Requirements for the culture of the Pacific white shrimp, *Litopenaeus vannamei*, reared in low salinity waters: water modification and nutritional strategies for improving production

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Abstract

The culture of shrimp utilizing low salinity water (LSW) has become common practice in a number of countries worldwide. The Pacific white shrimp, *Litopenaeus vannamei*, is the preferred shrimp species for culture in LSW, primarily due to its remarkable ability to effectively grow and survive at extreme salinities. In the last decade, significant strides have been made in the understanding of low salinity culture of this particular species. Improved understanding of the physiology of *L. vannamei* has translated into the development of effective culture techniques that have resulted in profits for farmers utilizing LSW. In general, two separate strategies have been employed by researchers to improve growth and survival of *L. vannamei* reared in LSW. These strategies include water modification approaches which alter the low salinity rearing medium to make it more acceptable for production of shrimp and nutritional strategies that focus on modification of diets offered to shrimp, usually with supplements that might theoretically improve osmoregulatory capacity. This article seeks to summarize research findings from both of these approaches and ultimately provide a recommendation to shrimp farmers using LSW as a rearing medium for the culture of *L. vannamei*.

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Introduction

The culture of shrimp in low salinity waters (LSW) is common practice in many countries throughout the world including China, Thailand, Vietnam, Ecuador, Brazil, Mexico, and the United States among others. Due to its remarkable ability to thrive in low salinity environments, the Pacific white shrimp, *L. vannamei*, is the primary candidate of choice for shrimp farmers utilizing LSW. Over the past ten years, significant advances have been made in the understanding of low salinity culture of this particular species. Improved understanding of the physiology of *L. vannamei* has resulted in the development of effective culture techniques and strategies for farmers utilizing LSW.

While *L. vannamei* is capable of tolerating a wide range of salinities (Roy, Davis, Saoud & Henry 2007a), proper acclimation to LSW is the first step in the production process for successfully rearing this species at low salinity. A large number of studies have been devoted to the proper acclimation of post-larval shrimp to LSW prior to stocking. Rate of salinity reduction, salinity endpoint, temperature, and age of post-larvae are all important factors to consider when acclimating shrimp to low salinity (Tsuzuki, Cavalli & Bianchini 2000; Laramore, Laramore & Scarpa 2001; McGraw, Davis, Teichert-Coddington & Rouse 2002, McGraw & Scarpa 2004; Davis, Samocha & Boyd 2004). When shrimp are properly acclimated, farmers and aquaculturists have been quite successful at rearing shrimp using a variety of different LSW sources which have varied greatly both in salinity and ionic profile (Cawthorne, Beard, Davenport & Wickens 1983; Smith & Lawrence 1990; Samocha, Lawrence & Pooser 1998; Davis, Saoud, McGraw & Rouse 2002; Samocha, Hamper, Emberson, Davis, McIntosh, Lawrence & Van Wyk 2002; Atwood, Young, Tomasso & Browdy 2003; McGraw & Scarpa 2003; Gong, Jiang, Lightner, Collins & Brock 2004; McNevin, Boyd, Silapajarn & Silapajarn 2004; Ur-Rahman, Jain, Reddy, Kumar & Raju 2005; Sowers & Tomasso 2006; Araneda, Perez & Gasca-Leyva 2008; Green 2008; Cuvin-Aralar, Lazartigue & Aralar 2009; Parmenter, Bisesi, Young, Klaine, Atwood, Browdy & Tomasso 2009; Roy, Bordinhon, Sookying, Davis, Brown & Whitis 2009b).

