., Charmantier, G., Charmantier-Daures, M. & Trilles, J.-P. (1991) Na+-K+ ATPase and carbonic anhydrase activities in larvae, postlarvae and adults of the shrimp *Penaeus japonicus* (Decapoda, Penaeidea). *Comparative Biochemistry and Physiology Part A: Physiology,* 100**,** 433-437.

Boyd, C.E. (1989) Water quality management and aeration in shrimp farming. *Fisheries and Allied Aquacultures Departmental series No. 2, Alabama agricultural Experiment Station, Auburn University, Auburn, AL, USA***,** 83.

Boyd, C.E. (2018) Revisiting ionic imbalance in low-salinity shrimp aquaculture. Global Aquaculture Alliance.

Bray, W., Lawrence, A. & Leung-Trujillo, J. (1994) The effect of salinity on growth and survival of *Penaeus vannamei,* with observations on the interaction of IHHN virus and salinity. *Aquaculture,* 122**,** 133-146.

Castilho, P.C., Martins, I.A. & Bianchini, A. (2001) Gill Na+, K+-ATPase and osmoregulation in the estuarine crab, *Chasmagnathus granulata* Dana, 1851 (Decapoda, Grapsidae). *Journal of Experimental Marine Biology and Ecology,* 256**,** 215-227.

Castille Jr, F.L. & Lawrence, A.L. (1981) The effect of salinity on the osmotic, sodium and chloride concentrations in the hemolymph of euryhaline shrimp of the genus *Penaeus*. *Comparative Biochemistry and Physiology Part A: Physiology,* 68**,** 75-80.

Cuzon, G., Lawrence, A., Gaxiola, G., Rosas, C. & Guillaume, J. (2004) Nutrition of *Litopenaeus vannamei* reared in tanks or in ponds. *Aquaculture,* 235**,** 513-551.

Davis, D.A., Boyd, C.E., Rouse, D.B. & Saoud, I.P. (2005) Effects of potassium, magnesium and age on growth and survival of *Litopenaeus vannamei* post-larvae reared in inland low salinity well waters in west Alabama. *Journal of the World Aquaculture Society,* 36**,** 416-419.

Flaherty, M. & Karnjanakesorn, C. (1995) Marine shrimp aquaculture and natural resource degradation in Thailand. *Environmental Management,* 19**,** 27-37.

Flaherty, M., Szuster, B. & Miller, P. (2000) Low salinity inland shrimp farming in Thailand. *Ambio: A Journal of the Human. Environment***,** 174-179.

Gao, W., Tian, L., Huang, T., Yao, M., Hu, W. & Xu, Q. (2016) Effect of salinity on the growth performance, osmolarity and metabolism-related gene expression in white shrimp *Litopenaeus vannamei*. *Aquaculture Reports,* 4**,** 125-129.

Gong, H., Jiang, D.H., Lightner, D.V., Collins, C. & Brock, D. (2004) A dietary modification approach to improve the osmoregulatory capacity of *Litopenaeus vannamei* cultured in the Arizona desert. *Aquaculture Nutrition,* 10**,** 227-236.

Gong, H., Lawrence, A.L., Jiang, D.-H., Castille, F.L. & Gatlin III, D.M. (2000) Lipid nutrition of juvenile *Litopenaeus vannamei*: I. Dietary cholesterol and de-oiled soy lecithin requirements and their interaction. *Aquaculture,* 190**,** 305-324.

Green, B.W. (2008) Stocking strategies for production of *Litopenaeus vannamei* (Boone) in amended freshwater in inland ponds. *Aquaculture Research,* 39**,** 10-17.

Hagerman, L. & Uglow, R.F. (1982) Effects of hypoxia on osmotic and ionic regulation in the brown shrimp *Crangon crangon* (L.) from brackish water. *Journal of Experimental Marine Biology and Ecology,* 63**,** 93-104.

Henry, R.P., Garrelts, E.E., McCarty, M.M. & Towle, D.W. (2002) Differential induction of branchial carbonic anhydrase and Na+/K+ ATPase activity in the euryhaline crab, *Carcinus maenas*, in response to low salinity exposure. *Journal of Experimental Zoology,* 292**,** 595-603.

Hou, C., Wang, F., Dong, S., Zhu, Y. & Yu, T. (2012) Effects of constant Ca 2+ concentration in salinity fluctuations on growth and energy budget of juvenile *Litopenaeus vannamei*. *Aquaculture International,* 20**,** 177-188.

Kuhn, D.D., Smith, S.A., Boardman, G.D., Angier, M.W., Marsh, L. & Flick Jr, G.J. (2010) Chronic toxicity of nitrate to Pacific white shrimp, *Litopenaeus vannamei*: impacts on survival, growth, antennae length, and pathology. *Aquaculture,* 309**,** 109-114.

Laramore, S., Laramore, C.R. & Scarpa, J. (2001) Effect of low salinity on growth and survival of postlarvae and juvenile *Litopenaeus vannamei*. *Journal of the World Aquaculture Society,* 32**,** 385-392.

Lester, L.J. & Pante, M.J.R. (1992) Penaeid temperature and salinity responses. *Developments in Aquaculture and Fisheries Science,* 23**,** 515-534.

Li, F. & Xiang, J. (2013) Recent advances in researches on the innate immunity of shrimp in China. *Developmental & Comparative Immunology,* 39**,** 11-26.

Lightner, D.V., Redman, R., Arce, S. & Moss, S.M. (2009) Specific pathogen-free shrimp stocks in shrimp farming facilities as a novel method for disease control in crustaceans In *Shellfish safety and quality*, pp. 384-424. Elsevier.

Lima, A.G., McNamara, J.C. & Terra, W.R. (1997) Regulation of hemolymph osmolytes and gill Na+/K+-ATPase activities during acclimation to saline media in the freshwater shrimp *Macrobrachium olfersii* (Wiegmann, 1836)(Decapoda, Palaemonidae). *Journal of Experimental Marine Biology and Ecology,* 215**,** 81-91.

Lucu, Č. (1990) Ionic regulatory mechanisms in crustacean gill epithelia. *Comparative Biochemistry and Physiology Part A: Physiology,* 97**,** 297-306.

Mair, J.M. (1980) Salinity and water-type preferences of four species of postlarval shrimp (*Penaeus*) from west Mexico. *Journal of Experimental Marine Biology and Ecology,* 45**,** 69-82.

McGraw, W., Davis, D., Teichert-Coddington, D. & Rouse, D. (2002) Acclimation of *Litopenaeus vannamei* postlarvae to low salinity: influence of age, salinity endpoint, and rate of salinity reduction. *Journal of the World Aquaculture Society,* 33**,** 78-84.

Morris, S. (2001) Neuroendocrine regulation of osmoregulation and the evolution of air-breathing in decapod crustaceans. *Journal of Experimental Biology,* 204**,** 979-989.

Moss, D.R., Arce, S.M., Otoshi, C.A., Doyle, R.W. & Moss, S.M. (2007) Effects of inbreeding on survival and growth of Pacific white shrimp *Penaeus (Litopenaeus) vannamei*. *Aquaculture,* 272**,** S30-S37.

Neufeld, G.J., Holliday, C.W. & Pritchard, J.B. (1980) Salinity adaption of gill Na, K-ATPase in the blue crab, *Callinectes sapidus*. *Journal of Experimental Zoology,* 211**,** 215-224.

Ogle, J.T., Beaugez, K. & Lotz, J.M. (1992) Effects of salinity on survival and growth of postlarval *Penaeus vannamei*. *Gulf and Caribbean Research,* 8**,** 415-421.

Panini, R.L., Freitas, L.E.L., Guimarães, A.M., Rios, C., da Silva, M.F.O., Vieira, F.N., Fracalossi, D.M., Samuels, R.I., Prudêncio, E.S. & Silva, C.P. (2017) Potential use of mealworms as an alternative protein source for Pacific white shrimp: Digestibility and performance. *Aquaculture,* 473**,** 115-120.

Parmenter, K., Bisesi Jr, J., Young, S., Klaine, S., Atwood, H., Browdy, C. & Tomasso, J. (2009) Culture of Pacific white shrimp *Litopenaeus vannamei* in a mixed-ion solution. *North American Journal of Aquaculture,* 71**,** 134-137.

Pequeux, A. (1995) Osmotic regulation in crustaceans. *Journal of Crustacean Biology,* 15**,** 1-60.

Quagrainie, K. (2015) *Profitability of Indoor Production of Pacific White Shrimp: Litopenaeus Vannamei, a Case Study of the Indiana Industry,* Purdue University Extension Fact Sheet.

Ray, A.J. (2015) Indoor-raised shrimp find potential market in Kentucky State University test In *The Global Aquaculture Advocate*.

Rocha, J.L., Guerrelhas, A.C., Teixeira, A.K., Farias, F.A. & Teixeira, A.P. (2010) SPF shrimp breeding in Brazil--genetic, phenotypic trends after generation of selection. *Global aquaculture advocate*.

Roy, L.A. & Davis, D.A. (2010) Requirements for the culture of the Pacific white shrimp, *Litopenaeus vannamei*, reared in low salinity waters: water modification and nutritional strategies for improving production In *Avances en Nutrición Acuicola X-Memorias del X Simposio Internacional de Nutrición Acuicola*, pp. 8-10.

Roy, L.A., Davis, D.A., Saoud, I.P., Boyd, C.A., Pine, H.J. & Boyd, C.E. (2010) Shrimp culture in inland low salinity waters. *Reviews in Aquaculture,* 2**,** 191-208.

Roy, L.A., Davis, D.A., Saoud, I.P. & Henry, R.P. (2007) Branchial carbonic anhydrase activity and ninhydrin positive substances in the Pacific white shrimp, *Litopenaeus vannamei*, acclimated to low and high salinities. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology,* 147**,** 404-411.

Samocha, T.M., Lawrence, A.L. & Pooser, D. (1998) Growth and survival of juvenile *Penaeus vannamei* in low salinity water in a semi-closed recirculating system. *Israeli Journal of Aquaculture/Bamidgeh,* 50**,** 55-59.

Saoud, I.P., Davis, D.A. & Rouse, D.B. (2003) Suitability studies of inland well waters for *Litopenaeus vannamei* culture. *Aquaculture,* 217**,** 373-383.

Sowers, A.D., Gatlin, D.M., Young, S.P., Isely, J.J., Browdy, C.L. & Tomasso, J.R. (2005) Responses of *Litopenaeus vannamei* (Boone) in water containing low concentrations of total dissolved solids. *Aquaculture Research,* 36**,** 819-823.

Sowers, A.D., Tomasso Jr, J.R., Browdy, C.L. & Atwood, H.L. (2006) Production characteristics of *Litopenaeus vannamei* in low-salinity water augmented with mixed salts. *Journal of the World Aquaculture Society,* 37**,** 214-217.

Stevenson, N. (1996) Disused shrimp ponds: Options for redevelopment of mangroves. *Coastal Management,* 25**,** 423-435.

Towle, D. (1981) Role of Na++ K+-ATPase in ionic regulation by marine and estuarine animals. *Marine Biology Letters,* 2**,** 107-122.

Towle, D.W. (1984) Membrane-bound ATPases in arthropod ion-transporting tissues. *American Zoologist,* 24**,** 177-185.

Towle, D.W., Palmer, G.E. & Harris III, J.L. (1976) Role of gill Na++ K+-dependent ATPase in acclimation of blue crabs (*Callinectes sapidus*) to low salinity. *Journal of Experimental Zoology,* 196**,** 315-321.

Towle, D.W. & Weihrauch, D. (2001) Osmoregulation by gills of euryhaline crabs: molecular analysis of transporters. *American Zoologist,* 41**,** 770-780.

Tsuzum, M.Y., Cavalli, R.O. & Bianchini, A. (2000) The effects of temperature, age, and acclimation to salinity on the survival of *Farfantepenaeus paulensis* postlarvae. *Journal of the World Aquaculture Society,* 31**,** 459-468.

Vargas-Albores, F. & Ochoa, J. (1992) Variation of pH, osmolality, sodium and potassium concentrations in the haemolymph of sub-adult blue shrimp (Ps) according to size. *Comparative Biochemistry and Physiology Part A: Physiology,* 102**,** 1-5.

CHAPTER II

LABORATORY AND ON-FARM EVALUATION OF LOW-COST SALT MIXTURES FOR USE DURING SALINITY ACCLIMATION AND THE NURSERY PHASE OF PACIFIC WHITE SHRIMP, *Litopenaeus vannamei*[1]

Published as Galkanda-Arachchige, H., Roy, L.A., Dahl, S., James, J., Kelly, A.M. and Allen Davis, D., 2020. Laboratory and on-farm evaluation of low-cost salt mixtures for use during salinity acclimation and the nursery phase of Pacific white shrimp (Litopenaeus vannamei). Aquaculture Research, 51:3460-3471 (https://doi.org/10.1111/are.14681).

Abstract

Current study evaluates the efficacy of a Low-cost salt mixture (LCSM) to replace expensive reconstituted sea salts (RSS) in the salinity acclimation and nursery phase of Pacific white shrimp under laboratory and farm conditions. LCSM was formulated to yield sodium, potassium, calcium, and magnesium concentrations closely comparable to that of diluted seawater. Laboratory based nursery trials were conducted at 2, 6 and 15 g/L salinities, incrementally replacing RSS with LCSM (25, 50, 75 and 100%) at four replicates per treatment. 30 postlarvae were reared for 7-days in 24L aquaria during the 2 and 6 g/L trials, while the nursery trial for 15 g/L salinity was conducted for 21-days with 400 postlarvae stocked in 150 L tanks. On-farm evaluation of LCSM was carried out in two tank-based systems installed on levees adjacent to shrimp production ponds. RSS was incrementally replaced with LCSM (0, 50, 75 and 100%) and 100 postlarvae stocked into each 800L tank. Salinity acclimation was done from 30 g/L to 6 or 1.5 g/L within 2-3 days by pumping water from adjacent shrimp production ponds. Following salinity acclimation, the S4 System maintained flow-through at 1.5 g/L, while N10 system was maintained static at 6 g/L salinity. At the conclusion, no significant differences were observed for either survival or growth of shrimp postlarvae between RSS and LCSM treatments at all salinities examined. Results reflect the potential use of LCSM to replace RSS, which could be an excellent solution to bring down the cost of production in inland shrimp aquaculture.

Key words: acclimation, alternative salt mixture, growth, low salinity, nursery phase, Pacific white shrimp

1. Introduction

Shrimp farming is one of the fastest growing segments of the global aquaculture industry (FAO, 2018). The importance of shrimp to the global economy has been overshadowed by persistent and intractable social and environmental impacts such as indiscriminate use of antibiotics, degradation of coastal waters, pond abandonment and destruction of mangrove forests (Flaherty and Karnjanakesorn, 1995; Stevenson, 1996). Therefore, there is considerable interest in culturing Pacific white shrimp, *Litopenaeus vannamei* far from coastal areas either in inland ponds filled with low-salinity well water (2–5 g/L), in indoor recirculating aquaculture systems (RAS) or in indoor bio-floc systems, which are usually operated at salinities less than 15 g/L (Atwood *et al.*, 2003; Roy and Davis, 2010; Ray *et al.*, 2017). A nursery phase between hatchery and grow-out is vital in low salinity culture of shrimp for the proper acclimation of postlarvae (PL) from full strength seawater (≥ 30 g/L salinity) to low salinity. The nursery phase provides additional benefits such as better control of water quality parameters and facilitated control of feed delivery. In low salinity culture, additional time in the nursery phase allows for further gill development for better osmoregulatory capacity. Collectively, a nursery phase allows immature PL time to grow to a larger size prior to stocking in grow out ponds (Sturmer *et al.*, 1992).

The ionic composition of culture water may be a more important limiting factor for shrimp growth and survival than the salinity itself. Several studies demonstrated that deficiencies in certain ions, such as sodium (Na^+), potassium (K^+), calcium (Ca^{2+}) and magnesium (Mg^{2+}), negatively impact growth and survival of shrimp (Saoud *et al.*, 2003; Davis *et al*., 2005; Hou *et al*., 2012). Some inland shrimp producers in Ecuador, the USA, Australia and China using low salinity groundwater have experienced low survival rates of Pacific white shrimp PL due to a low concentration of potassium (K^+) in the water (Boyd, 2002; Saoud *et al.*, 2003; McNevin *et al.*, 2004; Partridge *et al.*, 2008; Roy *et al.*, 2010). Additionally, a low concentration of aqueous magnesium (Mg^{2+}) has also resulted in less-than-ideal growth and survival of Pacific white shrimp reared in low salinity well water in west Alabama, USA (Davis *et al.*, 2005; Roy *et al.*, 2006; Roy *et al.*, 2007). Boyd (2018) explained how Na^+ uptake occurs as a replacement of K^+ in shrimp and vise-versa was due to their chemical similarities, such as holding a single negative charge. The

same logic applies to the Mg^{2+} and Ca^{2+} uptake by shrimp. While the importance of absolute concentrations and relative ratios of Na^{+}, K^{+}, Mg^{2+} and Ca^{2+} to meet the physiological demands of Pacific white shrimp PL have been highlighted, the specific requirements for individual ions and ionic ratios are not well known and can vary depending on the ionic composition of the culture water. Most nursery systems and indoor tank systems use commercially available reconstituted sea salt (RSS) products, as this ensures ionic concentrations and ratios similar to that of seawater, the optimal medium for the survival and growth of *L. vannamei* (Atwood *et al.*, 2003; Roy and Davis, 2010). However, the high price of RSS represents a considerable financial burden considering the volume of salt necessary for the acclimation process or nursery phase in inland farms. Therefore, an economically attractive salt solutions would reduce production costs of inland shrimp production facilities, thereby helping to stimulate the growth of the industry further.

Several studies have investigated various combinations of chlorides of sodium, potassium, calcium, and magnesium prepared in the same ionic ratios as found in dilute seawater to support survival and growth of Pacific white shrimp (Atwood *et al.*, 2003; Sowers *et al.*, 2005, 2006) and found them to be unsatisfactory unless mixed with RSS (Atwood *et al.*, 2003; Sowers *et al.*, 2005; Sowers *et al.*, 2006). However, a ion mix solution formulated to yield similar ionic concentrations of Na^{+}, K^{+}, Mg^{2+} and Ca^{2+} as in seawater using sodium chloride, magnesium chloride, magnesium sulfate, potassium chloride, calcium chloride and sodium bicarbonate, successfully replaced RSS, without compromising growth, survival and food conversion ratio (FCR) of juvenile Pacific white shrimp (Parmenter *et al.*, 2009). Following the success of this ion mix solution, the objective of our study was to evaluate the efficacy of the same salt formulation with a slight modification (hereafter; low-cost salt mixture/ LCSM) to replace RSS in the salinity acclimation and nursery phase of Pacific white shrimp cultured at different salinities under both laboratory and on-farm production conditions.

2. Materials and Methods

2.1 Low-cost salt mixture

Low-cost salt mixture consisting of Na^{+}, K^{+}, Ca^{2+}, and Mg^{2+} concentrations of 298, 9, 17, and 39 mg/L, respectively, in 1-g/L solution were prepared based on Parmenter *et al.* (2009). This mixture is similar to the major cations in 1-g/L dilute seawater (Na^{+} = 300 mg/L, K^{+} = 11 mg/L, Ca^{2+} = 11 mg/L, Mg^{2+} = 39 mg/L). Agriculture grade sodium chloride (Champion's Choice,

Cargill, Inc. Minneapolis, MN), magnesium chloride (Nedmag B.V., Veendam, Netherlands), magnesium sulfate (Giles Chemical, Waynesville, NC), Muriate of potash (potassium chloride) (Mosaic Global Sales, LLC, Lithia, FL), calcium chloride (Industrial Chemicals, Inc. Birmingham, AL), and sodium bicarbonate (Church and Dwight co., Inc. Ewing, NJ) were used as source compounds. At least two days before each experimental trial, salt compounds were weighed and mixed thoroughly with fresh water and carefully balanced to yield the aforementioned ionic ratios under the various salinities used in our studies.

2.2 Laboratory nursery trials

Nursery trials were conducted at the E.W. Shell Fisheries Center at Auburn University in Auburn, Alabama, and at the Alabama Fish Farming Center (AFFC) in Greensboro, Alabama, in compliance with the Auburn University animal care policy. Pacific white shrimp PL were obtained from SIS-Islamorada, Florida and American Mariculture, Fort Meyers, Florida. Trials were conducted in 150 L polyethylene tanks (at Auburn University) or 24 L glass aquaria (at AFFC), each equipped with a miniature fluidized bed biofilter. Four nursery trials were conducted at three different salinities of 2, 6, and 15 g/L in which RSS were gradually replaced with LCSM. The six treatment groups for each salinity tested at the AFFC were 100% RSS, 75% RSS mixed with 25% LCSM, 50% RSS mixed with 50% LCSM, 25% RSS mixed with 75% LCSM, 100% LCSM and pond water from a commercial shrimp farm. The same treatments were used in the trials at Auburn University except pond water was replaced with a mixed solution of 97.5% RSS mixed with 2.5% LCSM. The trial conducted in 15 g/L salinity was repeated due to an elevated cationic concentration (K^+, Mg^{2+} and Ca^{2+}) in LCSM than in control (reconstituted sea water), because of an error in calculation. Therefore, particular cationic concentrations were corrected individually and in combinations and repeated to verify the responses of shrimp PL. Each treatment at each salinity tested was quadruplicated. The pond water treatment for the 2 g/L salinity trials conducted at the AFFC was obtained from Greene Prairie Aquafarm Pond S3, while pond water for the 6 g/L salinity trials came from Sumter County Shrimp Pond 7.

For all experimental trials, PL were shipped in full strength seawater (32 g/L). The PL were placed into a nursery tank containing full strength seawater. Since the treatments were designed at low salinities, PL were acclimated to the target salinities of 15, 6 or 2 g/L by dripping freshwater into the nursery tank over time (by reducing approximately 2 g/L salinity per hour). After the PL were acclimated to the appropriate salinity, they were concentrated via draining water through a

small meshed and hand counted. Finally, PLs were acclimated to individual experimental tanks with test solutions by dripping the specific mixed ion solution for 30 minutes. In nursery trials at 2 g/L and 6 g/L, 30 PL were stocked into each tank at the AFFC and reared for seven days (initial weight of 0.006 ± 0.003g and 0.02 ± 0.01g, respectively), while the nursery trials at Auburn University were conducted for 21 days with 400 PL per 150 L tank (initial weight of 0.004 ± 0.003g and 0.006 ± 0.006g, respectively). During the nursery trials, PL were fed a commercial shrimp ration (Zeigler Bros. Inc. Gardners, PA, USA; protein ≥ 50%, fat ≥ 15%, fiber ≤ 1%) four times per day. Daily feed rations were calculated based on a percent bodyweight of animals (50% at start and gradually reduced to 15%); PL were expected to double in size every three days, and size of feed provided was gradually increased accordingly over time.

2.3 On-farm salinity acclimation and nursery trials

On-farm evaluation of LCSM was conducted at Greene Prairie Aquafarm (GPA), an inland, low-salinity shrimp farm that produces Pacific white shrimp. This farm is located about 5 km north of Forkland, Alabama, in southeastern Greene County (GPS coordinates 32°41'40.8" N, 87°54'10.0" W). Experimental trials were conducted in two separate tank systems (S4 and N10) installed on levees adjacent to shrimp production ponds. Each system consisted of twelve 800-L circular polypropylene tanks. The systems were designed to allow water from the adjacent ponds to be continuously pumped into the tanks, and via a central standpipe, drain back into the pond. The flow rates for the S4 and N10 tank systems were 3.5 L/min and 4 L/min, respectively. Aeration was supplied through two submersible air stones in each culture tank connected to a 0.5 HP Sweetwater® regenerative blower. Tanks were covered with a net to prevent shrimp from jumping out.

Reconstituted sea salt was incrementally replaced with LCSM (0, 50, 75 and 100%) at three replicates per treatment to evaluate the efficacy of LCSM for salinity acclimation and nursery phase of PL under commercial farm conditions. Two days before the experimental trials, LCSM was formulated to yield 30 g/L salinity; salts were weighed and mixed thoroughly with pond water in respective individual tanks. Pacific white shrimp were obtained from American Mariculture. These PL were received at Greene Prairie Aquafarm in full strength seawater (32 g/L) and were acclimated to pond water temperature upon arrival prior to any salinity adjustments. One hundred PL were hand counted and stocked into each tank in both the S4 and N10 systems (average stocking size= 0.009 ± 0.002g). Shrimp were acclimated in tanks for two hours before salinity

acclimation from 30 g/L to 6 or 1.5 g/L. Salinities were reduced within 2-3 days by controlling the inflow of pond water into each tank. These two salinity values were selected because these are within the most common range of salinities (1.5 – 6 g/L) obtained from low salinity artesian wells used by commercial shrimp producers in west Alabama. In the S4 system, target salinity was equivalent to the salinity of the adjacent shrimp production pond (~1.5 g/L) and maintained as a flow-through system following the acclimation process. After reducing the salinity to 6 g/L, the N10 system was maintained as static by ceasing the circulation of water from the pond. This procedure was followed to test the higher end of salinities encountered by commercial shrimp producers and because tank systems were not available on any of the commercial farms that had higher pond salinities. Hence, this approach allowed the comparison of two different salinities on one commercial farm. Shrimp PL were fed (INVE Frippak Raceway RW+400 (300-500 microns) *ad libitum* six times per day using 24-h Baby Belt feeders (Pentair Aquatic Ecosystems, Apopka, FL, USA) and were reared for 21 days following salinity acclimation in each system to determine survival and growth performance.

2.4 Water analyses

Dissolved oxygen, salinity and water temperature were measured twice daily using a YSI-55 digital oxygen/temperature meter (YSI Corporation, Yellow Springs, Ohio, USA), and total ammonia-nitrogen (TAN) and nitrite-nitrogen were measured twice per week according to the methods described by Solorzano (1969) and Spotte (1979), respectively. The pH of the water was measured twice weekly during the study period using a pHTestr30 (Oakton Instrument, Vernon Hills, IL, USA). Ionic profile of water (in each experimental trial) was determined using inductively coupled argon plasma (ICAP) spectrophotometry by the Soil Testing Laboratory at Auburn University (Auburn, AL, USA) and reported as mg/L (Clesceri *et al.*, 1998).

