Water Quality in Inland Saline Aquaculture Ponds and its Relationships to Shrimp Survival and Production

by

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Abstract

Survival and production of shrimp (*Litopenaeus vannamei*) in low-salinity pond waters at Greene Prairie Aquafarm (GAF) near Forkland in Green County Alabama varied greatly among ponds, but averages were higher in 2013 than in 2014. Examination of historical data (2001-2014) for this farm revealed that survival and production were extremely low in 2001, but following the adoption of potassium (K) augmentation in 2002, survival and production improved. Magnesium (Mg) augmentation also has been used since 2003, but the main benefit to survival and production is accrued from K augmentation. Nevertheless, much unexplained variation in survival and production occurred among ponds during a given year, in the same pond across years, and for the entire farm across years.

By making weekly analyses in 20 ponds at GAF in 2013 and 2014, a large amount of data for concentrations of K, Mg, sodium (Na), calcium (Ca) and total alkalinity (TA) was acquired. The concentrations of these variables differed considerably among ponds on individual sampling dates, and for a given pond, across sampling dates. Simple linear regression revealed significant relationship ($P<0.05$) between K concentration and survival in 2013 and between Ca concentration and survival in 2014. Production was positively correlated with increasing K and TA concentration in 2013 and with salinity, sodium, and TA concentration in 2014. Variation in salinity, cations, and TA concentrations was not clearly related to rainfall patterns during the two years.

Ordinary least squares analysis provided equations that used concentrations of the four major cations and TA as explanatory variables to account for about 49% and 55% of the variation in survival and production, respectively. However, Ca and TA concentrations had the greatest influence on the predictability of both survival and production by the OLS equations. The seawater equivalent concentrations of the cations, and the Na/K, Ca/K, Ca/Mg, and Alk/K ratios were not found to be reliable indicators of shrimp survival and production.

Two other low-salinity shrimp farms located near GAF were included in the study in 2014. Fifteen ponds of these farms had relatively similar average concentrations of cations and TA, but there was considerable variation in concentrations of these variables among ponds on each sampling date as also occurred at GAF. Salinity and concentrations of cations were higher than those found in ponds at GAF, but TA concentration was lower. The lower TA concentration may have resulted from greater calcium carbonate ($CaCO_3$) precipitation in waters with greater Ca concentration than found at GAF. Unfortunately, the pond owners did not provide records on stocking rates, survival, or production necessary for comparison with shrimp performance at GAF.

Potassium supplementation of pond water at GAF is extremely critical, because of the low concentration of this cation in the water supply. Magnesium concentration also is low in the water supply, but the importance of Mg augmentation is unclear. In ponds of especially low salinity, Na augmentation would possibly be beneficial. The possibility for increasing Ca and TA concentrations in ponds at GAF is questionable, because water often is at saturation with $CaCO_3$. Compounds like calcium sulfate ($CaSO_4{\cdot}2H_2O$) and sodium bicarbonate ($NaHCO_3$) being highly soluble may be used to increase Ca and TA, however there are chances that Ca will precipitate in either case.

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Table of Contents

List of Tables

List of Figures

Introduction

Shortage of fresh water is a global issue, and changing climatic conditions have made the availability of fresh water more unpredictable in many parts of the world. Many developing nations are persistently facing drought or near drought conditions, and sufficient fresh water is a distant dream to most of them. Many areas face drought every few years that results in loss of livestock and crops, and in some cases, precious human lives. This situation demands that additional water resources be found for food production. For increasing aquaculture production, saline groundwater is the most potential candidate in some countries. This resource is readily available in many countries, and saline soils occur in arid regions in more than 100 countries (Roy et al., 2010). Surface waters and groundwater in such areas often have more than 1 g/L salinity [(Keren (2000) as quoted by Roy et al. (2010)]. Saline groundwater may also occur in regions of greater rainfall as a result of underground salt deposits, connate water of marine origin and saltwater intrusion in coastal areas [Cook (1997) as cited by (C. A. Boyd et al., 2009)]. In the USA, saline groundwater can be found beneath two-thirds of the country (Feth, 1970). This situation warrants exploring the potential use of inland saline groundwater for culturing selected aquaculture species.

The utility of saline water for agriculture has long been studied, but traditional terrestrial crops are not salt-tolerant enough to be irrigated with saline water. On the other hand, a marine environment is suitable for production of many species of marine animals and plants all over the world (Jarwar, 2014). Thus, it should be possible to culture some of these marine species in inland saline water. Inland saline aquaculture may offer an opportunity for income diversification and a potentially productive use of land that can no longer support traditional agriculture (Doupé et al., 2003).

The culture of shrimp and marine fish in low salinity water (LSW) is common practice in many countries throughout the world including China, Thailand, Vietnam, Ecuador, Brazil, Mexico, and the United States (McNevin et al., 2004) and Israel, Australia and many other countries (Crespi et al., 2011). The culture of shrimp and other fish and crustaceans using low salinity water is a trend that continues to grow throughout the world (Roy et al., 2010). Most efforts to culture marine shrimp in inland ponds have focused on the use of saline groundwater (Roy et al., 2010). Inland culture of marine shrimp using saline well water (with salinities of 1–15 g/L) is becoming more widespread throughout the world (Roy et al., 2007). It has become rather common even 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 (Roy et al., 2010). Depending on their source, inland waters available for shrimp culture are usually of different salinities and possess different ionic compositions (Boyd and Thunjai, 2003). Alabama has several saltwater aquifers (Boyd et al., 2009), that are being utilized as sources of low salinity water for aquaculture (Saoud et al., 2003; Roy et al., 2007).

The great variation in salinity and ionic profiles of LSWs pose a problem in aquaculture (Boyd and Thunjai, 2003), (Saoud et al., 2003). Most LSWs, especially those from aquifers, usually have different proportions of major ions than found in seawater (Boyd and Thunjai, 2003). Ionic modification approaches alter the low salinity rearing medium to make it more acceptable for production of shrimp (McNevin et al., 2004) are being applied.

The ability of *Litopenaeus vannamei* to tolerate a wide range of salinities has made it a popular species for low salinity culture (McGraw et al., (2002); Samocha et al., (1998); Samocha et al., 2002). Over the past 10 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 LSWs (Roy et al. 2010). Because of its superiority in tolerance and adaptability, the production of *L. vannamei* in inland saline well water is a growing industry in coastal and inland regions of several countries including China (Cheng et al., 2005), United States (Roy et al., 2012), Thailand (Wudtisin and Boyd, 2011), Ecuador (Boyd and Thunjai, 2003), (Saoud et al., 2003), Mexico (Castillo-Soriano et al., 2010) and other countries with considerable areas where LSWs are available (Liu et al., 2014b).

Farmers in west Alabama have been successful in raising *L. vannamei* in inland low salinity waters by raising the dissolved potassium (K) and dissolved magnesium (Mg) concentrations of their pond waters to more ideal levels. McNevin et al. (2004) observed increased shrimp production in Alabama low salinity waters (2-4 g/L) by raising the levels of K and Mg from 6.2 and 4.6 mg/L, respectively to 40 and 20 mg/L, respectively. Such water treatment using muriate of potash and potassium-magnesium sulfate modify proportions to more nearly reflect those found in seawater.

Scientific and economic interest in shrimp culture in LSWs has stimulated numerous studies and, to date, considerable progress has been made in understanding the implications of rearing marine shrimp in this rather unusual environment. For example, it was found that the ionic composition of wellwaters may be a more important growth- and survival-determining factor than the salinity itself (Saoud et al., 2003). Various studies have demonstrated a benefit to having appropriate ratios of K and Mg and other minerals (Roy et al., 2007). McGraw and Scarpa (2003), and Saoud et al. (2003) observed that lack of K and/or Mg in some well waters could negatively affect survival and growth.

Davis et al., (2005) and Saoud et al. (2003) observed that deficiencies in specific ions such as K and Mg negatively impact shrimp growth and survival. Where as McGraw and Scarpa (2003) and McNevin et al. (2004) observed that such deficiencies could be amended, with relatively good success, by the addition of K and Mg fertilizers in field trials. Evidence also suggests that the sodium (Na) to potassium ratio (Na:K) of low salinity well-waters may be a critical factor for successful growth and survival of shrimp (Roy et al., 2007). Liu et al. (2014a) observed that adjusting the Na:K ratios to levels similar to natural seawater (Na:K = 28:1) by fortifying the aqueous K concentration with potash or potassium salts was an effective way of facilitating the culture of *L. vannamei* in K deficient inland saline water. Davis et al. (2005) suggested that low levels of K or K and Mg were correlated to poor shrimp survival.

