

Shrimp culture in inland low salinity waters

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Abstract

Inland aquaculture of shrimp in low salinity waters is widespread in many regions worldwide. Owing to its ability to grow and survive in low salinity environments the Pacific white shrimp, (*Litopenaeus vannamei* Boone) has become the candidate of choice for low salinity culture. Remediation techniques have been developed to improve the osmoregulatory capacity of shrimp reared in low salinity waters. These techniques have evaluated water modification strategies that improve low salinity waters used for production by adding potassium and magnesium fertilizers and dietary approaches that involve modification of the feeds offered to shrimp with supplements that might improve osmoregulatory capacity. Based on our own experience as well as what we found predominantly in the literature, it appears that modification of the rearing medium with potassium and magnesium fertilizers is more effective than dietary modification techniques at improving the growth, survival and osmoregulatory capacity of shrimp reared in low salinity waters.

Key words: *Litopenaeus vannamei*, low salinity water, magnesium, osmoregulation, potassium.

Introduction

The culture of shrimp and other fish and crustaceans using low salinity water is a trend that continues to grow throughout the world. In 2006, aquaculture accounted for 47% of the world's fish food supply (FAO 2009). Most fish, crustacean and mollusc aquaculture production (61%) occurs in inland waters. In the same year, brackish water production accounted for 8%. In most locations throughout the world the primary candidate of choice for shrimp culture in low salinity water is the Pacific white shrimp, *Litopenaeus vannamei*, which is native to the Pacific coast from Northern Peru to Mexico. In 2007, *L. vannamei* production worldwide was close to 2.3 million tonnes, which is roughly 70% of total shrimp and prawn production worldwide (FAO 2007). All indications are that the production of *L. vannamei* will continue to expand, particularly in countries such as China, Vietnam and Thailand.

The Pacific white shrimp is a euryhaline species that can tolerate a wide range of salinities (0.5–45 g L⁻¹) (Menz & Blake 1980; Bray *et al.* 1994). There are even some indications that it is capable of growing in waters of less than 0.5 g L⁻¹. (Araneda *et al.* 2008; Cuvin-Aralar

et al. 2009). The remarkable ability of *L. vannamei* to grow in less than ideal environments has made it the species of choice for culturing in low salinity water. For a more comprehensive review of penaeid biology and its relevance to aquaculture see reviews by Dall *et al.* (1990), Rothlisberg (1998) or Alday-Sanz (2010).

Water sources

Saline soils occur in arid regions in more than 100 countries and surface waters and groundwaters in such areas often have more than 1 g L⁻¹ salinity (Keren 2000). Saline groundwater may also occur in regions of greater rainfall as a result of salt deposits, connate water of marine origin and saltwater intrusion in coastal areas (Cook 1997; Boyd *et al.* 2009). Most efforts to culture marine shrimp in inland ponds have focused on the use of saline groundwater. For instance, in the USA saline groundwater can be found beneath two thirds of the country (Feth 1970). Smith and Lawrence (1990) demonstrated that marine shrimp could be successfully cultured in ponds supplied with saline groundwater and freshwater to maintain a salinity of 25 g L⁻¹. Inland culture of marine

shrimp using saline well water with salinities of 1–15 g L⁻¹ has become rather common in the USA with farms in Florida, Alabama, Texas, Arizona and other states. Some of these farms have been in production for more than 10 years.

A number of farmers in Ecuador initiated projects to culture marine shrimp in saline well water at sites more than 100 km inland near Palestina in the Guayas Province (Boyd 2002). Although good survival and growth of shrimp was possible, most of these facilities apparently are no longer in operation. There have been various reports of shrimp culture in saline well water in Brazil (Nunes & Lopez 2001) and in other South American nations, but no documentation of the current status of the use of well water for shrimp culture in these countries could be located.

In arid agricultural areas, irrigation may cause the water table to rise to within 1 m or less of the land surface. Because the evaporation rate exceeds precipitation, the shallow groundwater is saline. A pond excavated below the shallow water table will fill with groundwater to the level of the piezometric surface. In the early 1990s, a few farmers in northeastern Thailand successfully cultured black tiger prawns, *Penaeus monodon*, in excavated ponds where the groundwater contained 5–10 g L⁻¹ salinity (Yont Musig, pers. comm. 1990). However, the practice was not adopted in the region.

There are extensive areas in the delta and floodplain of the Yellow River in Shandong Province, China, where cultivation of traditional agricultural crops was not possible because of shallow, saline groundwater. One author (Claude Boyd) recently visited an area near Dong Ying, China, where approximately 3000 ha of land has been reclaimed for productive use by digging ponds and placing the excavated earth around the ponds and above the original land surface (Fig. 1). The completed effort consists of 40% ponds, 40% elevated land area for crops

and 20% roads and canals. Salt leaches from the excavated soil between the ponds, and after 2–3 years this soil can be used for cultivating crops. The ponds fill to a depth of approximately 1.5 m with saline groundwater of 2–3 g L⁻¹ salinity. Freshwater from the irrigation system may be added to ponds to dilute the salinity if necessary. Tilapia were originally cultured in the ponds, but because of its higher value *L. vannamei* is now the main species (S. Dong, pers. comm. 2010).

There are projects in the Murry-Darling River Basin of southern Australia and in the wheat belt of Western Australia in which saline groundwater is pumped with air-lift devices from a network of bore holes to lower the water table and reclaim the land for agriculture. The saline water is placed in large, shallow ponds for evaporation and salt recovery. Considerable research has been conducted on the suitability of this water for commercial aquaculture. A review of these studies (Partridge *et al.* 2008) suggests that the waters have considerable potential for the culture of several marine finfish species. However, the temperature climate in most of the salinity-affected areas of Australia is not considered conducive to marine shrimp culture (Partridge *et al.* 2008).

In Thailand, a different approach has been used to obtain low salinity water for inland shrimp culture. Brine solution from coastal, seawater evaporation ponds is mixed with freshwater. The brine solution is obtained from evaporation ponds before the salinity exceeds 250 g L⁻¹ and sodium chloride precipitation occurs (Limsuwan *et al.* 2002). Salt from seawater evaporation ponds may also be added to ponds in Thailand to increase salinity. Pond waters there typically have 2–5 g L⁻¹ salinity, and excellent survival and growth of shrimp is usually achieved (Boyd *et al.* 2002).

There has been an effort inland in the Jiangsu, Shanghai and Zhejiang Provinces of China to culture marine

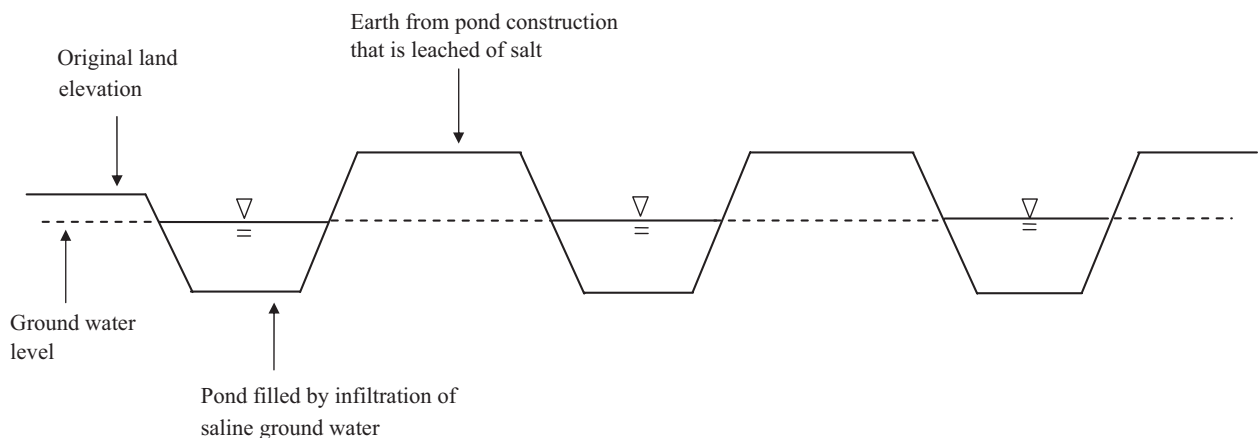


Figure 1 Procedure for reclaiming saline areas of the Yellow River floodplain in Shandong Province, China.

shrimp in inland waters with less than 1 g L^{-1} salinity (Boyd 2002; Boyd & Thunjai 2003). However, growth and production of shrimp has been highly variable and often low, and some producers in these provinces add crushed rock salt to the ponds to increase the salinity.