2.5 Statistical analyses

Statistical analyses were conducted using SAS (version 9.4; SAS Institute, Cary, North Carolina). Since different tank systems and different durations were used in the nursery trials conducted at AFFC and AU, statistical analyses were conducted by individual trial. Survival and growth performances of PL were statistically analyzed via one-way analysis of variance followed by Tukey pairwise comparison test to determine significant differences ($p < 0.05$) among treatment means according to Steel and Torrie (1980). In order to make a general comparison between static and flow-through systems used during the farm demonstration study, a two-sample t-test was used.

3. Results

3.1 Laboratory trials

AFFC

At the conclusion of the AFFC seven-day nursery trial at 2 g/L salinity, PL survival ranged from 89.2 - 92.5% with no significant differences between treatments (Table 1; *P*= 0.99). During the trial, DO, water temperature, salinity, pH, TAN, and nitrite concentrations were maintained within the acceptable ranges for *L. vannamei* (Table 2). All treatments containing LCSM had higher concentrations of cations than that of 100 % RSS treatment group (Table 2). The pond water from Greene Prairie Aquafarm had a significantly lower Mg^{2+} concentration of 24 $\pm$ 1 mg/L compared to 75 $\pm$ 3 mg/L in the 100% RSS treatment.

At the conclusion of the 7-day nursery trial at 6 g/L salinity, the overall PL survival ranged between 85 - 95% with no significant differences between treatments (*P*= 0.66; Table 3). Water quality parameters, including the ionic profiles of the various treatment groups are presented in Table 4. Like the observation for the 2 g/L trials, Mg^{2+} concentrations in pond water from Sumter County Shrimp (69 mg/L) was significantly less than that of the 100% RSS treatment (228 mg/L).

Auburn University

No significant differences in growth (*P*= 0.90) or survival (*P*= 0.12) of PL were observed when reared in 15 g/L salinity for 21-days (Table 5). Survival of PL ranged between 88 – 94% while weight gain was between 0.20 – 0.22 g following the 21-day nursery phase. However, due to elevated concentrations of K^+, Ca^{2+} and Mg^{2+} (1.7, 2.2 and 1.3 times respectively, to that of the100% RSS treatment) detected during this trial (Table 6), the trial was repeated by adjusting the cationic concentrations, individually and in combinations to detect possible effects of particular cations on the growth and survival of PL. No significant differences in final mean weight, weight gain, FCR or survival between treatments (Table 7) occurred. Survival percentages and weight gain data ranged from 89 – 94% and 0.25 – 0.30g, respectively (Table 7). Results revealed that the low Mg^{2+} or higher concentrations of K^+, Ca^{2+}, Mg^{2+} than that of 100% RSS treatment did not affect growth or survival of Pacific white shrimp PL.

3.2 On-farm evaluation

Survival of PL in 1.5 g/L and 6 g/L salinity water ranged from 89-94% and 96-100% in S4 and N10 systems, respectively. No significant differences were found between treatments within a system for survival or growth (Table 9). Significantly higher survival was detected in static N10 system (6 g/L) compared to the flow-through S4 system (1.5 g/L). A significantly higher final weight of PL was obtained in the flow-through S4 system (0.9 $\pm$ 0.09 g) compared to the static N10 system (0.77 $\pm$ 0.09 g) (Table 9).

All treatments containing the LCSM had slightly higher concentrations of cations than that of 100% RSS except for Mg^{2+}, which was slightly less than that in the 100% RSS treatment (Table 11). In the flow-through system S4, the Na^{+}, K^{+}, Ca^{2+}, and Mg^{2+} concentrations were 823, 33, 21 and 14 mg/L, respectively, at 1.5 g/L salinity and 1268, 50, 33 and 19 mg/L, respectively in static tank system N10, which was maintained at 6.0 g/L salinity). At the end of salinity acclimation period and nursery phase, the ionic profile of pond water was reflected in study tank system S4 (since this system was maintained flow through), while the water in tank system N10 had higher cationic concentrations than that in adjacent pond (since the circulation was ceased at 6 g/L salinity). The Na^{+}, K^{+}, and Ca^{2+} concentrations in all treatment waters were in the range of diluted seawater with respect to salinity. However, the Mg^{2+} concentration in treatment waters in system S4 (at 1.5 g/L salinity) were extremely low, ranging from 14-17 mg/L in system S4 and from 106-118 mg/L in system N10 (6 g/L salinity), when compared to the Mg^{2+} concentration in diluted seawater, which is around 50 mg/L (at 1.5 g/L salinity) and 198 mg/L (at 6 g/L salinity).

4. Discussion

The Pacific white shrimp can tolerate a wide range of salinity. They are capable of hypo- and hyper-osmoregulation when the ambient salinity is above and below the isotonic point of 718 mOsm/kg, which is equivalent to 25 g/L salinity (Castille Jr and Lawrence, 1981b; Lester and Pante, 1992; Roy *et al.*, 2010). Therefore, there has been considerable interest and success in culturing Pacific white shrimp far from coastal areas in either inland ponds filled with low-salinity ground water (2–5 g/L) or in indoor tank systems (~10-20 g/L) (Flaherty *et al.*, 2000; Atwood *et al.*, 2003; Green, 2008). However, inland shrimp farmers must use commercially available RSS for the acclimation process or nursery phase in inland aquaculture systems, because no other options currently exist. The use of RRS in indoor production systems require large amounts of

RSS to amend culture water throughout the entire production cycle. In the southeastern USA, shrimp farmers using semi-intensive low salinity ponds use RSS only during acclimation and nursery phases. However, large farms often must make large quantities of artificial seawater to house millions of shrimp PL in large scale nursery systems. Therefore, the present study examines the efficacy of an economically attractive salt solution that would reduce the cost of production in inland shrimp production facilities.

Numerous researchers have formulated different combinations of Na^+, K^+, Ca^{2+}, and Mg^{2+} in the form of chlorides to reflect ionic ratios found in dilute seawater. Unfortunately, these combinations did not supported survival and growth of Pacific white shrimp unless mixed with RSS (Atwood *et al.*, 2003; Sowers *et al.*, 2005; Sowers *et al.*, 2006). Assuming the absence of sulfur in the ion mix solutions to be the reason for significantly lower survival in previous experiments, Parmenter *et al.* (2009) slightly modified the formulation by adding magnesium sulfate to the mixture. This modified ion mix was tested in a 42-day growth trial (initial weight of 7.1g) and successfully replaced RSS without compromising growth, food conversion ratio (FCR) and survival of juvenile Pacific white shrimp. Through simplifying the formulation rather than balancing all the different anions and trace minerals, it is possible to reduce the cost of the salt mixture by approximately 50%. However, it is essential that any novel salt mixtures used to replace RSS at different salinities (especially very low salinity) and in different production phases used to rear Pacific white shrimp be tested before the transfer of this technology to commercial farming operations. This study demonstrated that LCSM replacement of RSS at 2, 6 and 15 g/L salinities to rear PL in nursery phases and for salinity acclimation is efficacious and economical. At all salinities tested during the nursery trials, LCSM successfully replaced RSS without compromising the survival of PL.

In general, the ~ 90% survival obtained during the nursery trials conducted at 2, 6, and 15 g/L salinities indicate that rearing Pacific white shrimp PL is possible using LCSM in all tested salinities. Our results are similar to those of Ogle *et al*. (1992) who found no differences in growth of 22 day-old PL between 2 and 16 g/L salinities (4-week exposure) and Atwood *et al.* (2003) who showed no differences in PL (initial weight of 0.218 g) growth or survival (80-90%) between 1, 2, 5 and 20 g/L salinity solutions (3-week exposure).

Marine shrimp that regulate the osmolality of body fluids encounter dual problems of internal dilution at low salinities, and concentration of body fluids at high salinities. Therefore, shrimp hemolymph is hyperosmotic to the environment at low salinities and hypoosmotic at high salinities (Castille Jr and Lawrence, 1981b). The Pacific white shrimp has been found to be one of the best hyperosmotic regulators among its family (Castille Jr and Lawrence, 1981b). Osmoregulatory abilities of *L. vannamei* at low salinities has been found to decline naturally when they reach subadult or adult stages and the smaller shrimp are considered to be the best at osmoregulation (Vargas-Albores and Ochoa, 1992; Gong *et al.*, 2004). This explains why mature shrimp are not usually found in low salinity environments (Castille Jr and Lawrence, 1981a). When shrimp are acutely transferred to a different salinity, there is a rapid change in hemolymph osmotic concentration as a new steady-state equilibrium between the animal and seawater is approached (Castille Jr and Lawrence, 1981b; Charmantier-Daures *et al.*, 1988; Chen *et al.*, 1995; Charmantier, 1998). This rapid change in the hemolymph osmotic concentration could be used as a stress indicator in extreme salinities. However, similar to the study conducted by Dall and Smith (1981), no attempt was made during the nursery trials or following salinity acclimations in this study to examine ionic regulation of hemolymph in PL, because they do not possess sufficient blood to enable a full analysis of osmolality and major ions.

In addition to the changes in osmolality, increase in amino acid metabolism (Pressley and Graves, 1983) and oxygen consumption (Siebers *et al.*, 1972; Dalla Via, 1986; Tsuzum *et al.*, 2000), oxidation rates of glucose and amino acids were observed in the gills of crabs and shrimp acclimated to diluted seawater. These oxidation rates indicate increased enzyme activity to accelerate ATP turnover as a result of increased energy consumption in gills following exposure to lower salinities (Engel *et al.*, 1975; Lucu, 1990). This in turn reduces gill permeability (both cuticle and epithelium) for specific ions and due to the increased enzymatic mechanisms of Na, K ATPase and carbonic anhydrase, both of which are involved directly and/or indirectly in the ionic transport mechanisms (Lucu, 1990). Therefore, age of Pacific white shrimp (Laramore *et al.*, 2001; McGraw *et al.*, 2002), the ionic composition of water (Davis *et al.*, 2002; McGraw and Scarpa, 2004), genetic differences (Chim *et al.*, 2003) and the length of the acclimation period (Pantastico and Oliveros, 1980) have been identified as vital factors in obtaining the maximum survival following salinity acclimation of PL. Pantastico and Oliveros (1980) observed significantly higher survival in *Penaeus monodon* PL (PL-20, 35, and 90) acclimated from 16 to 0 g/L salinity over a

period of 72 hours, compared to a 24 or 48 hour acclimation period. Similarly, McGraw and Scarpa (2004) demonstrated that *L. vannamei* PL (PL-15) mortality is significantly reduced by allowing an extended acclimation time from 48 to 72 hours or when a habituation period of 48 hours is provided after the 48-hour acclimation period (30 to <1 g/L salinity). During the on-farm evaluation trials in this study, the initial salinity of 30 g/L was reduced to 6 g/L within 96 hours, providing enough time for greater equalization of ions between shrimp hemolymph and the surrounding acclimation medium. This was expected to minimize osmoregulatory stress in PL during the salinity acclimation process and to increase survival when exposed to treatment waters with different ionic compositions. At the conclusion of the on-farm evaluation of LCSM for the salinity acclimation (30 g/L to 6 or 1.5 g/L) and nursery phase of PL (21-days), survival ranged from 89-100% and no significant differences were found between treatments for survival and growth. This confirmed the remarkable osmoregulatory capability of *L. vannamei* PLs over the range of salinities considered in the study and the efficacy of LCSM to use in salinity acclimation and in the nursery phase of inland shrimp production systems.

During the on-farm trials, no effort was made to induce bio-floc conditions in tanks, and post-larval feed was the only addition to the system during the experimental period. In the flow through system (S4) the same water quality conditions, i.e., ionic concentrations, organic and inorganic particulate matter including algae, phytoplankton and zooplankton, etc., existed in experimental tanks as in the adjacent pond. An equal amount of post-larval feed was distributed to shrimp in both the flow-through and the static systems. The observed differences in growth were likely related to water circulation (static versus flow-through), salinity (6 and 1.5 g/L), ionic concentrations and availability of live feeds, in the flow-through system following salinity acclimation. However, in order to make a general comparison between systems, a two-sample t-test was used. Despite the wider range of natural feeds available in the S4 system, significantly higher overall survival was detected in the static system (N10). Notably, significantly higher final weights of PL were detected in the flow-through (S4) system. Though it is difficult to make a firm conclusion, we suggest that the 6 g/L salinity had sufficient cationic concentrations (and perhaps ionic ratios) that better supported survival in the static system over the extremely low salinity (1.5 g/L) and ionic concentrations/ratios of the flow-through system (Table 11). Significantly higher growth of PL in the flow-through system was likely due to the availability of live feeds

(phytoplankton, zooplankton, etc.) from the adjacent pond whereas the static system only had access to commercial feed during the experimental period.

At the conclusion of the nursery trials and salinity acclimation demonstrations under commercial farm conditions, the efficacy of LCSM to completely replace RSS and thus providing a savings of approximately 50% of the cost of RSS for commercial farmers is confirmed (Maier and Quagrainie, 2020; Tierney *et al.*, 2020). However, additional research to further explore the suitability of other agriculture-based salt sources on survival and growth of Pacific white shrimp PL is warranted. Furthermore, minimum concentrations of the major ions (Na^+, K^+, Ca^{2+}, and Mg^{2+}) and the ionic ratios required for physiological functions in Penaeid shrimp are not yet well known (Boyd, 2018), and additional research could lead to further adjustments in the salt formulation of LCSM.

5. Conclusion

At the conclusion of laboratory and on-farm trials, no significant differences were observed in the survival or growth of Pacific white shrimp PL when RSS and LCSM where used at the salinities examined. Results reflect the potential of LCSM to replace RSS, which could reduce production costs of the nursery phase and the acclimation process in low salinity inland ponds and overall production costs in indoor production systems, thereby helping to stimulate growth of the shrimp industry further in the USA and other regions of the world.

Table 1: Survival (%) of Pacific white shrimp post-larvae (0.006±0.003 g) during a seven-day nursery phase conducted in different ion mix solutions at 2 g/L salinity. Values represent the mean of four replicates ± standard deviation.

Treatment	Survival (%)
100RSS	90.0±15.6
75RSS-25LCSM	89.2±5.7
50RSS-50LCSM	90.8±4.2
4 (25RSS-75LCSM)	90.0±9.8
100LCSM	90.0±9.8
Pond water*	92.5±8.8
PSE	9.69
P-value	0.99

RSS= Reconstituted sea salt, LCSM= Low cost salt mixture, PSE= Pooled standard error, *=from Pond S3 (2.5 g/L) at Greene Prairie Aquafarm, AL

Table 2: Dissolved oxygen (DO), temperature, salinity, pH, total ammonia nitrogen (TAN), nitrite nitrogen and ionic composition (mg/L) of waters (2 g/L salinity) used in a seven-day nursery trial with Pacific white shrimp post-larvae. Values represent the mean of four replicates ± standard deviation.

Treatment*	100RSS	75RSS-25LCSM	50RSS-50LCSM	25RSS-75LCSM	100LCSM	Pond Water*
Dissolved oxygen (mg/L)	7.2±0.8	7.1±0.8	7.3±0.8	6.9±0.9	7.1±0.8	7.1±0.8
Temperature (^{0}C)	25.5±2.3	25.2±1.5	25.2±1.5	25.2±1.5	25.2±1.5	25.3±1.5
Salinity (g/L)	1.9±0.1	2.1±0.2	2.2±0.4	2.1±0.0	2.3±0.2	2.5±0.0
pH	8.0±0.1	7.9±0.1	7.9±0.1	7.9±0.1	7.9±0.1	8.0±0.1
TAN (mg/L)	0.6±0.3	0.6±0.2	0.7±0.4	0.6±0.2	0.6±0.2	0.6±0.3
Nitrite (mg/L)	0	0.01±0.01	0	0	0.02±0.03	0
Ionic profile of water (mg/L)						
Na	702±27	732±71	754±87	751±20	747±59	992±23
K	29±1	33±1	37±2	45±3	49±1	33±1
Ca	26±1	31±3	40±3	47±3	50±2	54±2
Mg	75±3	89±8	92±6	108±3	111±9	24±1
Na/K	24±0	22±2	20±3	17±1	15±2	30±1
Mg/Ca	2.9±0.1	3.0±0.3	2.4±0.2	2.3±0.1	2.5±0.4	0.4±0

*from Pond S3 (2.5 g/L) at Greene Prairie Aquafarm, AL

Table 3: Survival of Pacific white shrimp post-larvae (0.02±0.01g) during a seven-day nursery trial in water of different ion mix solutions at 6 g/L salinity. Values represent the mean of four replicates ± standard deviation.

Treatment	Survival (%)
100RSS	87.5±9.6
75RSS-25LCSM	89.2±7.4
50RSS-50LCSM	87.8±10.2
25RSS-75LCSM	85.0±8.8
100LCSM	90.8±9.6
Pond water*	95.0±4.3
PSE	8.44
P-value	0.66

RSS= Reconstituted sea salt, LCSM= Low cost salt mixture, PSE= Pooled standard error, *= from Pond 7 (5.7 g/L) at Sumter County Shrimp, AL

Table 4: Dissolved oxygen (DO), temperature, salinity, pH, total ammonia nitrogen (TAN), nitrite nitrogen and ionic composition (mg/L) of waters (6 g/L salinity) used during a nursery phase of Pacific white shrimp post-larvae. Values represent the mean of four replicates ± standard deviation.

Treatment*	100RSS	75RSS-25LCSM	50RSS-50LCSM	25RSS-75LCSM	100LCSM	Pond Water*
Dissolved oxygen (mg/L)	7.7±0.5	7.6±0.6	7.6±0.5	7.5±0.5	7.6±0.5	7.5±0.5
Temperature (^{0}C)	25.5±1.4	25.7±1.3	25.5±1.3	25.6±1.3	25.6±1.3	25.5±1.4
Salinity (g/L)	5.5±0.1	5.5±0.5	6.0±0.5	6.1±0.7	6.0±0.3	5.6±0.2
pH	7.7±0.1	7.7±0.01	7.7±0.1	7.7±0.1	7.7±0.1	7.5±0.2
TAN (mg/L)	1.2±0.5	1.3±0.9	1.5±0.9	1.6±0.7	1.8±1.3	1.3±0.5
Nitrite (mg/L)	0.01±0.03	0.00±0.0	0.01±0.01	0.01±0.01	0.00±0.01	0.31±0.2
Ionic profile of water (mg/L)						
Na	1990±104	2046±81	2141±72	2236±124	2005±54	2191±133
K	81±7	96±4	108±4	126±7	121±5	78±10
Ca	69±6	85±2	108±6	134±10	131±9	131±11
Mg	228±13	260±10	276±22	320±36	315±12	69±6
Na/K	25±1	21±1	20±0	18±1	17±1	28±3
Mg/Ca	3.3±0.1	3.1±0.1	2.5±0.1	2.4±0.1	2.4±0.1	0.5±0.1

* from pond 7 (5.7 g/L) at Sumter County Shrimp, AL

Table 5: Response of Pacific white shrimp postlarvae (0.004 ± 0.0003 g) reared in different ionic solutions (15 g/L salinity) for 21 days. Values represent the mean of four replicates ± standard deviation.

Treatment	Final weight (g)	Weight gain (g)	Weight gain (%)	FCR	Survival (%)
100RSS	0.207±0.019	0.203±0.019	4579±428	1.4±0.44	92.3±3.50
97.5RSS-2.5LCSM	0.201±0.015	0.197±0.015	4439±330	1.3±0.08	88.0±3.54
75RSS-25LCSM	0.207±0.016	0.203±0.016	4576±353	1.2±0.13	88.5±3.61
50RSS-50LCSM	0.222±0.046	0.218±0.046	4908±1027	1.2±0.26	88.7±5.67
25RSS-75LCSM	0.210±0.025	0.206±0.025	4643±567	1.2±0.12	93.6±2.70
100LCSM	0.215±0.020	0.210±0.020	4745±440	1.1±0.13	93.8±2.30
PSE	0.026	0.026	575.68	0.23	3.72
P-value	0.897	0.897	0.90	0.70	0.115

RSS= Reconstituted sea salt, LCSM= Low cost salt mixture, PSE= Pooled standard error
Feed conversion ratio= feed offered/ (final weight-initial weight)
Weight gain%= ((final weight-initial weight)/initial weight) × 100

Table 6: Dissolved oxygen (DO), temperature, salinity, pH, total ammonia nitrogen (TAN), nitrite nitrogen and ionic composition (mg/L) of waters (15 g/L salinity) during a 21-day nursery phase of Pacific White shrimp post-larvae. Values represent the mean of four replicates ± standard deviation.

Treatment	100RSS	97.5RSS-2.5LCSM	75RSS-25LCSM	50RSS-50LCSM	25RSS-75LCSM	100LCSM
Dissolved oxygen (mg/L)	6.2±1.0	6.3±1.0	6.3±1.1	6.2±1.1	6.2±1.0	6.2±1.0
Temperature (^{0}C)	26.2±1.9	26.2±1.9	26.2±1.7	26.3±1.7	26.3±1.6	26.3±1.5
Salinity (g/L)	15.4±0.5	15.7±0.7	16.6±0.5	16.8±0.5	17.5±0.5	18.1±0.6
pH	7.5±0.1	7.5±0.1	7.4±0.1	7.5±0.1	7.5±0.1	7.4±0.1
TAN (mg/L)	0.9±0.07	0.8±0.02	0.8±0.05	0.9±0.06	0.9±0.08	0.9±0.10
Nitrite (mg/L)	0.1±0.05	0.1±0.05	0.1±0.05	0.1±0.05	0.1±0.05	0.1±0.05
Ionic profile of water (mg/L)						
Na	4672±160	4627±167	4829±220	4792±42	4949±107	5052±125
K	187±15	192±33	213±9	242±2	279±7	313±8
Ca	128±23	147±69	162±7	220±16	246±8	284±11
Mg	941±37	930±42	1063±38	1114±25	1203±15	1269±34
Na/K	25±2	25±3	23±0	20±0	18±0	16±0
Mg/Ca	7.5±1.0	7.1±2.1	6.6±0.5	5.1±0.3	4.9±0.1	4.5±0.2

Table 7: Response of Pacific white shrimp post-larvae (0.006± 0.00006 g) reared in different ion mix solutions at 15 g/L salinity for 21 days. Values represent the mean of four replicates ± standard deviation.

Treatment	Final mean weight (g)	Weight gain (g)	Weight gain (%)	FCR	Survival (%)
100RSS	0.260±0.03	0.254±0.03	4158±457	0.84±0.05[ab]	93±4
100LCSM[2]	0.305±0.02	0.299±0.02	4886±321	0.73±0.02b	91±7
Ca corrected[3]	0.264±0.03	0.258±0.03	4216±524	0.88±0.09a	89±3
K corrected[4]	0.292±0.02	0.286±0.02	4683±313	0.74±0.07ab	94±5
Ca & K corrected[5]	0.303±0.02	0.297±0.02	4863±348	0.73±0.05b	92±5
Ca, K & Mg corrected[6]	0.296±0.02	0.290±0.02	4736±314	0.73±0.05ab	94±1
PSE	0.02	0.02	377	0.06	4.8
P-value	0.09	0.09	0.09	0.01	0.70

[1]RSS= Reconstituted sea salt, [2]LCSM= Low cost salt mixture with elevated K, Ca and Mg ion concentrations than the concentrations in RSS (see Table 6- Treat. 1 and 6), [3]Ca level lowered to mimic the level in RSS, [4]K level lowered to mimic the level in RSS, [5]Ca and K concentrations lowered to mimic the level in RSS, [6]Ca, K and Mg concentrations were lowered to mimic the level in RSS, PSE= Pooled standard error.

Feed conversion ratio= feed offered/ (final weight-initial weight)

Weight gain%= ((final weight-initial weight)/initial weight) × 100

Table 8: Dissolved oxygen (DO), temperature, salinity, pH, total ammonia nitrogen (TAN), nitrite nitrogen and ionic composition (mg/L) of waters (15 g/L salinity) used to nurse Pacific white shrimp post-larvae. Values represent the mean of four replicates ± standard deviation.

Treatment*	1	2	3	4	5	6
Dissolved oxygen (mg/L)	6.2±0.3	6.0±0.4	6.2±0.3	6.3±0.3	6.1±0.3	6.1±0.3
Temperature (^{0}C)	28.0±1.0	28.1±1.2	27.8±1.0	27.8±1.2	28.3±1.0	28.3±1.1
Salinity (g/L)	16.0±0.9	18.3±0.9	18.3±0.8	17.9±0.8	17.5±0.8	17.0±0.8
pH	7.3±0.4	7.4±0.4	7.3±0.4	7.3±0.4	7.4±0.4	7.3±0.4
TAN (mg/L)	0.7±0.44	0.8±0.38	0.7±0.51	1.0±0.35	0.7±0.51	0.8±0.54
Nitrite (mg/L)	0.1±0.001	0.1±0.001	0.1±0.001	0.1±0.002	0.1±0.001	0.1±0.001
Ionic profile of water (mg/L)						
Na	4823±21	5362±39	5494±163	5359±189	5292±101	5348±174
K	219±11	363±32	344±11	239±7	228±5	231±6
Ca	205±2	379±29	197±5	352±8	190±5	191±4
Mg	665±23	850±17	849±27	831±23	817±20	511±14
Na/K	22.1±1.1	14.9±1.2	15.9±0.1	22.4±0.3	23.2±0.2	23.1±0.2
Mg/Ca	3.2±0.1	2.3±0.1	4.3±0.1	2.4±0.1	4.3±0.1	2.7±0.1

*Treatment 1= Reconstituted sea salt (RSS), 2= Low cost salt mixture (LCSM) with elevated K, Ca and Mg ion concentrations than the concentrations in RSS (see Table 6- Treat. 1 and 6), 3= Ca level lowered to reflect the concentration in RSS, 4= K level lowered to reflect the concentration in RSS, 5= Ca and K concentrations lowered to reflect the concentrations in RSS, 6= Ca, K and Mg concentrations were lowered to reflect the concentrations in RSS

Table 9: Response of juvenile Pacific white shrimp (0.009 ± 0.002 g) acclimated to low salinity pond conditions (1.5 g/L in S4 system; 6 g/L in N10 system) and reared for 21 days in different ion mix solutions.