In the past decade, numerous studies have examined growth and survival of *L. vannamei* in low-salinity water with various ion concentrations (Saoud et al., 2003; Davis et al., 2005; Zhu et al., 2006; Roy et al., 2007). And researchers at various institutions are working to identify the reasons for the differences in survival and growth among farms and to develop mitigation strategies (Smith and Lawrence, 1990; McGraw et al., 2002; Samocha et al., 2002; Saoud et al., 2003). Despite many years of farming and success in rearing shrimp in low salinity environments, variable growth and survival among ponds are still being reported on a regular basis Roy et al. (2009); (Chumnanka et al., 2015) and aquaculturists still face problems due to mineral deficiencies in the ionic profiles of pond waters (Atwood et al., 2003; Saoud et al., 2003). Atwood et al. (2003) and Saoud et al. (2003) observed that even though salinity may be adequate for shrimp, ionic imbalances may negatively impact survival and growth. Further, Boyd and Thunjai (2003), McGraw and Scarpa (2003), and Saoud et al. (2003) found that in particular potassium concentrations in well water often are too low for good survival and growth of shrimp.

More knowledge of these relationships is needed in order to successfully and consistently rear penaeids in low salinity well-waters (Perez-Velazquez et al., 2012). Consequently, the present study was designed to further evaluate the relationships of water quality variables and production, survival and growth with special emphasis on K and Na:K ratios on culture of *L. vanname*i in LSWs.

Literature Review

Saline soils occur in arid regions in more than 100 countries (Roy et al., 2010) and surface waters and groundwater in such areas often have more than 1 g/L salinity [Keren (2000) as quoted by Roy et al., (2010)]. 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) as cited by Boyd et al., (2009)]. In the USA approximately two-thirds of the country is known to have saline groundwater at some depth (Feth, 1970). Early researchers suggested exploring the productivity of saline groundwater and the suitability of organisms to be farmed in such waters. Smith and Lawrence, (1990) suggested that the use of saline ground water to grow shrimp would make good use of a natural resource that normally is unwanted. There is a large potential for inland shrimp farming, and it should be encouraged – especially in salt-affected land. Inland saline aquaculture, is relatively a new type of aquaculture and has progressed in many parts of the world (Roy and Davis, 2010; Crespi et al., 2011; Jarwar, 2014). Inland saline aquaculture may offer an opportunity for income diversification and a potentially productive use of land that can no longer support traditional agriculture (Doupé et al., 2003).

Saoud et al. (2003), Sowers et al. (2005), and Parmenter et al. (2009) reported that inland low salinity waters (LSWs) generally do not have the same ion profile as marine waters. Boyd and Thunjai (2003) and Saoud et al. (2003) reported that irrespective of its source, LSWs often exhibit large variations in salinity and ionic profile, and has different proportions of major ions than found in seawater. Roy and Davis (2010) reported that even variations occur in ionic profiles of waters derived from the same aquifer and even individual ponds on the same farm can vary in ionic concentrations from year to year, resulting in the need to evaluate pond levels of ions every year prior to stocking. Saoud et al. (2003), Gong et al. (2004), (Boyd et al., 2007a), and Roy et al. (2007) reported that inland low salinity well waters are generally deficient in potassium, magnesium and sulfate and many other ions. There is a need for water modification approaches which alter the low salinity rearing medium to make it more conducive for production of shrimp and other marine species (Roy and Davis, 2010).

At this moment, the culture of shrimp and marine fish in low salinity waters is common practice in many countries throughout the world including China, Thailand, Vietnam, Ecuador, Brazil, Mexico, Israel, Australia, India and the United States and many other countries (Roy and Davis, 2010; Crespi et al., 2011; Partridge et al., 2008; Fielder et al., 2001; Jain et al., 2006). The culture of shrimp and other fish and crustaceans using low salinity water is a trend that continues to grow throughout the world (Roy et al., 2010). However there are certain differences in utilization of LSW. In Thailand, shrimp are cultured in ponds containing water of 2 to 5 g/L salinity prepared by mixing brine solution from coastal seawater evaporation ponds with fresh water (Fast and Menasveta, 2000; Limsuwan et al., 2002). In other nations, the primary sources of low-salinity water for shrimp culture are saline groundwater from wells and surface water. In some cases, granular salt has been applied to ponds to increase salinity (Boyd and Thunjai, 2003).

Most efforts to culture marine shrimp in inland ponds have focused on the use of saline groundwater (Roy et al., 2010). Inland culture of marine shrimp using saline well water with salinities of 1– 15 g/L is becoming more widespread throughout the world. In the USA inland marine shrimp farms have been established in Texas, Alabama, Arkansas, Arizona, Florida, Indiana, Illinois and and possibly other states (Samocha et al., 2002; Boyd, 2006). Roy et al. (2010) observed that some of these farms have been in production for more than 10 years (15 years as of today). Depending on their source, inland waters available for shrimp culture are usually of different salinities and possess different ionic compositions (Boyd and Thunjai, 2003). Alabama has several saltwater aquifers which are being utilized as sources of low salinity water for aquaculture (Saoud et al., 2003; C. A. Boyd et al., 2009).

The ability of *L. vannamei* (Pacific white shrimp) to tolerate a wide range of salinities has made it a popular species for low salinity culture (McGraw et al., 2002; Samocha et al., 1998, 2002). The Pacific white shrimp is a euryhaline species that can tolerate wide fluctuations in salinity throughout its life cycle (Atwood et al., 2003). There are even some instances that it is capable of growing in waters of less than 0.5 g/L (Laramore et al., 2001); however, this practice was not found to be commercially viable (Araneda et al., 2008; Cuvin-Aralar et al., 2009). Over the past few 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 (Roy and Davis, 2010). Because of this species' superiority in tolerance and adaptability, the production of *L. vannamei* in inland saline well water is a growing industry in coastal and inland regions of several countries including China (Cheng et al., 2005), United States (Roy et al., 2012), Thailand (Wudtisin and Boyd, 2011), Ecuador (Boyd and Thunjai, 2003; Saoud et al., 2003), Mexico (Castillo-Soriano et al., 2010) and other countries with considerable sizes of inland regions. Although *L. vannamei* can survive in low salinity water, it cannot survive cool water for extended periods of time. At low temperatures, metabolic processes slow down to the point that cellular processes do not work fast enough for survival (Lester and Pante, 1992). Boyd,

(2003) suggested that groundwater used to fill production ponds should have salinity greater than 2 g/L, and the water should be tested to determine ionic deficiencies compared to that of seawater. Both potassium and magnesium are necessary for several physiological processes (Roy et al., 2007). In low salinity water, potassium has been shown to increase growth and survival of shrimp, whereas magnesium has been demonstrated to increase survival (Saoud et al., 2003; Davis et al., 2005; Sowers et al., 2005; Roy et al., 2007). Hooge and Cummings, (1995) reported that although K is a minor constituent of brackish and fresh water but plays a pivotal role in biological processes such as acid-base equilibrium, ionic and osmotic balance, carbon dioxide transfer, and amino acid synthesis. Potassium is the primary intracellular cation and is also important in the activation of the N-K-ATPase (Mantel and Farmer, 1983), lack of adequate levels of aqueous K could thus be potentially detrimental in terms of the ability to effectively osmoregulate, because enzyme activity can be directly related to K concentration (Bursey and Lane, 1971).

Farmers in west Alabama have been successful in raising *L. vannamei* in inland low salinity waters by raising the K and Mg levels of their pond waters to more ideal levels. McNevin et al. (2004) observed increased shrimp production in Alabama low salinity waters (2-4 g/L) by raising the levels of K from 6.2 mg/L and Mg from 4.6 mg/L to 40 and 20 mg/L, respectively. 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 concentration (Saoud et al., 2003; McNevin et al., 2004). Davis et al., (2005) found that the addition of K improved *L. vanname*i postlarval survival and growth in inland low-salinity well water in west Alabama. Boyd et al., (2002) and Atwood et al., (2003) reported that ionic deficiencies in brackish water have led to poor growth and survival of marine shrimp. Potassium is the primary intracellular cation and is also important in the activation of the Na-K-ATPase (Mantel and Farmer, 1983). Lack of adequate levels of aqueous K could thus be potentially detrimental in terms of the ability to effectively osmoregulate, because enzyme activity can be directly related to K concentration (Bursey and Lane, 1971). Roy et al. (2010) reported that remediation techniques have been developed to improve the osmoregulatory capacity of shrimp reared in low salinity waters. It might therefore be more favorable for the growth of juvenile *L. vannamei* if the water K concentration was comparatively higher than that of oceanic seawater of the same salinity (Zhu et al., 2004).