Location of saline groundwater

In many countries, useful information on the depth and specific conductivity or chloride concentration of groundwater is available from records that are based on well driller's logs. These data can be examined to identify areas where saline groundwater may be found. For example, Boyd *et al.* (2009) examined 2527 well records of the Geological Survey of Alabama and the United States Geological Survey. Two hundred and thirty-eight wells with water chloride concentrations of 125 mg L^{-1} or more were found in 11 counties of central and west-central Alabama. The chloride concentration varied from 136 to $94\,000 \text{ mg L}^{-1}$. Saline groundwater in Washington, Choctaw and Clarke Counties was not considered ideal for inland shrimp culture because of great depth and an extremely high chloride concentration. In Dallas, Hale, Greene, Marengo, Wilcox and Sumter Counties, water from wells supplied by the Eutaw, Gordo, McShan and undifferentiated Eutaw–McShan formations had an average and standard deviation of $1238 \pm 615 \text{ mg L}^{-1}$ chloride. Based on the frequency of saline-water wells, the number of wells containing water with chloride concentrations above 700 mg L^{-1} (approximately 2 g L^{-1} salinity) and relatively shallow well depths, it was concluded that Hale, Greene, Marengo and Sumter Counties have the greatest potential for saline-water aquaculture in Alabama. Contour maps for chloride concentrations and depths of wells in these four counties were prepared to better delineate this potential water source for aquaculture. There were, however, areas in Lowndes, Tuscaloosa and Wilcox Counties with saline groundwater suitable for inland aquaculture (Boyd *et al.* 2009).

Ionic composition of the water

Interest in inland, low-salinity shrimp culture appears to have been stimulated mainly by the success of this practice in Thailand where pond waters were made saline by the addition of brine solution. Shrimp producers from Thailand encouraged Ecuadorian producers to use saline groundwater for shrimp culture. Initial attempts to produce shrimp in low-salinity groundwater in Ecuador resulted in low survival of postlarvae. Analysis of the water revealed a low potassium concentration and supplementation with potassium resulted in improved survival (Boyd *et al.* 2002). Similar observations have been made

in the USA (Saoud *et al.* 2003; McNevin *et al.* 2004), Australia (Partridge *et al.* 2008) and China (S. Dong, pers. comm. 2010).

A survey of the ionic composition of waters for inland, low-salinity culture of marine shrimp revealed that the proportions of the major ions were similar to those in normal seawater in Thailand where brine solution was used to raise salinity. Naturally, saline surface water and groundwater often have different ionic proportionalities than those in seawater diluted to the same salinity because of differential precipitation of salts as water evaporates and removal of ions by reactions with soil and other geological material (Gong *et al.* 2004). Thus, it is not surprising that ponds filled with saline groundwater in China, Ecuador and the USA are quite different in ionic proportions when compared with seawater. Saline water tended to be low in potassium, magnesium and sulfate relative to the concentrations expected in seawater diluted to the same salinity (Boyd *et al.* 2002; Boyd & Thunjai 2003; Saoud *et al.* 2003).

Marine shrimp are native in coastal waters in which the proportionality of major anions and cations resemble those of normal seawater. *Litopenaeus vannamei* can survive and grow well at salinities as low as 1 g L^{-1} , but the requirements for individual ions are not known exactly. However, practical experience has provided enough information about this issue to allow adequate culture conditions to be maintained and more exact information is sought through research.

The concentrations of major ions necessary to be equivalent to those in seawater diluted to the same salinity may be estimated using the equation presented by Boyd and Thunjai (2003) as follows:

$$SEC_x = (S_p)(R_x),$$

where SEC_x is the seawater equivalent concentration of ion x ; S_p is the salinity of pond water; R_x is the ratio of the concentration of ion x in seawater to the salinity of normal seawater. Values for R_x are provided in Table 1.

Table 1 Factors used to estimate acceptable concentrations of individual ions for inland shrimp culture from the salinity

Ion	Factor†
Calcium	11.6
Magnesium	39.1
Potassium	10.7
Sodium	304.5
Bicarbonate‡	–
Chloride	551.0
Sulfate	78.3

†Example: $\text{Na} (\text{mg L}^{-1}) = 304.5 (\text{salinity in } \text{g L}^{-1})$.

‡Should not be below 75 mg L^{-1} total alkalinity, which is equivalent to 92 mg L^{-1} bicarbonate.

Table 2 Concentrations of salinity, total alkalinity and major ions in well water used to supply ponds at an inland shrimp farm in Alabama and the concentrations of those variables in pond water at this farm in late April before potassium and magnesium salts were applied

Variable	Well water† (n = 4)	Pond water (n = 5)	Seawater diluted to 2.56 g L ⁻¹ salinity
Salinity (g L ⁻¹)	3.70 ± 0.34 ^a	2.56 ± 0.51 ^b	2.56
Total alkalinity (mg L ⁻¹ as CaCO ₃)‡	272.6 ± 30.3 ^a	119.9 ± 55.7 ^b	10.5 ²
Chloride (mg L ⁻¹)	1982 ± 177 ^a	1460 ± 117 ^b	1410
Sulfate (mg L ⁻¹)	0.46 ± 0.56 ^a	33.8 ± 18.7 ^b	200
Calcium (mg L ⁻¹)	118.2 ± 5.6 ^a	59.8 ± 5.1 ^b	29.7
Magnesium (mg L ⁻¹)	5.46 ± 0.92 ^a	4.61 ± 1.18 ^a	100
Potassium (mg L ⁻¹)	11.6 ± 1.8 ^a	6.25 ± 2.44 ^a	27.4
Sodium (mg L ⁻¹)	1402 ± 110 ^a	971 ± 208 ^b	77.9

†Average of four samples collected between January and April 2002.

‡Minimum recommended total alkalinity is 75 mg L⁻¹ for shrimp ponds (Boyd *et al.* 2002). Concentrations of major ions in seawater diluted to the same salinity as the pond water are also presented. For each variable, values with different letters (a/b) are significantly different ($P < 0.05$) between well water and pond water.

Source: McNevin *et al.* (2004).

Data on well-water composition, the composition of water after equilibrium with conditions in the ponds and the seawater equivalent concentrations for pond water are provided for a shrimp farm in Alabama (Table 2). The loss of calcium and alkalinity from the well water after equilibration in the pond resulted from calcium carbonate precipitation. Salinity declined because of rainfall and soil uptake of ions. The pond water was particularly low in potassium, magnesium and sulfate relative to the seawater equivalent concentration.

There is evidence from short-term exposures in culture tanks that both potassium and magnesium additions to well water will enhance survival and growth of postlarvae (Davis *et al.* 2005; Roy *et al.* 2007a). Roy *et al.* (2007a) reported an increase in shrimp growth when potassium levels were raised in low salinity waters. To date, several authors have established that when raising shrimp and other marine species in low salinity waters it is important to maintain sodium to potassium ratios (Na : K) at levels similar to seawater diluted to the same salinity (Fielder *et al.* 2001; Davis *et al.* 2004; Zhu *et al.* 2004; Roy *et al.* 2007a). Treatment of pond waters on one shrimp farm in west Alabama with potassium was as effective in increasing shrimp production as was treatment of the ponds with both potassium and magnesium. During 2001, no potassium was applied and shrimp survival was only 27% and production averaged 750 kg ha⁻¹ (Boyd *et al.* 2007a). Potassium was applied to maintain the concentration between 35 and 50 mg L⁻¹ in 2002 and survival increased

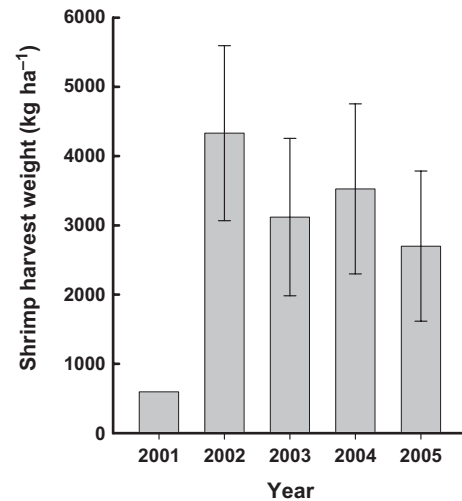


Figure 2 Production of *Litopenaeus vannamei* at an inland shrimp farm in Alabama. The bars represent standard deviation. Source: Boyd *et al.* (2007a).

to 67% and production to 3500 kg ha⁻¹. In 2003, 2004 and 2005, both potassium and magnesium were applied, but production was no better than that achieved in 2002 (Fig. 2).