Treatment	S4 system[1]		N10 system[2]	
	Final mean weight (g)	Survival (%)	Final mean weight (g)	Survival (%)
100 RSS	0.89	90	0.73	98
50 RSS/50 LCSM	0.88	94	0.70	97
25 RSS/75 LCSM	0.89	89	0.80	100
100 LCSM	0.94	90	0.86	96
PSE	0.11	3.57	0.08	2.60
P-value	0.90	0.30	0.18	0.34

General comparison between S4 and N10 systems (2-sample t-test)

	Survival (%)	Final mean weight (g)
S4 system[1]	91±3.8	0.90±0.09
N10 system[2]	98±3.4	0.77±0.09
P-value	0	0.002

[1]Acclimated to 1.5 g/L salinity and maintained flow-through with the adjacent shrimp production pond for the experimental period

[2]Acclimated to 6 g/L salinity and maintained static by ceasing water circulation with adjacent shrimp production pond for the experimental period

Table 10: Water quality data (mean ± SD) for the 21-day on-farm trials.

	S4 System[1]	N10 System[2]
Salinity acclimation (g/L)		
Day 01	30.5	31.2
Day 02	19.8	20.5
Day 03	11.5	10.4
Day 04	6.1	5.9
Day 05	1.9	
Day 06	1.5	
Nursery phase		
DO (mg/L)	6.7±0.5	7.5±0.1
Temperature (°C)	28.4±1.4	28.1±0.5
Salinity (g/L)	1.5±0.04	5.9±0.1
pH	9.1±0.2	8.4±0.3
TAN (mg/L)	0.3±0.1	1.2±0.5
Nitrite (mg/L)	0	0.2±0.1
Total Alkalinity (mg/L)	76	110
Total Hardness (mg/L)	92	680

[1]Acclimated to 1.5 g/L salinity and maintained flow-through with the adjacent shrimp production pond for the experimental period

[2]Acclimated to 6 g/L salinity and maintained static by ceasing water circulation from the adjacent shrimp production pond for the experimental period

Table 11: Ionic composition (mg/L) of culture water at the start[1] of salinity acclimation and end[2] of the on-farm trials during the nursery phase of Pacific White shrimp post-larvae. Values represent the mean of three replicates ± standard deviation.

Sodium (Na^+)	**S4**		**N10**		**Potassium (K^+)**	**S4**		**N10**	
Treatment	Start	End	Start	End	Treatment	Start	End	Start	End
100 RSS	9029±59	813±3	8830±63	2482±181	100 RSS	349±42	34±9	302±5	76±5
50 RSS/50 LCSM	9259±85	816±1	9082±82	2592±80	50 RSS/50 LCSM	376±4	37±5	366±3	84±3
25 RSS/75 LCSM	9345±59	820±7	9187±69	2539±5	25 RSS/75 LCSM	389±2	34±2	395±3	82±1
100 LCSM	9410±58	824±10	9265±79	2596±73	100 LCSM	428±2	33±1	426±2	87±6
Pond water	823		1268		Pond water	33		50	
Calcium (Ca^{2+})	**S4**		**N10**		**Magnesium (Mg^{2+})**	**S4**		**N10**	
Treatment	Start	End	Start	End	Treatment	Start	End	Start	End
100 RSS	199±16	25±5	193±5	60±4	100 RSS	1525±39	15±0	1534±34	118±15
50 RSS/50 LCSM	259±6	24±2	278±3	97±10	50 RSS/50 LCSM	1442±16	17±12	1458±14	117±6
25 RSS/75 LCSM	294±3	23±1	308±2	102±4	25 RSS/75 LCSM	1455±17	15±1	1449±7	106±2
100 LCSM	332±3	22±1	340±11	115±4	100 LCSM	1415±14	14±0	1411±17	106±4
Pond water	21		33		Pond water	14		19	

[1]Salinity was set at 30 g/L through different treatments at the start of acclimation process

[2]S4 system: PLs were acclimated to 1.5 g/L salinity and maintained flow-through with the adjacent shrimp production pond for the experimental period while in N10 system, PLs were acclimated to 6 g/L salinity and maintained static by ceasing water circulation from the adjacent shrimp production pond for the experimental period.

References

Atwood, H., Young, S., Tomasso, J. & Browdy, C. (2003) Survival and growth of Pacific white shrimp *Litopenaeus vannamei* postlarvae in low-salinity and mixed-salt environments. *Journal of the World Aquaculture Society,* 34**,** 518-523.

Boyd, C. (2002) Inland farming of marine shrimp: examples for China, Ecuador, Thailand and the United States. Report prepared under the World Bank, NACA, WWF and FAO Consortium Program on Shrimp Farming and the Environment. *World Wildlife Fund, Washington, DC, USA*.

Boyd, C.E. (2018) Revisiting ionic imbalance in low-salinity shrimp aquaculture. Global Aquaculture Alliance.

Castille Jr, F.L. & Lawrence, A.L. (1981a) A comparison of the capabilities of juvenile and adult *Penaeus setiferus* and *Penaeus stylirostris* to regulate the osmotic, sodium, and chloride concentrations in the hemolymph. *Comp. Biochem. Physiol. A,* 68**,** 677-680.

Castille Jr, F.L. & Lawrence, A.L. (1981b) The effect of salinity on the osmotic, sodium and chloride concentrations in the hemolymph of euryhaline shrimp of the genus *Penaeus*. *Comparative Biochemistry and Physiology Part A: Physiology,* 68**,** 75-80.

Charmantier-Daures, M., Thuet, P., Charmantier, G. & Trilles, J.-P. (1988) Tolérance à la salinité et osmorégulation chez les post-larves de *Penaeus japonicus* et *P. chinensis*. Effet de la température. *Aquatic living resources,* 1**,** 267-276.

Charmantier, G. (1998) Ontogeny of osmoregulation in crustaceans: a review. *Invertebrate Reproduction & Development,* 33**,** 177-190.

Chen, J.-C., Lin, M.-N., Ting, Y.-Y. & Lin, J.-N. (1995) Survival, haemolymph osmolality and tissue water of *Penaeus chinensis* juveniles acclimated to different salinity and temperature levels. *Comparative Biochemistry and Physiology Part A: Physiology,* 110**,** 253-258.

Chim, L., Bouveret, R., Lemaire, P. & Martin, J. (2003) Tolerance of the shrimp *Litopenaeus stylirostris*, Stimpson 1894, to environmental stress: interindividual variability and selection potential for stress-resistant individuals. *Aquaculture research,* 34**,** 629-632.

Clesceri, L.S., Greenberg, A.E. & Eaton, A.D. (1998) Standard methods for the examination of water and wastewater, American Public Health Association. *Washington, DC***,** 4-415.

Dall, W. & Smith, D. (1981) Ionic regulation of four species of penaeid prawn. *Journal of Experimental Marine Biology and Ecology,* 55**,** 219-232.

Dalla Via, G. (1986) Salinity responses of the juvenile penaeid shrimp *Penaeus japonicus*: I. Oxygen consumption and estimations of productivity. *Aquaculture,* 55**,** 297-306.

Davis, D.A., Boyd, C.E., Rouse, D.B. & Saoud, I.P. (2005) Effects of potassium, magnesium and age on growth and survival of *Litopenaeus vannamei* post-larvae reared in inland low salinity well waters in west Alabama. *Journal of the World Aquaculture Society,* 36**,** 416-419.

Davis, D.A., Saoud, I.P., McGraw, W.J. & Rouse, D.B. (2002) Considerations for *Litopenaeus vannamei* reared in inland low salinity waters. *Avances en Nutrición Acuicola VI. Memorias del VI Simposium Internacional de Nutrición Acuicola*.

Engel, D., Ferguson, R. & Eggert, L. (1975) Respiration rates and ATP concentrations in the excised gills of the blue crab as a function of salinity. *Comparative Biochemistry and Physiology Part A: Physiology,* 52**,** 669-673.

FAO (2018) The State of World Fisheries and Aquaculture 2018-Meeting the sustainable development goals. FAO Rome, Italy.

Flaherty, M. & Karnjanakesorn, C. (1995) Marine shrimp aquaculture and natural resource degradation in Thailand. *Environmental Management,* 19**,** 27-37.

Flaherty, M., Szuster, B. & Miller, P. (2000) Low salinity inland shrimp farming in Thailand. *Ambio: A Journal of the Human. Environment***,** 174-179.

Gong, H., Jiang, D.H., Lightner, D.V., Collins, C. & Brock, D. (2004) A dietary modification approach to improve the osmoregulatory capacity of *Litopenaeus vannamei* cultured in the Arizona desert. *Aquaculture Nutrition,* 10**,** 227-236.

Green, B.W. (2008) Stocking strategies for production of *Litopenaeus vannamei* (Boone) in amended freshwater in inland ponds. *Aquaculture Research,* 39**,** 10-17.

Hou, C., Wang, F., Dong, S., Zhu, Y. & Yu, T. (2012) Effects of constant Ca 2+ concentration in salinity fluctuations on growth and energy budget of juvenile *Litopenaeus vannamei*. *Aquaculture international,* 20**,** 177-188.

Laramore, S., Laramore, C.R. & Scarpa, J. (2001) Effect of low salinity on growth and survival of postlarvae and juvenile *Litopenaeus vannamei*. *Journal of the World Aquaculture Society,* 32**,** 385-392.

Lester, L.J. & Pante, M.J.R. (1992) Penaeid temperature and salinity responses. *Developments in Aquaculture and Fisheries Science,* 23**,** 515-534.

Lucu, Č. (1990) Ionic regulatory mechanisms in crustacean gill epithelia. *Comparative Biochemistry and Physiology Part A: Physiology,* 97**,** 297-306.

Maier, P. & Quagrainie, K. (2020) A bioeconomic model of indoor marine shrimp *Litopenaeus Vannamei* farms with low-cost salt mixtures In *Aquaculture America 2020 Conference proceedings*, pp. 413Honolulu, Hawaii, USA.

McGraw, W., Davis, D., Teichert-Coddington, D. & Rouse, D. (2002) Acclimation of *Litopenaeus vannamei* postlarvae to low salinity: influence of age, salinity endpoint, and rate of salinity reduction. *Journal of the World Aquaculture Society,* 33**,** 78-84.

McGraw, W.J. & Scarpa, J. (2004) Mortality of freshwater-acclimated *Litopenaeus vannamei* associated with acclimation rate, habituation period, and ionic challenge. *Aquaculture,* 236**,** 285-296.

McNevin, A.A., Boyd, C.E., Silapajarn, O. & Silapajarn, K. (2004) Ionic supplementation of pond waters for inland culture of marine shrimp. *Journal of the World Aquaculture Society,* 35**,** 460-467.

Ogle, J.T., Beaugez, K. & Lotz, J.M. (1992) Effects of salinity on survival and growth of postlarval *Penaeus vannamei*. *Gulf and Caribbean Research,* 8**,** 415-421.

Pantastico, J.B. & Oliveros, E.N. (1980) Acclimation of Penaeus monodon postlarvae to fresh water. *Fisheries Research Journal of the Philippines,* 5**,** 33-38.

Parmenter, K., Bisesi Jr, J., Young, S., Klaine, S., Atwood, H., Browdy, C. & Tomasso, J. (2009) Culture of Pacific white shrimp *Litopenaeus vannamei* in a mixed-ion solution. *North American Journal of Aquaculture,* 71**,** 134-137.

Partridge, G., Lymbery, A. & George, R. (2008) Finfish mariculture in inland Australia: a review of potential water sources, species, and production systems. *Journal of the World Aquaculture Society,* 39**,** 291-310.

Pressley, T.A. & Graves, J.S. (1983) Increased amino acid oxidation in the gills of blue crabs acclimated to dilute seawater. *Journal of Experimental Zoology,* 226**,** 45-51.

Ray, A.J., Drury, T.H. & Cecil, A. (2017) Comparing clear-water RAS and biofloc systems: Shrimp (*Litopenaeus vannamei*) production, water quality, and biofloc nutritional contributions estimated using stable isotopes. *Aquacultural Engineering,* 77**,** 9-14.

Roy, L.A. & Davis, D.A. (2010) Requirements for the culture of the Pacific white shrimp, *Litopenaeus vannamei*, reared in low salinity waters: water modification and nutritional strategies for improving production In *Avances en Nutrición Acuicola X-Memorias del X Simposio Internacional de Nutrición Acuicola*, pp. 8-10.

Roy, L.A., Davis, D.A. & Saoud, I.P. (2006) Effects of lecithin and cholesterol supplementation to practical diets for *Litopenaeus vannamei* reared in low salinity waters. *Aquaculture,* 257**,** 446-452.

Roy, L.A., Davis, D.A., Saoud, I.P., Boyd, C.A., Pine, H.J. & Boyd, C.E. (2010) Shrimp culture in inland low salinity waters. *Reviews in Aquaculture,* 2**,** 191-208.

Roy, L.A., Davis, D.A., Saoud, I.P. & Henry, R.P. (2007) Effects of varying levels of aqueous potassium and magnesium on survival, growth, and respiration of the Pacific white shrimp, *Litopenaeus vannamei*, reared in low salinity waters. *Aquaculture,* 262**,** 461-469.

Saoud, I.P., Davis, D.A. & Rouse, D.B. (2003) Suitability studies of inland well waters for *Litopenaeus vannamei* culture. *Aquaculture,* 217**,** 373-383.

Siebers, D., Lucu, C., Sperling, K.-R. & Eberlein, K. (1972) Kinetics of osmoregulation in the crab Carcinus maenas. *Marine Biology,* 17**,** 291-303.

Solorzano, L. (1969) Determination of ammonia in natural waters by the phenolhypochlorite method. *Limnology and Oceanography,* 14**,** 799-801.

Sowers, A.D., Gatlin, D.M., Young, S.P., Isely, J.J., Browdy, C.L. & Tomasso, J.R. (2005) Responses of *Litopenaeus vannamei* (Boone) in water containing low concentrations of total dissolved solids. *Aquaculture Research,* 36**,** 819-823.

Sowers, A.D., Tomasso Jr, J.R., Browdy, C.L. & Atwood, H.L. (2006) Production characteristics of *Litopenaeus vannamei* in low-salinity water augmented with mixed salts. *Journal of the World Aquaculture Society,* 37**,** 214-217.

Spotte, S. (1979) *Fish and Invertebrate Culture: Water Managment in Closed Systems, 2nd Edition.,* Wiley, New York.

Stevenson, N. (1996) Disused shrimp ponds: Options for redevelopment of mangroves. *Coastal Management,* 25**,** 423-435.

Sturmer, L., Samocha, T.M. & Lawrence, A.L. (1992) Intensification of penaeid nursery systems. *Developments in aquaculture and fisheries science,* 23**,** 321-344.

Tierney, T., Fleckenstein, L.J. & Ray, A.J. (2020) Using alternative low cost artificial sea salt mixtures for intensive shrimp (*Litopenaeus vannamei*) production. In *Aquaculture America 2020 Conference proceedings*, pp. 662Honolulu, Hawaii, USA.

Tsuzum, M.Y., Cavalli, R.O. & Bianchini, A. (2000) The effects of temperature, age, and acclimation to salinity on the survival of *Farfantepenaeus paulensis* postlarvae. *Journal of the World Aquaculture Society,* 31**,** 459-468.

Vargas-Albores, F. & Ochoa, J. (1992) Variation of pH, osmolality, sodium and potassium concentrations in the haemolymph of sub-adult blue shrimp (Ps) according to size. *Comparative Biochemistry and Physiology Part A: Physiology,* 102**,** 1-5.

Chapter III

EVALUATION OF AN ALTERNATIVE SALT MIXTURE TO CULTURE PACIFIC WHITE SHRIMP (*Litopenaeus vannamei*) IN INLAND AQUACULTURE

Published as Galkanda-Arachchige, H.S., Roy, L.A. and Davis, D.A., 2020. Evaluation of an alternative salt mixture to culture Pacific white shrimp (Litopenaeus vannamei) in inland aquaculture. Aquaculture Research, 51(9):3540-3550 (https://doi.org/10.1111/are.14691).

Abstract

Pacific white shrimp, *Litopenaeus vannamei* exhibit a remarkable ability to tolerate low salinity environments, facilitating its culture far from coastal areas using various production systems at salinities less than 15 g/L. Recirculating aquaculture systems (RAS) and bio-floc systems are usually operated using reconstituted sea salt (RSS), which is a considerable financial burden to commercial producers due to its higher price. Current study was carried out with the objective of testing the efficacy of a low-cost salt solution to replace expensive RSS to grow shrimp under laboratory conditions. Low-cost salt mixture (LCSM) was formulated to yield sodium, potassium, calcium, and magnesium concentrations closely comparable to that of diluted seawater using agriculture grade sodium chloride, magnesium chloride, magnesium sulfate, potassium oxide, calcium chloride, and sodium bicarbonate. Growth trials were conducted at three different salinities of 3, 6 and 15 g/L, incrementally replacing RSS with LCSM (0, 2.5, 25, 50, 75 and 100%) at four replicates per treatment. Twenty juvenile shrimp were reared for 42-days in 150 L polyethylene tanks. Ionic profile of water, ionic profile and osmolality of shrimp hemolymph were determined to justify growth and survival data through analyzing ionic variations and osmoregulatory capacity of shrimp. At the conclusion, no significant differences were observed in survival, growth, osmoregulation and levels of cations in shrimp hemolymph between RSS and LCSM treatments at all salinities examined. Results reflect the potential use of LCSM to replace

RSS which could be an excellent solution to bring down the cost of production in inland shrimp aquaculture.

Key words: alternative salt mixture, growth, inland aquaculture, low salinity, osmoregulation, Pacific white shrimp

1. Introduction

Due to the remarkable ability to tolerate a wide range of salinities (Castille Jr and Lawrence, 1981; Lester and Pante, 1992; Roy *et al.*, 2010) and ease of culture, there is considerable interest in the culture of Pacific white shrimp, *Litopenaeus vannamei,* far from coastal areas in inland ponds filled with low-salinity ground water (1–6 g/L) or in indoor tank systems (~10-20 g/L) (Flaherty *et al.*, 2000; Atwood *et al.*, 2003; Green, 2008). Inland production of shrimp has attracted substantial attention globally, due to the criticism developed over coastal culture of shrimp, such as degradation of coastal waters, pond abandonment and destruction of mangrove forests (Flaherty and Karnjanakesorn, 1995; Stevenson, 1996; Páez-Osuna, 2001; Valiela, 2006). In addition, several advantages of inland aquaculture such as year-round production, increased biosecurity, diverse locations for shrimp farming close to various markets, reduced transportation cost, and ability to provide fresh products to consumers, all play a vital role in promoting this culture practice (Ray, 2015).

One of the primary challenges faced by U.S. inland shrimp farmers today is the higher cost of reconstituted sea salts (RSS) which are scientifically formulated to contain the major, minor, and trace elements to support delicate marine life including fish, corals and invertebrates. Due to higher costs of production in the U.S., profit margins are much less robust than in other regions worldwide. Some of the commercially available RSS contain dechlorinating agents to ensure instant removal of chlorine from tap water. Therefore, there is no doubt about their efficacy for use in public aquariums, aquaculture, university research, environmental studies, ornamental fish exhibits, and reef aquaria. Currently RSS is used in inland shrimp production systems as well, assuming that ionic levels and ratios similar to that of sea water are best for optimum survival and growth of *L. vannamei* (Atwood *et al.*, 2003; Roy and Davis, 2010). Farmers using semi-intensive ponds for low salinity culture use RSS during the nursery phase to acclimate shrimp from full

strength seawater down to the salinity of their ponds during acclimation prior to stocking. Commercial producers utilizing indoor RAS and bio-floc, use culture water formulated with RSS throughout their entire production cycle. Unfortunately, the high price of RSS represents a considerable financial burden to commercial producers considering the volume of salt necessary for the acclimation process in outdoor pond culture or for use in indoor production systems (Quagrainie, 2015). As a result, indoor shrimp producers are forced to re-use brackish water prepared with RSS for as many growing cycles as possible to minimize cost. However, over time nitrate accumulates in culture water, and after 3-4 shrimp crops, can become high enough to suppress shrimp growth or even cause mortality (Kuhn *et al.*, 2010). If less expensive salts options were available, indoor shrimp producers could justify exchanging more water to help dilute toxic nitrate levels.

Saoud et al. (2003), Davis et al. (2005) and Hou et al. (2012) reported that the ionic composition of culture water may be a more important limiting factor for shrimp growth and survival than the salinity itself, since the deficiencies in certain ions such as sodium (Na^+), potassium (K^+), calcium (Ca^{2+}) and magnesium (Mg^{2+}) have a negative impact on the growth and survival of shrimp (Boyd, 2002; Saoud *et al*., 2003; McNevin *et al*., 2004; Davis *et al*., 2005; Roy *et al*., 2006, 2007b, 2010, Partridge *et al*., 2008). While the importance of absolute concentrations and relative rations of Na^+, K^+, Mg^{2+} and Ca^{2+} to meet physiological demands of Pacific white shrimp have been highlighted, the specific requirements for individual ions and ionic ratios are still not well known and can vary considering the ionic composition of the rearing medium (Boyd, 2018). Several studies have investigated the ability of combinations of chlorides of sodium, potassium, calcium, and magnesium prepared in the same ionic ratios as found in dilute seawater to support survival and growth of Pacific white shrimp (Atwood *et al.*, 2003; Sowers *et al.*, 2005, 2006) and found them to be unsatisfactory unless mixed with RSS (Atwood *et al*., 2003; Sowers *et al.*, 2005, 2006). However, a mixed ion solution formulated to yield similar ionic concentrations of Na^+, K^+, Ca^{2+} and Mg^{2+} as in seawater using sodium chloride, magnesium chloride, magnesium sulfate, potassium chloride, calcium chloride and sodium bicarbonate, successfully replaced RSS, without compromising the growth, survival and food conversion ratio (FCR) of juvenile Pacific white shrimp with mean initial weight of 7.1 ± 0.26 g in a laboratory study (Parmenter *et al.*, 2009). Following the success of this mixed ion solution, the current study was designed to evaluate the efficacy of the same salt formulation with a slight modification (hereafter; low-cost salt mixture/

LCSM) to replace RSS under different growth phases of *L. vannamei* at different salinities under laboratory conditions for potential application to the commercial shrimp industry.

2. Material and methods

2.1 Low cost salt mixture

Low-cost salt mixture (LCSM) constitutes of Na^+, K^+, Ca^{2+}, Mg^{2+}concentrations of 298, 9, 17, and 39 mg/L respectively in 1-g/L solution were prepared based on Parmenter *et al.* (2009), which is closely comparable with the major cations in 1-g/L dilute seawater (Na^+ = 300 mg/L, K^+ = 10.7 mg/L, Ca^{2+} = 11.6 mg/L, Mg^{2+} = 39.1 mg/L). Agriculture grade sodium chloride (Champion's Choice, Cargill, Inc. Minneapolis, MN), magnesium chloride (Nedmag B.V., Veendam, Netherlands), magnesium sulfate (Giles Chemical, Waynesville, NC), Muriate of potash (potassium chloride) (Mosaic Global Sales, LLC, Lithia, FL), calcium chloride (Industrial Chemicals, Inc. Birmingham, AL), and sodium bicarbonate (Church and Dwight co., Inc. Ewing, NJ) were used as source compounds. At least two days prior to each experimental trial, salt compounds were carefully balanced to yield aforementioned ionic ratios under required salinity, weighed and mixed thoroughly with fresh water sourced from Water Works Board of the City of Auburn (salinity= 0.1 g/L, pH= 7.3, alkalinity= 29 ppm, hardness=38.7 ppm, Ca^{2+}=10 ppm, Mg^{2+}=2.8 ppm, Na^+=5.8 ppm, K^+=2.9 ppm).

2.2 Growth trials

Growth trials were conducted at E.W. Shell Fisheries Center at Auburn University, Auburn, Alabama, in 150 L polyethylene tanks, each equipped with a miniature fluidized bed bio-filter. Post larval Pacific white shrimp (~0.003 g) for the experiments were obtained from SIS-Islamorada, Florida and American Mari-culture, Fort Meyers, Florida and reared (usually for 3-4 weeks) using commercial feed (Zeigler Bros. Inc. Gardners, PA, USA; protein ≥ 50%, fat ≥ 15%, fiber ≤ 1%) to an appropriate size (>0.1g). Since the treatments were designed at low salinities, PL were acclimated to the target salinities of 15, 6 or 2 g/L by dripping freshwater into the nursery tank over time (by reducing approximately 2 g/L salinity per hour). Growth trials were conducted in compliance with the Auburn University animal care policy, at 3, 6 and 15 g/L salinities, while the trial conducted in 15 g/L salinity was repeated due to an elevated cationic concentration (K, Mg and Ca) in LCSM than in control (reconstituted sea water), because of an error in calculation.

Therefore, cationic concentrations were corrected individually and in combinations and repeated to verify the responses of shrimp. At 6 and 15 g/L salinities, RSS was incrementally replaced with LCSM (2.5, 25, 50, 75 and 100%) at four replicates (n) per treatment while only 100% RSS and 100% LCSM were compared at 3 g/L trial.