In the past, studies have examined growth and survival of *L. vannamei* in low-salinity water with various ion concentrations (Saoud et al., 2003; Davis et al., 2005; Zhu et al., 2006; Roy et al., 2007). Researchers at various institutions are working to identify the reasons for the difference in survival and growth among farms and to develop mitigation strategies (Smith and Lawrence, 1990; McGraw et al., 2002; Samocha et al., 2002; Saoud et al., 2003). Roy et al. (2009) and Chumnanka et al., (2015) reported that despite many years of farming and success in rearing shrimp in low salinity environments, variable growth and survival among ponds are reported on a regular basis; and aquaculturists still face problems with ionic profile imbalances in pond waters (Atwood et al., 2003; Saoud et al., 2003). Atwood et al. (2003) and Saoud et al. (2003) observed that even though salinity may be adequate for shrimp, ionic imbalances may negatively impact survival and growth. Further, Boyd and Thunjai (2003), McGraw and Scarpa (2003), and Saoud et al. (2003) found that particularly potassium concentrations in well water often are too low for good survival and growth of shrimp. K, Na, Ca, and Mg are essential ionic elements for aquatic animals (Roy et al., 2010). Calcium, magnesium, potassium, sodium, chloride, and sulfate are the ions that are involved in shrimp osmoregulation and are the most important for shrimp culture (Boyd, 2006). Concentrations of these ions needed by shrimp in low-salinity culture are not known (Boyd et al., 2002). However, it is assumed that shrimp will survive and grow best if the ionic proportions are similar to those for seawater diluted to the same salinity as the low-salinity culture water (Boyd and Thunjai, 2003; Boyd, 2006). Roy and Davis, (2010), while referring to (Saoud et al., 2003, Zhu et al., 2004; L. A. Roy et al., 2007); and many other researchers noted that there is sufficient evidence to suggest that less than ideal ionic profiles are indeed responsible for many of the observed mortalities. Boyd and Thunjai, (2003) suggested that until data on ionic requirements of water for inland shrimp culture are developed through laboratory studies, it seems prudent to assume that concentrations of calcium, magnesium, potassium, and sodium should be similar to those of normal seawater diluted to the same salinity. Moreover, calcium and bicarbonate concentration should not be lower than 30 mg/L and 90 mg/L, respectively (Boyd and Thunjai, 2003).

Fortification of water with K has been advantageous with a number of other species of shrimp and fish cultured in low salinity environments; such as Australian snapper *(Pagrus auratus)*, Western king prawns (*Penaeus latisulcatus)*, Mulloway (*Argyrosomus japonicas),* Barramundi (*Lates calcarifer)*, and Tiger Prawn *(Penaeus monodon)* (Fielder et al., 2001; Prangnell and Fotedar, 2006; Partridge et al., 2008). After application of mineral amendments, levels of K and Mg are lost due to soil uptake, shrimp harvest, draining at harvest, seepage, or overflow (Boyd et al., 2007b; Pine and Boyd, 2010), which requires repeated applications of fertilizers containing these minerals.

Roy et al. (2007) noted that magnesium levels are also important for shrimp well-being, and can be maintained by regulating ratios of divalent cations in the water. In regards to magnesium, levels in pond water should equal at least 25% of the magnesium level in seawater diluted to the same salinity. 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, Mg levels in west Alabama LSW are naturally so low that the financial cost of raising Mg levels to such an extent with K-Mag can be cost prohibitive (Roy and Davis, 2010). Both K and Mg are essential for normal growth, survival, and osmoregulatory function of crustaceans (Mantel and Farmer, 1983).

Evidence also suggests that the sodium Na and K ratio of low salinity well-waters may be a critical factor for successful growth and survival of shrimp (Roy et al., 2007). 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., 2007). Many studies have revealed that the ratio between Na and K concentration strongly influences the survival of *L. vannamei* juveniles when K is added to coastal saline-alkaline groundwater, or low-salinity well water (Pan et al., 2006; Roy et al., 2007).

Liu et al., (2014a) observed that adjusting the sodium to potassium (Na/K) ratios to levels similar to natural seawater by fortifying the aqueous potassium (K) concentration with potash or other potassium salts is an effective way of facilitating the culture of *L. vannamei* in K deficient inland saline waters. Roy and Davis (2010) recommended a ratio of 40: 1, however they emphasized on the ratio to be closer to 28:1.1. Liu et al. (2014) concluded in their recent study that the Na/K ratios ranging from 23:1 to 33:1 might improve survival and growth. They further suggested that too high or too low Na /K ratios in low-salinity well water are a limiting factor to *L. vannamei*. They were of the view that immunity and disease resistance are also closely related to the Na/K ratio of the low-salinity well water. Roy and Davis (2010) suggested that 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. They further suggested that ratios of Mg:Ca should also approximate those found in natural seawater (3.1:1) to ensure adequate survival of *L. vannamei* reared under low salinity conditions.

Perez-Velazquez et al. (2012) cited the work of (Pan et al., 2006; Prangnell and Fotedar, 2006; Romano and Zeng, 2007a, 2007b, 2011), and (Tantulo and Fotedar, 2006) who showed that high Na:K ratios elicited decreased growth, survival, gill Na/K ATPase activity, hemolymph osmolality, or increased ammonia toxicity in penaeid shrimp and other crustaceans. Perez-Velazquez et al. (2012) demonstrated a strong sensitivity of *L. vannamei* survival to the aqueous Na:K ratio at low temperature.

Roy et al. (2007) found that the closer the Na:K ratio is to 28:1 the better the growth of the animals. Roy et al., (2006) reported that better growth and survival were obtained in low salinity water of (1.4 g/L) at a farm with the Na:K ratio most similar to full strength seawater. Fielder et al., (2001) also reported an influence of Na:K ratio on growth of Australian snapper (*Pagrus auratus*) cultured in saline groundwater deficient in K. Zhu et al., (2004), Zhu et al., (2006) reported that high Na:K levels can have an effect on shrimp growth even at salinities as high as 30 g/L. Davis et al. (2005) suggested that previous work has correlated low levels of potassium and/or magnesium to poor shrimp survival. Saoud et al., (2003) demonstrated a positive correlation between survival and the K concentration in the water. Roy et al. (2007) found increased individual weight, specific growth rate, and percent weight with increasing K concentration (decreasing Na:K ratios).

Prapaiwong and Boyd, (2012a) reported that water variables in their study never reached actual toxic levels; however, they may have stressed shrimp and negatively influenced survival, feeding activity, growth rate, and production. Perez-Velazquez et al. (2012) believed that water temperature interacts with salinity and ionic ratios to determine growth and survival of shrimp reared in low salinity water. Ponce-Palafox et al., (1997) and Wyban et al., (1995) observed that shrimp growth increases with increase in temperature. Pine and Boyd, (2010), while referring to a 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. Saoud et al. (2003) found that besides K, Mg, manganese (Mn) and sulfate (SO_4) also have an effect on shrimp survival. Shrimp survival and growth in outdoor ponds are affected by a variety of factors such as soil type (Ritvo et al., 1998); shrimp strain (Kumlu and Jones, 1995); and PL health (Samocha et al., 1998c). Perez-Velazquez et al. (2012) emphasized the need of additional knowledge in understanding these relationships for successful and long term aquaculture of penaeids in low salinity well-waters.

Materials and Methods

The Study Area: The majority of this effort was based on data collected from 20 ponds filled with low-salinity water at the Green Prairie Aquafarm (GAF) during 2013 and 2014. The farm (Fig. 1) is located in the Blackland Prairie region of Alabama about 6 km north of Forkland on State Highway 43 in Greene County (GPS coordinates 32°41'43.35"N, 87°54'25.50"W). Pond size ranged from 0.50 ha to 2.02 ha in water surface area with average water depths when full to levels of overflow structures of 1.19 to 1.77 m. The water source is a well that draws water from a saline aquifer that has a salinity of 3.7 g/L (McNevin et al., 2004).