A recent study in Thailand revealed that bottom soils quickly removed magnesium from pond waters made saline by the addition of brine solution. Periodic application of magnesium chloride to maintain the magnesium concentration above 100 mg L⁻¹ in ponds with salinities of 1–6 g L⁻¹ resulted in a significant increase in shrimp survival and production (Idsariya Wuditisin, pers. comm. 2010).

Total alkalinity in pond waters should be 75 mg L⁻¹ or more to provide adequate buffering capacity, regardless of salinity; this equates to 90 mg L⁻¹ bicarbonate in water with a pH less than 8.3 (Boyd & Tucker 1998). Low concentrations of other ions may negatively impact shrimp growth and survival to a lesser degree, and further research on this topic is needed. However, practical experience suggests that the addition of potassium alone provides excellent remediation of the ionic imbalance in many ponds filled with groundwater from wells or other sources.

Potassium and magnesium fertilizers

The most common material for increasing the potassium concentration in pond water is fertilizer-grade potassium chloride (KCl), often called muriate of potash. This material is approximately 50% potassium, so a treatment rate of 2 g m⁻³ provides a potassium increase of 1 g m⁻³ (1 mg L⁻¹). Another fertilizer, sulfate of potash magnesia (K₂SO₄·2MgSO₄), sold under the trade name K-Mag, has been used as a source of both potassium and magnesium

for treating inland shrimp ponds (Boyd 2003). This product contains 10.5% magnesium, 17.8% potassium and 63.6% sulfate. A treatment rate of 5.6 g m^{-3} is necessary to cause a 1 mg L^{-1} increase in potassium; the resulting increases in magnesium and sulfate would be 0.59 mg L^{-1} and 3.5 mg L^{-1} , respectively.

The costs of muriate of potassium and K-Mag are similar per tonne, but the use of K-Mag to increase the potassium concentration is much more expensive than applications of muriate of potash. To illustrate, ponds on a shrimp farm in Alabama are approximately 1.2 m deep and contain 8 mg L^{-1} potassium and 20 mg L^{-1} magnesium (Table 2). To increase the potassium concentration to a seawater equivalent concentration of 41.7 mg L^{-1} would require 67.4 mg L^{-1} (809 kg ha^{-1}) of muriate of potash, but it would require 2480 kg ha^{-1} of K-Mag. Attaining the seawater equivalent concentration of magnesium would require 9000 kg ha^{-1} of K-Mag, much more than necessary to adjust the potassium concentration to a seawater equivalent concentration. In Alabama, shrimp producers often use both muriate of potash and K-Mag to increase the potassium concentration slightly above the seawater equivalent concentration and to provide a modest increase in magnesium concentration, usually approximately 25% of the seawater equivalent.

Potassium and magnesium uptake by soils

Potassium and magnesium applied to pond waters are lost through overflow, seepage, draining at harvest, soil uptake and shrimp harvest. During the grow-out period in Alabama, there was little outflow of water until the ponds were harvested, and the observed, gradual decrease in potassium and magnesium concentration (McNevin *et al.* 2004; Boyd *et al.* 2007a) was believed to result from adsorption of these ions on cation exchange sites in bottom soil. However, Boyd *et al.* (2007a) found that the exchangeable potassium concentration in bottom soils did not increase appreciably after the first growing season. Thus, the continued loss of potassium, and possibly of magnesium, was related to soil adsorption processes other than ion exchange. Potassium and magnesium budgets (Boyd *et al.* 2007a; Pine & Boyd 2010a) were made for ponds treated with muriate of potash and K-Mag and were determined by assessing the inputs and outputs of the two cations (Table 3). The increase in exchangeable potassium and magnesium in bottom soil was not sufficient to account for the removal of the two ions from the water, especially for potassium.

To answer some of these questions a laboratory study was devised using soil–water systems established in plastic tanks. Soil from a farm that was similar to pond bottom soil, but never exposed to saline water was used. Saline

Table 3 Average potassium and magnesium budgets measured for three ponds in Alabama used for inland culture of marine shrimp in low salinity water

Variable	Potassium Mean \pm SD (kg ha^{-1})	Magnesium Mean \pm SD (kg ha^{-1})
Inputs		
Muriate of potash	596.8 \pm 45.0	
Kmag	220.6 \pm 16.6	142.9 \pm 16.8
Well water	130.2 \pm 2.7	275.1 \pm 69.7
Feed	55.0 \pm 10.3	17.0 \pm 9.7
Rainfall and runoff	18.6 \pm 0.23	6.5 \pm 0.2
Sum	1021.2 \pm 65.2	441.5 \pm 78.2
Outputs		
Harvest effluent	347.9 \pm 49.1	212.8 \pm 67.4
Seepage	101.2 \pm 16.2	74.6 \pm 88.9
Shrimp	5.4 \pm 1.5	5.3 \pm 3.1
Overflow	2.0 \pm 3.4	
Sum	456.5 \pm 53.2	292.6 \pm 24.2

The studies were done on the same farm, but in different years. Source: Boyd *et al.* (2007b); Pine (2008). SD, standard deviation.

water was obtained from the well that supplied the ponds on the farm. The systems were treated with either potassium chloride to give 50 mg L^{-1} potassium (Boyd *et al.* 2007b) or magnesium sulfate to provide 80 mg L^{-1} magnesium (Pine & Boyd 2010a). Concentrations of the ions were measured at intervals and retreatment was made when concentrations approached the original concentrations in the well water. The rate of loss of ions from the water declined over time, but the uptake rate of potassium was still substantial after three treatments over a 10-month period.

During the course of this study, potassium and magnesium loss from water in the tanks revealed that soils took up $4982 \text{ mg tank}^{-1}$ potassium and $1568 \text{ mg tank}^{-1}$ magnesium (Table 4). The exchangeable potassium increase in

Table 4 Average potassium and magnesium loss from water and adsorption by soil in laboratory soil–water systems

Variable	Potassium Mean \pm SD	Magnesium Mean \pm SD
Loss from water		
(mg L^{-1})	89.0 \pm 10.0	28.0 \pm 2.8
(mg tank^{-1})	4982 \pm 559	1568 \pm 155
Soil weight (kg tank^{-1})	9.60 \pm 1.32	5.5 \pm 0.4
Exchangeable ion absorption by soil		
(mg kg^{-1})	136 \pm 83	263.0 \pm 67.4
(mg tank^{-1})	1284 \pm 792	1440 \pm 274

Source: Boyd *et al.* (2007a); Pine (2008). SD, standard deviation.

the soil accounted for 25.8% of the potassium loss from the water. The remainder of the potassium was obviously absorbed by the soil through a process other than ion exchange. Soils in the area where inland shrimp farming is conducted in Alabama have a high concentration of 2:1 layered, smectite clay. Such soil can fix potassium between the adjacent tetrahedral layers of the clay, and potassium fixed by this mechanism is not available to the surrounding solution (Yuan *et al.* 1976; Sparks 2000).

Most of the magnesium lost from the water (91.8%) was present in the soil as exchangeable magnesium. Thus, the soils did not fix appreciable amounts of magnesium within the interlayers of the clay. The laboratory soil–water study suggested that magnesium uptake by bottom soils should decline quickly over time and possibly become insignificant after 1–2 years. However, potassium uptake by bottom soils can be expected to be an important factor for a much longer period of time.