Despite the success of many farmers in rearing shrimp in low salinity environments, variable growth and survival among ponds are still being reported on a regular basis (Roy *et al.* 2009a). The problem is compounded by the fact that no LSW is the same with large variations in source, salinity, and ionic profile being reported (Boyd & Thunjai 2003; Saoud, Davis & Rouse 2003). While it is suspected that some of the "mortality" and variability is due to stocking errors, environmental factors, and farm management, there is sufficient evidence to suggest that less than ideal ionic profiles are indeed responsible for much of the observed mortality (Saoud *et al.* 2003; Zhu, Dong, Wang & Huang 2004; Roy, Davis & Saoud 2006; Roy, Davis, Saoud & Henry 2007b). In order to improve growth, survival, and production of shrimp reared in LSW two different strategies have been employed by researchers and farmers. These strategies include water modification approaches which alter the low salinity rearing medium to make it more acceptable for production of shrimp and nutritional strategies that focus on modification of diets offered to shrimp, usually with supplements that might provide an osmoregulatory advantage at low salinity. This article seeks to summarize research findings from these two different approaches.

Water Modification

The sources of LSW used to culture *L. vannamei* can vary greatly. For instance, in west Alabama LSW is obtained from inland low salinity aquifers and pumped into production ponds (Saoud *et al.* 2003; Boyd, Chaney, Boyd & Rouse 2009). In places such as Thailand, a high salinity brine solution is transported inland and diluted with freshwater from ponds (Boyd & Thunjai 2003). In other areas of the world, brackish water derived from estuaries is utilized as the LSW source (Boyd & Thunjai 2003). Irrespective of the source, there is a huge amount of variation in both the salinity and ionic profiles of LSW sources utilized for shrimp production

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(Boyd, Thunjai & Boonyaratpalin 2003, Saoud *et al.* 2003). Thus, no LSW will be the same, with variations in ionic profiles common even among ponds from the same farm that are derived from the same aquifer.

Farmers initially began raising shrimp utilizing low salinity artesian well water in Alabama in 1999. Initial efforts resulted in high mortality and poor growth of post-larval and juvenile shrimp. Further investigation revealed deficiencies in potassium (K) and magnesium (Mg) in inland LSW utilized for shrimp culture (Saoud *et al.* 2003; Davis, Saoud, Boyd & Rouse 2005; Roy *et al.* 2007b). These variations in pond K and Mg levels have resulted in large variations in survival and growth in shrimp raised in different ponds on the same farm (Roy *et al.* 2009a). Deficiencies in K and Mg were corrected by pond supplementation of fertilizers containing sources of K and Mg, including muriate of potash (potassium chloride; 50% K) and K-Mag® (potassium magnesium sulfate, 17.8% K and 10.5% Mg) (McNevin *et al.* 2004). Ionic concentrations in individual ponds can vary from year to year (Table 1), resulting in the need to evaluate pond levels of ions every year prior to stocking. Following application of fertilizers, levels of K and Mg are lost due to soil uptake, shrimp harvest, draining at harvest, seepage, or overflow (Boyd, Boyd & Rouse *2007a*). Throughout the production cycle, large amounts of K and Mg are lost to soil adsorption (Boyd *et al.* 2007a; Boyd, Boyd & Rouse 2007b; Pine 2008) and as a precaution Alabama farmers have ion levels checked once or twice during the production cycle to make sure levels of K and Mg are maintained at high enough concentrations to allow for adequate growth and survival of *L. vannamei*. Fortification of water with K has also been advantageous with a number of other species of shrimp and fish cultured in low salinity environments (Forsberg & Neil 1997; Fielder, Bardsley & Allen 2001; Prangnell & Fotedar 2006; Partridge & Lymberry 2008; Partridge, Lymbery & George 2008).