Ponds were filled to about 15 cm below the overflow structures before stocking, and well water was added as necessary to maintain this level. The practice provided storage volume to prevent overflow after rainfall. Ponds were stocked with postlarval Pacific white shrimp *Litopenaeus vannamei* during the last week of May each year. The postlarvae were acclimatized in indoor tanks for 1 wk and then stocked in ponds. The means ± standard deviations and (ranges) for pond stocking rates were 25.0 ± 2.1/m^2 (22-30/m^2) and 29.5 ± 4.6/m^2 (18.5-34.2/m^2) in 2013 and 2014, respectively. Ponds were treated with muriate of potash (potassium chloride) and K-Mag (potassium magnesium sulfate) before shrimp were stocked, and ponds were treated once or twice more during the crop for the purpose of K and Mg augmentation. The average treatment rate was about 500 kg/ha of muriate of potash and 900 kg/ha of K-Mag. About two-thirds of the quantities of mineral amendments were applied initially, and the remainders were applied later. The shrimp were fed twice daily with 35% crude protein, pelleted feed. Daily feed input averaged around 1.5 to 2.0% of shrimp body weight per day and was applied with a truck-mounted, mechanical feeder. Ponds were aerated with floating, electric, 5- and 10-hp paddlewheels aerators as necessary to avoid low dissolved oxygen (DO) concentrations. These mechanical aerators were connected to a DO monitoring system that triggered the aerators to turn on when DO concentration fell below 3 mg/L. It also turned aerators off at 6 mg/L of DO. Thus, aeration usually was applied at night, but ponds were aerated around noon daily for about 1 hr in order to mix the water columns, avoid thermal stratification, and assure adequate DO in deeper water where shrimp dwell. Water exchange was not applied either year. Ponds were harvested in September and October each year by using a modified shrimp pump and de-watering tower, and to facilitate harvest, the water level in each pond was reduced by pumping water to one or more nearby ponds that already had been harvested; in this way most of the water is retained on the farm. A portion of the water from some ponds had to be discharged from the farm into a nearby stream in order to provide storage volume for water from adjacent ponds at the beginning of harvest in different sections of the farm. Prapaiwong and Boyd, (2012b), found that roughly 50% of the total farm water volume at the beginning of harvest was discharged each year. Retention of pond water lessened the cost of pumping well water and conserved the K and Mg added in mineral amendments.

In 2014, two other farms were added; the Dickie Odom Farm (DOF) and the Forkland Springs Farm (FSF) depicted in Figs. 2 and 3. The DOF is located about 11 km north of Forkland on Alabama State Highway 43 in Greene County (GPS coordinates 32°44'28.12"N; 87°54'16.86"W). The FSF is located on Alabama State Highway 48 about 10 km north-west of Forkland (GPS coordinates 32°40'5.67"N; 87°56'58.46"W). Eight ponds were sampled at each of the two farms. These two farms also are located in the Blackland Prairie region and rear both channel catfish and shrimp – but in separate ponds. Only shrimp are produced at GAF.

Ponds at DOF and FSF also were filled with saline well water. Potassium and Mg augmentation, stocking, feeding, aeration, water management, and harvesting practices at DOF and FSF were similar to those used at GAF.

Sampling: The water quality monitoring began on 6 June and ended on 12 September in 2013, while in 2014 it began on 5 June and ended on 23 September. Samples were collected weekly from a pier in each pond by dipping water from approximately 50 cm below the surface. Samples were confined in 500-mL plastic bottles each of which had been washed thoroughly in the laboratory and again with water from the pond being sampled. Samples were transported on ice in insulated chests to the E.W. Shell Fisheries Research Center, Auburn University, Alabama, for chemical analysis. Other preservation methods were not applied, because the samples were in transit for only around 3 hr and analyzed soon after they arrived at the laboratory.

Water Quality Analysis: Samples were passed through Whatman No. 42 filter paper, and concentrations of K, Na, total alkalinity (TA), total hardness (TH), calcium hardness (CaH), and salinity were measured. Salinity was determined with an Orion 3-Star Conductivity Benchtop (Thermo Scientific, Singapore). Total alkalinity concentration was determined by sulfuric acid titration to the methyl orange endpoint. Total hardness was measured by EDTA titration to the eriochrome black-T endpoint, while CaH was analyzed by EDTA titration to the murexide endpoint (Eaton et al., 2005). Magnesium hardness (MgH) was calculated by subtracting CaH from TH. Calcium and Mg concentrations were estimated as follows:

$$\text{Ca (mg/L)} = \text{CaH} \div 2.5$$

$$\text{Mg (mg/L)} = \text{MgH} \div 4.12$$

Aliquots of filtered samples for K analysis were diluted to 1:10 with distilled water, while those for Na analysis were diluted 1:100. A Cole-Parmer Model 2655-00 flame photometer was used for Na and K analyses of the diluted aliquots.

The seawater equivalent concentration (SEC) of a cation – the concentration of a cation that would result if normal seawater was diluted to the salinity of the low-salinity water being considered (Boyd et al., 2010) – was calculated as follows:

$$\text{SEC (mg/L)} = \text{Salinity in pond (g/L)} \times \text{F}$$

where F = a factor for a particular cation (mg/L per g/L). Factors for Na, K, Ca, and Mg are 304.35, 11.01, 11.59, and 39.13 mg/L per g/L, respectively.

The pH values at which calcium carbonate ($CaCO_3$) saturation would occur in the study ponds under various conditions were estimated by calculating the saturation pH (pH_{sat}) for the Langelier saturation index (LSI). The LSI equation (Langelier, 1936) has the form:

$$\text{LSI} = \text{pH of water} - pH_{sat}.$$

The pH_{sat} term was obtained by the following equation of Langelier (1936):

$$pH_{sat} = (9.3 + A + B) - (C + D)$$

in which A = [$\log_{10}$ total dissolved solids (mg/L) – 1] ÷ 10; B = -13.12 × [$\log_{10}$ water temperature (°C) + 2.73] + 34.55; C = $\log_{10}$[Ca^{2+} (mg/L) × 2.5]; D = $\log_{10}$[total alkalinity (mg/L)].

Shrimp production and survival data were collected by the farmer at GAF and provided at the end of each growing season. This farmer also provided historical survival and production data for each pond from the first year of operation (2001) until the present. Farmers at DOF and FSF did not keep organized records, and could not provide reliable data. Daily rainfall data were obtained for a weather station near Demopolis, Alabama and located about 20 km from GAF http://www.usclimatedata.com/climate/demopolis/alabama/united-states/usa10155/2013/5.

Statistical Analysis: Averages, standard deviations (SD), coefficient of variation and ranges were calculated for water quality data for individual ponds as well as for farms. The relationships between water quality variables and shrimp survival and production were assessed through simple, linear regression analysis and multiple regression. Analysis of variance (one-way ANOVA) was used to detect if significant differences in mean concentrations of water quality variables at GAF and among the three farms occurred among ponds. T-test and Tukey’s test were then conducted to further identify the differences ($P \leq 0.05$). Microsoft Excel and StatPlus:mac, statistical software for Mac OS, were used for statistical analysis.

Multiple regression analysis of relationships between water quality and survival and production were conducted by ordinary least squares (OLS) regression. The OLS was processed in (ArcMap 10.7.1) that couples traditional OLS with ArcGIS. The explanatory or independent variables (water quality data) and the dependent variables (survival and production data) from GAF in 2013 and 2014 was used to make equations for predicting survival and production from the explanatory variables. The details of OLS regression are explained by (Rossi, 2010) and (Mansour, 2015). The ArcMap allowed the results of OLS regression to be displayed on maps of GAF.

Results and Discussion

Survival and Production: The ponds at GAF were stocked at similar average rates of 25 ± 2.1/m^2 in 2013 and 28.9 ± 4.0/m^2 in 2014, but in 2013, survival was greater ($t = 3.97$; $P = 0.0003$) than in 2014 (Table 1). Thus, production per pond averaged higher ($t = 2.86$; $P = 0.0068$) in 2013 than in 2014 (Table 1). The R^2 values between survival and production were 0.906 and 0.763 in 2013 and 2014 respectively. This finding agrees with an earlier study at GAF in 2012 in which production had a high, positive correlation ($R^2 = 0.926$) with increasing survival (Chumnanka et al., 2015). Nevertheless, there was great variation among ponds in survival and production during 2013 and 2014 as indicated by the large coefficients of variation (Table 1). In 2013, survival ranged from 8.5% to 104%, while in 2014, the range was between 13.3% and 65.4%. Survival above 100% resulted in errors in counting the number of postlarvae stocked into ponds or in enumerating the number of harvested shrimp. Considering the small size of postlarvae, it is most likely that the errors in enumeration occurred at stocking. It is also possible for a pond to be double stocked unintentionally; in either case the error affected the survival estimate. Production in individual ponds ranged from 681 to 6,550 kg/ha in 2013 and from 1,166 to 5,008 in 2014.