Owing to the unique abilities of the pond environment to assimilate both potassium and magnesium, farmers should measure potassium and magnesium concentrations before each crop and apply fertilizers as indicated by the results. However, the potassium concentration should be monitored at 4–6-week intervals and retreatment with muriate of potash made as necessary. The magnesium concentration can be measured on farms using water analysis kits for total hardness and calcium hardness. The magnesium ion concentration may be estimated as follows:

$$\text{Mg} = (\text{TH} - \text{CaH})/2.5,$$

where Mg is the magnesium concentration (mg L^{-1}); TH is the total hardness (mg L^{-1} as CaCO_3); CaH is the calcium hardness (mg L^{-1} as CaCO_3); 2.5 is the ratio of weights of CaCO_3 : Ca.

The potassium concentration in water is normally measured using a flame emission spectrophotometer. Farmers must send samples to a laboratory for analysis, and it may not be possible to obtain results in a timely manner. HANNA Instruments (Ann Arbor, MI, USA) manufactures a relatively inexpensive potassium meter (Model HI 93750 Ion Specific Meter, Potassium) that measures the potassium concentration by a colorimeter method. Comparisons of the potassium concentration made with the meter and by flame emission spectrophotometry on samples of low salinity water have revealed that the meter provides sufficiently accurate results for use by inland shrimp farmers in assessing potassium concentrations in ponds (Chainark & Boyd 2009).

Inland shrimp culture is accomplished using water from various origins and sources. Inland shrimp farmers are facing several different culture scenarios depending on a number of factors, including geographical location, the length of the production season, climate and the availability of

saline water. Because environmental factors vary greatly with geographical location, there is no magic recipe that can be applied to all inland shrimp culture scenarios. When culturing shrimp in inland saline waters, farmers must tailor their production strategies to their specific culture conditions, and thus to their specific water. As a result, a number of different production strategies have been utilized worldwide to culture shrimp in low salinity waters.

The culture of shrimp in inland ponds is perhaps the most widespread production strategy for raising shrimp in inland low salinity waters. In Thailand, for instance, shrimp are cultured in ponds ($2\text{--}5 \text{ g L}^{-1}$) prepared by mixing freshwater and brine solution from coastal seawater evaporation ponds (Boyd & Thunjai 2003). In much of the USA and South America, inland low salinity ponds are filled from saline aquifers located under ground (Saoud *et al.* 2003). In this case, low salinity water is generally pumped from artesian wells and used to fill production ponds with water containing salinities ranging from 2 to 10 g L^{-1} . In addition, some producers mix granular salt (either from evaporation ponds or mines) with freshwater to prepare low salinity water (Boyd & Thunjai 2003).

The water used to culture shrimp in inland low salinity waters can vary considerably both in salinity and ionic composition. Saoud *et al.* (2003) reported differences in the ionic composition among west Alabama shrimp farms. These differences were not only observed among pond waters located on different farms, but also among ponds from the same farm that draw water from the same low salinity artesian well (Saoud *et al.* 2003). Pond salinity and ionic composition is dependent on a number of factors, including initial salinity, initial concentrations of dissolved ions, different adsorption rates in pond bottom sediments, runoff, evaporation, rain and other contributing factors. These and other factors have resulted in large variation in ionic profiles among farms. As a result, farmers are forced to analyze the water in individual ponds prior to stocking to assess the viability of the specific pond water for growing shrimp.

In most of the west Alabama low salinity water used for shrimp culture, the ionic ratios are not the same as those found in full strength seawater (Roy *et al.* 2007a). This has resulted in large variations in survival, growth and production among ponds (Roy *et al.* 2009a).

Environmental regulations and control

Utilization of saline water sources has allowed for the expansion of shrimp aquaculture into inland areas; however, there are concerns about bringing water containing high concentrations of total dissolved solids (TDS) into freshwater ecosystems (Boyd 2006; Boyd *et al.* 2006; Pine 2008). Although problems associated with salinization are

typically associated with arid regions that experience irrigation, induced salinization and rising water tables, recent studies have revealed that inland low salinity water aquaculture may lead to stream salinization in humid climates.

In the Chachoengsao Province of Thailand low salinity culture of shrimp occupies more than 8400 ha of land and uses saline water transported inland from the coast (Flaherty *et al.* 2000). Braaten and Flaherty (2001) investigated the salt balance of these operations as they were currently managed and through modeling a zero-discharge scenario. Under the then current management system, which used water exchanges to maintain water quality and further discharges at harvest, the electrical conductivity of the irrigation canal water peaked well above levels that would negatively impact rice (2.0 mS cm^{-1}) and orchard crops ($1.0\text{--}1.8 \text{ mS cm}^{-1}$) several times throughout the study. Using a zero-discharge management system was still expected to result in a nearly 50% loss of salts to the environment, mainly through lateral seepage. Currently the Government of Thailand has placed a moratorium on inland marine shrimp farms in areas designated as freshwater ecosystems by provincial governments because of the potential elevation of salts.

In the USA effluent salinity, specific conductance and TDS criteria and standards specific to aquaculture do not exist. However, United States Environmental Protection Agency (USEPA) regulations specify that in-stream chloride concentrations should not exceed 230 mg L^{-1} . Recent investigations into salt discharges by marine shrimp farms in the Blackland Prairie region of Alabama revealed elevated electrical conductivity and chloride concentrations, above 230 mg L^{-1} , in both the groundwater and nearby streams (Boyd *et al.* 2006; Pine & Boyd 2010b). Electrical conductivity and chlorides remained elevated in streams near these production facilities throughout the year, despite the absence of direct discharges into the streams, and appear to be the result of lateral seepage. Elevated chloride concentrations persisted as far as 4 km downstream at some locations, but were highly dependent on freshwater inputs from base flow and runoff from storm events. A potential solution to this problem is to locate inland mariculture facilities away from freshwater streams, and to properly construct ponds to limit seepage of saline water (Boyd 2001, 2002; Boyd *et al.* 2006; Pine & Boyd 2010b). In addition, farmers can recycle pond water to reduce discharge into streams (Boyd *et al.* 2006).

Osmoregulation of shrimp in low salinity waters

Penaeid shrimp are a large and diverse family of decapod crustaceans whose members inhabit saline waters ranging from full strength seawater to relatively dilute estuarine

brackish water. Regardless of salinity, penaeids are typically strong hypo-hyperosmoregulators (Pequeux 1995) that maintain a relatively stable haemolymph osmolality, yet they are not all euryhaline. Penaeid osmoregulation in low salinity waters has been reviewed thoroughly by various authors (Mantel & Farmer 1983; Pequeux 1995; Álvarez *et al.* 2004) and we refer the reader to those manuscripts for an understanding of osmoregulatory processes. In the present document, we will only review basic hyperosmoregulation and discuss osmoregulation in low salinity inland waters.

Although osmoregulation is a complex physiological process involving body surface, digestive system, gills and renal organs, most sodium (Na^+) and chloride (Cl^-) regulation takes place in the gills (Mantel & Farmer 1983; Pequeux 1995). When euryhaline marine crustaceans are exposed to low salinities, body permeability decreases, water excretion via renal organs increases and organic osmolytes in the body fluids are regulated to decrease the osmotic pressure differential between the environment and the haemolymph (Pequeux 1995). As haemolymph osmolality decreases, so does that of the cells, causing them to swell. Swelling then decreases intercellular space causing an increase in tonicity that in turn slows water flow into the body. The renal organ (green gland; antennal gland) ultra filters haemolymph and then reabsorbs Na^+ and Cl^- in the tubule, thus excreting a dilute urine (Mantel & Farmer 1983; Pequeux 1995). Divalent ions, such as calcium (Ca^{++}), magnesium (Mg^{++}) and sulfate (SO_4^-), are regulated by the antennal gland. The main osmoregulatory organs responsible for Na^+ and Cl^- regulation are the gills (Lucu & Towle 2003).