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Table 1: Variation in water concentrations of selected ions (mg L^{-1}) and production (lbs/acre) of four shrimp production ponds from the same west Alabama shrimp farm in 2006 and 2007 following fertilization with muriate of potash and K-Mag® (adapted from Roy *et al.* 2009a)

2006	**Pond 1**	**Pond 2**	**Pond 3**	**Pond 4**
Potassium	41.2	36.9	34.4	40.3
Magnesium	65.1	62.7	49.2	44.7
Calcium	85.4	103.3	67.0	71.5
Sodium	981.4	891.2	666.3	704.4
Salinity (ppt)	3.7	3.3	2.6	2.4
Production (lbs/acre)	1732	1884	1359	2360

2007	**Pond 1**	**Pond 2**	**Pond 3**	**Pond 4**
Potassium	90.7	70.1	86.7	94.2
Magnesium	46.5	42.5	35.7	40.5
Calcium	99.1	66.6	73.2	87.5
Sodium	2492.5	2650.5	1996.5	1921.5
Salinity (ppt)	4.9	5.3	4.1	4.0
Production (lbs/acre)	4482	1105	4707	1158

The application of fertilizers, containing sources of K and Mg, has dramatically increased growth, survival, and overall production of shrimp in LSW where these ions are deficient. Several studies have focused on the importance of Na:K ratios on survival and growth of *L. vannamei* in LSW. In full strength seawater, the Na:K ratio is approximately 28:1. In K deficient waters, such as those used by farmers to grow shrimp in west Alabama, lowering the Na:K ratio (by increasing water K concentrations by the addition of fertilizers) dramatically increases growth and survival of shrimp reared in LSW. Zhu *et al.* (2004) and Zhu, Dong and Wang (2006) reported that high Na:K levels can have an effect on shrimp growth even at salinities as high as 30 ppt. In a laboratory study conducted with juvenile *L. vannamei* in a recirculating system with artificial LSW (4 ppt) in Alabama, growth, survival, and weight gain, were affected

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by Na:K ratio following seven weeks of culture (Table 2). In this study, poorest growth and survival were observed in the treatment with the highest Na:K ratio. Increased growth of *L. vannamei* raised in intensive systems was observed when ionic ratios more closely resembled those found in full strength seawater (Esparza-Leal, Ponce-Palafox, Valenzuela-Quiňónez, Beltrán & Figueroa 2009). In west Alabama, we recommend that our farmers lower their Na:K ratios to a minimum of 40:1, but preferably the ratio should be closer to 28:1.

Table 2: Final weight (g) and survival (%) of juvenile *L. vannamei* (0.28 g mean initial weight) reared in 4 ppt artificial seawater containing different Na:K (Sodium: Potassium) ratios for seven weeks. Values represent the mean of four replicates. Letters that are different are significantly different (P<0.05) (adapted from Roy *et al.* 2007b)

Na:K ratio	**Final weight (g)**	**Survival (%)**	**Weight gain (%)**
119:1	2.40[c]	23.3[b]	852.8[c]
68:1	2.79[bc]	95.0[a]	1064.8[bc]
48:1	3.18[b]	96.7[a]	1145.5[b]
29:1	3.30[b]	93.3[a]	1208.9[b]
30:1 (4 ppt Crystal Sea Salt)	4.90[a]	93.3[a]	1854.6[a]
PSE[1]	0.211	4.1	63.4
P-value	<0.0001	<0.0001	<0.0001

[1]Pooled Standard Error

There has also been a number of studies evaluating Mg supplementation to LSW used for shrimp production (McNevin *et al.* 2004, Davis *et al.* 2005, Roy *et al.* 2007b, Pine 2008). In a 42-day laboratory study conducted with juvenile *L. vannamei* in a recirculating system with artificial LSW (4 ppt) supplemented with different levels of Mg (10, 20, 40, 80, and 160 mg L^{-1}), survival was significantly lower in the 10 mg L^{-1} compared to the other treatments examined (Roy *et al.* 2007b). In the same study, growth increased with increasing Mg level, however, results were not significantly different. Supplementation of Mg and K to LSW in a four week study with post-larval *L. vannamei* increased survival compared to treatments that did not receive mineral supplements (Davis *et al.* 2005). In west Alabama LSW, we typically recommend our shrimp farmers that they should raise the Mg levels in their low salinity ponds to a minimum of 25% of what the Mg concentration is at seawater diluted to the same salinity as their production ponds. For our west Alabama shrimp farmers, this equates to raising Mg levels to 25-50 mg L^{-1}, depending on salinity and ionic concentration of specific farms and ponds. It would be ideal if Mg levels could be raised to 100% of what the Mg levels are at a given salinity (Davis *et al.* 2004), however, since Mg levels in west Alabama LSW are so low to begin with, the financial cost of raising Mg levels to such an extent with K-Mag® (only 10.5% Mg) can be cost prohibitive.