Historically, survival and production in ponds at GAF were lower during the first year of production (2001) than any year since (Table 1). The especially low survival in 2001 deserves mention. That year, dying shrimp were noted soon after stocking, and analyses revealed low K concentrations (<10 mg/L) in pond waters. Application of muriate of potash increased K concentration and mortality subsided, but it was too late to stock more postlarvae (David Teichert-Coddington, owner of GAF and C. E. Boyd, Auburn University, personal communications). In 2002, all ponds were treated with muriate of potash to maintain at least 30 mg/L of K and survival and production were much greater than in 2001 (McNevin et al., 2004). In addition to muriate of potash, K-Mag was applied to ponds beginning in 2003, because laboratory studies by (Saoud et al., 2003) suggested that greater Mg concentration improved growth of postlarvae. McNevin et al., (2004) concluded, however, that benefits to survival and production of cation augmentation in ponds at GAF were almost entirely from K. Since 2003, ponds at GAF have been treated two to three times annually to increase K and Mg concentrations, because the two cations are absorbed from the water by bottom soil (Boyd et al., 2007a; Pine and Boyd, 2010), and lost from the ponds in water discharged at harvest (Prapaiwong and Boyd, 2012b). Potassium and Mg augmentation has allowed the farms to be successful in producing shrimp.

The results for 2001 and 2002 were included in the estimates of survival and production despite the fact that mineral amendments were not applied in the same manner as in other years. However it was determined that leaving these two years from the calculation would have improved survival by less than 1% and production by less than 100 kg. In 2013, average survival in ponds at GAF was only slightly less than the best ever achieved, while mean production was the best ever realized (Table 1). The following year, survival and production were similar to the historical averages. These differences are based on farm averages for all ponds, and any given pond often differed considerably in survival and production between 2013 and 2014. For example, pond S-7 had 93% survival and produced 6,170 kg/ha in 2013, but it had survival and production of 15.5% and 1,166 kg/ha, respectively, in 2014. Pond N-12 had 8.5% survival and

681 kg/ha production in 2013, but it had 59.8% survival and 2,707 kg/ha production in 2014. Of course, some ponds had similar survival and production both years.

Sixteen ponds at GAF have been operated annually since 2001. Annual, historical survival rates in these ponds averaged from 37.3% in N-2 to 71.5% in S-5 (Table 2). Five of the 16 ponds had low survival (<50%), eight had intermediate survival (50-60%), and three had high survival (>60%) based on historical averages. Yet, on some years, ponds with a high, average, historical survival had low survival and vise-versa. The same phenomenon also occurred for production. The average, historical production range was 2,297 to 4,831 kg/ha (Table 2), but as with survival, on a given year, production in a particular pond might deviate greatly from its average, historical production. Classifying production <3,000 kg/ha as low, 3,000 to 4,000 kg/ha as intermediate, and >4,000 kg/ha as high, there were three, six, and one pond in common, respectively, among the three categories of average, historical survival and production between 2013 and 2014. It is not surprising that complete agreement was not obtained between the three categories. In ponds stocked at roughly the same rate, when survival is low, the remaining shrimp often grow to a larger size than they do in ponds with greater survival and more shrimp per unit area. Nevertheless, there was a high positive correlation between survival and growth at GAF as mentioned earlier.

The survival and production in 2013 and 2014 of the 16 ponds used annually since 2001 were plotted versus historical averages for annual survival and production in these ponds (Fig. 4). No trends occurred between historical averages and 2013 and 2014 results for either survival or production. Although data in Table 2 revealed that some ponds historically had better survival and production than other ponds, the plots in Fig. 4 showed that the survival and production in an individual pond could not be predicted from the historical performance of that pond.

The variation in survival and production apparently is related to factors operating in a given pond on a specific year rather than to basic characteristics of a particular pond. This hypothesis is supported by the work of (Chumnanka et al., 2015), in which differences in pond sediment characteristics among ponds could not be consistently related to variation in survival and production. Also, water for supplying all ponds was from the same aquifer or from rainfall and runoff into ponds. The composition of well water typically is constant over time, and rainfall and surface runoff are not highly mineralized (Boyd, 2015). The chemical composition of the water supply for ponds apparently was rather constant from year to year. The ponds also received similar rates of K and Mg inputs in mineral amendments, but the farm owner could not provide the exact quantities of K and Mg applied to each pond in 2014, 2013, or during earlier years. Nevertheless, K and Mg were measured weekly in ponds, and the affects of different application rates of the amendments were reflected in the increased concentrations. In summary the K and Mg amendments in these ponds have increased the production and survival. However still there is a lot variation in production and survival among ponds and among years which needs further investigation.

Water Quality Data: Previous studies did not reveal relationships between low DO, concentrations or high concentrations of potentially toxic metabolites on survival and production of shrimp in ponds at GAF (Prapaiwong and Boyd, 2012a). These investigators concluded that aeration rates were adequate to avoid stressfully low DO concentration and to stimulate the oxidation of ammonia and nitrite to nitrate by nitrifying bacteria. Aeration was also considered to adequate to maintain an elevated redox potential at the sediment-water interface to prevent hydrogen sulfide from entering the water column. Trace elements were also at acceptable concentration in ponds at GAF (Prapaiwong and Boyd, 2014). Nevertheless, these investigators suggested that Ca and TA concentration might affect survival and production. Water temperature also may be responsible for variation in survival among years, but water temperature is relatively similar among ponds during a given year (Prapaiwong and Boyd, 2012c). Because of these previous findings the focus of the present study on concentrations of salinity, major ions, and TA concentrations seems logical.

Potassium and Mg augmentation has been practiced regularly, but the farmers have not been monitoring K and Mg concentration and responding with applications of amendments on an individual pond basis as regularly as in the past. Thus, K and Mg concentrations were measured in the present study to ascertain if differences in concentrations of the two cations might occur among ponds and affect survival or production. This had not been done previously because it was assumed that concentrations of K and Mg were adequate because of their addition in potassium chloride and K-Mag. The Na/K ratio was computed, because the farmer at GAF feels that this ratio rather than the K concentration alone is most indicative of the suitability of low-salinity water for shrimp culture. Chloride was not measured because its concentration had been measured several times in the past and found to be directly related to the salinity (Boyd et al., 2006). Sulfate was not determined, because its concentration was low (<10 mg/L) in all previous assessments, and discussions with physiologists revealed that low sulfate should not be expected to lessen shrimp survival and growth (C. E. Boyd, Auburn University, personal communications).

The grand means for concentrations of measured water quality variables each year were calculated by averaging the individual pond averages of the entire sampling period for each variable. The grand means of cations, salinity, TA, and the Na/K ratio in ponds at GAF did not differ (t-values of 0.44 to 1.73; P-values of 0.10 to 0.62) between 2013 and 2014 (Table 3). Nevertheless, this was not grounds for dismissing these variables as possible causes of the variation in survival and production between years or among ponds during each year. There could have been differences in concentrations of these variables among ponds during both years that influenced survival and production.

Water quality data collected in 2014 for ponds at DOF and FSF also are summarized as grand means in Table 4. Salinity averaged 3.7 and 5.1 g/L at FSF and DOF, respectively, as compared to 1.6 g/L at GAF. Grand means for concentrations of salinity, Na, Ca, and Mg in ponds at DOF were greater ($P<0.05$) than those in ponds at FSF, but other variables did not differ between the two farms. When compared with GAF (Table 3), both DOF and FSF had greater ($P<0.05$) grand means of salinity, Na, K, Na/K, Ca, and Mg, but a lower grand mean for TA, than were measured at GAF. Greater concentrations of K and Mg at DOF and FSF might have been related to greater K and Mg augmentation at these farms than at GAF, but data on the amounts of muriate of potash and K-mag applied at DOF and FSF were unavailable. The greater concentrations of cations at DOF and FSF than at GAF reflect the greater salinity at DOF and FSF. The lower TA concentration at DOF and FSF than at GAF likely resulted because of more $CaCO_3$ precipitation in ponds at these two farms than at GAF – Ca concentrations were more than twice as great at DOF and FSF than at GAF.