All four growth trials were conducted for 42 days at a stocking density of 20 shrimp per 150 L tank, with average initial weights of 0.11±0.01, 0.26±0.01, 0.17±0.01, 0.37±0.01g, respectively in 3, 6 and the two trials at 15 g/L salinity. Daily feed ration, including the initial daily ration, was calculated based on expected growth assuming a feed conversion ratio of 1.8 and a doubling in size (approximately every 7 days) until the estimated shrimp weights were in excess of 1 g. Thereafter, a growth rate of 1 g/week was assumed. Shrimp were fed commercial shrimp feed (Zeigler Bros. Inc Gardners, PA, USA; protein ≥ 35%, fat ≥ 7%, fiber ≤ 3%) four times daily (8AM, 11AM, 2PM and 5PM). Following six weeks of culture, shrimp were counted and group-weighed by replicate tank. The average final weight, final biomass, survival (%), and feed conversion ratio (FCR) were determined.

2.3 Hemolymph analysis

Samples of hemolymph were obtained from shrimp collected at the end of the trial. Hemolymph was withdrawn from shrimp via the pericardial cavity using a 25-gauge needle and 1-cc syringe inserted beneath the carapace at the cephalothorax-abdominal junction (Roy *et al.*, 2009). Hemolymph samples were withdrawn from all shrimp in the experiment (one composite sample obtained per tank) and stored at -20 ºC. In order to determine hemolymph osmolality, samples were thawed on ice and sonicated (25 W, 30 S, Branson Sonifier Model 150, Branson Ultrasonic Corporation, Dansbury, CT) to disrupt the clot according to Henry et al. (2003). Following sonication, hemolymph samples were centrifuged at 10,000 x g for 60 s to separate the clot from the serum. Total osmolality was measured using 10 µL of sample by dewpoint depression using an osmometer (Wescor Vapro 5520 Vapor Pressure Osmometer, Logan, Utah). Additional hemolymph sample (40-µL) from each treatment was collected and diluted in 40 ml distilled water to bring ionic concentrations to within working limits and subjected to inductively coupled argon plasma (ICAP) spectrophotometry at the Soil Testing Laboratory, Auburn University (Clesceri *et al.*, 1998) to determine hemolymph ionic concentrations.

2.4 Water analysis

Dissolved oxygen was maintained near saturation using air stones in each culture tank and the sump tank using a common airline connected to a regenerative blower. Dissolved oxygen, salinity and water temperature was measured twice daily using a YSI-55 digital oxygen/temperature meter (YSI corporation, Yellow Springs, Ohio, USA), and total ammonia N (TAN) and nitrite-N were measured twice per week according to the methods described by Solorzano (1969) and Spotte (1979), respectively. The pH of the water was measured two times per week during the experimental period using a pHTestr30 (Oakton Instrument, Vernon Hills, IL, USA). The ionic profile of culture water (onset of each experimental trial) was determined using inductively coupled argon plasma (ICAP) spectrophotometry by the Soil Testing Laboratory at Auburn University according to established techniques (Clesceri *et al.*, 1998).

2.5 Statistical analysis

Statistical analyses were conducted using SAS (version 9.4; SAS Institute, Cary, North Carolina). Since different initial sizes of shrimp were used in the different trials, statistical analyses were conducted by individual trial (no statistical comparisons across trials were made). Growth performance of shrimp, hemolymph osmolality, and hemolymph ions were statistically analyzed via one-way analysis of variance followed by Tukey's pairwise comparison test to determine significant differences ($P < 0.05$) among treatment means according to Steel and Torrie (1980).

3. Results

At the conclusion of the trial conducted at 3 g/L salinity, no significant differences ($P > 0.05$) were found in shrimp between 100RSS and 100LCSM treatments for survival (93 and 93%, respectively), weight gain (7.0 and 6.6 g, respectively), FCR (1.1 and 1.1 respectively), hemolymph osmolality (610 and 592 mmol/kg, respectively) and osmoregulatory capacity (537 and 522 mmol/kg, respectively) (Table 1). There were also no differences in growth or hemolymph osmolality reflected in Na^+, K^+, Ca^{2+}, and Mg^{2+} levels of hemolymph (Table 1). Water quality parameters recorded during the trial are presented in Table 2. Water quality parameters recorded during trials (presented in Tables 2, 5, 7, and 10) remained within acceptable limits for the culture of this species throughout the study (Garcia III and Brune, 1991, Wyban *et al.*, 1995, Van Wyk and Scarpa, 1999); for the case of the TAN and nitrites-N, these were below of the safe

concentrations in low (Gross *et al.*, 2004, Ramírez-Rochín *et al.*, 2017, Valencia-Castañeda *et al.*, 2019) and brackish (Lin and Chen 2001, 2003; Huang *et al*. 2006) salinities.

No significant differences in weight gain (P-value: 0.59), FCR (P-value: 0.27), survival (P-value: 0.89), hemolymph osmolality (P-value: 0.55) and osmoregulatory capacity (P-value: 0.10) of shrimp were detected at 6 g/L salinity, which ranged from 5.9-6.2 g, 1.2-1.3, 95-98 %, 545-565 mmol/kg and 300-335 mmol/kg, respectively (Table 3). Growth data of shrimp were supported by the Na^+, K^+, Ca^{2+}, and Mg^{2+} levels of hemolymph, which were not different ($P>0.05$) among treatments (Table 4). Water quality parameters recorded during the trial are presented in Table 5.

In the trial at 15 g/L salinity, significantly higher growth and significantly lower FCR was recorded in 100LCSM than that of 100RSS and 97.5RSS-2.5LCSM, while no significant differences were detected in survival between the treatments (Table 6). Unfortunately, analysis for hemolymph osmolality and ionic levels were not carried out at the conclusion of this trial. However, elevated levels of K^+, Ca^{2+} and Mg^{2+} were detected in 100LCSM treatment (1.7, 2.2 and 1.3 times higher than that of RSS control) during the trial (Table 7). As a result, this trial was repeated by adjusting cationic concentrations, individually and in combination to detect possible effects of particular cations on the growth performance and survival of shrimp. Water quality parameters recorded during the trial are presented in Table 7.

At the conclusion of the repeated trial at 15 g/L salinity, no significant differences were detected for survival (91-100 %), weight gain (5.9-6.8 g), FCR (1.9-2.1), hemolymph osmolality (555-602 mmol/kg), osmoregulatory capacity (25-111 mmol/kg) and levels of cations in shrimp hemolymph reared in different ionic solutions (Table 8 and 9). Water quality parameters recorded during the trial are presented in Table 10. Alkalinity values of culture water were remained within the range of 80-100 mg/L (as $CaCO_3$) during all four trials.

4. Discussion

The Pacific white shrimp has a documented ability to tolerate a wide range of salinities from 0.5 to 45 g/L and higher (Castille Jr and Lawrence, 1981; Lester and Pante, 1992; Roy *et al.*, 2010). Therefore, there is a considerable interest in culturing Pacific white leg shrimp far from coastal areas in inland ponds filled with low-salinity ground water or in indoor tank systems, which

is deemed by many to be more viable and less damaging to the environment than coastal culture (Flaherty *et al.*, 2000; Atwood *et al.*, 2003; Green, 2008; Roy and Davis, 2010; Roy *et al.*, 2010).

The euryhaline nature of Pacific white shrimp is truly remarkable, but that does not mean that this species can achieve maximum growth and survival throughout the entire salinity spectrum. Numerous researchers have investigated the optimum salinity for Pacific white shrimp post larvae and juveniles (Mair, 1980; Boyd, 1989; Ogle *et al.*, 1992; Bray *et al.*, 1994; Samocha *et al.*, 1998; Tsuzum *et al.*, 2000; Laramore *et al.*, 2001; Atwood *et al.*, 2003; Sowers *et al.*, 2005). Ogle et al. (1992) found no differences in 22-day old post larvae in terms of growth and survival between 2 and 16 g/L (4-wk exposures), which was confirmed by Atwood *et al.* (2003) by exposing 0.22 g post larvae (initial weight of 0.218 g) to 1, 2, 5, 20 g/L salinity solutions in a 3-week nursery trial using artificial sea salt (Atwood *et al.*, 2003). However, Laramore *et al.* (2001) observed a treatment effect of salinity after culturing 0.05 and 0.3 g post larvae for 40 days in to 0, 2, 4 and 30 g/L dilute seawater. In both post larval stages, 0 and 2 g/L salinity yielded significantly lower survival (<29%) compared to 4 and 30 g/L (>86%). Though the argument is still there on suitability of 2 or less than 2 g/L salinity for post larvae (smaller than PL_{15}), it is clear that salinities higher than 2 g/L have no detrimental effect on survival and growth of Pacific white shrimp post larvae, once they reached to PL15 stage (McGraw *et al.*, 2002).

The data pertaining to the effect of salinity on larger shrimp is fairly inconsistent and could be due to the between-study variance of experimental design, water quality parameters (temperature, TAN, nitrite, etc.), initial size of animal, experimental duration, handling and acclimation procedures, ionic composition of the culture medium, or other factors. The salinity range of 15-25 g/L was considered ideal to culture Pacific white leg shrimp by Boyd (1989), while Bray et al. (1994) observed superior growth of shrimp at 5 and 15 g/L salinity compared to 25, 35, and 49 g/L salinities (35-day growth trial; 1.6 g initial size). During the present study salinities of 3, 6 and 15 g/L were used to evaluate growth and survival of shrimp in different mix ion solutions, with the lower end being practical for inland low salinity ponds (3 and 6 g/L) in west Alabama (Roy *et al.*, 2010), while 15 g/L salinity is common in indoor bio-floc and RAS systems used to grow shrimp in the U.S. (Ray, 2015).

The high price of RSS represents a considerable financial burden on inland shrimp producers and an alternative solution to replace RSS is needed (Quagrainie, 2015; Maier and

Quagrainie, 2020; Tierney *et al.*, 2020). Several studies have investigated the ability of combinations of chlorides of Na^+, K^+, Ca^{2+}, and Mg^{2+} prepared in the same ionic ratios as found in dilute seawater to support survival and growth of Pacific white shrimp (Atwood *et al.*, 2003; Sowers *et al.*, 2005, 2006) and found them to be unsatisfactory unless mixed with RSS (Atwood *et al.*, 2003; Sowers *et al.*, 2005; Sowers *et al.*, 2006). However, by adding magnesium sulfate and sodium bicarbonate to the previous salt formulation developed by Sowers et al., (2005), Parmenter *et al.* (2009) was able to successfully replace RSS, which was demonstrated in 42-day growth trial conducted in 15 g/L salinity (mean initial weight of 7.1 ± 0.26 g). The current study extends these findings by evaluating the same salt formula (with a slight modification using potassium oxide instead of potassium chloride) at 3, 6 and 15 g/L salinities which is common in the commercial inland shrimp production sector. Contrary to Parmenter *et al.* (2009) we initiated our trials with a much smaller shrimp (0.11-0.37 g compared to 7.1 g) which is a size more similar, albeit still larger, to the stocking size of commercial producers in Alabama.

Verifying the results of Parmenter *et al.* (2009), no significant differences were observed in survival, growth performance, or FCR of shrimp between RSS and LCSM treatments in all salinities examined during the current study. However, significantly higher growth and significantly lower FCR was observed in 100LCSM compared to100RSS and 97.5RSS-2.5LCSM in the first trial at 15 g/L salinity was assumed to be due to an anomaly or due to elevated level of cations in water than that of RSS that might have conferred an additional performance advantage. However, the results of the repeated trial at 15 g/L salinity with adjustments of particular ionic concentrations revealed that the higher levels of K^+, Ca^{2+}, Mg^{2+} than that of RSS did not result in an adverse or beneficial effect on growth performance or survival of Pacific white shrimp.

Changes in blood osmolality, which is a measure of all dissolved ions in hemolymph/blood (Kültz, 2015), is considered to be an early indicator of stress (McCormick *et al.*, 1987). The Pacific white shrimp has been found to be one of the best osmotic regulators among its family (Castille Jr and Lawrence, 1981) due to their exceptional ability of making a new steady state equilibrium with a new medium (comprised of different salinity) by rapidly changing its osmotic concentration in the hemolymph (Castille Jr and Lawrence, 1981; Roy *et al.*, 2007). Shrimp hemolymph functions hyperosmotic at low salinities to avoid internal dilution and is hypoosmotic at high salinities to avoid concentration of body fluids (Castille Jr and Lawrence, 1981). However, the energy requirement for maintaining hemolymph concentrations can be a considerable proportion

of total energy expenditure (Hagerman and Uglow, 1982; Lucu, 1990), which could lead to molt-associated mortality problems partly due to the shortage of available energy under the circumstances of low salinity. Therefore, changes in salinity and ionic concentrations in the rearing medium not only induce modifications in the activity of processes directly related to ion transport mechanisms, but also in the processes related to energy consumption of shrimp (Pequeux, 1995; Gong *et al.*, 2000). Based on this phenomenon, once the change in salinity or ionic composition of the medium is greater than that of the osmoregulation range of shrimp, effects on growth and survival of shrimp are expected to occur accordingly (Gao *et al.*, 2016). Osmoregulatory abilities of *L. vannamei* at low salinities has been found to decline naturally when they reach subadult or adult stages and juvenile shrimp are considered to be the best osmoregulators (Vargas-Albores and Ochoa, 1992; Gong *et al.*, 2004). During the present study, a final individual weight of 7.1g was attained in 3 g/L salinity solution with no effect of low salinity on survival or growth of shrimp compared to higher salinities tested. Hemolymph osmolality of shrimp reared in 3, 6 and 15 g/L salinity solutions were found to be within the same range despite the large differences in salinities between trials which confirms the remarkable osmoregulatory capability of juvenile shrimp.

Sodium and chloride are the principal osmotically active solutes (76-94%) in shrimp hemolymph, which are not assumed to be affected by the external salinity in the species of the subgenus *Litopenaeus,* due to their ion regulatory capability (McFarland and Lee, 1963, Castille Jr and Lawrence, 1981, Gong *et al.*, 2004). This is in agreement with the hemolymph sodium (Na^+) content of shrimp reared in different salinities during the present study, which ranged from 380-393, 401-428 and 416-470 mEq/L, respectively in 3, 6 and 15 g/L salinity solutions. McFarland and Lee (1963) documented a marked fall in muscle K^+, but not in serum K^+, at low salinities in *Penaeus setiferus*. This seems true in *L. vannamei* with respect to hemolymph K^+ levels, which ranged from 10.6-12.5, 8.5-10.8 and 8.9-13.8 meq/L respectively in 3, 6 and 15 g/L salinities. Unlike Na^+ and K^+, serum Mg^{2+} declines with external salinity, which is equal between two mediums at about 7 g/L salinity (McFarland and Lee, 1963). Hall and Van Ham (1998) highlighted Mg^{2+} as the best cation in shrimp hemolymph having correlation with stress, where significant changes in the level of Mg^{2+} was used as an indicator of damaged gill surfaces or osmoregulatory dysfunction in shrimp (Dall, 1964). Non-significant changes in the level of Na^+, K^+, Ca^{2+}, and Mg^{2+} within each trial (between treatments) is an indication of equal efficacy of LCSM to rear shrimp similar to RSS, while approximately similar hemolymph cationic levels noted between

trials (different salinities) are indicative of the remarkable ion regulatory capability of juvenile Pacific white shrimp.

At the conclusion of shrimp growth trials conducted at 3, 6 and 15 g/L salinities, the efficacy of LCSM to completely replace RSS saving approximately 50% of the cost of RSS for commercial farmers is confirmed (economics studies by Maier and Quagrainie (2020) and Tierney *et al.*, (2020)). However, additional research is recommended in order to further explore the suitability of different agriculture-based salt sources to check their efficacy in salt formulation and to determine the effects of impurities (ions, substances, remains in the salt sources, etc., in addition to the target cations) on survival and growth performance of shrimp. Furthermore, it is important to determine the minimum concentrations of major ions (Na^+, K^+, Ca^{2+}, and Mg^{2+}) and ionic ratios required for physiological functions in Penaeid shrimp with certainty (Boyd, 2018), which could lead to further adjustments in the salt formulation of LCSM.

5. Conclusion

The results of these trials are quite promising and point to the potential use of LCSM to replace RSS, which could be an excellent alternative to reduce the cost of production in indoor shrimp aquaculture, thereby helping to further stimulate the growth of shrimp industry in the U.S. and other regions of the world. The use of LCSM needs to be tested in the field on commercial shrimp operations using various production systems (semi-intensive outdoor ponds, bio-floc systems, RAS) to validate results obtained in laboratory trials. Field validation could lead to reduced costs associated with annual purchase of RSS products and lead to further profitability and sustainability of the U.S. shrimp industry.

Table 1: Response of juvenile Pacific white shrimp (0.11 ± 0.01 g) reared in reconstituted sea salt (RSS) and low-cost salt mixture (LCSM) solutions (3 g/L salinity) for 6-weeks. Values represent the mean of three replicates ± standard deviation.

Treatment	100RSS	100LCSM	P-value
Final weight (g)	7.1±0.6	6.7±0.2	0.30
Weight gain (g)	7.0±0.6	6.6±0.2	0.31
Weight gain (%)	6589±929	6277±408	0.62
FCR	1.1±0.1	1.1±0.1	0.36
Survival (%)	93±8	93±8	1.00
Hemolymph osmolality (mmol/kg)	610±21	592±42	0.53
Osmolality difference from water (mmol/kg)	537±18	522±31	0.52
Hemolymph ion levels (mEq/L)			
Na	380±18	393±51	0.70
K	11±1	13±2	0.21
Ca	14±1	11±2	0.14
Mg	7±1	9±4	0.34

Feed conversion ratio= feed offered/ (final weight-initial weight)

Weight gain%= ((final weight-initial weight)/initial weight) × 100

Table 2: Dissolved oxygen (DO), temperature, salinity, pH, total ammonia nitrogen (TAN), nitrite nitrogen, ionic composition (mg/L) and osmolality of waters (3 g/L salinity) used to rear Pacific white shrimp. Values represent the mean of three replicates ± standard deviation.

Treatment	100RSS	100LCSM
Dissolved oxygen (mg/L)	5.9±1.0	6.0±0.9
Temperature (^{0}C)	29.3±0.9	29.1±0.9
Salinity (ppt)	3.1±0.2	3.2±0.1
pH	7.3±1.0	7.7±0.4
TAN (mg/L)	0.27±0.10	0.25±0.12
Nitrite-N (mg/L)	0.07±0.02	0.05±0.02
Ionic profile of water (mg/L)		
Na	1120±9	1055±31
K	38±4	45±1
Ca	49±1	51±2
Mg	129±1	100±2
Na:K ratio	29.4±2.8	23.2±0.1
Mg:Ca ratio	2.6±0.1	2.0±0.1
Osmolality (mmol/kg)	73.5±2.3	69.8±11.3

Table 3: Response of juvenile Pacific white shrimp (0.26 ± 0.01 g) reared in different ionic solutions (6 g/L salinity) for 6-weeks. Values represent the mean of four replicates ± standard deviation.

Treatment	Final weight (g)	Weight gain (g)	Weight gain (%)	FCR	Survival (%)	Hemolymph osmolality (mmol/kg)	Osmolality difference from water (mmol/kg)
100RSS	6.5±0.4	6.2±0.4	2414±172	1.23±0.05	95.0±7.1	565±15	335±12
97.5RSS-2.5LCSM	6.2±0.3	5.9±0.3	2352±103	1.27±0.03	96.3±4.8	554±14	324±16
75RSS-25LCSM	6.2±0.3	5.9±0.3	2247±122	1.25±0.03	97.5±2.9	564±±16	325±15
50RSS-50LCSM	6.4±0.2	6.1±0.2	2412±107	1.24±0.02	95.0±4.1	545±18	300±12
25RSS-75LCSM	6.3±0.1	6.1±0.1	2440±49	1.21±0.04	98.3±2.9	551±22	313±19
100LCSM	6.3±0.2	6.0±0.2	2313±121	1.24±0.03	96.3±2.5	561±20	310±23
PSE	0.26	0.26	121.03	0.04	4.40	17.66	16.67
P-value	0.35	0.59	0.28	0.27	0.89	0.55	0.10

RSS= Reconstituted sea salt, LCSM= Low cost salt mixture, PSE= Pooled standard error

Feed conversion ratio= feed offered/ (final weight-initial weight)

Weight gain%= ((final weight-initial weight)/initial weight) × 100

Table 4: Hemolymph ion levels (mEq/L) of Pacific white shrimp reared in different ionic solutions at 6 g/L salinity. Values represent the mean of four replicates ± standard deviation.

Treatment	Na	K	Ca	Mg
100RSS	412±10	10±1	11±1	12±4
97.5RSS-2.5LCSM	426±16	9±2	10±2	13±8
75RSS-25LCSM	428±7	11±2	12±3	15±3
50RSS-50LCSM	418±16	9±2	12±3	11±1
25RSS-75LCSM	402±31	11±5	12±4	13±7
100LCSM	407±14	10±2	11±2	9±2
PSE	35.8	2.5	2.5	5.1
P-value	0.88	0.74	0.86	0.72

RSS= Reconstituted sea salt, LCSM= Low cost salt mixture, PSE= Pooled standard error

Table 5: Dissolved oxygen (DO), temperature, salinity, pH, total ammonia nitrogen (TAN), nitrite nitrogen, ionic composition (mg/L) and osmolality of waters (6 g/L salinity) used to rear Pacific white shrimp. Values represent the mean of four replicates ± standard deviation.

Treatment	100RSS	97.5RSS-2.5LCSM	75RSS-25LCSM	50RSS-50LCSM	25RSS-75LCSM	100LCSM
Dissolved oxygen (mg/L)	6.4±0.6	6.3±0.6	6.4±0.6	6.3±0.6	6.4±0.6	6.4±0.6
Temperature (^{0}C)	28.5±1.0	28.5±2.6	28.4±2.6	28.5±2.6	28.2±2.7	28.2±2.6
Salinity (ppt)	7.3±0.4	7.5±2.2	7.8±2.2	8.0±2.2	8.1±2.2	8.5±2.1
pH	7.6±0.4	7.5±0.4	7.6±0.4	7.6±0.4	7.6±0.4	7.6±0.4
TAN (mg/L)	0.5±0.2	0.4±0.2	0.4±0.3	0.4± 0.3	0.4±0.3	0.4±0.3
Nitrite-N (mg/L)	0.07 ± 0.02	0.07 ± 0.01	0.07 ± 0.02	0.07 ± 0.02	0.07 ± 0.02	0.07 ± 0.01
Ionic profile of water (mg/L)						
Na	1974±126	1880±29	1969±26	1983±57	2034±35	2484±769
K	71±5	67±2	87±8	106±15	115±5	129±5
Ca	57±5	55±3	73±13	98±16	119±22	125±2
Mg	414±31	394±8	438±16	464±34	490±10	591±123
Na:K ratio	27.8±2.2	28.1±0.5	22.8±1.7	18.9±2.0	17.7±0.7	19.5±7.0
Mg:Ca ratio	7.3±0.3	7.1±0.2	6.1±0.7	4.8±0.4	4.2±0.7	4.7±1.0
Osmolality (mmol/kg)	231±8	230±6	239±4	245±9	238±8	251±7

Table 6: Response of juvenile Pacific white shrimp (0.17 ± 0.01 g) reared in different ionic solutions (15 g/L salinity) for 6-weeks. Values represent the mean of four replicates ± standard deviation.

Treatment	Final weight (g)	Weight gain (g)	Weight gain (%)	FCR	Survival (%)
100RSS	3.1±0.7^{bc}	2.9±0.7^{bc}	1775±433^{bc}	2.3±0.6^{a}	100±0
97.5RSS-2.5LCSM	2.9±0.2^{c}	2.8±0.2^{c}	1594±120^{c}	2.4±0.3^{a}	98±3
75RSS-25LCSM	3.7±0.4^{abc}	3.5±0.4^{abc}	2124±237^{abc}	1.9±0.2^{ab}	98±3
50RSS-50LCSM	3.4±0.4^{abc}	3.3±0.4^{abc}	1991±257^{abc}	2.0±0.2^{ab}	99±3
25RSS-75LCSM	4.1±0.6^{ab}	3.9±0.6^{ab}	2276±381^{ab}	1.8±0.3^{ab}	94±8
100LCSM	4.4±0.4^{a}	4.3±0.4^{a}	2518±228^{a}	1.5±0.1^{b}	100±0
PSE	0.49	0.49	294.7	0.30	3.63
P-value	0.003	0.003	0.004	0.007	0.20

Values with different superscripts within the same column are significantly different based on Tukey pairwise comparisons.

RSS= Reconstituted sea salt, LCSM= Low cost salt mixture, PSE= Pooled standard error

Feed conversion ratio= feed offered/ (final weight-initial weight)

Weight gain%= ((final weight-initial weight)/initial weight) × 100

Table 7: Dissolved oxygen (DO), temperature, salinity, pH, total ammonia nitrogen (TAN), nitrite nitrogen and ionic composition (mg/L) of waters (15 g/L salinity) used to rear Pacific white shrimp. Values represent the mean of four replicates ± standard deviation.

Treatment	100RSS	97.5RSS-2.5LCSM	75RSS-25LCSM	50RSS-50LCSM	25RSS-75LCSM	100LCSM
Dissolved oxygen (mg/L)	6.6±3.4	6.6±3.4	6.6±3.4	6.6±3.4	6.6±3.4	6.5±3.4
Temperature (^{0}C)	26.4±2.1	26.3±2.1	26.3±2.1	26.4±2.0	26.4±1.9	26.4±1.8
Salinity (ppt)	15.8±0.9	15.5±1.0	15.9±0.9	16.8±1.0	16.9±0.8	17.4±0.8
pH	7.1±0.8	7.1±0.8	7.0±0.6	7.0±0.7	7.0±0.7	7.0±0.7
TAN (mg/L)	0.3±0.4	0.2±0.3	0.3±0.3	0.3±0.4	0.2±0.3	0.3±0.3
Nitrite-N (mg/L)	0.09±0.21	0.08±0.18	0.07±0.22	0.07±0.17	0.06±0.19	0.07±0.21
Ionic profile of water (mg/L)						
Na	4934±39	4832±133	4878±279	5048±56	5051±89	5236±113
K	178±6	179±12	201±11	238±5	269±7	304±8
Ca	172±6	162±6	214±10	300±6	370±14	401±13
Mg	869±9	851±25	913±43	1017±11	1099±22	1193±22
Na:K ratio	27.7±0.9	27.9±0.3	24.3±0.3	21.2±0.4	18.8±0.2	17.2±0.1
Mg:Ca ratio	5.0±0.2	5.2±0.0	4.3±0.1	3.4±0.1	3.0±0.1	3.0±0.1

Table 8: Response of juvenile Pacific white shrimp (0.37 ± 0.01 g) reared in different ionic solutions (repeated trial at 15 g/L salinity) for 6-weeks. Values represent the mean of four replicates ± standard deviation.