Dietary Modification

Dietary supplementation of ingredients that might improve the osmoregulatory capacity of shrimp have also been examined by a number of researchers as a potential means of improving growth and survival in low salinity waters (Table 3). Fertilizers utilized by farmers to supplement K and Mg to pond water can be costly, ranging over the last several years from $400 - $800 U.S. dollars per ton for muriate of potash and K-Mag®. Depending on the ion profile and size of each pond as well as the number of ponds on a given farm, fertilization to correct ionic deficiencies can be a large financial cost for some inland commercial shrimp operations that rely on low salinity artesian well water. Inclusion of dietary supplements such as K and Mg in the feed, if successful, could provide a considerable cost-savings for inland shrimp operations.

Table 3: Studies evaluating different dietary supplements to improve growth and survival of shrimp reared in low salinity waters

Dietary Supplement	**Reference**
Potassium	Gong *et al.* 2004, Roy *et al.* 2007c, Saoud *et al.* 2007
Magnesium	Gong *et al.* 2004, Cheng *et al.* 2005, Roy *et al.* 2007c, Roy *et al.* 2009c
Sodium Chloride	Gong *et al.* 2004, Roy *et al.* 2007c
Betaine	Saoud & Davis 2005
Arginine	Saoud *et al.* 2007
Threonine	Huai *et al.* 2009
Cholesterol	Gong *et al.* 2004, Roy *et al.* 2006
Lecithin	Roy *et al.* 2006
HUFAs	Palacios *et al.* 2004, González-Félix *et al.* 2009
Carbohydrates	Wang *et al.* 2004
Astaxanthin	Flores *et al.* 2007
Prebiotics	Li *et al.* 2010

Adequate dietary minerals are necessary for normal growth and survival of shrimp (Davis & Lawrence 1997). Minerals that play a role in the osmoregulatory process, such as K, Mg, sodium (Na) and chloride (Cl) have been suggested as dietary supplements for *L. vannamei* reared in LSW. The basic premise behind this strategy is that a lack of minerals, such as K, in the water column for absorption at the gill might be offset by increased availability, and thus absorption in the digestive tract of the shrimp. This strategy has also been employed in juvenile red drum (*Sciaenops ocellatus*) and chinook salmon (*Oncorhynchus tshawytscha*) (Zaugg, Roley, Prentice, Gores & Waknitz 1983; Holsapple 1990; Gatlin, MacKenzie, Craig & Neil 1992). Gong *et al.* (2004) reported a benefit of a diet supplemented with K, Mg, Na, cholesterol, and phospholipids offered to *L. vannamei* reared in LSW in Arizona, however, the diet contained all five supplements and it was unclear which or what combination provided the observed benefit. Shiau and Hseih (2001) reported a beneficial effect of dietary K supplementation for *Penaeus monodon* reared in brackish water. In Alabama, a number of dietary supplementation studies were conducted with chloride salts and chelated minerals (Roy, Davis, Saoud & Henry 2007c; Saoud, Roy & Davis 2007). Dietary supplementation of sodium chloride salt at levels of 1% and 2% did not increase growth or survival of shrimp reared in 4 ppt LSW (Roy *et al.* 2007c). While chelated K supplemented at 1% of the diet was promising in reconstituted LSW (4 ppt) in the laboratory (Roy *et al.* 2007c), when supplemented at the same level in a tank trial conducted at a low salinity shrimp farm in west Alabama no benefit was observed (Saoud *et al.* 2007). Likewise supplementation of magnesium in excess of the requirement for shrimp did not provide any benefit in laboratory trials (4 ppt) using magnesium chloride (150 mg kg^{-1}; 300 mg kg^{-1}) or farm trials using magnesium chelates (0.15%, 0.30%, 0.60%) (Roy *et al.* 2007c; Roy, Davis, Nguyen & Saoud 2009c). A dietary magnesium requirement for shrimp reared in LSW was reported by Cheng, Hu, Liu, Zheng and Qi (2005). Unfortunately, as already previously discussed, shrimp are reared in a wide range of different LSW and the requirement reported by Cheng *et al.* (2005) is most likely valid only for the salinity and ionic composition of the water they utilized in their study. In our experience in west Alabama supplementation in excess of the dietary requirement of Mg provided no advantage to shrimp survival, growth, or osmoregulatory capacity, especially when aqueous deficiencies were accounted for by fertilizers containing Mg.