Survival and growth of shrimp in inland low salinity well water of Alabama is affected mainly by the potassium concentration in the water and to a lesser degree by magnesium (Davis *et al.* 2002; Saoud *et al.* 2003; McNevin *et al.* 2004). These findings have been corroborated by various authors including Davis *et al.* (2005), Ur-Rahman *et al.* (2005), Roy *et al.* (2006), Sowers *et al.* (2006a) and Roy *et al.* (2007a). Later, we will suggest a process by which potassium and magnesium might be affecting shrimp well-being. Our discussion of hyper-regulation is based mainly on information from Pequeux (1995), Lucu and Towle (2003) and Mantel and Farmer (1983). Some information is borrowed from studies of teleostean gill osmoregulation by Evans *et al.* (2005), Hirose *et al.* (2003), Evans (2008) and Marshall and Bryson (1998).

As stated above, the main solutes that are regulated in the body are Na^+ and Cl^- . The rate of transport of these ions in or out of cells appears to exhibit saturation kinetics, implying that they do not diffuse freely across the plasma membrane. Moreover, these ions are regulated mainly by the posterior pairs of gills in most crustaceans

as well as teleosts. Specialized cells in the epithelium of the posterior gills work to regulate Na^+ and Cl^- uptake from the external medium. The potassium concentration in the haemolymph does not change appreciably when environmental potassium decreases (Dall & Smith 1981; Roy *et al.* 2007a), regardless of whether the surrounding water is dilute seawater or inland low salinity well water. These specialized cells are called mitochondria rich cells (MRC) or chloride cells. As the names imply, they have a large number of mitochondria in the plasmalemma closely associated to a tubular system composed of invaginations of the basolateral membrane, and appear to contain a lot of chloride. The apical membrane also develops an intricate network of infoldings that form an extracellular compartment between the cell and the cuticle when in saline water. This corrugation of the apical membrane is greatly reduced in very low salinity waters, suggesting that they serve the organism in removing salts rather than absorbing salts.

Sodium permeability of the posterior gills is very low and diffusive efflux of these ions decreases as the media becomes more dilute and the transepithelial gradient increases. However, inward movement of Na^+ and Cl^- is high in dilute media. There is ample evidence that Na^+ is exchanged for NH_4^+ or H^+ and Cl^- for HCO_3^- across the apical membrane thus maintaining electroneutrality. The proton and bicarbonate are produced by carbonic anhydrase hydration of CO_2 . Accordingly, salt uptake is partially coupled with respiration in aquatic organisms (see Figure 3). On the basolateral membrane, an energy consuming membrane bound enzyme, Na^+/K^+ -ATPase (NKA) exports three sodium ions out of the cell in exchange for two potassium ions. Potassium then leaks back into the intercellular medium down its concentration gradient through special potassium channels on

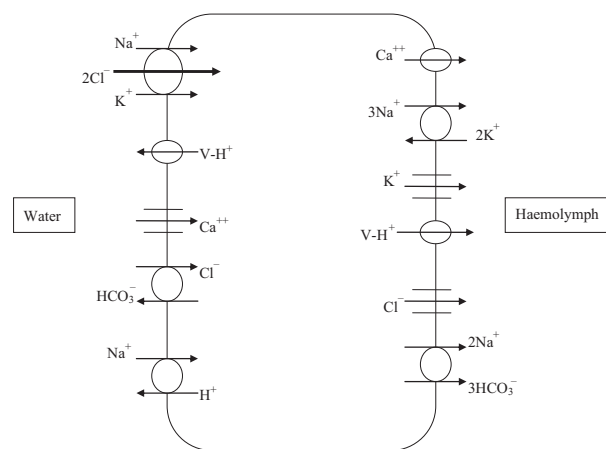


Figure 3 Mitochondria rich cell including the transporters and channels involved in osmoregulation.

the basolateral membrane. In addition, Cl^- leaks into the haemolymph through special chloride channels also in the basolateral membrane. The driving force behind Cl^- movement is an electropositive charge produced on the serosal side of the basolateral membrane by the NKA pump. It might seem intuitive for readers that the Na^+ exchange for NH_4^+ or H^+ and Cl^- for HCO_3^- across the apical membrane must be linked to maintain electroneutrality. However, Pequeux (1995) cites various authors that have found a rheogenic effect of the exchanges and have rejected a link between the anion exchanger and the cation antiporter (i.e. a change in the concentration of Cl^- does not affect Na^+ uptake and vice versa). Finally, there is some evidence for a Na-K-2Cl co-transporter on the apical membrane of some crustaceans that allows ion movement into the cell down the concentration gradient.

The movement of ions in and out of chloride cells is dependent on NKA activity in the basolateral membrane. Activity of NKA has been demonstrated to depend on the Na^+/K^+ ratio and on the osmolality of the external medium. Moreover, the external K^+ concentration often does not affect NKA activity because the enzyme is located on the basolateral membrane of the MRC. Long-term NKA activity is increased with acclimation to dilute media owing to an increase in the expression of NKA in posterior gills concomitant with differentiation of new MRC. Short-term increases in NKA appear to result from a reduction in the concentration of the basolateral medium. A reduction in the sodium or chloride concentrations while maintaining iso-osmolality does not increase NKA activity.

We can now describe what happens at the gill level when a shrimp is placed in a dilute medium. First, the permeability of the gills decreases. Second, an increase in NKA activity, owing to a decrease in haemolymph osmolality, decreases the Na^+ in the cell and changes the potential difference across the basolateral membrane, making the serosal side more positive. Furthermore, a $2\text{Na}^+/\text{HCO}_3^-$ co-transporter on the basolateral membrane could also remove Na^+ from the cell. A V-type H^+ ATPase transporter possibly maintains electroneutrality without affecting osmolality. The electrochemical gradient pushes the Cl^- out of the cell into the haemolymph through special chloride channels on the basolateral side. Thus, Na^+ , K^+ and Cl^- are transported into the cell using an Na-K-2Cl co-transporter on the apical membrane. Chloride is also exchanged for bicarbonate and Na^+ for H^+ via a rheogenic antiporter that brings in more Na^+ than H^+ secreted. Electrochemical stability is maintained via a V-type H^+ ATPase transporter on the apical membrane. Finally, Ca^{++} is transported actively across the basolateral membrane into the haemolymph and diffuses into the cell through apical divalent cation channels.

The above description of hyperosmoregulation in euryhaline crustaceans summarizes the gill processes involved in acclimation and acclimatization of *L. vannamei* to low salinity marine waters. However, inland low salinity waters do not have the same ionic ratios as marine waters, often having low concentrations of potassium and magnesium. This causes an increase in NKA activity, retarded growth and mortality. Because *L. vannamei* can tolerate very low salinity waters provided that the calcium hardness is adequate, we need to try to explain why some inland low salinity waters are not suitable for shrimp survival, despite suitable water hardness. The following is a speculation that has not been empirically tested. With movement into low salinity waters, stretch-activated potassium channels in the apical membrane are opened to allow potassium to migrate out of the cell to lower cytosol osmolality and thus reduce water inflow. This, coupled with potassium loss via anterior gills and the rest of the body surface, reduces the haemolymph potassium and disturbs the potassium to sodium ratio, which in turn disrupts Na–K–2Cl and NKA activity. Magnesium is necessary for NKA activity and a reduction in magnesium ions would disrupt NKA, which is the driving force behind most other transport mechanisms in the cell. Accordingly low potassium and/or low magnesium disrupts normal functions of MRC, affecting organismal osmoregulation.

Remediation techniques

Water modification

Shrimp farmers typically acclimate postlarvae to pond water before stocking. An immediate transferral of shrimp from full strength seawater to low salinity waters without acclimation will generally kill the shrimp, depending on endpoint salinity. Acclimation, in shrimp farming, typically means that the farmer slowly adds pond water to the water that the postlarvae have been transported in, so that the shrimp do not get a temperature or salinity shock. Typical acclimation procedures have been described by various authors, including Villalon (1991), Kumlu and Jones (1995), Tsuzuki *et al.* (2000) and Davis *et al.* (2004). These methods work well for shrimp postlarvae being acclimated to marine waters. Because transferring shrimp from hatchery water to pond water instantaneously would stress the animals and possibly kill them, Samocha *et al.* (1998) used rapid salinity shifts as a stress test to evaluate shrimp hardness before stocking. All environmental differences between the two waters remained constant in these stress tests, except for salinity. However, in inland low salinity waters, the ionic composition of the water could be as much a stressor as the salinity itself.