Treatment*	Final weight (g)	Weight gain (g)	Weight gain (%)	FCR	Survival (%)	Hemolymph osmolality (mmol/kg)	Osmolality difference from water (mmol/kg)
1 (100RSS[1])	7.2±0.6	6.8±0.6	1817±160	1.9±0.2	91±6	602±49	111±48
2 (100LCSM[2])	6.7±1.1	6.3±1.1	1684±305	1.9±0.5	98±3	555±27	57±26
3 (Ca corrected[3])	6.3±0.6	5.9±0.6	1599±161	2.1±0.2	93±6	573±29	25±4
4 (K corrected[4])	6.6±0.4	6.3±0.4	1678±112	1.9±0.1	100±0	562±39	30±48
5 (Ca & K corrected[5])	6.4±1.0	6.0±1.0	1592±273	2.0±0.2	98±5	575±10	74±53
6 (Ca, K & Mg corrected[6])	6.6±1.4	6.2±1.3	1667±354	2.0±0.3	98±5	576±32	53±57
PSE	0.91	0.91	244	0.28	4.82	33	48
P-value	0.79	0.79	0.81	0.77	0.13	0.49	0.09

*Treatment 1= Reconstituted sea salt (RSS), 2= Low cost salt mixture (LCSM) with elevated K, Ca and Mg ion concentrations than the concentrations in RSS (see Table 6- Treat. 1 and 6), 3= Ca level lowered to reflect the concentration in RSS, 4= K level lowered to reflect the concentration in RSS, 5= Ca and K concentrations lowered to reflect the concentrations in RSS, 6= Ca, K and Mg concentrations were lowered to reflect the concentrations in RSS

Feed conversion ratio= feed offered/ (final weight-initial weight)

Weight gain%= ((final weight-initial weight)/initial weight) × 100

Table 9: Hemolymph ion levels (mEq/L) of Pacific white shrimp reared in different ionic solutions (repeated trial at 15 g/L salinity). Values represent the mean of four replicates ± standard deviation.

Treatment*	Na	K	Ca	Mg
1 (100RSS[1])	453±37	12±4	39±10	19±8
2 (100LCSM[2])	458±63	14±7	34±14	14±4
3 (Ca corrected[3])	416±15	11±1	23±2	16±10
4 (K corrected[4])	470±89	11±3	36±6	20±4
5 (Ca & K corrected[5])	428±5	9±1	29±11	12±1
6 (Ca, K & Mg corrected[6])	462±58	11±2	34±14	18±7
PSE	53.3	3.8	10.5	6.5
p-value	0.68	0.62	0.46	0.51

*Treatment 1= Reconstituted sea salt (RSS), 2= Low cost salt mixture (LCSM) with elevated K, Ca and Mg ion concentrations than the concentrations in RSS (see Table 6- Treat. 1 and 6), 3= Ca level lowered to reflect the concentration in RSS, 4= K level lowered to reflect the concentration in RSS, 5= Ca and K concentrations lowered to reflect the concentrations in RSS, 6= Ca, K and Mg concentrations were lowered to reflect the concentrations in RSS

Table 10: Dissolved oxygen (DO), temperature, salinity, pH, total ammonia nitrogen (TAN), nitrite nitrogen, ionic composition (mg/L) and osmolality of waters (repeated trial at 15 g/L salinity) used to rear Pacific white shrimp. Values represent the mean of four replicates ± standard deviation.

Treatment*	1	2	3	4	5	6
Dissolved oxygen (mg/L)	5.7±0.6	5.8±0.7	5.6±0.7	5.8±0.6	5.7±0.7	5.8±0.5
Temperature (^{0}C)	28.5±0.9	27.4±1.6	28.4±0.9	28.2±1.0	28.1±1.0	28.4±1.7
Salinity (ppt)	15.6±1.9	17.1±2.3	18.1±2.0	17.7±1.8	17.0±1.6	16.2±1.8
pH	7.1 ± 0.4	7.1 ± 0.4	7.1 ± 0.4	7.1 ± 0.4	7.1 ± 0.3	7.1 ± 0.4
TAN (mg/L)	0.31±0.01	0.27±0.01	0.29±0.02	0.31±0.03	0.32±0.01	0.27±0.03
Nitrite-N (mg/L)	0.41±0.01	0.57±0.01	0.53±0.06	0.51±0.01	0.59±0.05	0.58±0.03
Ionic profile of water (mg/L)						
Na	4901±232	5163±149	5347±195	5279±67	5130±71	5254±165
K	172±23	337±27	328±14	233±5	218±3	224±9
Ca	186±6	376±13	201±7	380±9	199±5	204±5
Mg	612±30	820±36	835±26	827±19	800±4	509±19
Na:K ratio	28.9±4.6	15.3±0.8	16.3±0.6	22.7±0.5	23.5±0.5	23.4±0.6
Mg:Ca ratio	3.3±0.1	2.2±0.1	4.2±0.1	2.2±0.0	4.0±0.1	2.5±0.1
Osmolality (mmol/kg)	459±25	549±47	563±31	534±62	501±55	523±26

*Treatment 1= Reconstituted sea salt (RSS), 2= Low cost salt mixture (LCSM) with elevated K, Ca and Mg ion concentrations than the concentrations in RSS (see Table 6- Treat. 1 and 6), 3= Ca level lowered to reflect the concentration in RSS, 4= K level lowered to reflect the concentration in RSS, 5= Ca and K concentrations lowered to reflect the concentrations in RSS, 6= Ca, K and Mg concentrations were lowered to reflect the concentrations in RSS

References

Atwood, H., Young, S., Tomasso, J. & Browdy, C. (2003) Survival and growth of Pacific white shrimp *Litopenaeus vannamei* postlarvae in low-salinity and mixed-salt environments. *Journal of the World Aquaculture Society,* 34**,** 518-523.

Boyd, C.E. (1989) Water quality management and aeration in shrimp farming. *Fisheries and Allied Aquacultures Departmental series No. 2, Alabama agricultural Experiment Station, Auburn University, Auburn, AL, USA***,** 83.

Boyd, C.E. (2018) Revisiting ionic imbalance in low-salinity shrimp aquaculture. Global Aquaculture Alliance.

Bray, W., Lawrence, A. & Leung-Trujillo, J. (1994) The effect of salinity on growth and survival of *Penaeus vannamei,* with observations on the interaction of IHHN virus and salinity. *Aquaculture,* 122**,** 133-146.

Castille Jr, F.L. & Lawrence, A.L. (1981) The effect of salinity on the osmotic, sodium and chloride concentrations in the hemolymph of euryhaline shrimp of the genus *Penaeus*. *Comparative Biochemistry and Physiology Part A: Physiology,* 68**,** 75-80.

Clesceri, L.S., Greenberg, A.E. & Eaton, A.D. (1998) Standard methods for the examination of water and wastewater, American Public Health Association. *Washington, DC***,** 4-415.

Dall, W. (1964) Studies on the physiology of a shrimp, *Metapenaeus mastersii* (Haswell)(Crustacea: Decapoda: Peneidae). I. Blood constituents. *Marine and Freshwater Research,* 15**,** 145-161.

Davis, D.A., Boyd, C.E., Rouse, D.B. & Saoud, I.P. (2005) Effects of potassium, magnesium and age on growth and survival of *Litopenaeus vannamei* post-larvae reared in inland low salinity well waters in west Alabama. *Journal of the World Aquaculture Society,* 36**,** 416-419.

Flaherty, M. & Karnjanakesorn, C. (1995) Marine shrimp aquaculture and natural resource degradation in Thailand. *Environmental Management,* 19**,** 27-37.

Flaherty, M., Szuster, B. & Miller, P. (2000) Low salinity inland shrimp farming in Thailand. *Ambio: A Journal of the Human. Environment***,** 174-179.

Gao, W., Tian, L., Huang, T., Yao, M., Hu, W. & Xu, Q. (2016) Effect of salinity on the growth performance, osmolarity and metabolism-related gene expression in white shrimp *Litopenaeus vannamei*. *Aquaculture Reports,* 4**,** 125-129.

Garcia III, A. & Brune, D.E. (1991) Transport limitation of oxygen in shrimp culture ponds. *Aquacultural Engineering,* 10**,** 269-279.

Gong, H., Jiang, D.H., Lightner, D.V., Collins, C. & Brock, D. (2004) A dietary modification approach to improve the osmoregulatory capacity of *Litopenaeus vannamei* cultured in the Arizona desert. *Aquaculture Nutrition,* 10**,** 227-236.

Gong, H., Lawrence, A.L., Jiang, D.-H., Castille, F.L. & Gatlin III, D.M. (2000) Lipid nutrition of juvenile *Litopenaeus vannamei*: I. Dietary cholesterol and de-oiled soy lecithin requirements and their interaction. *Aquaculture,* 190**,** 305-324.

Green, B.W. (2008) Stocking strategies for production of *Litopenaeus vannamei* (Boone) in amended freshwater in inland ponds. *Aquaculture Research,* 39**,** 10-17.

Gross, A., Abutbul, S. & Zilberg, D. (2004) Acute and chronic effects of nitrite on white shrimp, *Litopenaeus vannamei*, cultured in low-salinity brackish water. *Journal of the World Aquaculture Society,* 35**,** 315-321.

Hagerman, L. & Uglow, R.F. (1982) Effects of hypoxia on osmotic and ionic regulation in the brown shrimp *Crangon crangon* (L.) from brackish water. *Journal of Experimental Marine Biology and Ecology,* 63**,** 93-104.

Hall, M.R. & Van Ham, E.H. (1998) The effects of different types of stress on blood glucose in the giant tiger prawn *Penaeus monodon*. *Journal of the World Aquaculture Society,* 29**,** 290-299.

Henry, R.P., Gehnrich, S., Weihrauch, D. & Towle, D.W. (2003) Salinity-mediated carbonic anhydrase induction in the gills of the euryhaline green crab, *Carcinus maenas*. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology,* 136**,** 243-258.

Hou, C., Wang, F., Dong, S., Zhu, Y. & Yu, T. (2012) Effects of constant Ca^{2+} concentration in salinity fluctuations on growth and energy budget of juvenile *Litopenaeus vannamei*. *Aquaculture International,* 20**,** 177-188.

Huang, X., Li, C., Zheng, L., Liu, C. & Zhou, J. (2006) The toxicity of NO2-N on *Litopenaeus vannamei* and effects of NO2-N on factors relating to the anti-disease ability. *Acta Hydrobiologica Sinica,* 30**,** 471.

Kuhn, D.D., Smith, S.A., Boardman, G.D., Angier, M.W., Marsh, L. & Flick Jr, G.J. (2010) Chronic toxicity of nitrate to Pacific white shrimp, *Litopenaeus vannamei*: impacts on survival, growth, antennae length, and pathology. *Aquaculture,* 309**,** 109-114.

Kültz, D. (2015) Physiological mechanisms used by fish to cope with salinity stress. *Journal of Experimental Biology,* 218**,** 1907-1914.

Laramore, S., Laramore, C.R. & Scarpa, J. (2001) Effect of low salinity on growth and survival of postlarvae and juvenile *Litopenaeus vannamei*. *Journal of the World Aquaculture Society,* 32**,** 385-392.

Lester, L.J. & Pante, M.J.R. (1992) Penaeid temperature and salinity responses. *Developments in Aquaculture and Fisheries Science,* 23**,** 515-534.

Lin, Y.C. & Chen, J.C. (2001) Acute toxicity of ammonia on *Litopenaeus vannamei* Boone juveniles at different salinity levels. *Journal of Experimental Marine Biology and Ecology,* 259**,** 109-119.

Lin, Y.C. & Chen, J.C. (2003) Acute toxicity of nitrite on *Litopenaeus vannamei* (Boone) juveniles at different salinity levels. *Aquaculture,* 224**,** 193-201.

Lucu, Č. (1990) Ionic regulatory mechanisms in crustacean gill epithelia. *Comparative Biochemistry and Physiology Part A: Physiology,* 97**,** 297-306.

Maier, P. & Quagrainie, K. (2020) A bioeconomic model of indoor marine shrimp *Litopenaeus Vannamei* farms with low-cost salt mixtures In *Aquaculture America 2020 Conference proceedings*, pp. 413Honolulu, Hawaii, USA.

Mair, J.M. (1980) Salinity and water-type preferences of four species of postlarval shrimp (Penaeus) from west Mexico. *Journal of Experimental Marine Biology and Ecology,* 45**,** 69-82.

McCormick, J., Stokes, G., Jensen, K. & Leino, R. (1987) Fish blood osmolality, gill histology and oocyte atresia as early warning acid stress indicators. *Annales de la Societe Royale Zoologique de Belgique (Belgium). Annalen van de Koninklijke Belgische Vereniging voor Dierkunde. v. 117 (suppl. 1).*

McFarland, W. & Lee, B.D. (1963) Osmotic and ionic concentrations of *penaeidean* shrimps of the Texas coast. *Bulletin of Marine Science,* 13**,** 391-417.

McGraw, W., Davis, D., Teichert-Coddington, D. & Rouse, D. (2002) Acclimation of *Litopenaeus vannamei* postlarvae to low salinity: influence of age, salinity endpoint, and rate of salinity reduction. *Journal of the World Aquaculture Society,* 33**,** 78-84.

Ogle, J.T., Beaugez, K. & Lotz, J.M. (1992) Effects of salinity on survival and growth of postlarval *Penaeus vannamei*. *Gulf and Caribbean Research,* 8**,** 415-421.

Páez-Osuna, F. (2001) The environmental impact of shrimp aquaculture: causes, effects, and mitigating alternatives. *Environmental Management,* 28**,** 131-140.

Parmenter, K., Bisesi Jr, J., Young, S., Klaine, S., Atwood, H., Browdy, C. & Tomasso, J. (2009) Culture of Pacific white shrimp *Litopenaeus vannamei* in a mixed-ion solution. *North American Journal of Aquaculture,* 71**,** 134-137.

Pequeux, A. (1995) Osmotic regulation in crustaceans. *Journal of Crustacean Biology,* 15**,** 1-60.

Quagrainie, K. (2015) *Profitability of Indoor Production of Pacific White Shrimp: Litopenaeus Vannamei, a Case Study of the Indiana Industry,* Purdue University Extension Fact Sheet.

Ramírez-Rochín, J., Frías-Espericueta, M.G., Fierro-Sañudo, J.F., Alarcón-Silvas, S.G., Fregoso-López, M.G. & Páez-Osuna, F. (2017) Acute toxicity of nitrite on white shrimp *Litopenaeus vannamei* (Boone) juveniles in low-salinity water. *Aquaculture Research,* 48**,** 2337-2343.

Ray, A.J. (2015) Indoor-raised shrimp find potential market in Kentucky State University test In *The Global Aquaculture Alliance*.

Roy, L.A. & Davis, D.A. (2010) Requirements for the culture of the Pacific white shrimp, *Litopenaeus vannamei*, reared in low salinity waters: water modification and nutritional strategies for improving production In *Avances en Nutrición Acuicola X-Memorias del X Simposio Internacional de Nutrición Acuicola*, pp. 8-10.

Roy, L.A., Davis, D.A., Nguyen, T.N. & Saoud, I.P. (2009) Supplementation of chelated magnesium to diets of the pacific white shrimp, *Litopenaeus vannamei,* reared in low-salinity waters of west Alabama. *Journal of the World Aquaculture Society,* 40**,** 248-254.

Roy, L.A., Davis, D.A., Saoud, I.P., Boyd, C.A., Pine, H.J. & Boyd, C.E. (2010) Shrimp culture in inland low salinity waters. *Reviews in Aquaculture,* 2**,** 191-208.

Roy, L.A., Davis, D.A., Saoud, I.P. & Henry, R.P. (2007) Branchial carbonic anhydrase activity and ninhydrin positive substances in the Pacific white shrimp, *Litopenaeus vannamei*, acclimated to low and high salinities. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology,* 147**,** 404-411.

Samocha, T.M., Lawrence, A.L. & Pooser, D. (1998) Growth and survival of juvenile *Penaeus vannamei* in low salinity water in a semi-closed recirculating system. *Israeli Journal of Aquaculture/Bamidgeh,* 50**,** 55-59.

Saoud, I.P., Davis, D.A. & Rouse, D.B. (2003) Suitability studies of inland well waters for *Litopenaeus vannamei* culture. *Aquaculture,* 217**,** 373-383.

Solorzano, L. (1969) Determination of ammonia in natural waters by the phenolhypochlorite method. *Limnology and Oceanography,* 14**,** 799-801.

Sowers, A.D., Gatlin, D.M., Young, S.P., Isely, J.J., Browdy, C.L. & Tomasso, J.R. (2005) Responses of *Litopenaeus vannamei* (Boone) in water containing low concentrations of total dissolved solids. *Aquaculture Research,* 36**,** 819-823.

Sowers, A.D., Tomasso Jr, J.R., Browdy, C.L. & Atwood, H.L. (2006) Production characteristics of *Litopenaeus vannamei* in low-salinity water augmented with mixed salts. *Journal of the World Aquaculture Society,* 37**,** 214-217.

Spotte, S. (1979) *Fish and Invertebrate Culture: Water Managment in Closed Systems, 2nd Edition.,* Wiley, New York.

Steel, R.G. & Torrie, J.H. (1980) *Principles and Procedures of Statistics,* McGraw-Hill Book Co. Inc., New York.

Stevenson, N. (1996) Disused shrimp ponds: Options for redevelopment of mangroves. *Coastal Management,* 25**,** 423-435.

Tierney, T., Fleckenstein, L.J. & Ray, A.J. (2020) Using alternative low cost artificial sea salt mixtures for intensive shrimp (*Litopenaeus vannamei*) production. In *Aquaculture America 2020 Conference proceedings*, pp. 662Honolulu, Hawaii, USA.

Tsuzum, M.Y., Cavalli, R.O. & Bianchini, A. (2000) The effects of temperature, age, and acclimation to salinity on the survival of *Farfantepenaeus paulensis* postlarvae. *Journal of the World Aquaculture Society,* 31**,** 459-468.

Valencia-Castañeda, G., Frías-Espericueta, M.G., Vanegas-Pérez, R.C., Chávez-Sánchez, M.C. & Páez-Osuna, F. (2019) Toxicity of ammonia, nitrite and nitrate to *Litopenaeus vannamei* juveniles in low-salinity water in single and ternary exposure experiments and their environmental implications. *Environmental Toxicology and Pharmacology,* 70.

Valiela, I. (2006) *Global Coastal Change,* John Wiley & Sons, MA, USA.

Van Wyk, P. & Scarpa, J. (1999) Water quality requirements and management. *Farming Marine Shrimp in Recirculating Freshwater Systems***,** 141-162.

Vargas-Albores, F. & Ochoa, J. (1992) Variation of pH, osmolality, sodium and potassium concentrations in the haemolymph of sub-adult blue shrimp (Ps) according to size. *Comparative Biochemistry and Physiology Part A: Physiology,* 102**,** 1-5.

Wyban, J., Walsh, W.A. & Godin, D.M. (1995) Temperature effects on growth, feeding rate and feed conversion of the Pacific white shrimp (*Penaeus vannamei*). *Aquaculture,* 138**,** 267-279.

CHAPTER IV

THE EFFECTS OF MAGNESIUM CONCENTRATION IN LOW SALINITY WATER ON GROWTH OF PACIFIC WHITE SHRIMP (*Litopenaeus vannamei*)

Published as Galkanda-Arachchige, H.S., Roy, L.A. and Davis, D.A., 2021. The effects of magnesium concentration in low-salinity water on growth of Pacific white shrimp (Litopenaeus vannamei). Aquaculture Research, 52(2), pp.589-597 (https://doi.org/10.1111/are.14916).

Abstract

The current study evaluates the effect of different Mg levels in low salinity (3 g/L) water on growth, survival, hemolymph osmolality, cationic composition in hemolymph, carapace and whole-body of Pacific white shrimp, *Litopenaeus vannamei*. A low-cost salt mixture (LCSM) comprised of Na, K, Ca, Mg concentrations of 298, 9, 17, and 39 mg/L, respectively in a 1-g/L solution was modified by decreasing the Mg level of LCSM to allow different Mg levels in the culture medium (100, 78, 55, 30, 17, 13 and 12 mg/L). Reconstituted sea salt (RSS) was used as the control treatment with Mg level of 129mg/L. A 42-day growth trial (initial weight: 0.11 ± 0.01g and stocking density:20 shrimp/tank) was carried out in 150 L plastic tanks, each equipped with a fluidized bed biofilter. As Mg^{2+} level was reduced, there was a subsequent reduction ($P<0.05$) in shrimp performance reflected in final weight, weight gain, hemolymph osmolality, osmoregulatory capacity, Mg^{2+} concentration in hemolymph, carapace and whole body of shrimp paralleled by an increase in FCR. Low hemolymph osmolality and Mg^{2+} concentrations ($P<0.05$) in shrimp hemolymph were found to be indicative of stress, which was ultimately reflected as reduced growth and increased FCR in low Mg^{2+} concentrations at 3 g/L salinity.

Key words: growth, low salinity, magnesium level in water, osmoregulation, Pacific white shrimp, survival

1. Introduction

The Pacific white shrimp, *Litopenaeus vannamei* are capable of hypo- and hyper-osmoregulation when the ambient salinity is above and below the iso-osmotic point of 718 mOsm/kg (equivalent of 25 g/L salinity), which provides them with a remarkable ability to tolerate a wide range of salinities from 0.5 to 45 g/L and higher (Castille Jr and Lawrence, 1981; Roy *et al.*, 2010). Therefore, there is considerable interest by the commercial aquaculture industry in culturing Pacific white shrimp far from coastal areas either in inland ponds filled with low-salinity well water (1–6 g/L), in indoor recirculating aquaculture systems (RAS) or in indoor bio-floc systems, which usually operate at salinities less than 15 g/L (Atwood *et al.*, 2003; Roy and Davis, 2010; Ray *et al.*, 2017).

One of the major problems with inland low salinity aquaculture is suboptimal ionic profiles in low salinity ground water. Culture water with suboptimal ionic profiles have resulted in negative impacts on feed consumption, growth and survival of shrimp (Saoud *et al.*, 2003; Davis *et al.*, 2005; Hou *et al.*, 2012). Some inland shrimp producers in Ecuador, USA, Australia and China using low salinity groundwater have experienced low survival rates of Pacific white shrimp post larvae (PL) due to low concentrations of potassium (K^+) in the water (Boyd, 2002; Saoud *et al.*, 2003; McNevin *et al.*, 2004; Partridge *et al.*, 2008; Roy *et al.*, 2010). Low concentration of aqueous magnesium (Mg^{2+}) resulted in less-than-ideal growth and survival of Pacific white shrimp reared in low-salinity well water in west Alabama, USA (Davis *et al.*, 2005; Roy *et al.*, 2006; Roy *et al.*, 2007). In addition, high environmental calcium levels (Ca^{2+}) have been shown to be detrimental to fishes and crustaceans (Kline and Stekoll, 2000; Pillard *et al.*, 2000).

Magnesium (Mg^{2+}) is the most abundant intracellular divalent cation with a central role in many cellular processes such as activation of a large number of enzymes, hormonal signaling, protein synthesis, cell division, etc. (Alvarez-Leefmans *et al.*, 1987). In addition, Mg^{2+} comprises a major portion of the exoskeleton of crustaceans (Greenaway, 1993), and greatly affects molting frequency (Wilder *et al.*, 2009). $Mg^{2+}$is a cofactor for the Na^+-K^+-ATPase enzyme involved in osmoregulatory mechanisms (Wilder *et al.*, 1998; Roy *et al.*, 2007) and is also important for carapace mineralization (Brown *et al.*, 1991) and survival (Adhikari *et al.*, 2007; Rafiee *et al.*, 2015). However, inland low salinity aquifers that provide the water for Pacific white shrimp raised in earthen ponds in west Alabama have Mg^{2+} concentrations that vary widely and is extremely

deficient on most farms. While the addition of magnesium salt (typically K-Mag® which is a commercial grade potassium magnesium sulfate) is used by commercial shrimp producers to raise the Mg^{2+} levels in pond water (Saoud *et al.*, 2003; Davis *et al.*, 2005; Roy *et al.*, 2009b) there is limited information on the effect of different Mg^{2+} levels in low salinity water and its effect on physiological processes such as survival or growth performance of Pacific white shrimp. This is a major concern of inland low-salinity shrimp farmers today, particularly due to mortality occurring late in the production season with larger shrimp cultured in water with much lower Mg levels than found in diluted seawater of the same salinity (Roy *et al*. 2020 in press). The current study was carried out to determine the effect of different Mg levels on growth, survival, hemolymph osmolality, cationic composition of hemolymph, carapace and whole-body mineral levels of Pacific white shrimp reared in low salinity water (3 g/L).