Due to the role that free amino acids play in shrimp osmoregulation (Mantel & Farmer 1983; Pequeux 1995; Roy *et al.* 2007a), dietary supplementation of amino acids has been suggested to improve osmoregulatory capacity of shrimp reared in low salinity. Saoud and Davis (2005) reported that dietary betaine supplementation (0.4%) to juvenile shrimp reared in 0.5% salinity did not improve growth after 8 weeks. Saoud *et al.* (2007) determined that 0.41% arginine did not improve growth or survival of juvenile *L. vannamei* reared in LSW after a nine week farm trial. At the levels tested, supplementation of amino acids to shrimp reared in LSW did not improve growth or survival. A dietary requirement for threonine for *Litopenaeus vannamei* has also been reported for LSW (Huai, Tian, Liu, Xu, Liang & Yang 2009). Further studies are needed to investigate whether other amino acids might be beneficial to *L. vannamei* reared in LSW, particularly in the post-larval stages when shrimp are initially stocked into production ponds.

Phospholipids and cholesterol are essential for normal growth of crustaceans. Dietary deficiencies in cholesterol have been linked to mortality in crustaceans (D'Abramo, Wright, Wright, Bordner & Conklin 1985; Teshima, Kanazawa & Kakuta1986). Cholesterol plays a role as a necessary precursor for steroid and molting hormones (Teshima, Ishikawa, Koshio & Kanazawa 1997) and phospholipids are involved in gill membrane function, lipid metabolism, and in the incorporation of cholesterol into hemolymph proteins (Teshima 1986; Teshima *et al.* 1986). Due to their role in lipid mobilization and storage, supplementation in excess of the dietary requirement has been suggested as a potential strategy to increase growth and survival of shrimp in LSW (Gong *et al.* 2004). Roy *et al.* (2006) investigated supplementation of cholesterol and lecithin (as a phospholipid source) by conducting laboratory trials with reconstituted seawater (4 ppt) and farm trials conducted at two low salinity shrimp farms. Results from this study revealed no increase in growth and survival of *L. vannamei* when cholesterol and lecithin were supplemented above the dietary requirement.