Weekly Variation in Pond Water Quality at GAF: The wide standard deviations, coefficients of variation, and ranges for measured water quality variables (Tables 3 and 4) revealed that there was considerable variation in average concentrations of each measured variable among ponds over the entire study period each year. Moreover, concentrations of water quality variables for all ponds differed from week to week within the same pond. The weekly averages, standard deviations, and ranges for the concentrations of each variable in ponds at GAF were plotted in Figs. 5 to 11 to depict variation among ponds on each sampling date in 2013 and 2014. Such plots were not made for ponds at DOF and FSF, because no survival and production data were available from these two farms to allow relationships of water quality with shrimp performance to be evaluated.

Before discussing weekly variation of measured water quality variables, rainfall patterns will be discussed as the amount of rainfall might influence water quality. Normal, annual rainfall and rainfall in 2013 and 2014 are contrasted in Table 5. Clearly, neither year had normal rainfall; 2013 was a wet year while 2014 was a dry year. Rainfall during the period beginning 1 wk before sampling was initiated and extending until the last sampling date also was greater in 2013 than in 2014 – 46.7 cm vs 30.3 cm, respectively (Fig. 12).

Evaporation from pond water surfaces in Alabama from late spring until mid-fall is almost always greater than direct precipitation into ponds (Boyd, 1985a). Using average pond evaporation for Alabama (Boyd, 1985b), evaporation for the period of rainfall shown in Fig. 12 averaged about 55 cm. Evaporation from pond surfaces in 2013 and 2014 offset the gain in water accrued from direct precipitation into ponds. Nevertheless, heavy rain would temporarily dilute concentrations of water quality variables in ponds – possibly for long enough to affect shrimp. For example, the average depth of water in some ponds was no more than 1.0 to 1.2 m, because water levels were maintained below overflow structures. The heaviest weekly rainfall in 2012 (≈10 cm) likely diluted the shallower ponds by 8-10%.

Some ponds at GAF are true embankment ponds, while others have watershed areas in addition to side slopes and tops of embankments. In Alabama, runoff usually is restricted to only the largest rainfall events between mid-May and mid-November (Boyd et al., 2009). Nevertheless, some ponds at GAF likely received additional dilution by runoff water during some weeks.

Seepage from ponds in the Blackland Prairie is modest, because the heavy clay soil resist infiltration (Yoo and Boyd, 1994). Moreover, seepage does not directly affect ion concentrations in pond water, because ions move downward with the infiltrating water. There was a deficit between direct precipitation and evaporation plus seepage, and it was necessary to add saline well water to ponds to maintain adequate water levels. Ponds that seeped more or obtained lesser amounts of runoff likely received greater additions of well water than did other ponds. Variation in saline well water additions would have resulted in greater inputs of ions into some ponds than others.

The average salinity of all ponds at GAF in 2013 was 1.5 g/L on week 1, but it declined to about 1.5 g/L by week 3 and remained near this concentration (Fig. 5). In 2014, the initial salinity was about 1.2 g/L, but it gradually increased reaching 2.1 g/L on week 17. During 2014, salinity usually was above 1.5 g/L. Regardless of greater rainfall in 2013 than 2014, the average salinity of all ponds at GAF did not differ between the two years (Table 3). Thus, salinity did not appear to be associated with better survival and production for the farm in 2013.

The farm averages for K concentration were similar both years despite greater rainfall in 2013. Moreover, the averages did not differ between weeks by more than 10 mg/L during the entire study either year (Fig. 6) – this consistency resulted from K augmentation. Variation among ponds on individual sampling dates, nonetheless, was appreciable – usually at least 30 mg/l and sometimes more. On some dates, K concentrations <20 mg/L were measured.

Sodium concentration (Fig. 7) began at about 600 mg/L and increased to around 800 mg/L in 2013. There was appreciable rainfall during the first 11 weeks of sampling, and average Na concentration fell to around 300 mg/L. Near the end of the grow-out period, less rainfall occurred and Na concentration increased to about 500 mg/L. In 2014, Na concentration began at about 550 mg/L. There was an unexplained dip to nearly 200 mg/L between weeks 1 and 3, but afterwards, the Na concentration steadily increased to slightly over 600 mg/L on week 17. Sodium concentration varied more among ponds on the same date in 2014 than in 2013, but the minimum concentrations tended to be less in 2013 than in 2014. The minimum concentration measured was below 200 mg/L on week 2 in 2014, but minimum concentrations often were near 200 mg/L in 2013. Rainfall appears to have been the cause of low concentrations of Na and presumably of other ions. Despite more variable weekly Na concentrations among ponds on individual sampling dates and lower minimum levels of Na, survival and production were better in 2013 than 2014.

The average Na/K ratio fluctuated between 9 and 21 in 2013 and 7.5 and 17.5 in 2014 (Fig. 8). There also was considerable variation in the Na/K ratio among ponds on each sampling date, but overall, there was more variation over time in the ratio in 2013 than in 2014. The Na/K ratio was consistently below the ratio of 27.6 for normal seawater in most ponds both years.

The Ca and TA concentrations followed similar trends of change. The Ca concentration usually averaged between 30 and 40 mg/L both years (Fig. 9). There was tremendous variation in Ca concentration on individual sampling dates with differences among ponds usually being 20 to 30 mg/L in 2013 and even greater in 2014. Total alkalinity concentration in ponds at GAF in 2013 averaged 120 mg/l at the beginning of the study, increased to about 150 mg/L on week 6, and remained at this concentration (Fig. 10). On individual sampling dates, there was a difference of 75 to over 150 mg/L TA among the ponds. In 2014, the pattern in TA concentrations was quite similar to the previous year. The similarity between trends in Ca and TA concentrations was not surprising as these two variables often are closely related (Boyd et al., 2015).

Aquaculture ponds in the Blackland Prairie region of Alabama filled by runoff often have TA and Ca concentrations in excess of 100 mg/L and 30 mg/L, respectively, because of dissolution of limestone in bottom soil (Silapajarn et al., 2004). Thus, pond waters at GAF are not dependent upon saline well water as the main source of these two variables.

The wide variation in TA and Ca concentrations likely resulted from fluctuations in the availability of carbon dioxide (CO_2) in pond water to re-dissolve $CaCO_3$ suspended in the water column or contained in sediment and bottom soil. The reaction of CO_2 with $CaCO_3$ is:

$$CaCO_3 + CO_2 + H_2O = Ca^{2+} + 2HCO_3^-.$$

This reaction yields chemically equivalent quantities of Ca and HCO_3 (bicarbonate). Bicarbonate is the major source of alkalinity in natural waters (Boyd et al., 2015). Increasing CO_2 concentration enhances $CaCO_3$ solubility, while decreasing CO_2 concentration causes $CaCO_3$ precipitation.

In aquaculture ponds receiving large daily feed applications, dense phytoplankton blooms wax and wane during intervals of a few weeks (Boyd, 1973; Boyd and Scarsbrook, 1974; Tucker and Lloyd, 1984). The ponds at GAF were constructed on soils containing limestone of a high $CaCO_3$ content (Chumnanka et al., 2015). When phytoplankton blooms wax there likely would be a shortage of CO_2, pH would rise above 8.3, and phytoplankton would use HCO_3 as a carbon source. As explained by (Boyd et al., 2015), when aquatic plants depend upon HCO_3 as a carbon source, CO_2 for use in photosynthesis is removed from HCO_3 in a process mediated by the enzyme carbonic anhydrase, and carbonate (CO_3) is released into the surrounding water. The following apparent equation illustrates the process:

$$2HCO_3^- \xrightarrow[\text{anhydrase}]{\text{carbonic}} CO_2 + CO_3^{2-} + H_2O$$

Carbonate released into the water hydrolyzes according to the following reaction:

$$CO_3^- + H_2O = HCO_3^- + OH^-.$$

This reaction reaches an equilibrium rather than progressing completely to the right, but both CO_3 concentration and pH rise as photosynthesis proceeds. When the saturation pH for $CaCO_3$ is exceeded, $CaCO_3$ precipitates.

$$Ca + CO_3 = CaCO_3\downarrow$$

Precipitation of $CaCO_3$ causes a decrease in both TA and Ca concentration. When phytoplankton blooms wane, there is more CO_2 as a result of CO_2 release from decomposition at a time of less CO_2 demand by phytoplankton for photosynthesis. Increased CO_2 concentration in water reverses the process, pH declines, and limestone dissolves to increase TA and Ca concentration. Strong mixing of ponds at night by the aerators favors the movement of water containing CO_2 across the pond bottom to cause $CaCO_3$ dissolution.