2. Material and Methods

2.1 Ionic ratios in water

A low-cost salt mixture (LCSM) comprised of Na^+, K^+, Ca^{2+}, Mg^{2+} concentrations of 298, 9, 17, and 39 mg/L, respectively in a 1-g/L solution, which is closely comparable to the major cations in 1-g/L dilute seawater, was used to formulate waters with different Mg levels in low salinity water. LCSM was used here, as this has been successful in replacing reconstituted sea salt (RSS) in both nursery and growth phases of Pacific white shrimp at 3, 6 and 15 g/L salinities (Parmenter *et al.*, 2009, Galkanda-Arachchige *et al.*, 2020a, Galkanda-Arachchige *et al.*, 2020b). Agriculture grade sodium chloride (Champion's Choice, Cargill, Inc. Minneapolis, MN), magnesium chloride (Nedmag B.V., Veendam, Netherlands), magnesium sulfate (Giles Chemical, Waynesville, NC), muriate of potash (potassium chloride) of 62% potassium oxide equivalence (Mosaic Global Sales, LLC, Lithia, FL), calcium chloride (Industrial Chemicals, Inc. Birmingham, AL), and sodium bicarbonate (Church and Dwight co., Inc. Ewing, NJ) were used as source compounds of LCSM. Mg levels of 100, 78, 55, 30, 17, 12 and 13 mg/L at 3 g/L salinity were achieved by decreasing the level of magnesium chloride inclusion in LCSM while the Na^+, K^+ and Ca^{2+} levels remained constant. Reconstituted sea salt (RSS) was used as the control treatment with an Mg^{2+} level of 129 mg/L at 3 g/L salinity, which is closely comparable to that of sea water. At least two days prior to each experimental trial, salt compounds were carefully balanced to yield aforementioned ionic levels at 3 g/L salinity, weighed and mixed thoroughly with fresh water

sourced from Water Works Board of the City of Auburn (salinity= 0.1 g/L, pH= 7.3, alkalinity= 29 ppm, hardness=38.7 ppm, Ca^{2+}=10 ppm, Mg^{2+}=2.8 ppm, Na^{+}=5.8 ppm, K^{+}=2.9 ppm).

2.2 Growth trial

Post larval (PL) Pacific white shrimp (~0.003 g) for the experiment were obtained from American Mari-culture, Fort Meyers, Florida and nursed in an indoor recirculating system. PLs were fed a commercial feed (Zeigler Bros. Inc. Gardners, PA, USA; protein ≥ 50%, fat ≥ 15%, fiber ≤ 1%) using an automatic feeder for ~1 week and then switched to crumbled commercial shrimp feed (Zeigler Bros. Inc., Gardners, PA, USA; protein ≥ 40%, fat ≥ 9%, fiber ≤ 3%) for ~1 week. At the end of the nursery phase, 20 juvenile shrimp were stocked per tank with a mean initial weight of 0.11±0.01 g.

In compliance with the Auburn University animal care policy, a 42-day growth trial was conducted at E.W. Shell Fisheries Center at Auburn University, Auburn, Alabama, in 150 L polyethylene tanks, each equipped with a fluidized bed bio-filter. Daily feed ration, including the initial daily ration, was calculated based on expected growth assuming a feed conversion ratio of 1.8 and a doubling in size (approximately every 7 days) until the estimated shrimp weight was in excess of 1 g. Thereafter, a growth rate of 1 g/week was assumed. Shrimp were fed a commercial shrimp feed (Zeigler Bros. Inc., Gardners, PA, USA; protein ≥ 35%, fat ≥ 7%, fiber ≤ 3%) four times daily. Six weeks after the start of the experiment, shrimp were counted and group-weighed by replicate tank (n=3). The average final weight, final biomass, survival (%), and feed conversion ratio (FCR) were determined.

2.3 Hemolymph analysis

Samples of hemolymph were obtained from shrimp collected at the end of the trial. Hemolymph was withdrawn from shrimp via the pericardial cavity using a 25-gauge needle and 1-cc syringe inserted beneath the carapace at the cephalothorax-abdominal junction (Roy *et al.*, 2009a). Hemolymph samples were withdrawn from all groups of shrimp in the experiment (one composite sample obtained from four shrimp per tank) and stored at -20 ºC. In order to determine hemolymph osmolality, samples were thawed on ice and sonicated (25 W, 30 S, Branson Sonifier Model 150, Branson Ultrasonic Corporation, Dansbury, CT) to disrupt the clot according to Henry et al. (2003). Following sonication, hemolymph samples were centrifuged (Fisher Scientific: Marathon 16km, USA) at 10,000 rpm for 60 s to separate the clot from the serum. Total osmolality

was measured using 10 µL of sample by dewpoint depression using an osmometer (Wescor Vapro 5520 Vapor Pressure Osmometer, Logan, Utah). An additional hemolymph sample (40-µL) from each treatment was collected and diluted in 40 ml deionized water to bring ionic concentrations to within working limits and subjected to inductively coupled argon plasma (ICAP) spectrophotometry at the Soil Testing Laboratory, Auburn University and reported as mEq/L (Clesceri *et al.*, 1998).

2.4 Shrimp carapace and whole-body analysis

Frozen shrimp were rinsed with deionized water and the carapace was dissected. One composite sample of four carapaces from each tank was oven-dried and then dry-ashed (in a muffle furnace at 550^0C overnight) according to the procedures described by the Association of Official Analytical Chemists (1984). The percentage ash content of the carapace sample was determined as follows:

$$\% \text{ Ash} = \frac{\text{Ash weight (g)}}{\text{Sample weight (g)}} \times 100$$

Consequently, ash samples were dissolved in 100% HCL which was subsequently diluted (1:10) with deionized water to a final volume of 25 ml and subjected to inductively coupled argon plasma (ICAP) spectrophotometry at the Soil Testing Laboratory, Auburn University. A separate sample of four shrimp (whole-body) from each tank was oven-dried (90^0C until constant weight), ground and powdered to determine the whole-body shrimp ionic profile through inductively coupled argon plasma (ICAP) spectrophotometry at the Soil Testing Laboratory, Auburn University.

2.5 Water analysis

Dissolved oxygen was maintained near saturation using air stones in each culture tank via a common airline connected to a regenerative blower. Dissolved oxygen, salinity and water temperature were measured twice daily using a YSI-55 digital oxygen/temperature meter (YSI Corporation, Yellow Springs, Ohio, USA), and total ammonia N (TAN) and nitrite-N were measured twice per week according to the methods described by Solorzano (1969) and Spotte (1979), respectively. The pH of the water was measured two times per week during the experimental period using a pHTestr30 (Oakton Instrument, Vernon Hills, IL, USA). Ionic profile of water (in each experimental trial) was determined using inductively coupled argon plasma (ICAP) spectrophotometry by the Soil Testing Laboratory at Auburn University (Clesceri *et al.*, 1998).

2.6 Statistical analysis

Statistical analyses were conducted using SAS (version 9.4; SAS Institute, Cary, North Carolina). Growth performance of shrimp, hemolymph osmolality, ash content in shrimp carapace, ionic profile of hemolymph, carapace and shrimp-whole body were statistically analyzed via one-way analysis of variance (one-way ANOVA) followed by Tukey pairwise comparison test to determine significant differences ($P < 0.05$) between treatment means according to Steel and Torrie (1980). Additionally, linear regression was performed to identify the relationships between weight gain, osmoregulatory capacity, Mg^{2+} content in hemolymph, carapace and whole-body with Mg^{2+} level in 3 g/L salinity rearing medium.

3. Results

Growth performance, survival, hemolymph osmolality and osmoregulatory capacity of juvenile *L. vannamei* reared in 3 g/L salinity water comprised of different Mg^{2+} levels are presented in Table 1. At the conclusion of the six week culture period, waters with Mg^{2+} levels of 129 and 100 mg/L yielded significantly higher final weight, weight gain and percentage weight gain compared to the growth performance of shrimp reared in Mg^{2+} level of 55 mg/L or below ($P<0.05$). Based on the statistical outcomes of one-way ANOVA, no significant differences were noted for survival, hemolymph osmolality and osmoregulatory capacity (difference in osmolality between the hemolymph and of the culture water (Charmantier *et al.*, 1989)) of shrimp between treatments, which ranged from 83-95%, 525-610 mmol/kg and 456-537 mmol/kg, respectively (Table 1). However, as per the results of linear regression, significantly positive associations ($P<0.05$) were observed for final weight, weight gain, percentage weight gain, hemolymph osmolality, and osmoregulatory capacity of shrimp while a significantly inverse relationship was observed for FCR corresponding to the Mg^{2+} level in water (Table 1; Figure 1).

No significant differences were noted for Na^{+}, K^{+}, and Ca^{2+} concentrations in shrimp hemolymph except for Mg^{2+} (Table 2), which showed a significant decrease (P-value: 0.003; $R^2=0.38$) corresponding to lowering levels of Mg^{2+} in the rearing medium (Figure 2-a). Carapace ash level ranged from 64.6-71.4%, which showed no significant differences between treatments (Table 3). However, in line with the observation of cationic concentrations in shrimp hemolymph, carapace Mg^{2+} levels showed significant dose response (P-value: 0.00; $R^2=0.71$) to the level of

Mg^{2+} level in water (Figure 2-b), while no differences (P>0.05) were noted for Na^{+}, K^{+}, and Ca^{2+} concentrations between treatments.

There were no differences (P>0.05) in Na^{+}, K^{+}, and Ca^{2+} whole-body concentrations in shrimp between treatments (Table 4). However, significantly higher Mg^{2+} levels in shrimp were noted in treatments with Mg^{2+} levels of 129 and 100 mg/L, while the lowest whole-body Mg^{2+} levels occurred in the treatment in which the Mg^{2+} levels were lowered below that of 55 mg/L (Table 4). Based on the results of linear regression, a strong positive association (P-value: 0.00; R^2=0.92) was noted between whole-body Mg^{2+} level and the level of Mg^{2+} in water (Figure 2-c).

During the growth trial, DO, temperature, salinity, pH, TAN, and nitrite levels were maintained within acceptable ranges for *L. vannamei* at 6.0±0.4 mg/L, 29.1±0.5 C, 3.1±0.3 g/L, 7.4±0.4, 0.27±0.18 mg/L, and 0.08±0.22 mg/L, respectively (Table 5). Alkalinity values of culture water remained within the range of 80-100 mg/L (as $CaCO_3$) during the trial.

4. Discussion

There are numerous commercial shrimp farms in the USA and other regions of the world producing shrimp in low-salinity water with less-than-ideal levels of K^{+} and/or Mg^{2+} compared to sea water of the corresponding salinity. Though the importance of absolute concentrations and relative rations of Na^{+}, K^{+}, Mg^{2+} and Ca^{2+} to meet physiological demands of Pacific white shrimp have been highlighted, the specific requirements for individual ions and ionic ratios are still not well known (Boyd, 2018). These circumstances continuously force inland shrimp farmers using low salinity water sources to experiment with modification of ionic levels and ratios of culture water to be similar to that of seawater (Na^{+}, K^{+}, Ca^{2+} and Mg^{2+} levels at 1 g/L salinity as 304.35, 11.01, 11.59 and 39.13 mg/L, respectively). The levels and ratios found in seawater are perhaps the safest reference values to attain optimal survival and growth of *L. vannamei* (Boyd, 2018). As a result, K^{+} and Mg^{2+} fertilizers have been found to enhance the survival and growth of shrimp by increasing aqueous levels of K^{+} and Mg^{2+} in low-salinity pond water. However, due to the high cost of supplementation to large commercial earthen ponds, the particular levels achieved on commercial farms are often far less than that of sea water diluted to the same salinity (McNevin *et al.*, 2004).

The findings of the current study confirm that the Mg^{2+} level in low salinity water (3 g/L) had a significant effect on final growth performance, feed conversion ratio, hemolymph osmolality and osmoregulatory capacity of shrimp. The performance of shrimp observed in reconstituted sea salt declined as the Mg level was reduced from 129 to 12 mg/L. Pan *et al.* (2006) reported significant reduction in the survival rates and weight gain of *Marsupenaeus japonicus* postlarvae due to lower Mg^{2+}/Ca^{2+} ratios than the same ratio found in sea water. During their particular study, Mg^{2+} level was gradually changed to prepare culture mediums with different Mg/Ca ratios, while Ca^{2+} level remained constant. Therefore, depressed growth of *M. japonicus* postlarvae observed by Pan *et al.* (2006) could be interpreted to be a result of deficient Mg^{2+}/Ca^{2+} ratios or due to low Mg^{2+} levels in culture water. On the contrary, Roy *et al.* (2007) and Zacaris *et al*. (2019) reported no significant differences in final weights of Pacific white shrimp reared in low salinity (4 g/L) waters containing various levels of Mg^{2+}, albeit a significant reduction in survival was observed in the lowest (10 mg/L) Mg^{2+} treatment in the Roy *et al.* (2007) study. Roy *et al.* (2007) observed a clear trend between Mg^{2+} level and growth performance of shrimp in low salinity culture water with the highest concentration of Mg^{2+} (160 mg/L) yielding the largest weight gain, while the treatment with the lowest concentration of Mg^{2+} (10 mg/L) resulted in the lowest weight gain of shrimp. Insignificant differences in growth performances of shrimp in Zacaris *et al*., (2019) might be due to no Mg^{2+} deficiencies in treatments (167-205 mg/L) compared to the level of Mg^{2+} in diluted sea water (~156 mg/L) at the same salinity.

Though the exact reasons for the reduced performance of shrimp reared in low salinity water with low Mg^{2+} levels are not clear, high energy expenditure to maintain osmoregulation due to low Mg^{2+} level in low salinity water could play a vital role. It's is well established that Na^{+}–K^{+}-ATPase (NKA) plays a major role in osmoregulation of decapod crustaceans, which is dependent on Mg^{2+} concentration in the water (Towle, 1981; Towle, 1984; Pan *et al.*, 2006; Romano and Zeng, 2011). Reduction in Mg^{2+} concentration in the medium could disrupt the activity of NKA in gills, leading to osmoregulation dysfunction in shrimp (Pan *et al.*, 2006; Roy *et al.*, 2010). Significantly lower hemolymph osmolality and osmoregulatory capacity of shrimp reared in culture water with suboptimal Mg^{2+} concentrations during the current study support this argument. In addition, significantly low growth performances of shrimp at low Mg^{2+} concentrations in low salinity water could be due to the deficiency of Mg^{2+} in the hemolymph to run various physiological processes in the body including growth (Alvarez-Leefmans *et al.*, 1987).

Rosas *et al*. (1999) documented an increase in energy demand in *L. setiferus* to regulate the osmotic pressure and ionic concentration in response to a change in environmental salinity, while Dalla Via (1986) noted an increase in oxygen consumption of 300% in *M. japonicus* due a sudden change in water salinity. Roy *et al*. (2007) observed a significant increase in respiration rate of *L. vannamei* corresponding to low aqueous Mg^{2+} levels in low salinity water while Jiann-Chu and Nan, (1992) reported a decrease in total ATP of *Fenneropenaeus chinensis*, when shrimp suffered from physiological stress. Based on this phenomenon, stress due to changes in salinity or ionic composition of the culture water could modify energy partitioning of the animal and ultimately lead to variability in growth and survival (Gao *et al.*, 2016). Shrimp survival was not significantly different at the end of the 6-week growth trial between treatments in this laboratory study. However, adverse effects on shrimp survival in later stages of the production cycle on commercial shrimp farms in west Alabama might result from prolonged exposure (>16-weeks) of shrimp to extremely low Mg^{2+} levels under low salinity conditions.

Magnesium is one of the key cations in shrimp hemolymph. Aqueous deficiencies in Mg^{2+} can be related to stress and significant changes in the level of Mg^{2+} can be used as an indicator of damaged gill surfaces or osmoregulatory dysfunction in shrimp (Dall, 1964, Hall and Van Ham, 1998). Significant reduction in Mg^{2+} content in hemolymph, in response to a reduction in the Mg^{2+} level in the culture medium observed during this study, is in agreement with the findings of (Hall and Van Ham, 1998), which suggest that osmoregulatory dysfunction could be the product of stress experienced by shrimp. The significant reduction of Mg^{2+} content in the carapace and whole-body shrimp in parallel with declining levels of Mg^{2+} level in water may be connected to the osmoregulatory dysfunction as well. Under this circumstance where Mg^{2+} is barely available from the external environment, shrimp may be forced to use stored sources of Mg^{2+} to carry out required chemical reactions to sustain life functions (Proverbio *et al.*, 1990; Roy *et al.*, 2007). This may be a result of the reduced availability of Mg^{2+} in the water column or reduced capacity for absorption through the gills in waters with suboptimal Mg^{2+} levels.

5. Conclusion

Alabama shrimp farmers using inland low salinity water to culture shrimp have recently been experiencing poor survival and production even in culture waters amended with K^{+} and Mg^{2+}

fertilizers. While farmers routinely amend waters to reflect an Na:K ratio similar to that of seawater, typically Mg^{2+} levels are only supplemented to a small degree due to the large expense of raising Mg^{2+} enough in low salinity pond water to reflect the Mg/Ca ratio and Mg^{2+} levels found in seawater. Mg^{2+} levels in shrimp production ponds on west Alabama shrimp farms can be below 30 mg/L (Mg/Ca ratios < 1) even after addition of fertilizers containing magnesium, which is much different than the level in seawater diluted to the same salinity (~117 mg/L Mg^{2+} at 3 g/L). As Mg^{2+} level was reduced in this study, there was a subsequent reduction in shrimp performance reflected in final weight, weight gain, hemolymph osmolality, and osmoregulatory capacity paralleled by an increase in FCR. Reductions in hemolymph osmolality and Mg^{2+} concentration in hemolymph are likely indicative of stress, which is assumed to be due to the dysfunction of osmoregulation in shrimp caused by low levels of Mg^{2+} in culture water. Future studies should perhaps evaluate the performance of enzymes involved in osmoregulation, such as the Na^{+}–K^{+}-ATPase, in low salinity culture mediums with suboptimal Mg^{2+} levels (<30 mg/L) as well as the performance of larger shrimp, which have been reported to have less osmoregulatory capacity than juvenile shrimp at low salinity and could perhaps be more susceptible to deficiencies of Mg^{2+} in the culture medium.

Table 1: Response of juvenile Pacific white shrimp (0.11 ± 0.01 g) reared in low salinity water of different Mg levels (salinity = 3 g/L, Ca=48-54 mg/L, K=38-53 mg/L and Na=1055-1120 mg/L) for 6-weeks. Values represent the mean of three replicates ± standard deviation.

Mg level in water (mg/L)	Final weight (g)	Weight gain (g)	Weight gain (%)	FCR	Survival (%)	Hemolymph osmolality (mmol/kg)	Osmoregulatory capacity (mmol/kg)
129 (control)	7.1±0.2^{a}	7.0±0.2^{a}	6589±408^{a}	1.1±0.1^{c}	93±8	610±21	537±18
100	6.7±0.2^{a}	6.6±0.2^{a}	6277±386^{ab}	1.1±0.1^{c}	93±5	592±42	522±31
78	6.1±0.9^{ab}	6.0±0.9^{ab}	5340±957^{abc}	1.2±0.4^{bc}	95±3	585±22	511±22
55	4.6±0.6^{bc}	4.5±0.6^{bc}	4207±929^{c}	1.9±0.0^{ab}	83±8	546±46	468±46
30	5.0±1.1^{bc}	4.9±1.1^{bc}	4492±940^{bc}	1.7±0.4^{abc}	87±6	544±43	465±40
17	4.1±0.2^{c}	4.0±0.2^{c}	3883±125^{c}	2.0±0.3^{a}	88±13	557±23	480±41
12	4.4±0.4^{c}	4.3±0.4^{c}	4074±574^{c}	1.7±0.2^{abc}	93±6	525±39	456±43
13	4.0±0.1^{c}	3.9±0.1^{c}	3761±426^{c}	2.0±0.3^{a}	90±10	545±27	471±35
PSD	0.6	0.6	662	0.3	8	34	36
p-value	0.00	0.00	0.00	0.00	0.58	0.09	0.10
Linear regression outcomes (between measured variable and Mg level in culture water)							
r^2	0.78	0.79	0.72	0.60	0.05	0.42	0.41
P-value	0.00	0.00	0.00	0.00	0.32	0.00	0.00

Values with different superscripts within the same column are significantly different based on Tukey Pairwise Comparisons. PSD= Pooled standard deviation.
Feed conversion ratio= feed offered/ (final weight-initial weight)
Weight gain%= ((final weight-initial weight)/initial weight) × 100
Osmoregulatory capacity = osmolality of culture media – osmolality of hemolymph

Table 2: Hemolymph ion levels (mEq/L) of Pacific white shrimp reared in culture water of different Mg levels in low salinity water (3 g/L). Values represent the mean of three replicates ± standard deviation.

Mg level in water (mg/L)	Na	K	Ca	Mg
129 (control)	380±18	11±1	14±1	7±1[ab]
100	393±51	13±2	11±2	9±4[a]
78	415±19	13±3	12±2	9±4[ab]
55	404±65	15±8	12±3	4±3[ab]
30	371±67	10±2	12±2	4±2[ab]
17	374±34	11±1	13±1	3±1[ab]
12	358±48	13±5	11±2	2±2[ab]
13	360±27	11±2	11±1	1±1[b]
PSD	44.8	3.8	1.98	2.9
P-value	0.72	0.71	0.57	0.02

Values with different superscripts within the same column are significantly different based on Tukey Pairwise Comparisons. PSD= Pooled standard deviation.

Table 3: Carapace ash (%) and ionic levels (mEq/L) of Pacific white shrimp reared in different Mg levels in low salinity (3 g/L) water. Values represent the mean of three replicates ± standard deviation.

Mg level in water (mg/L)	Ash	Na	K	Ca	Mg
129 (control)	70.3±1.4	30±4	13±2	167±33	24±1[a]
100	67.9±5.5	20±2	6±2	178±59	21±7[ab]
78	64.6±0.9	19±7	6±4	157±80	12±6[abc]
55	69.9±5.3	15±7	4±1	137±77	11±3[bc]
30	69.3±2.2	16±5	5±2	131±70	9±4[bc]
17	70.9±3.6	15±2	4±1	146±58	8±3[c]
12	69.7±2.1	11±2	4±1	91±12	4±1[c]
13	71.4±4.4	22±8	7±4	119±22	6±1[c]
PSD	3.58	9.66	5.28	56.80	4.40
P-value	0.42	0.166	0.101	0.698	0.001

Values with different superscripts within the same column are significantly different based on Tukey Pairwise Comparisons. PSD= Pooled standard deviation.

Table 4: Whole-body cationic levels (mEq/L) of Pacific white shrimp reared in culture water of different Mg levels in low salinity (3 g/L) water. Values represent the mean of three replicates ± standard deviation.

Mg level in water (mg/L)	Na	K	Ca	Mg
129 (control)	537±31	0.030±0.001	0.063±0.004	0.020±0.000[a]
100	537±14	0.031±0.000	0.060±0.004	0.018±0.000[a]
78	551±6	0.030±0.002	0.066±0.006	0.015±0.001[b]
55	629±57	0.030±0.001	0.064±0.021	0.016±0.000[b]
30	557±36	0.030±0.001	0.058±0.015	0.013±0.001[c]
17	585±52	0.029±0.002	0.066±0.006	0.013±0.000[c]
12	549±10	0.030±0.000	0.065±0.007	0.012±0.000[c]
13	601±46	0.029±0.000	0.078±0.020	0.012±0.001[c]
PSD	36.53	0.001	0.01	0.001
P-value	0.062	0.356	0.67	0.00

Values with different superscripts within the same column are significantly different based on Tukey Pairwise Comparisons. PSD= Pooled standard deviation.

Table 5: Dissolved oxygen (DO), temperature, salinity, pH, total ammonia nitrogen (TAN), nitrite nitrogen, ionic composition (mg/L) and osmolality of waters (3 g/L salinity) with different Mg levels used to rear Pacific white shrimp. Values represent the mean of three replicates ± standard deviation.

Treatments (Mg level in mg/L)	129 (control)	100	78	55	30	17	12	13
Dissolved oxygen (mg/L)	6.0 ± 0.9	6.0 ± 0.9	6.0 ± 0.9	5.9 ± 1.0	6.0 ± 0.9	6.0 ± 1.0	6.1 ± 1.0	6.0 ± 1.0
Temperature (^{0}C)	29.1 ± 0.9	29.4 ± 1.1	29.3 ± 1.0	29.3 ± 0.9	29.1 ± 1.0	29.2 ± 1.0	29.0 ± 1.1	29.1 ± 1.0
Salinity (g/L)	3.2 ± 0.1	3.2 ± 0.2	3.1 ± 0.1	3.1 ± 0.2	3.1 ± 0.1	3.1 ± 0.1	3.0 ± 0.1	3.0 ± 0.1
pH	7.7 ± 0.4	7.6 ± 0.4	7.7 ± 0.4	7.3 ± 1.0	7.6 ± 0.4	7.7 ± 0.4	7.7 ± 0.4	7.6 ± 0.5
TAN (mg/L)	0.25 ± 0.12	0.26 ± 0.11	0.28 ± 0.08	0.27 ± 0.10	0.37 ± 0.08	0.34 ± 0.03	0.27 ± 0.10	0.28 ± 0.09
Nitrite (mg/L)	0.05 ± 0.02	0.05 ± 0.02	0.07 ± 0.03	0.07 ± 0.02	0.13 ± 0.12	0.14 ± 0.10	0.10 ± 0.06	0.10 ± 0.06
Ionic profile of water (mg/L)								
Na	1120±9	1055±31	1074±30	1061±8	1076±6	1090±10	1063±18	1065±18
K	38±4	45±1	45±1	45±1	45±0	46±1	45±0	53±14
Ca	49±0	51±2	54±2	50±2	52±1	52±2	48±2	51±5
Mg	129±1	100±2	78±2	55±0	30±1	17±1	12±1	13±7
Na:K Ratio	26.3±1.4	23.2±0.1	23.7±0.4	23.8±0.3	23.9±0.1	23.9±0.3	23.9±0.2	23.9±1.0
Mg:Ca Ratio	2.63±0.0	1.96±0.1	1.44±0.0	1.11±0.1	0.58±0.0	0.33±0.0	0.26±0.0	0.25±0.1
Osmolality (mmol/kg)	74±2	70±11	74±6	79±13	79±8	77±17	70±12	74±10

Figure 1: Effect of Mg level in low salinity water (3 g/L) on the growth (a) and osmoregulatory capacity (b) of juvenile Pacific white shrimp.