Dietary supplementation of highly unsaturated fatty acids and/or shifting the n3/n6 ratio of the diet has also been suggested as a strategy to increase growth and survival of shrimp reared in LSW. It has been established that HUFA can have an effect on osmoregulatory mechanisms in post-larval and juvenile shrimp (Palacios, Bonilla, Pérez, Racotta & Civera 2004). Shrimp offered diets with high levels of HUFA have demonstrated an increased tolerance to acute salinity challenges (Rees, Cure, Piyatiratitivorakul, Sorgeloos & Menasveta 1994; Wouters, Vanhauwaert, Naessens, Pedrazolli & Lavens 1997; Palacios *et al.* 2004). This is primarily a direct result of changes in gill fatty acid composition and a larger gill surface area, which have a profound influence on osmoregulatory mechanisms (Palacios *et al.* 2004; Hurtado, Racotta, Arjona, Hernández-Rodríguez, Goytortúa, Civera & Palacios 2006; Hurtado, Racotta, Civera, Ibarra, Hernández-Rodríguez & Palacios 2007). Palacios *et al.* (2004) observed increased survival of post-larvae that were fed diets supplemented with EPA and DHA following a stress test. In another study conducted in LSW (4.1 ppt), however, no differences were observed in juvenile *L. vannamei* offered diets supplemented with DHA and AA and different n3/n6 ratios following six weeks of culture (González-Félix, Perez-Velazquez, Quintero-Alvarez & Davis 2009). While there appears to be some promise in this strategy, further studies are necessary, particularly on-farm studies conducted in LSW that track the growth and survival of post-larval and juvenile shrimp offered diets supplemented with high levels of HUFAs over entire production periods.

Other dietary supplements tested to improve the growth of *L. vannamei* reared in LSW include dietary carbohydrates and astaxanthin. Higher dietary carbohydrates have been suggested as a means for shrimp to offset the higher energetic cost of osmoregulation in LSW. Wang, Ma, Dong, and Cao (2004) evaluated the effect of dietary carbohydrate at a range of low salinities and found that specific growth rate of shrimp was higher at low salinity in the shrimp offered diets containing higher carbohydrate levels. This is in contrast to a previous study, which reported that growth rate was optimum when shrimp were fed diets containing low dietary carbohydrate levels at low salinity (Rosas, Cuzon, Taboada, Pascual, Gaxiola & Van Wormhoudt 2001a. Another study (Rosas, Cuzon, Gaxiola, Le Priol, Pascual, Rossignyol, Contreras, Sanchez & Wan Wormhoudt 2001b) also reported that growth rate was higher when shrimp were offered a low carbohydrate diet at low salinity than a low carbohydrate diet at high salinity.

Dietary astaxanthin, a carotenoid pigment, has been suggested as an effective means to reduce stress in shrimp (Darachai, Piyatiratitivorakul & Menasveta 1999; Chien, Pan & Hunger 2003). Flores, Diaz, Medina, Re and Licea (2007) reported that 80 mg kg^{-1} astaxanthin enhanced daily growth coefficient as well as hemolymph concentrations of glucose, lactate, and haemocyanin compared to the other diets tested in the 6 week study conducted in 3 ppt LSW (0, 40, and 150 mg kg^{-1} astaxanthin). Increased survival of *L. vannamei* when offered dietary supplementation

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of a prebiotic (GroBiotic®, International Ingredient Corporation, St. Louis, MO, USA) for *L. vannamei* cultured in LSW (2 ppt) has also been reported (Li, Wang, Murthy, Gatlin III, Castille & Lawrence 2010).

Conclusion

Our experience in west Alabama suggests that water modification approaches that improve pond ionic profiles, specifically by adding K and Mg, are more effective at improving growth, survival, and production of *Litopenaeus vannamei* than dietary modification strategies. While several studies have observed modest benefits using dietary supplementation of various minerals, amino acids, and other ingredients in excess of the dietary requirement, optimum growth and survival are rarely achieved if water profiles are not adjusted. Further studies are needed on diets that contain combinations of different supplements as few studies have evaluated multiple ingredients and their effects on growth and survival of *L. vannamei* reared in LSW. Based on the last decade of research and farm production of *L. vannamei* in LSW of west Alabama we have found that modification of the pond water with fertilizers containing K and Mg is the most effective solution for our farmers. Farmers should adjust Na:K ratios in their ponds to closely reflect the ratio found in seawater (28:1) to achieve maximum growth, survival, and production of shrimp reared in LSW. In regards to magnesium, levels in pond water should equal at least 25% of the magnesium level in seawater diluted to the same salinity.

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