The average Mg concentration in pond waters was low (<15 mg/L) both years (Fig. 11). There was also great variation in Mg concentration. The limestone in the pond soils contains some $MgCO_3$, and the increases in Mg concentration were probably the result of increased limestone solubility under conditions explained above.

Water Quality versus Survival and Production: The correlations of determination (R^2) for simple linear regressions between the averages of salinity, Na/K ratio, and concentrations of cations and TA and survival and production of shrimp in each pond at GAF during 2013 and 2014 were calculated (Table 6). Some of the variables were significant at $P<0.05$. It may be seen from the R^2 values that K concentration – in spite of augmentation of this ion – accounted for about 20% of the variation in survival and production in 2013 and even less during 2014. Differences in TA concentration was responsible for about 30% of variation during 2013 and 20% of variation during 2014 in production, but TA concentration was not correlated with survival either in 2013 or 2014. Salinity was positively correlated with production in 2014, while Ca concentration was positively correlated with survival in 2014. Significant relationships could not be found between salinity and production and between Ca concentration and survival in 2013. The simple regression analysis did not account for more than 20% in survival and production.

There could be interactions among the concentrations of water quality variables and survival and growth not detectable by simple correlation. Therefore, the data were analyzed by ordinary least squares analysis (OLS) which is a kind of multiple regression. The regression analysis is more consistent when the variables do not have perfect multicollinearity, e.g., one or more linear relationships exist within the set of independent variables (Neeleman, 2014). The

OLS analysis provides a variance inflation factor (VIF), and variables with a VIF greater than 7.5 should be removed (Mansour, 2015). In the present analysis, salinity and the Na/K ratio had VIF above 7.5 and were removed from both the survival and production assessments. The coefficient of the OLS reflects the direction (negative or positive) of the expected change caused in the dependent variable for each unit change in a particular independent variable (ArcGIS Help 10.1 - Interpreting OLS results").

The OLS was conducted using five explanatory variables, Na, K, TA, Ca, and Mg, and the resulting coefficients, VIF values, and P-values are given in Table 7. Coefficients of determination for Na, K, and Mg had $P>0.05$. Potassium and Mg amendments are added to pond waters on a regular basis to supplement their naturally low concentrations. As a result, relationships of K and Mg and any of their ratios with other cations to survival and production would be minimized or completely masked. Whether Mg augmentation is actually needed at GAF is not clear, but this cation is applied regularly nonetheless. The weak relationship for Na to shrimp performance also is not surprising, because Na is a major component of the saline well water used to fill and maintain the ponds. The salinity in waters at GAF is highly dependent upon Na concentration, and the ratios of K and Mg with Na would be affected by salinity. The strongest relationships ($P<0.05$) were found to be for TA and CA; $P = 0.02$ and 0.01 for survival and 0.02 and 0.04 for production (Table 7).

The OLS prediction equations for survival and production are provided below:

Survival (%) = $-116.0 + 0.035\ \text{Na} - 0.40\ \text{K} + 0.605\ \text{TA} + 2.97\ \text{Ca} - 0.28\ \text{Mg}$ ($R^2 = 0.487$)

where Na, K, TA, Ca, and Mg = concentrations of these variables (mg/L).

Production (kg/ha) = $-7{,}313.8 + 4.1\ \text{Na} - 16.4\ \text{K} + 39.6\ \text{TA} + 153.7\ \text{Ca} - 28.2\ \text{Mg}$ ($R^2 = 0.547$)

Thus, the OLS regression equation accounted for roughly 50% of the variation in survival and production.

The correlation analyses were conducted using the average concentrations of water quality variables and final survival and production for each pond. This approach can ascertain if there is a relationship between average concentrations of a measured water quality variable during the entire production period and survival and production. Of course, periods when the concentration of a water quality variable is either low or high enough to kill or stress and lessen growth of shrimp may occur. A deviant concentration existing for a few days might affect shrimp survival and production without appreciably affecting the average concentration of a water quality variable calculated over the duration of the grow-out period. Moreover, a temporary period of deviant concentration could be responsible for post larval mortality soon after stocking that might go undetected yet severely affect production. The survival and production in a pond may also decline because of mortality during the crop resulting from a disease or a water quality imbalance such as low DO concentration or high ammonia nitrogen concentration. The temporary declines in water quality variables could have been assessed if the weekly growth and survival data would be available as promised by the farmer. In absence of such data temporary declines in concentrations of variables are hard to account for; and there is no way of accurately estimating weekly survival.

The correlation effort would have been much more definitive if weekly survival and growth rate estimates had been available. Nevertheless, the correlation analyses revealed that salinity, Na, K, TA, and Ca concentrations had a positive correlation to survival, growth, or both in ponds at GAF. Moreover, the OLS analysis showed that about 50% of the variation in survival and production could be explained by differences in concentrations of the four cations and TA.

The importance of maintaining K and Mg concentrations was masked in the OLS regressions by augmentation of these two cations. However, the OLS regressions clearly confirmed the importance of TA and CA concentration.

It is unfortunate that managers at DOF and FSF did not provide survival and production records for ponds. Ponds at DOF and FSF had similar concentrations of measured variables, but the concentrations of water quality variables at these two farms were strikingly different from those measured at GAF. It would have been helpful to determine if lower TA concentration and greater salinity and cation concentrations observed at DOF and FSF as compared to those found at GAF resulted in differences in survival and production of shrimp.

The variation not accounted for by the OLS equation could result from a variety of factors to include the condition of postlarvae at stocking, errors in stocking density, water temperature, disease, toxic algae, stress and mortality cause by low DO concentration, high concentration of ammonia nitrogen, and hydrogen sulfide production in bottom soil, as well as unknown factors. Because the many variables that can affect shrimp survival and production vary in concentration or intensity among ponds and years, it seems remarkable that cation and TA concentrations explained almost half of the variation. The maps generated by OLS in ArcGIS are at Fig. 13-14.

The Seawater Equivalent Concentration (SEC) and Water Quality Ratios: Optimal ranges of cation concentrations at different salinity levels for survival and growth of *L. vannamei* have not been ascertained. The SEC was suggested as an unproven but seemingly logical way of determining if a particular low-salinity water had a suitable cation balance for shrimp culture (Boyd et al., 2002). The basic assumption for the utility of the SEC was that *L. vannamei* grow in seawater as well as in estuarine water that typically resembles seawater in proportions of cations. Thus, it was recommended that low-salinity water for culturing shrimp should have concentrations of cations similar to those in seawater diluted to the salinity of the water in question. Of course, (Boyd et al., 2002), mentioned that shrimp might have a minimum requirement for one or more cations. If so, at a salinity of a few grams per liter, the SEC might be less than the minimum requirement for one or more cations. In fact average Na, K and Ca concentrations were found to be in excess of SEC at all three farms, except average Mg concentration which was found less than SEC at all these farms. Furthermore, it is not known if a concentration of an individual cation much greater than its SEC might be directly harmful or result in an undesirable imbalance of cationic proportions (ratios). Despite these limitations, the SEC has been used to assess water for cation balance with some degree of success.

Saline well water before and after standing for several weeks in ponds without K and Mg augmentation diverged greatly with respect to SEC values (Table 8). In the present study, only Na concentration was near its SEC value (Table 9). The SEC for K is quite low in waters of low salinity – about 17 mg/L at 1.5 g/L salinity. Thus, K augmentation increased K concentration above the SEC – usually more than 100% greater. Calcium concentration also averaged well above its SEC both years; because the well water was relatively high in Ca concentration. At a salinity of 1.5 g/L, the SEC for Ca is around 18 mg/L. The Mg concentration was roughly 300-400% less than its SEC, because even at 1.5 g/L salinity, the SEC for Mg is nearly 60 mg/L. The amendment K-Mag contains 22% K and 10.8% Mg. The amount of this amendment applied to ponds to cause a modest increase in Mg raised the K concentration well above the SEC, while the Mg concentration remained far below the SEC.