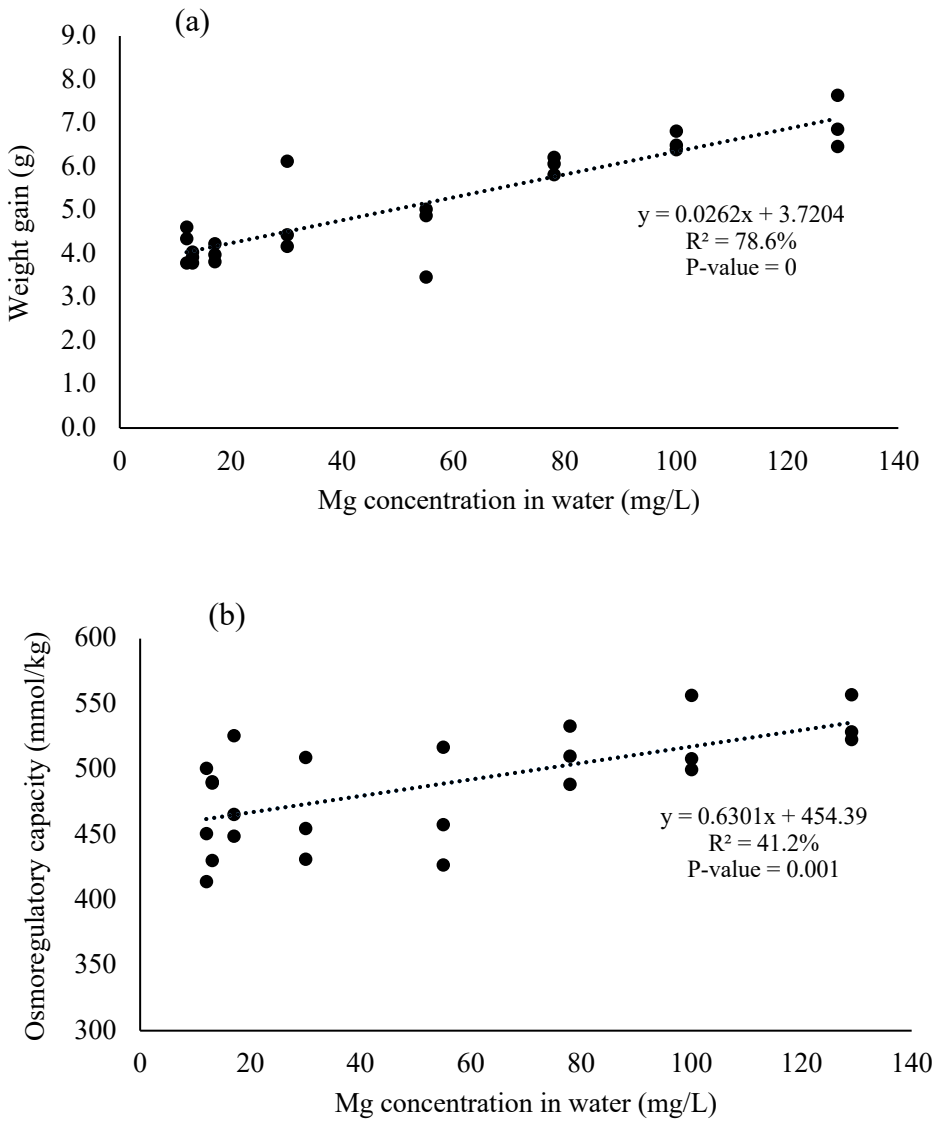
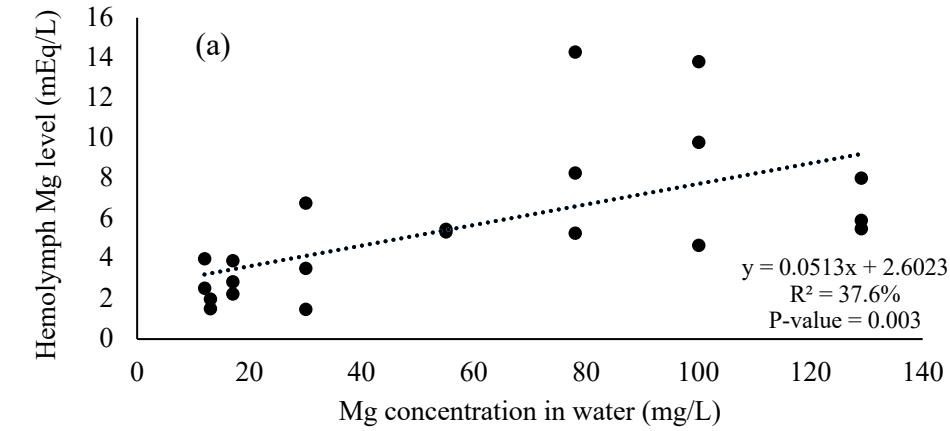
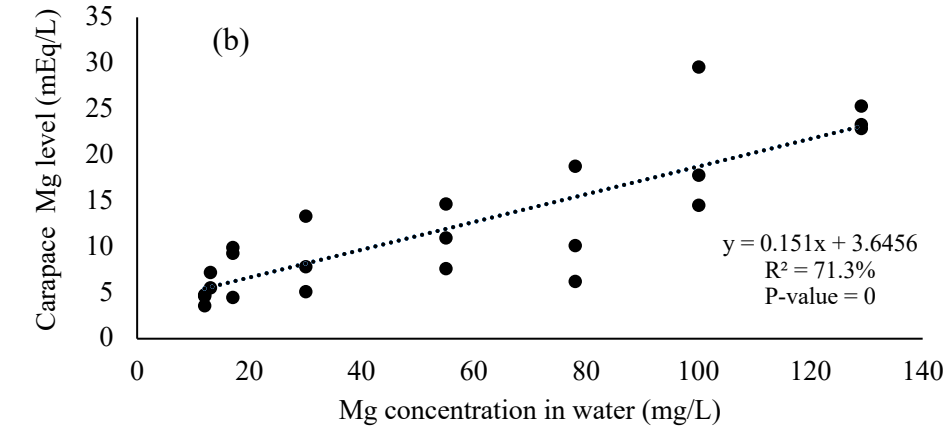
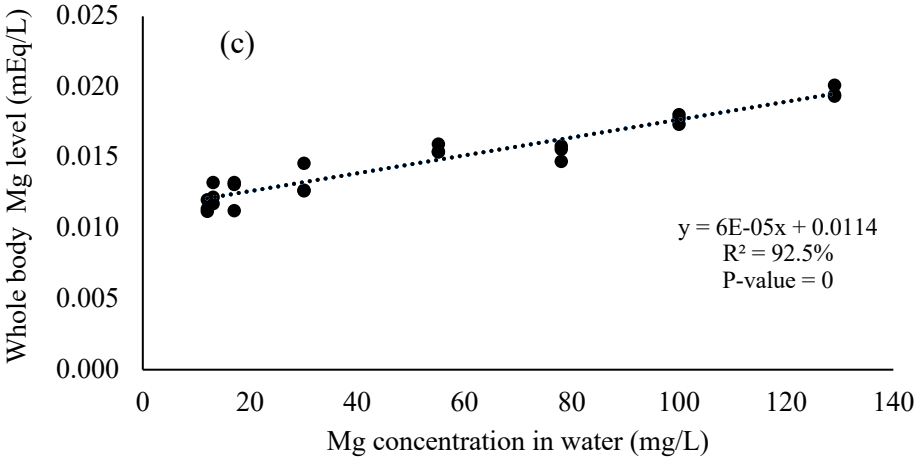


Figure 2: Effect of Mg level in low salinity water (3 g/L) on Mg level (mEq/L) in hemolymph (a), carapace (b) and whole body (c) of juvenile Pacific white shrimp.







References

Adhikari, S., Chaurasia, V., Naqvi, A.A. & Pillai, B. (2007) Survival and growth of *Macrobrachium rosenbergii* (de Man) juvenile in relation to calcium hardness and bicarbonate alkalinity. *Turkish Journal of Fisheries and Aquatic Sciences,* 7**,** 23-26.

Alvarez-Leefmans, F., Giraldez, F. & Gamino, S. (1987) Intracellular free magnesium in excitable cells: its measurement and its biologic significance. *Canadian Journal of Physiology and Pharmacology,* 65**,** 915-925.

AOAC (1984) *Official methods of analysis,* Association of Official Analytical Chemists, Aelington, Virginia, USA.

Atwood, H., Young, S., Tomasso, J. & Browdy, C. (2003) Survival and growth of Pacific white shrimp *Litopenaeus vannamei* postlarvae in low-salinity and mixed-salt environments. *Journal of the World Aquaculture Society,* 34**,** 518-523.

Boyd, C. (2002) Inland farming of marine shrimp: examples for China, Ecuador, Thailand and the United States. Report prepared under the World Bank, NACA, WWF and FAO Consortium Program on Shrimp Farming and the Environment. *World Wildlife Fund, Washington, DC, USA*.

Boyd, C.E. (2018) Revisiting ionic imbalance in low-salinity shrimp aquaculture. Global Aquaculture Alliance.

Brown, J.H., Wickins, J.F. & MacLean, M.H. (1991) The effect of water hardness on growth and carapace mineralization of juvenile freshwater prawns, *Macrobrachium rosenbergii* de Man. *Aquaculture,* 95**,** 329-345.

Castille Jr, F.L. & Lawrence, A.L. (1981) The effect of salinity on the osmotic, sodium and chloride concentrations in the hemolymph of euryhaline shrimp of the genus *Penaeus*. *Comparative Biochemistry and Physiology Part A: Physiology,* 68**,** 75-80.

Charmantier, G., Bouaricha, N., Charmantier-Daures, M., Thuet, P. & Trilles, J. (1989) Salinity tolerance and osmoregulatory capacity as indicators of the physiological state of peneid shrimps. *European Aquaculture Society Special Publication,* 10**,** 65-66.

Clesceri, L.S., Greenberg, A.E. & Eaton, A.D. (1998) Standard methods for the examination of water and wastewater, American Public Health Association. *Washington, DC***,** 4-415.

Dall, W. (1964) Studies on the physiology of a shrimp, *Metapenaeus mastersii* (Haswell)(Crustacea: Decapoda: Peneidae). I. Blood constituents. *Marine and Freshwater Research,* 15**,** 145-161.

Dalla Via, G. (1986) Salinity responses of the juvenile penaeid shrimp *Penaeus japonicus*: I. Oxygen consumption and estimations of productivity. *Aquaculture,* 55**,** 297-306.

Davis, D.A., Boyd, C.E., Rouse, D.B. & Saoud, I.P. (2005) Effects of potassium, magnesium and age on growth and survival of *Litopenaeus vannamei* post-larvae reared in inland low salinity well waters in west Alabama. *Journal of the World Aquaculture Society,* 36**,** 416-419.

Galkanda-Arachchige, H., Roy, L.A., Dahl, S., James, J., Kelly, A.M. & Allen Davis, D. (2020a) Laboratory and on-farm evaluation of low-cost salt mixtures for use during salinity acclimation and the nursery phase of Pacific white shrimp (*Litopenaeus vannamei*). *Aquaculture Research*.

Galkanda-Arachchige, H.S., Roy, L.A. & Davis, D.A. (2020b) Evaluation of an alternative salt mixture to culture Pacific white shrimp (*Litopenaeus vannamei*) in inland aquaculture. *Aquaculture Research*.

Gao, W., Tian, L., Huang, T., Yao, M., Hu, W. & Xu, Q. (2016) Effect of salinity on the growth performance, osmolarity and metabolism-related gene expression in white shrimp *Litopenaeus vannamei*. *Aquaculture Reports,* 4**,** 125-129.

Greenaway, P. (1993) Calcium and magnesium balance during molting in land crabs. *Journal of Crustacean Biology,* 13**,** 191-197.

Hall, M.R. & Van Ham, E.H. (1998) The effects of different types of stress on blood glucose in the giant tiger prawn *Penaeus monodon*. *Journal of the World Aquaculture Society,* 29**,** 290-299.

Henry, R.P., Gehnrich, S., Weihrauch, D. & Towle, D.W. (2003) Salinity-mediated carbonic anhydrase induction in the gills of the euryhaline green crab, *Carcinus maenas*. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology,* 136**,** 243-258.

Hou, C., Wang, F., Dong, S., Zhu, Y. & Yu, T. (2012) Effects of constant Ca^{2+} concentration in salinity fluctuations on growth and energy budget of juvenile *Litopenaeus vannamei*. *Aquaculture International,* 20**,** 177-188.

Jiann-Chu, C. & Nan, F.-H. (1992) Effect of ambient ammonia on ammonia-N excretion and ATPase activity of *Penaeus chinensis*. *Aquatic Toxicology,* 23**,** 1-10.

Kline, E.R. & Stekoll, M.S. (2000) The role of calcium and sodium in toxicity of an effluent to mysid shrimp (*Mysidopsis bahia*). *Environmental Toxicology and Chemistry: An International Journal,* 19**,** 234-241.

McNevin, A.A., Boyd, C.E., Silapajarn, O. & Silapajarn, K. (2004) Ionic supplementation of pond waters for inland culture of marine shrimp. *Journal of the World Aquaculture Society,* 35**,** 460-467.

Pan, L.-Q., Luan, Z.-H. & Jin, C.-X. (2006) Effects of Na+/K+ and Mg2+/Ca2+ ratios in saline groundwaters on Na+–K+-ATPase activity, survival and growth of *Marsupenaeus japonicus* postlarvae. *Aquaculture,* 261**,** 1396-1402.

Parmenter, K., Bisesi Jr, J., Young, S., Klaine, S., Atwood, H., Browdy, C. & Tomasso, J. (2009) Culture of Pacific white shrimp *Litopenaeus vannamei* in a mixed-ion solution. *North American Journal of Aquaculture,* 71**,** 134-137.

Partridge, G., Lymbery, A. & George, R. (2008) Finfish mariculture in inland Australia: a review of potential water sources, species, and production systems. *Journal of the World Aquaculture Society,* 39**,** 291-310.

Pillard, D.A., DuFresne, D.L., Caudle, D.D., Tietge, J.E. & Evans, J.M. (2000) Predicting the toxicity of major ions in seawater to mysid shrimp (*Mysidopsis bahia*), sheepshead minnow (*Cyprinodon variegatus*), and inland silverside minnow (*Menidia beryllina*). *Environmental Toxicology and Chemistry: An International Journal,* 19**,** 183-191.

Proverbio, T., Zanders, I.P., Marín, R., Rodríguez, J. & Proverbio, F. (1990) Effects of Na+ and/or K+ on the Mg2+-dependent ATPase activities in shrimp (*Macrobrachium amazonicum*) gill homogenates. *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry,* 97**,** 383-390.

Rafiee, G., Tavabe, K.R., Frinsko, M. & Daniels, H. (2015) Effects of various sodium adsorption ratio (SAR) mediums on larval performance of the freshwater prawn *Macrobrachium rosenbergii* (de Man). *Aquaculture Research,* 46**,** 725-735.

Ray, A.J., Drury, T.H. & Cecil, A. (2017) Comparing clear-water RAS and biofloc systems: Shrimp (*Litopenaeus vannamei*) production, water quality, and biofloc nutritional contributions estimated using stable isotopes. *Aquacultural Engineering,* 77**,** 9-14.

Romano, N. & Zeng, C. (2011) Importance of balanced Na+/K+ ratios for blue swimmer crabs, *Portunus pelagicus*, to cope with elevated ammonia-N and differences between in vitro and in vivo gill Na+/K+-ATPase responses. *Aquaculture,* 318**,** 154-161.

Rosas, C., Martinez, E., Gaxiola, G., Brito, R., Sanchez, A. & Soto, L.A. (1999) The effect of dissolved oxygen and salinity on oxygen consumption, ammonia excretion and osmotic pressure of *Penaeus setiferus* (*Linnaeus*) juveniles. *Journal of Experimental Marine Biology and Ecology,* 234**,** 41-57.

Roy, L.A. & Davis, D.A. (2010) Requirements for the culture of the Pacific white shrimp, Litopenaeus vannamei, reared in low salinity waters: water modification and nutritional strategies for improving production In *Avances en Nutrición Acuicola X-Memorias del X Simposio Internacional de Nutrición Acuicola*, pp. 8-10.

Roy, L.A., Davis, D.A., Nguyen, T.N. & Saoud, I.P. (2009a) Supplementation of chelated magnesium to diets of the pacific white shrimp, *Litopenaeus vannamei,* reared in low-salinity waters of west Alabama. *Journal of the World Aquaculture Society,* 40**,** 248-254.

Roy, L.A., Davis, D.A. & Saoud, I.P. (2006) Effects of lecithin and cholesterol supplementation to practical diets for Litopenaeus vannamei reared in low salinity waters. *Aquaculture,* 257**,** 446-452.

Roy, L.A., Davis, D.A., Saoud, I.P., Boyd, C.A., Pine, H.J. & Boyd, C.E. (2010) Shrimp culture in inland low salinity waters. *Reviews in Aquaculture,* 2**,** 191-208.

Roy, L.A., Davis, D.A., Saoud, I.P. & Henry, R.P. (2007) Effects of varying levels of aqueous potassium and magnesium on survival, growth, and respiration of the Pacific white shrimp, *Litopenaeus vannamei*, reared in low salinity waters. *Aquaculture,* 262**,** 461-469.

Roy, L.A., Davis, D.A. & Whitis, G.N. (2009b) Pond-to-pond variability in post-larval shrimp, *Litopenaeus vannamei*, survival and growth in inland low-salinity waters of west Alabama. *Aquaculture Research,* 40**,** 1823-1829.

Saoud, I.P., Davis, D.A. & Rouse, D.B. (2003) Suitability studies of inland well waters for *Litopenaeus vannamei* culture. *Aquaculture,* 217**,** 373-383.

Solorzano, L. (1969) Determination of ammonia in natural waters by the phenolhypochlorite method. *Limnology and Oceanography,* 14**,** 799-801.

Spotte, S. (1979) *Fish and Invertebrate Culture: Water Managment in Closed Systems, 2nd Edition.,* Wiley, New York.

Steel, R.G. & Torrie, J.H. (1980) *Principles and Procedures of Statistics,* McGraw-Hill Book Co. Inc., New York.

Towle, D. (1981) Role of Na++ K+-ATPase in ionic regulation by marine and estuarine animals. *Marine Biology Letters,* 2**,** 107-122.

Towle, D.W. (1984) Membrane-bound ATPases in arthropod ion-transporting tissues. *American Zoologist,* 24**,** 177-185.

Wilder, M.N., Ikuta, K., Atmomarsono, M., Hatta, T. & Komuro, K. (1998) Changes in osmotic and ionic concentrations in the hemolymph of *Macrobrachium rosenbergii* exposed to varying salinities and correlation to ionic and crystalline composition of the cuticle. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology,* 119**,** 941-950.

Wilder, M.N., Jasmani, S., Jayasankar, V., Kaneko, T., Aida, K., Hatta, T., Nemoto, S. & Wigginton, A. (2009) Hemolymph osmolality, ion concentrations and calcium in the structural organization of the cuticle of the giant freshwater prawn *Macrobrachium rosenbergii:* Changes with the molt cycle. *Aquaculture,* 292**,** 104-110.

Zacarias, S., Schveitzer, R., Arantes, R., Galasso, H., Pinheiro, I., Espirito Santo, C. & Vinatea, L. (2019) Effect of different concentrations of potassium and magnesium on performance of Litopenaeus vannamei postlarvae reared in low-salinity water and a biofloc system. *Journal of Applied Aquaculture,* 31**,** 85-96.

CHAPTER V

SUMMARY AND CONCLUSION

Considerable interest in culturing Pacific white shrimp, *Litopenaeus vannamei* exists far from coastal areas either in inland ponds filled with low-salinity well water (2–5 g/L), in indoor recirculating aquaculture systems (RAS) or in indoor bio-floc systems, which are usually operated at salinities less than 15 g/L. One of the primary challenges faced by inland shrimp farmers today is the higher cost of reconstituted sea salts (RSS), which are scientifically formulated to contain the major, minor, and trace elements to support delicate marine life. The high price of RSS represents a considerable financial burden considering the volume of salt necessary for the acclimation process or nursery and growth phases in inland shrimp farming making the profit margins much less robust. An economically attractive salt solutions would reduce production costs of inland shrimp production facilities, thereby helping to stimulate the growth of the industry.

Filling research gaps, the current line of research was designed to test the efficacy of a low-cost salt solution (LCSM) to replace expensive RSS in the process of salinity acclimation, nursery phases, and the growth phase of Pacific white shrimp in different salinities (3, 6 and 15 g/L) under laboratory and farm conditions. In addition, LCSM was modified by decreasing the Mg level in the mixture to allow different Mg levels in the culture medium (100, 78, 55, 30, 17, 13 and 12 mg/L) to determine the effect of different Mg levels in low salinity (3 g/L) water on growth, survival, hemolymph osmolality, cationic composition in hemolymph, carapace and whole-body of Pacific white shrimp. The LCSM was formulated to yield sodium, potassium, calcium, and magnesium concentrations closely comparable to those of diluted seawater using agriculture grade sodium chloride, magnesium chloride, magnesium sulfate, muriate of potash (potassium chloride) of 62% potassium oxide equivalence, calcium chloride, and sodium bicarbonate. Through simplifying the formulation rather than balancing all the different anions and trace minerals, it reduced the cost of LCSM by approximately 50% compared to RSS.

At the conclusion of laboratory and farm-based nursery and acclimation trials, no significant differences were observed for either survival or growth of shrimp post larvae between RSS and LCSM treatments at all salinities examined. At the conclusion of growth trials, no significant differences were observed in survival, growth, osmoregulation, and levels of cations in shrimp hemolymph between RSS and LCSM treatments at all salinities (3, 6 and 15 g/L) examined. Results reflect the potential use of LCSM to replace RSS at the commercial level which could be an excellent solution to bring down the cost of production for inland low salinity shrimp aquaculture, thereby helping to further stimulate industry growth. However, additional research is recommended in order to further explore the suitability of different agriculture-based salt sources to check their efficacy in salt formulation and to determine the effects of impurities (ions, substances, remains in the salt sources, etc., in addition to the target cations) on survival and growth performance of shrimp.

As Mg^{2+} level was reduced, a subsequent reduction ($P<0.05$) in shrimp performance reflected in final weight, weight gain, hemolymph osmolality, osmoregulatory capacity, Mg^{2+} concentration in hemolymph, carapace and whole body of shrimp paralleled by an increase in FCR. Reductions in hemolymph osmolality and Mg^{2+} concentration in hemolymph are likely indicative of stress, which is assumed to be due to the dysfunction of osmoregulation in shrimp caused by low levels of Mg^{2+} in culture water. Future studies are recommended to evaluate the performance of enzymes involved in osmoregulation, such as the Na^{+}–K^{+}-ATPase, in low salinity culture mediums with suboptimal Mg^{2+} levels (<30 mg/L) as well as the performance of larger shrimp, which have been reported to have less osmoregulatory capacity than juvenile shrimp at low salinity. Furthermore, LCSM could be used to determine the minimum concentrations of rest of the major ions (Na^{+}, K^{+}, and Ca^{2+}) and ionic ratios required (Mg^{2+}:Ca^{2+}, Na^{+}:Ca^{2+}, etc.) for physiological functions in Penaeid shrimp with certainty which could lead to further adjustments in the salt formulation of LCSM.

LITERATURE CITED

Adhikari, S., Chaurasia, V., Naqvi, A.A. & Pillai, B. (2007) Survival and growth of *Macrobrachium rosenbergii* (de Man) juvenile in relation to calcium hardness and bicarbonate alkalinity. *Turkish Journal of Fisheries and Aquatic Sciences,* 7**,** 23-26.

Alvarez-Leefmans, F., Giraldez, F. & Gamino, S. (1987) Intracellular free magnesium in excitable cells: its measurement and its biologic significance. *Canadian Journal of Physiology and Pharmacology,* 65**,** 915-925.

AOAC (1984) *Official methods of analysis,* Association of Official Analytical Chemists, Aelington, Virginia, USA.

Atwood, H., Young, S., Tomasso, J. & Browdy, C. (2003) 2283981. Survival and growth of Pacific white shrimp *Litopenaeus vannamei* postlarvae in low-salinity and mixed-salt environments. *Journal of the World Aquaculture Society,* 34**,** 518-523.

Bouaricha, N., Thuet, P., Charmantier, G., Charmantier-Daures, M. & Trilles, J.-P. (1991) Na+-K+ ATPase and carbonic anhydrase activities in larvae, postlarvae and adults of the shrimp *Penaeus japonicus* (Decapoda, Penaeidea). *Comparative Biochemistry and Physiology Part A: Physiology,* 100**,** 433-437.

Boyd, C. (2002) Inland farming of marine shrimp: examples for China, Ecuador, Thailand and the United States. Report prepared under the World Bank, NACA, WWF and FAO Consortium Program on Shrimp Farming and the Environment. *World Wildlife Fund, Washington, DC, USA*.

Boyd, C.E. (1989) Water quality management and aeration in shrimp farming. *Fisheries and Allied Aquacultures Departmental series No. 2, Alabama agricultural Experiment Station, Auburn University, Auburn, AL, USA***,** 83.

Boyd, C.E. (2018) Revisiting ionic imbalance in low-salinity shrimp aquaculture. Global Aquaculture Alliance.

Bray, W., Lawrence, A. & Leung-Trujillo, J. (1994) The effect of salinity on growth and survival of *Penaeus vannamei,* with observations on the interaction of IHHN virus and salinity. *Aquaculture,* 122**,** 133-146.

Brown, J.H., Wickins, J.F. & MacLean, M.H. (1991) The effect of water hardness on growth and carapace mineralization of juvenile freshwater prawns, *Macrobrachium rosenbergii* de Man. *Aquaculture,* 95**,** 329-345.

Castilho, P.C., Martins, I.A. & Bianchini, A. (2001) Gill Na+, K+-ATPase and osmoregulation in the estuarine crab, *Chasmagnathus granulata* Dana, 1851 (Decapoda, Grapsidae). *Journal of Experimental Marine Biology and Ecology,* 256**,** 215-227.