As previously discussed, inland low salinity waters generally do not have the same ion profile as marine waters (Saoud *et al.* 2003; Sowers *et al.* 2004, 2005, 2006b; Parmenter *et al.* 2009). Forchhammer's principle of constancy of the composition of seawater does not apply to inland low salinity waters (see Saoud *et al.* 2003), and thus shrimp need to be acclimated to new water ion profiles as well as to new salinities. Such a situation imposes a reduction on the rate of water exchange during acclimation. Furthermore, ionic profiles of inland low salinity waters are not similar among water sources and thus acclimation procedures will differ among waters.

A further variable to consider when penaeid postlarvae are transferred to inland low salinity water is the age of the animals (McGraw *et al.* 2002; Saoud *et al.* 2003). Pequeux (1995) cites many studies that suggest that salinity tolerance changes over the course of larval development. McGraw *et al.* (2002) showed that 10-day old *L. vannamei* postlarvae (PL₁₀) could be acclimated to 4 g L⁻¹ seawater, whereas PL₁₅ and PL₂₀ could tolerate salinities down to 1 g L⁻¹ Saoud *et al.* (2003) also found an effect of age on postlarval survival after acclimation attempts to low salinity water. They reported that PL₁₅ and PL₂₀ shrimp tolerate low salinity water acclimation better than PL₁₀ *L. vannamei*. Similar improvements to low salinity tolerance with postlarval age have been reported by many authors (Mair 1980; Cawthorne *et al.* 1983; Olin & Fast 1992; Kumlu & Jones 1995; Rosas *et al.* 1999; Tsuzuki *et al.* 2000), but Álvarez *et al.* (2004) found that osmoregulation was directly correlated with gill size, which tends to be more developed in older individuals. In addition to survival, growth rate might be affected by the age of acclimation to low salinity water (Davis *et al.* 2005).

Inland low salinity well waters are generally deficient in potassium (Smith & Lawrence 1990; Fielder *et al.* 2001; Samocha *et al.* 2002; Atwood *et al.* 2003; Boyd & Thunjai 2003; McGraw & Scarpa 2003; Saoud *et al.* 2003; Gong *et al.* 2004; McNevin *et al.* 2004; Sowers *et al.* 2004, 2006b; Ur-Rahman *et al.* 2005; Prangnell & Fotedar 2006; Roy 2006; Roy *et al.* 2006, 2009a,b,c; Araneda *et al.* 2008; Green 2008; Partridge & Lymbery 2008; Partridge *et al.* 2008; Cuvin-Aralar *et al.* 2009; Esparza-Leal *et al.* 2009). Furthermore, the concentration of magnesium and sulfate in inland low salinity waters tends to be lower than in diluted seawater (Boyd & Thunjai 2003; Saoud *et al.* 2003). Accordingly, when postlarvae are acclimated to low salinity waters supplemented with potassium, magnesium and sulfate, survival and growth are improved when compared with postlarvae acclimated to low salinity water with no supplemental minerals. Saoud *et al.* (2003) demonstrated a positive correlation between survival and the concentrations of potassium, magnesium and sulfate in the water, and Davis *et al.* (2005) showed that both

potassium and magnesium improved postlarvae survival in low salinity waters, whereas only potassium improved the growth of the same postlarvae.

Having made a case for postlarvae acclimation to low salinity water and for supplementation of potassium, magnesium and sulfate to acclimation water, we will now suggest an acclimation process. Postlarvae should be received at the farm and assessed for hardiness, disease and general health as described by Villalon (1991). They should then be maintained in well-aerated and filtered marine water and offered brine shrimp nauplii and artificial feed until an age of PL₁₅. Inland well water should then be added gradually to the acclimation tank. Postlarvae are tolerant of rapid salinity reductions down to 4 g L⁻¹ (see Saoud *et al.* 2003), but as a precaution we advise a maximum reduction rate of 4 g L⁻¹ h⁻¹ until a salinity equal to the low salinity water is reached. If the salinity of the low salinity water is less than 4 g L⁻¹, then refrain from adding more water when 4 g L⁻¹ is reached. Maintain the postlarvae at the new salinity endpoint for 72 h before continuing with the acclimation. In the meantime, mix low salinity pond water with potassium, magnesium and sulfate so that the concentrations of the ions equal their levels in seawater at the same salinity. An example would be to add muriate of potash (KCl) and magnesium sulfate (MgSO₄) to increase potassium, magnesium and sulfate levels to $0.38 \times S/35$, $1.35 \times S/35$ and $0.89 \times S/35$ g L⁻¹, respectively (calculated from Bidwell & Spotte 1985), where S is the salinity of the well water. After 72 h, start adding the supplemented well water to the acclimation tank until thrice the container volume has been added. The shrimp can now be held in this water until they are ready to be transferred to grow-out ponds. The water in the grow-out ponds should have a similar composition to the water used during the acclimation. Many farmers add potassium, magnesium and sulfate to their ponds and then use pond water to acclimate the shrimp. This method is acceptable if the potassium in the pond does not get lost in the sediment (see Boyd *et al.* 2007a). The acclimation rate is also an important factor to consider when stocking postlarvae into low salinity production ponds (Davis *et al.* 2004; McGraw & Scarpa 2004). Typically, farms hold postlarvae in a nursery system until they are the proper size (age) to stock into ponds. The ionic composition and temperature of the nursery system might vary considerably from the pond water and this needs to be taken into consideration. Enough time needs to be allowed to permit proper acclimation of postlarvae to the actual pond water. It is not uncommon in Alabama for shrimp ponds on the same farm to have a 1–2 g L⁻¹ difference in salinity. Thus, the nursery water will not be exactly the same as the water in all of the production ponds. To acclimate shrimp held in

the nursery to pond water in Alabama shrimp are held on the pond bank for 1–2 h prior to stocking. Water is pumped from the pond to the holding tank (which is supplied with aeration) until the same water temperature and exact salinity of the pond are achieved.

Feed

Owing to the success inland farmers have had with supplementing fertilizers rich in potassium and magnesium directly to pond waters, several researchers have also explored dietary supplementation of ingredients that might potentially improve the osmoregulatory capacity of shrimp reared in low salinity water. Adding K-Mag and muriate of potash directly to pond water has dramatically improved the survival and growth of *L. vannamei*; however, adding these fertilizers on an annual basis can be costly. In west Alabama, fertilizers containing potassium and magnesium must be added to waters annually prior to the stocking of postlarvae and occasionally during the production cycle to offset losses of ions as a result of adsorption by pond bottom soils. As a result, multiple applications of fertilizers are sometimes necessary throughout the production cycle, adding an additional expense to the farmer. As fertilizers are expensive, there has been considerable interest in determining if dietary manipulations could mediate the stress.

Minerals

Shrimp, like other crustaceans, require certain minerals to maintain basal metabolism and growth. Crustaceans utilize minerals as components of hard tissues, soft tissues, metalloproteins, enzymatic cofactors and enzymatic activators (Davis & Lawrence 1997). In addition to being important for acid–base balance and for the maintenance of membrane potentials, soluble minerals such as calcium, sodium, potassium and chloride also play a role in osmoregulatory function (Mantel & Farmer 1983; Pequeux 1995). Although dietary mineral requirements are well documented in penaeid shrimp, few studies have addressed whether dietary requirements of key minerals change under low salinity conditions.

Supplementation of the minerals necessary for normal osmoregulatory function into diets has been suggested for a number of fish and crustaceans. It has been hypothesized that a lack of ions at the gill–water interface could possibly be mitigated by dietary supplementation of these ions by increasing their availability and absorption in the digestive tract. Holsapple (1990) and Gatlin *et al.* (1992) report that the growth and feed efficiency of juvenile red drum (*Sciaenops ocellatus* Linnaeus) reared in freshwater improved following dietary supplementation of NaCl. Dietary supplementation of NaCl, magnesium chloride, potassium chloride, cholesterol and lecithin improved the

osmoregulatory capacity of *L. vannamei* reared in low salinity waters in Arizona (Gong *et al.* 2004). A dietary source of potassium was beneficial (1.2 g potassium 100 g⁻¹) for *Penaeus monodon* (Fabricius) reared in brackish water ranging in salinity from 19 to 21 g L⁻¹ (Shiau & Hsieh 2001). Dietary requirements for minerals in low salinity waters, such as magnesium, have also been published in the literature for *L. vannamei* (Cheng *et al.* 2005). Cheng *et al.* (2005) reported a dietary magnesium requirement of 2.60–3.46 g magnesium kg⁻¹. Unfortunately it is more likely that the actual requirement for minerals such as potassium and magnesium in low salinity waters will vary depending on the water source used by the farmer. It has already been established that individual ponds have markedly different ion profiles, including variations in profiles from ponds on the same farm (Saoud *et al.* 2003; Roy *et al.* 2009a; Pine & Boyd 2010b). Thus, the actual dietary mineral 'requirement' will also be a function of the aqueous concentration of the mineral in the rearing medium. As the aqueous concentration of potassium and magnesium varies greatly depending on the water source, it is likely that dietary mineral requirements could also vary depending on the salinity and ion profile. For instance, although some authors have reported dietary requirements of magnesium for *L. vannamei* in low salinity waters, several other studies have confirmed no benefit of supplementing dietary magnesium in excess of the requirement for culture of *L. vannamei* in actual inland low salinity waters (Roy *et al.* 2007b, 2009b).

Recently, a number of studies evaluating the impact of dietary mineral supplementation for improved growth and survival were conducted in west Alabama. Two laboratory trials conducted in 4 g L⁻¹ low salinity water reported improved growth in *L. vannamei* fed diets supplemented with chelated potassium (1.0% potassium), whereas no response was observed when using potassium chloride (0.5% potassium, 1.0% potassium), magnesium chloride (150 and 300 p.p.m. magnesium) or sodium chloride (1% and 2% NaCl) (Roy *et al.* 2007b). However, multiple studies conducted on site at low salinity shrimp farms in west Alabama failed to support the laboratory results obtained using chelated minerals. Furthermore, in a tank study utilizing a flow-through system supplied with low salinity water (1.3 g L⁻¹) from a shrimp production pond, chelated potassium did not improve the growth or survival of *L. vannamei* in low salinity waters of west Alabama (Saoud *et al.* 2007). Improved growth and survival were not observed in *L. vannamei* fed a diet supplemented with chelated magnesium in laboratory and farm studies in west Alabama (Roy *et al.* 2009b). In our experience, feed formulations (Tables 5 and 6) with chloride salts or chelated minerals have not produced consistent improvements in growth and survival at the

Table 5 Ingredient composition of diets formulated for *L. vannamei* reared in low salinity waters (g 100 g⁻¹ dry weight) supplemented with a potassium chelate (KC)

Ingredient	Diet 1 (basal)	Diet 2 (0.5% KC)	Diet 3 (1.0% KC)
Fish meal	3.00	3.00	3.00
Poultry meal	15.30	15.30	15.30
Soybean meal	33.60	33.60	33.60
Menhaden fish oil	4.52	4.52	4.52
Wheat starch	9.15	6.65	5.20
Whole wheat	19.60	19.60	19.60
Cellulfil	5.00	5.00	3.95
KC	0.00	2.50	5.00

Source: Saoud *et al.* (2007).

Table 6 Ingredient composition of diets formulated for *L. vannamei* reared in low salinity waters (g 100 g⁻¹ dry weight) supplemented with a magnesium chelate (MgC)

Ingredient	Diet 1 (basal)	Diet 2 (0.15% MgC)	Diet 3 (0.30% MgC)	Diet 4 (0.60% MgC)
Fish meal	3.00	3.00	3.00	3.00
Poultry meal	15.30	15.30	15.30	15.30
Soybean meal	33.60	33.60	33.60	33.60
Menhaden fish oil	4.52	4.52	4.52	4.52
Wheat starch	10.78	10.63	12.98	15.18
Whole wheat	19.60	19.60	19.60	19.60
Cellulfil	5.00	5.00	2.50	0.00
MgC	0.00	0.15	0.30	0.60

Source: Roy *et al.* (2009b).

farm level. In the absence of a similar ionic profile to seawater, in terms of ionic ratios such as Na : K, mineral supplementation of potassium and magnesium above the dietary requirement does not appear to be an effective way of increasing the growth, survival or osmoregulatory capacity of shrimp reared in low salinity waters. Thus, presently it is recommended that diets should be supplemented with magnesium to meet the requirement of most freshwater species (0.15% diet) and the use of a chelated K (1% diet) may be helpful, although results have been inconsistent (Roy *et al.* 2007b; Saoud *et al.* 2007). To date, the vast majority of findings, with shrimp reared in low salinity waters, suggest that there is no evidence that dietary mineral manipulations will offset stressful ionic conditions, and farmers would be better off supplementing minerals directly to culture waters rather than relying on mineral assimilation from feed sources.

Amino acids

Supplementation of free amino acids (FAA) in shrimp feed has also been suggested as a potential way to improve the osmoregulatory capacity of shrimp reared in low salinity

water (Saoud & Davis 2005; Saoud *et al.* 2007). Transfer of shrimp and other crustaceans from high to low salinity water results in a diffusive loss of salts from the haemolymph to the medium and, consequently, uptake of water from the medium (Mantel & Farmer 1983). As water intake increases in response to low salinity, tissues and cells take up water and increase their volume (Mantel & Farmer 1983; Henry 1995; Pequeux 1995). Most euryhaline organisms, such as *L. vannamei*, possess a cell volume regulatory response to counteract abrupt changes in salinity that involves certain non-essential amino acids (NAA) and quarternary ammonium compounds (QAC). These NAA and QAC are present in the cytoplasm of cells and can make up a large proportion of the total intracellular osmolality (Florkin & Schoffeniels 1969; Henry 1995). Following increased water uptake and subsequent cell swelling, these compounds are released into the haemolymph to rapidly decrease intracellular osmotic pressure. This phenomenon results in the exit of osmotically obligated water from the cell (Pierce & Amende 1981), restoring cell volume to normal. Gainey and Greenberg (1977) suggest that the degree to which an organism is able to reduce its intracellular FAA pool is the lower limit of survival with respect to salinity.

Dietary supplementation of FAA, such as betaine and arginine, has been suggested as a potential means to improve the osmoregulatory capacity of *L. vannamei* reared in low salinity environments (Saoud & Davis 2005; Saoud *et al.* 2007). The intracellular volume in *L. vannamei* is adjusted by changing the size of the intracellular pool of organic osmolytes (Roy *et al.* 2007c). In theory, the increased availability of FAA in the digestive tract via supplemented feed sources could potentially provide an advantage to shrimp reared in low salinity environments. Saoud and Davis (2005) reported that betaine supplementation at low (0.5 g L⁻¹ salinity) or high (50.0 g L⁻¹) salinity did not influence shrimp growth. Likewise, Saoud *et al.* (2007) reported no significant effects on growth or survival of *L. vannamei* fed diets supplemented with arginine when reared in low salinity waters (1.3 g L⁻¹). Huai *et al.* (2009) reported a dietary threonine requirement of 13.6 g kg⁻¹ dry diet for *L. vannamei* reared in low salinity water (0.5–1.5 g L⁻¹). Although the results to date are not conclusive this area of research merits further investigation. There has been no conclusive evidence correlating dietary supplementation of amino acids to improved osmoregulatory capacity of *L. vannamei* reared in low salinity environments. It is possible that other FAA not examined could play an important role (e.g. proline, glycine or taurine) (Roy *et al.* 2007c). Most studies have been designed primarily as nutrition studies and the actual intracellular pool of organic osmolytes was rarely measured by investigators to quantify an actual physiological

response. In addition, most studies (Saoud & Davis 2005; Saoud *et al.* 2007) were conducted with juvenile shrimp. Shrimp farmers typically stock postlarvae to low salinity waters and it is during these early developmental stages that FAA might provide the greatest advantage. To our knowledge, no studies to date have investigated the advantage of dietary supplementation of FAA to feeds for postlarvae immediately prior and during acclimation to stressful environments such as low salinity waters. This is an area that requires further study.