Castille Jr, F.L. & Lawrence, A.L. (1981) The effect of salinity on the osmotic, sodium and chloride concentrations in the hemolymph of euryhaline shrimp of the genus *Penaeus*. *Comparative Biochemistry and Physiology Part A: Physiology,* 68**,** 75-80.

Castille Jr, F.L. & Lawrence, A.L. (1981a) A comparison of the capabilities of juvenile and adult *Penaeus setiferus* and *Penaeus stylirostris* to regulate the osmotic, sodium, and chloride concentrations in the hemolymph. *Comp. Biochem. Physiol. A,* 68**,** 677-680.

Castille Jr, F.L. & Lawrence, A.L. (1981b) The effect of salinity on the osmotic, sodium and chloride concentrations in the hemolymph of euryhaline shrimp of the genus *Penaeus*. *Comparative Biochemistry and Physiology Part A: Physiology,* 68**,** 75-80.

Charmantier, G. (1998) Ontogeny of osmoregulation in crustaceans: a review. *Invertebrate Reproduction & Development,* 33**,** 177-190.

Charmantier, G., Bouaricha, N., Charmantier-Daures, M., Thuet, P. & Trilles, J. (1989) Salinity tolerance and osmoregulatory capacity as indicators of the physiological state of *peneid* shrimps. *European Aquaculture Society Special Publication,* 10**,** 65-66.

Charmantier-Daures, M., Thuet, P., Charmantier, G. & Trilles, J.-P. (1988) Tolérance à la salinité et osmorégulation chez les post-larves de *Penaeus japonicus* et P. chinensis. Effet de la température. *Aquatic living resources,* 1**,** 267-276.

Chen, J.-C., Lin, M.-N., Ting, Y.-Y. & Lin, J.-N. (1995) Survival, haemolymph osmolality and tissue water of *Penaeus chinensis* juveniles acclimated to different salinity and temperature levels. *Comparative Biochemistry and Physiology Part A: Physiology,* 110**,** 253-258.

Chim, L., Bouveret, R., Lemaire, P. & Martin, J. (2003) Tolerance of the shrimp *Litopenaeus stylirostris*, Stimpson 1894, to environmental stress: interindividual variability and selection potential for stress-resistant individuals. *Aquaculture research,* 34**,** 629-632.

Clesceri, L.S., Greenberg, A.E. & Eaton, A.D. (1998) Standard methods for the examination of water and wastewater, American Public Health Association. *Washington, DC***,** 4-415.

Cuzon, G., Lawrence, A., Gaxiola, G., Rosas, C. & Guillaume, J. (2004) Nutrition of *Litopenaeus vannamei* reared in tanks or in ponds. *Aquaculture,* 235**,** 513-551.

Dall, W. & Smith, D. (1981) Ionic regulation of four species of penaeid prawn. *Journal of Experimental Marine Biology and Ecology,* 55**,** 219-232.

Dall, W. (1964) Studies on the physiology of a shrimp, *Metapenaeus mastersii* (Haswell)(Crustacea: Decapoda: Peneidae). I. Blood constituents. *Marine and Freshwater Research,* 15**,** 145-161.

Dalla Via, G. (1986) Salinity responses of the juvenile penaeid shrimp *Penaeus japonicus*: I. Oxygen consumption and estimations of productivity. *Aquaculture,* 55**,** 297-306.

Davis, D.A., Boyd, C.E., Rouse, D.B. & Saoud, I.P. (2005) Effects of potassium, magnesium and age on growth and survival of *Litopenaeus vannamei* post-larvae reared in inland low salinity well waters in west Alabama. *Journal of the World Aquaculture Society,* 36**,** 416-419.

Davis, D.A., Saoud, I.P., McGraw, W.J. & Rouse, D.B. (2002) Considerations for *Litopenaeus vannamei* reared in inland low salinity waters. *Avances en Nutrición Acuicola VI. Memorias del VI Simposium Internacional de Nutrición Acuicola*.

Engel, D., Ferguson, R. & Eggert, L. (1975) Respiration rates and ATP concentrations in the excised gills of the blue crab as a function of salinity. *Comparative Biochemistry and Physiology Part A: Physiology,* 52**,** 669-673.

FAO (2018) The State of World Fisheries and Aquaculture 2018-Meeting the sustainable development goals. FAO Rome, Italy.

Flaherty, M. & Karnjanakesorn, C. (1995) Marine shrimp aquaculture and natural resource degradation in Thailand. *Environmental Management,* 19**,** 27-37.

Flaherty, M., Szuster, B. & Miller, P. (2000) Low salinity inland shrimp farming in Thailand. *Ambio: A Journal of the Human. Environment***,** 174-179.

Galkanda-Arachchige, H., Roy, L.A., Dahl, S., James, J., Kelly, A.M. & Allen Davis, D. (2020a) Laboratory and on-farm evaluation of low-cost salt mixtures for use during salinity acclimation and the nursery phase of Pacific white shrimp (*Litopenaeus vannamei*). *Aquaculture Research*.

Galkanda-Arachchige, H.S., Roy, L.A. & Davis, D.A. (2020b) Evaluation of an alternative salt mixture to culture Pacific white shrimp (*Litopenaeus vannamei*) in inland aquaculture. *Aquaculture Research*.

Gao, W., Tian, L., Huang, T., Yao, M., Hu, W. & Xu, Q. (2016) Effect of salinity on the growth performance, osmolarity and metabolism-related gene expression in white shrimp *Litopenaeus vannamei*. *Aquaculture Reports,* 4**,** 125-129.

Garcia III, A. & Brune, D.E. (1991) Transport limitation of oxygen in shrimp culture ponds. *Aquacultural Engineering,* 10**,** 269-279.

Gong, H., Jiang, D.H., Lightner, D.V., Collins, C. & Brock, D. (2004) A dietary modification approach to improve the osmoregulatory capacity of *Litopenaeus vannamei* cultured in the Arizona desert. *Aquaculture Nutrition,* 10**,** 227-236.

Gong, H., Lawrence, A.L., Jiang, D.-H., Castille, F.L. & Gatlin III, D.M. (2000) Lipid nutrition of juvenile *Litopenaeus vannamei*: I. Dietary cholesterol and de-oiled soy lecithin requirements and their interaction. *Aquaculture,* 190**,** 305-324.

Green, B.W. (2008) Stocking strategies for production of *Litopenaeus vannamei* (Boone) in amended freshwater in inland ponds. *Aquaculture Research,* 39**,** 10-17.

Greenaway, P. (1993) Calcium and magnesium balance during molting in land crabs. *Journal of Crustacean Biology,* 13**,** 191-197.

Gross, A., Abutbul, S. & Zilberg, D. (2004) Acute and chronic effects of nitrite on white shrimp, *Litopenaeus vannamei*, cultured in low-salinity brackish water. *Journal of the World Aquaculture Society,* 35**,** 315-321.

Hagerman, L. & Uglow, R.F. (1982) Effects of hypoxia on osmotic and ionic regulation in the brown shrimp *Crangon crangon* (L.) from brackish water. *Journal of Experimental Marine Biology and Ecology,* 63**,** 93-104.

Hall, M.R. & Van Ham, E.H. (1998) The effects of different types of stress on blood glucose in the giant tiger prawn *Penaeus monodon*. *Journal of the World Aquaculture Society,* 29**,** 290-299.

Henry, R.P., Garrelts, E.E., McCarty, M.M. & Towle, D.W. (2002) Differential induction of branchial carbonic anhydrase and Na+/K+ ATPase activity in the euryhaline crab, *Carcinus maenas*, in response to low salinity exposure. *Journal of Experimental Zoology,* 292**,** 595-603.

Henry, R.P., Gehnrich, S., Weihrauch, D. & Towle, D.W. (2003) Salinity-mediated carbonic anhydrase induction in the gills of the euryhaline green crab, *Carcinus maenas*. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology,* 136**,** 243-258.

Hou, C., Wang, F., Dong, S., Zhu, Y. & Yu, T. (2012) Effects of constant Ca 2+ concentration in salinity fluctuations on growth and energy budget of juvenile *Litopenaeus vannamei*. *Aquaculture international,* 20**,** 177-188.

Huang, X., Li, C., Zheng, L., Liu, C. & Zhou, J. (2006) The toxicity of NO2-N on *Litopenaeus vannamei* and effects of NO2-N on factors relating to the anti-disease ability. *Acta Hydrobiologica Sinica,* 30**,** 471.

Jiann-Chu, C. & Nan, F.-H. (1992) Effect of ambient ammonia on ammonia-N excretion and ATPase activity of *Penaeus chinensis*. *Aquatic Toxicology,* 23**,** 1-10.

Kline, E.R. & Stekoll, M.S. (2000) The role of calcium and sodium in toxicity of an effluent to mysid shrimp (*Mysidopsis bahia*). *Environmental Toxicology and Chemistry: An International Journal,* 19**,** 234-241.

Kuhn, D.D., Smith, S.A., Boardman, G.D., Angier, M.W., Marsh, L. & Flick Jr, G.J. (2010) Chronic toxicity of nitrate to Pacific white shrimp, *Litopenaeus vannamei*: impacts on survival, growth, antennae length, and pathology. *Aquaculture,* 309**,** 109-114.

Kültz, D. (2015) Physiological mechanisms used by fish to cope with salinity stress. *Journal of Experimental Biology,* 218**,** 1907-1914.

Laramore, S., Laramore, C.R. & Scarpa, J. (2001) Effect of low salinity on growth and survival of postlarvae and juvenile *Litopenaeus vannamei*. *Journal of the World Aquaculture Society,* 32**,** 385-392.

Lester, L.J. & Pante, M.J.R. (1992) Penaeid temperature and salinity responses. *Developments in Aquaculture and Fisheries Science,* 23**,** 515-534.

Li, F. & Xiang, J. (2013) Recent advances in researches on the innate immunity of shrimp in China. *Developmental & Comparative Immunology,* 39**,** 11-26.

Lightner, D.V., Redman, R., Arce, S. & Moss, S.M. (2009) Specific pathogen-free shrimp stocks in shrimp farming facilities as a novel method for disease control in crustaceans In *Shellfish safety and quality*, pp. 384-424. Elsevier.

Lima, A.G., McNamara, J.C. & Terra, W.R. (1997) Regulation of hemolymph osmolytes and gill Na+/K+-ATPase activities during acclimation to saline media in the freshwater shrimp *Macrobrachium olfersii* (Wiegmann, 1836)(Decapoda, Palaemonidae). *Journal of Experimental Marine Biology and Ecology,* 215**,** 81-91.

Lin, Y.C. & Chen, J.C. (2001) Acute toxicity of ammonia on *Litopenaeus vannamei* Boone juveniles at different salinity levels. *Journal of Experimental Marine Biology and Ecology,* 259**,** 109-119.

Lin, Y.C. & Chen, J.C. (2003) Acute toxicity of nitrite on *Litopenaeus vannamei* (Boone) juveniles at different salinity levels. *Aquaculture,* 224**,** 193-201.

Lucu, Č. (1990) Ionic regulatory mechanisms in crustacean gill epithelia. *Comparative Biochemistry and Physiology Part A: Physiology,* 97**,** 297-306.

Maier, P. & Quagrainie, K. (2020) A bioeconomic model of indoor marine shrimp *Litopenaeus Vannamei* farms with low-cost salt mixtures In *Aquaculture America 2020 Conference proceedings*, pp. 413Honolulu, Hawaii, USA.

Mair, J.M. (1980) Salinity and water-type preferences of four species of postlarval shrimp (*Penaeus*) from west Mexico. *Journal of Experimental Marine Biology and Ecology,* 45**,** 69-82.

McCormick, J., Stokes, G., Jensen, K. & Leino, R. (1987) Fish blood osmolality, gill histology and oocyte atresia as early warning acid stress indicators. *Annales de la Societe Royale Zoologique de Belgique (Belgium). Annalen van de Koninklijke Belgische Vereniging voor Dierkunde. v. 117 (suppl. 1).*

McFarland, W. & Lee, B.D. (1963) Osmotic and ionic concentrations of *penaeidean* shrimps of the Texas coast. *Bulletin of Marine Science,* 13**,** 391-417.

McGraw, W., Davis, D., Teichert-Coddington, D. & Rouse, D. (2002) Acclimation of *Litopenaeus vannamei* postlarvae to low salinity: influence of age, salinity endpoint, and rate of salinity reduction. *Journal of the World Aquaculture Society,* 33**,** 78-84.

McGraw, W.J. & Scarpa, J. (2004) Mortality of freshwater-acclimated *Litopenaeus vannamei* associated with acclimation rate, habituation period, and ionic challenge. *Aquaculture,* 236**,** 285-296.

McNevin, A.A., Boyd, C.E., Silapajarn, O. & Silapajarn, K. (2004) Ionic supplementation of pond waters for inland culture of marine shrimp. *Journal of the World Aquaculture Society,* 35**,** 460-467.

McNevin, A.A., Boyd, C.E., Silapajarn, O. & Silapajarn, K. (2004) Ionic supplementation of pond waters for inland culture of marine shrimp. *Journal of the World Aquaculture Society,* 35**,** 460-467.

Morris, S. (2001) Neuroendocrine regulation of osmoregulation and the evolution of air-breathing in decapod crustaceans. *Journal of Experimental Biology,* 204**,** 979-989.

Moss, D.R., Arce, S.M., Otoshi, C.A., Doyle, R.W. & Moss, S.M. (2007) Effects of inbreeding on survival and growth of Pacific white shrimp *Penaeus (Litopenaeus) vannamei*. *Aquaculture,* 272**,** S30-S37.

Neufeld, G.J., Holliday, C.W. & Pritchard, J.B. (1980) Salinity adaption of gill Na, K-ATPase in the blue crab, *Callinectes sapidus*. *Journal of Experimental Zoology,* 211**,** 215-224.

Ogle, J.T., Beaugez, K. & Lotz, J.M. (1992) Effects of salinity on survival and growth of postlarval *Penaeus vannamei*. *Gulf and Caribbean Research,* 8**,** 415-421.

Páez-Osuna, F. (2001) The environmental impact of shrimp aquaculture: causes, effects, and mitigating alternatives. *Environmental Management,* 28**,** 131-140.

Pan, L.-Q., Luan, Z.-H. & Jin, C.-X. (2006) Effects of Na+/K+ and Mg2+/Ca2+ ratios in saline groundwaters on Na+–K+-ATPase activity, survival and growth of *Marsupenaeus japonicus* postlarvae. *Aquaculture,* 261**,** 1396-1402.

Panini, R.L., Freitas, L.E.L., Guimarães, A.M., Rios, C., da Silva, M.F.O., Vieira, F.N., Fracalossi, D.M., Samuels, R.I., Prudêncio, E.S. & Silva, C.P. (2017) Potential use of mealworms as an alternative protein source for Pacific white shrimp: Digestibility and performance. *Aquaculture,* 473**,** 115-120.

Pantastico, J.B. & Oliveros, E.N. (1980) Acclimation of Penaeus monodon postlarvae to fresh water. *Fisheries Research Journal of the Philippines,* 5**,** 33-38.

Parmenter, K., Bisesi Jr, J., Young, S., Klaine, S., Atwood, H., Browdy, C. & Tomasso, J. (2009) Culture of Pacific white shrimp *Litopenaeus vannamei* in a mixed-ion solution. *North American Journal of Aquaculture,* 71**,** 134-137.

Partridge, G., Lymbery, A. & George, R. (2008) Finfish mariculture in inland Australia: a review of potential water sources, species, and production systems. *Journal of the World Aquaculture Society,* 39**,** 291-310.

Pequeux, A. (1995) Osmotic regulation in crustaceans. *Journal of Crustacean Biology,* 15**,** 1-60.

Pillard, D.A., DuFresne, D.L., Caudle, D.D., Tietge, J.E. & Evans, J.M. (2000) Predicting the toxicity of major ions in seawater to mysid shrimp (*Mysidopsis bahia*), sheepshead minnow (*Cyprinodon variegatus*), and inland silverside minnow (*Menidia beryllina*). *Environmental Toxicology and Chemistry: An International Journal,* 19**,** 183-191.

Pressley, T.A. & Graves, J.S. (1983) Increased amino acid oxidation in the gills of blue crabs acclimated to dilute seawater. *Journal of Experimental Zoology,* 226**,** 45-51.

Proverbio, T., Zanders, I.P., Marín, R., Rodríguez, J. & Proverbio, F. (1990) Effects of Na+ and/or K+ on the Mg2+-dependent ATPase activities in shrimp (*Macrobrachium amazonicum*) gill homogenates. *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry,* 97**,** 383-390.

Quagrainie, K. (2015) *Profitability of Indoor Production of Pacific White Shrimp: Litopenaeus Vannamei, a Case Study of the Indiana Industry,* Purdue University Extension Fact Sheet.

Rafiee, G., Tavabe, K.R., Frinsko, M. & Daniels, H. (2015) Effects of various sodium adsorption ratio (SAR) mediums on larval performance of the freshwater prawn *Macrobrachium rosenbergii* (de Man). *Aquaculture Research,* 46**,** 725-735.

Ramírez-Rochín, J., Frías-Espericueta, M.G., Fierro-Sañudo, J.F., Alarcón-Silvas, S.G., Fregoso-López, M.G. & Páez-Osuna, F. (2017) Acute toxicity of nitrite on white shrimp *Litopenaeus vannamei* (Boone) juveniles in low-salinity water. *Aquaculture Research,* 48**,** 2337-2343.

Ray, A.J. (2015) Indoor-raised shrimp find potential market in Kentucky State University test In *The Global Aquaculture Advocate*.

Ray, A.J., Drury, T.H. & Cecil, A. (2017) Comparing clear-water RAS and biofloc systems: Shrimp (*Litopenaeus vannamei*) production, water quality, and biofloc nutritional contributions estimated using stable isotopes. *Aquacultural Engineering,* 77**,** 9-14.

Rocha, J.L., Guerrelhas, A.C., Teixeira, A.K., Farias, F.A. & Teixeira, A.P. (2010) SPF shrimp breeding in Brazil--genetic, phenotypic trends after generation of selection. *Global aquaculture advocate*.

Romano, N. & Zeng, C. (2011) Importance of balanced Na+/K+ ratios for blue swimmer crabs, *Portunus pelagicus*, to cope with elevated ammonia-N and differences between in vitro and in vivo gill Na+/K+-ATPase responses. *Aquaculture,* 318**,** 154-161.

Rosas, C., Martinez, E., Gaxiola, G., Brito, R., Sanchez, A. & Soto, L.A. (1999) The effect of dissolved oxygen and salinity on oxygen consumption, ammonia excretion and osmotic pressure of *Penaeus setiferus* (*Linnaeus*) juveniles. *Journal of Experimental Marine Biology and Ecology,* 234**,** 41-57.

Roy, L.A. & Davis, D.A. (2010) Requirements for the culture of the Pacific white shrimp, *Litopenaeus vannamei*, reared in low salinity waters: water modification and nutritional strategies for improving production In *Avances en Nutrición Acuicola X-Memorias del X Simposio Internacional de Nutrición Acuicola*, pp. 8-10.

Roy, L.A., Davis, D.A. & Saoud, I.P. (2006) Effects of lecithin and cholesterol supplementation to practical diets for *Litopenaeus vannamei* reared in low salinity waters. *Aquaculture,* 257**,** 446-452.

Roy, L.A., Davis, D.A. & Whitis, G.N. (2009b) Pond-to-pond variability in post-larval shrimp, *Litopenaeus vannamei*, survival and growth in inland low-salinity waters of west Alabama. *Aquaculture Research,* 40**,** 1823-1829.

Roy, L.A., Davis, D.A., Nguyen, T.N. & Saoud, I.P. (2009) Supplementation of chelated magnesium to diets of the pacific white shrimp, *Litopenaeus vannamei,* reared in low-salinity waters of west Alabama. *Journal of the World Aquaculture Society,* 40**,** 248-254.

Roy, L.A., Davis, D.A., Saoud, I.P. & Henry, R.P. (2007) Branchial carbonic anhydrase activity and ninhydrin positive substances in the Pacific white shrimp, *Litopenaeus vannamei*, acclimated to low and high salinities. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology,* 147**,** 404-411.

Roy, L.A., Davis, D.A., Saoud, I.P. & Henry, R.P. (2007) Effects of varying levels of aqueous potassium and magnesium on survival, growth, and respiration of the Pacific white shrimp, *Litopenaeus vannamei*, reared in low salinity waters. *Aquaculture,* 262**,** 461-469.

Roy, L.A., Davis, D.A., Saoud, I.P., Boyd, C.A., Pine, H.J. & Boyd, C.E. (2010) Shrimp culture in inland low salinity waters. *Reviews in Aquaculture,* 2**,** 191-208.

Samocha, T.M., Lawrence, A.L. & Pooser, D. (1998) Growth and survival of juvenile *Penaeus vannamei* in low salinity water in a semi-closed recirculating system. *Israeli Journal of Aquaculture/Bamidgeh,* 50**,** 55-59.

Saoud, I.P., Davis, D.A. & Rouse, D.B. (2003) Suitability studies of inland well waters for *Litopenaeus vannamei* culture. *Aquaculture,* 217**,** 373-383.

Siebers, D., Lucu, C., Sperling, K.-R. & Eberlein, K. (1972) Kinetics of osmoregulation in the crab *Carcinus maenas*. *Marine Biology,* 17**,** 291-303.

Solorzano, L. (1969) Determination of ammonia in natural waters by the phenolhypochlorite method. *Limnology and Oceanography,* 14**,** 799-801.

Sowers, A.D., Gatlin, D.M., Young, S.P., Isely, J.J., Browdy, C.L. & Tomasso, J.R. (2005) Responses of *Litopenaeus vannamei* (Boone) in water containing low concentrations of total dissolved solids. *Aquaculture Research,* 36**,** 819-823.

Sowers, A.D., Tomasso Jr, J.R., Browdy, C.L. & Atwood, H.L. (2006) Production characteristics of *Litopenaeus vannamei* in low-salinity water augmented with mixed salts. *Journal of the World Aquaculture Society,* 37**,** 214-217.

Spotte, S. (1979) *Fish and Invertebrate Culture: Water Managment in Closed Systems, 2nd Edition.,* Wiley, New York.

Steel, R.G. & Torrie, J.H. (1980) *Principles and Procedures of Statistics,* McGraw-Hill Book Co. Inc., New York.

Stevenson, N. (1996) Disused shrimp ponds: Options for redevelopment of mangroves. *Coastal Management,* 25**,** 423-435.

Sturmer, L., Samocha, T.M. & Lawrence, A.L. (1992) Intensification of penaeid nursery systems. *Developments in aquaculture and fisheries science,* 23**,** 321-344.

Tierney, T., Fleckenstein, L.J. & Ray, A.J. (2020) Using alternative low cost artificial sea salt mixtures for intensive shrimp (*Litopenaeus vannamei*) production. In *Aquaculture America 2020 Conference proceedings*, pp. 662Honolulu, Hawaii, USA.

Towle, D. (1981) Role of Na++ K+-ATPase in ionic regulation by marine and estuarine animals. *Marine Biology Letters,* 2**,** 107-122.

Towle, D.W. & Weihrauch, D. (2001) Osmoregulation by gills of euryhaline crabs: molecular analysis of transporters. *American Zoologist,* 41**,** 770-780.

Towle, D.W. (1984) Membrane-bound ATPases in arthropod ion-transporting tissues. *American Zoologist,* 24**,** 177-185.

Towle, D.W., Palmer, G.E. & Harris III, J.L. (1976) Role of gill Na++ K+-dependent ATPase in acclimation of blue crabs (*Callinectes sapidus*) to low salinity. *Journal of Experimental Zoology,* 196**,** 315-321.

Tsuzum, M.Y., Cavalli, R.O. & Bianchini, A. (2000) The effects of temperature, age, and acclimation to salinity on the survival of *Farfantepenaeus paulensis* postlarvae. *Journal of the World Aquaculture Society,* 31**,** 459-468.

Valencia-Castañeda, G., Frías-Espericueta, M.G., Vanegas-Pérez, R.C., Chávez-Sánchez, M.C. & Páez-Osuna, F. (2019) Toxicity of ammonia, nitrite and nitrate to *Litopenaeus vannamei* juveniles in low-salinity water in single and ternary exposure experiments and their environmental implications. *Environmental Toxicology and Pharmacology,* 70.

Valiela, I. (2006) *Global Coastal Change,* John Wiley & Sons, MA, USA.

Van Wyk, P. & Scarpa, J. (1999) Water quality requirements and management. *Farming Marine Shrimp in Recirculating Freshwater Systems***,** 141-162.

Vargas-Albores, F. & Ochoa, J. (1992) Variation of pH, osmolality, sodium and potassium concentrations in the haemolymph of sub-adult blue shrimp (Ps) according to size. *Comparative Biochemistry and Physiology Part A: Physiology,* 102**,** 1-5.

Wilder, M.N., Ikuta, K., Atmomarsono, M., Hatta, T. & Komuro, K. (1998) Changes in osmotic and ionic concentrations in the hemolymph of *Macrobrachium rosenbergii* exposed to varying salinities and correlation to ionic and crystalline composition of the cuticle. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology,* 119**,** 941-950.

Wilder, M.N., Jasmani, S., Jayasankar, V., Kaneko, T., Aida, K., Hatta, T., Nemoto, S. & Wigginton, A. (2009) Hemolymph osmolality, ion concentrations and calcium in the structural organization of the cuticle of the giant freshwater prawn *Macrobrachium rosenbergii:* Changes with the molt cycle. *Aquaculture,* 292**,** 104-110.

Wyban, J., Walsh, W.A. & Godin, D.M. (1995) Temperature effects on growth, feeding rate and feed conversion of the Pacific white shrimp (*Penaeus vannamei*). *Aquaculture,* 138**,** 267-279.

Zacarias, S., Schveitzer, R., Arantes, R., Galasso, H., Pinheiro, I., Espirito Santo, C. & Vinatea, L. (2019) Effect of different concentrations of potassium and magnesium on performance of *Litopenaeus vannamei* postlarvae reared in low-salinity water and a biofloc system. *Journal of Applied Aquaculture,* 31**,** 85-96.