Phospholipids and cholesterol

Gong *et al.* (2004) suggested that dietary phospholipids and cholesterol probably are important for shrimp cultured in low salinity water because of their role in lipid mobilization and storage in the hepatopancreas. Phospholipids are necessary for normal cell structure and function because of their role as polar lipids that make up part of the cell membrane (Teshima 1986; Teshima *et al.* 1997). Phospholipids are also involved in gill membrane function and lipid metabolism, and they serve as secondary messengers in cell signalling. In addition, phospholipids might also facilitate the incorporation of cholesterol into haemolymph proteins (Teshima *et al.* 1997). Although shrimp are capable of synthesizing their own phospholipids, they are unable to synthesize enough to meet their dietary requirement, thus making their inclusion in commercial diets essential. Shrimp and other crustaceans use cholesterol as a precursor for steroid and molting hormones (Teshima *et al.* 1997). D'Abramo *et al.* (1985) and Teshima *et al.* (1986) concluded that insufficient transport of cholesterol was responsible for reduced weight gain in crustaceans receiving diets deficient in phospholipids. Suboptimal dietary levels of cholesterol have also been responsible for mortality syndrome observed in both juvenile lobsters and crayfish (Conklin *et al.* 1980; Bowser & Rosemark 1981; D'Abramo *et al.* 1985). As shrimp are unable to synthesize cholesterol *de novo*, it is considered an essential nutrient (Gong *et al.* 2004).

Field studies conducted on two separate low salinity shrimp farms in west Alabama by Roy *et al.* (2006) concluded that increasing the dietary cholesterol and lecithin requirement from 0.2% to 0.4% and from 0.5% to 1.0%, respectively, did not improve growth or survival of *L. vannamei*. In the same study, similar results were reported for a laboratory trial conducted with 4.0 g L⁻¹ reconstituted low salinity water. Gong *et al.* (2004) reported increased osmoregulatory capacity and larger shrimp in *L. vannamei* reared in low salinity water in Arizona when offered a diet containing supplements of cholesterol (0.1%), lecithin (1.5%), potassium (0.5%), magnesium oxide (0.8%) and sodium chloride (0.5%) compared with a diet without any of these five supplements. However, it is unclear

which supplemented ingredient or combination of ingredients was responsible for the observed effects in this study. Based on the evidence to date, it does not appear that any advantage is conferred to *L. vannamei* reared in low salinity water when offered feeds supplemented with cholesterol or lecithin above the dietary requirement.

Highly unsaturated fatty acids

As a result of the role that fatty acids play in membrane fluidity, a number of investigators have evaluated the supplementation of highly unsaturated fatty acids (HUFA) and/or shifting the n-3/n-6 ratio of the diets for *L. vannamei* reared at low salinity. Both postlarval and juvenile shrimp have increased tolerance to acute exposure to low salinity when fed diets high in HUFA (Rees *et al.* 1994; Wouters *et al.* 1997; Chim *et al.* 2001; Palacios *et al.* 2004). Increased tolerance to low salinity exposure results from the effect of HUFA on osmoregulatory mechanisms in *L. vannamei* (Palacios *et al.* 2004), particularly $\text{Na}^+ - \text{K}^+ - \text{ATPase}$ and carbonic anhydrase activities. *Litopenaeus vannamei* reared at low salinity have a high osmoregulatory capacity (Roy *et al.* 2007c) that they must regulate constantly to maintain homeostasis. The energetic cost of osmotic and ionic regulation can result in less energy being devoted to growth. It has been hypothesized that supplementation of diets high in HUFA will make osmoregulation in low salinity waters more efficient for *L. vannamei* (Palacios *et al.* 2004; González-Félix *et al.* 2009). Dietary supplementation of HUFA increases the fatty acid composition of shrimp gills (Hurtado *et al.* 2006). Changes in gill fatty acid composition can influence osmoregulation via modulation of water and ion permeability (Di Costanzo *et al.* 1983; Porter *et al.* 1996) as well as the activity of the $\text{Na}^+ - \text{K}^+ - \text{ATPase}$ (Hulbert & Else 1999; Turner *et al.* 2003). The primary theoretical benefits associated with HUFA supplementation are directly related to modification of the gill fatty acid composition and a larger gill area. These factors enhance both $\text{Na}^+ - \text{K}^+ - \text{ATPase}$ and carbonic anhydrase activities, which are both osmoregulatory mechanisms used by *L. vannamei* in low salinity environments (Palacios *et al.* 2004; Roy *et al.* 2007c). In a 6-week study with juvenile *L. vannamei* raised in low salinity water (4.1 g L^{-1}), dietary supplementation of docosahexaenoic acid (DHA) and arachidonic acid (AA) and different n-3/n-6 ratios resulted in no differences in final weight, weight gain or survival. In contrast, Palacios *et al.* (2004) observed a benefit of supplementing EPA and DHA on the survival of postlarval *L. vannamei* following a stress test. Increased survival of postlarvae in this case was attributed to a modification of the fatty acid composition of gills and a larger gill area, which enhanced the activities of $\text{Na}^+ - \text{K}^+ - \text{ATPase}$ and carbonic anhydrase.

Unfortunately, most studies in the literature concerning the influence of dietary HUFA supplementation on shrimp exposed to low salinity environments have been conducted over short-term periods. Although these studies provide an insight into the responses of postlarval and juvenile shrimp to low salinity acclimation, very little data are available in terms of long-term responses to low salinity exposure, such as a normal 20–24 week production cycle. In addition, most studies in the literature have evaluated HUFA supplementation at salinities higher than those typically utilized by low salinity shrimp farmers in Alabama and other regions of the world. As the salinity and ionic profile can vary considerably even among ponds from the same farm, much of the information learned has not been practical at the farm level.

Other nutritional supplements

Dietary carbohydrate levels have also been evaluated for *L. vannamei* cultured in low salinity waters. In a 5-week study evaluating the effect of dietary carbohydrate levels at different salinities (1%, 2%, 4%, 8% and 16%) Wang *et al.* (2004) reported that the specific growth rate was higher at lower salinities when shrimp were offered higher dietary carbohydrate levels. In this study, the authors surmise that higher dietary carbohydrate levels could help counteract the higher energetic cost of osmoregulation at low salinity.

Another dietary supplement that has been suggested as a means to improve the growth and survival of shrimp acclimated to low salinity water is the carotenoid pigment astaxanthin. Flores *et al.* (2007) added astaxanthin to diets offered to *L. vannamei* as a potential means to reduce stress (Merchie *et al.* 1998; Darachai *et al.* 1999; Chien *et al.* 2003) and monitored various haemolymph metabolites to assess stress. Following a 6-week study using 3 g L^{-1} low salinity water, supplementation of $80 \text{ mg astaxanthin kg}^{-1}$ enhanced shrimp haemolymph concentrations of glucose, lactate, haemocyanin and the total haemocyte count (Flores *et al.* 2007). The same diet yielded significant improvements in daily growth coefficients, but not survival when compared with other test diets (0, 40 and $150 \text{ mg astaxanthin kg}^{-1}$). Recently, a prebiotic (GroBiotic; International Ingredient Corporation, St Louis, MO, USA) was reported to increase survival in *L. vannamei* in low salinity water (2 g L^{-1}) (Li *et al.* 2010).

Conclusions

Inland culture of marine shrimp will likely continue to expand worldwide, particularly where viable inland low salinity waters are available. As remediation techniques are further refined, better survival, growth and production

of shrimp will be achieved. Based on our review of the literature there appears to be an information gap with regard to long-term production studies. The vast majority of information regarding low salinity aquaculture with *L. vannamei* is focused on short-term bioassays with post-larvae or 4–10 week growth trials using juvenile shrimp. Many of the remediation techniques reviewed here have not been tested over the course of an entire production season. Water modification and dietary modification approaches have been explored by researchers and farmers to improve the growth and survival of marine shrimp in low salinity waters. Based on our own experience in Alabama as well as what we found predominantly in the literature, it appears that modification of the rearing medium with potassium and magnesium fertilizers is more effective than dietary modification techniques at improving the growth, survival and osmoregulatory capacity of marine shrimp reared in low salinity water. Nevertheless, there are some dietary additions that have shown promise in laboratory studies, and future studies should seek to verify these findings in actual pond production trials in low salinity waters.

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