Survival and production in 2013 at GAF were excellent (production was the best ever) and comparable to that achieved in coastal ponds filled with seawater or brackishwater and stocked at similar rates (Boyd and Tucker, 2014a). This occurred in spite of great deviations in cation concentrations from their SEC values. Moreover, in 2014, survival and production were only average, yet the SEC values and Na/K ratio were similar to those recorded in 2013. These observations certainly cast serious doubt on the usefulness of the SEC and the Na/K ratio.

The Ca/Mg ratio has received scant attention in low-salinity shrimp farming, despite the Ca/Mg ratio of 0.30 in normal seawater being much less than that found in most inland, low-salinity shrimp ponds. For example, (Boyd and Thunjai, 2003) reported ionic concentrations for 77 ponds from 40 inland, low-salinity shrimp farms in China, Ecuador, Thailand, and the US. The Ca/Mg ratios calculated from these data ranged from 0.61 to 5.74 with an average and standard deviation of 2.48 ± 1.85. Although data could not be obtained on survival and production in the study by (Boyd and Thunjai, 2003), farm managers reported that they were successfully producing shrimp. In 2013 and 2014, Ca/Mg ratios averaged 2.46 and 3.12, respectively in ponds at GAF. The Ca/Mg ratio was 4.05 at DOF and 4.81 at FSF in 2014. Of course, shrimp were produced successfully at these farms. The better results between 2013 and 2014 at GAF did not appear related to a difference in the average Ca/Mg ratio because it was similar between years.

The OLS regression was conducted using various combinations of water quality ratios. The ratios Na/K, Ca/K, Ca/Mg, and TA/Ca had VIF values below 7.5 (Table 7), and were used as explanatory variables; P value ranged between 0.12 to 0.78 for survival and production, respectively. The regression equations for survival and production were not included because the variables p values had $P>0.05$. The OLS regressions revealed that simple ratios of different two-variable combinations of water quality variables, at least in this case, are not reliable indicators of shrimp survival and production. This is also favored by best survival and production in 2013 as compared to average survival and production during 2014, in spite of similar average ratios during both the years. This is further supported by an earlier study during 2012 which revealed an average Na/K ratio of 132.4, Ca/Mg ratio of 198.7, Ca/K ratio of 141.7, TA/Ca ratio of 0.0031 (Chumnanka et al., 2015). During 2012 the average survival and production at GAF was 60.1 ± 9.5 and 4524 ± 1378, respectively, in spite of water quality ratios that deviated greatly from normal seawater.

Minimum Acceptable Concentrations of Ions: Data from the three ponds with the best survival and production at GAF in 2013 and in 2014 provided insight into the average and minimum salinity and concentrations of cations required for excellent survival and production (Table 10). Salinity was not below 1.0 mg/L in the six selected ponds. Moreover, minimum concentrations of most variables were not appreciably less than their average concentrations for more than one or two sampling dates each year. The lowest mean K concentration was 33.8 mg/L, but ponds with low survival and production had K concentrations well below 30 mg/L on several sampling dates. These observations certainly suggest that there is a minimum, optimum K concentration and likely for certain other measured variables.

Based on data in Table 10, the following minimum acceptable concentrations for optimum survival and production could be recommended: Na, 170 mg/L; K, 28 mg/L, TA, 50 mg/L; Ca, 5 mg/L; Mg, 5 mg/L. But, such low values probably could not be tolerated for more than 1 or 2 weeks without seriously stressing shrimp. The average values for variables in Table 10 would be safer lower limits for assuring good survival and production. The following rounded concentrations might be suitable lower limits:: Na, 500 mg/L; K, 40 mg/L; TA, 150 mg/L; Ca, 30 mg/L; Mg, 12 mg/L. Notice that the minimum TA concentration given above is much greater than the 90 mg/L minimum TA concentration recommended by (Boyd et al., 2002).

Calcium Carbonate Saturation: Daily, minimum and maximum temperatures differed by 1°C or less between surface water and bottom water in ponds at GAF during the 2010 growing season. The lack of strong thermal stratification was the result of daily mixing of the water column by the aerators (Prapaiwong and Boyd, 2012c). Average daily water temperature was about 25°C in mid-May, 32 to 34°C from late May until the end of August, near 30°C until late September, and 20°C or less by early October in ponds at GAF in 2010. The farm owner has measured pH frequently in ponds, and reported that pH usually ranged from slightly above 7.0 to about 7.5 in the early morning and reached 8.5-9.2 in the afternoon. These observations agree well with measurements of pH in other aquaculture ponds in the Blackland Prairie region of Alabama with TA concentrations similar to those at GAF (Zhou and Boyd, 2015). The TDS concentration in ponds at GAF, as estimated from salinity, was between 1,000 and 2,500 mg/L during 2013 and 2014. These ranges in temperature, pH and TDS concentration along with concentrations of Ca and TA in ponds measured in the present study were used to estimate the likely range in pH_{sat} for $CaCO_3$ saturation in ponds at GAF.

The pH_{sat} values in Table 11 indicate that at 30°C, $CaCO_3$ precipitated at pH between 7.39 and 7.58 in 2013 and between 7.41 and 7.60 in 2014. Increasing the TDS, TA, or Ca concentration lowered the pH at which $CaCO_3$ saturation would occur. Also, an increase in temperature would lower the pH for $CaCO_3$ precipitation.

The well water at GAF contained more Ca and TA than could be maintained at equilibrium between pond water and atmospheric carbon dioxide concentration – TA and Ca concentrations declined markedly after standing in ponds for 2 or 3 weeks (Table 8). The pond water contains a high but fluctuating abundance of phytoplankton during the growing season because of nitrogen and phosphorus additions in feed not sequestered in shrimp biomass. The water often would have a pH below pH_{sat} in the morning hours, but because of CO_2 removal by phytoplankton during the day. The pH usually would be well above 8.0 in the afternoon as noted by (Prapaiwong and Boyd, 2012a). Thus, the pond waters at GAF often will be saturated with $CaCO_3$ during the daytime, and on some days, they are likely $CaCO_3$-saturated for an entire 24-hr period.

The significance of the water being saturated with $CaCO_3$ is that it probably would not be possible to raise TA and Ca concentration by adding traditional liming materials because they would not dissolve well. Moreover, Ca supplied in a more soluble form such as calcium sulfate ($CaSO_4{\cdot}2H_2O$) might initially dissolve, but the resulting Ca would likely precipitate. Thus, even though low Ca concentration is responsible for a portion of the variation in survival and production in ponds at GAF, it might not be possible to increase Ca concentration because the pH_{sat} is rather low. The same is true at DOF and FSF where pH_{sat} values for average concentrations of TA and Ca in the ponds at 25, 30, and 35°C were estimated as 7.36 and 7.46, 7.26 and 7.36, and 7.16 and 7.26, respectively. It would be possible to increase TA by addition of sodium bicarbonate ($NaHCO_3$) because this compound is highly water soluble. But, raising TA might result in precipitation of Ca as $CaCO_3$.

Conclusions

The OLS multiple regression analysis revealed that about 50% of the variation in survival and production of shrimp in ponds at GAF could be explained by concentrations of TA and the four major cations (Na, K, Ca, and Mg). The ponds at GAF had received K and Mg augmentation, and the TA and Ca concentrations were most important as explanatory variables. However, it likely would be difficult to increase Ca and TA concentrations in ponds at GAF, because water often would be at or near saturation with $CaCO_3$.

The present study revealed that the Na/K, Ca/Mg ratio, and several other simple water quality ratios as well as the SEC of cations were not reliable indicators of shrimp survival and production under such conditions. Nevertheless, there may be a minimum concentration of most variables, and especially K, Ca, and TA, below which shrimp performance declines.

The K concentration likely is the most important single variable, and the concentration of this cation is likely below optimum in many of the ponds at GAF despite the regular addition of muriate of potash and K-Mag. It also is possible that Na concentration may fall below its minimum acceptable concentration in some ponds. The Mg concentration is less important than the K concentration, but the Mg concentration may fall below its minimum, acceptable concentration in some ponds. The observation that rainfall diluted Na concentration – which was not augmented as were K and Mg – suggested that heavy rainfall might be an important factor related to minimum concentrations of other cations.

The present study was based primarily on a single low-salinity shrimp farm. However, the findings should be generally applicable to other farms in Alabama and in other regions under similar circumstances. Some general recommendations based on the findings of this research follow:

- The K concentration in ponds at GAF should not be allowed to fall below 40 mg/L. Low K concentration could be prevented by more frequent K analyses and appropriate additions of muriate of potash.

- Although Mg concentration was not as important as K concentration, the Mg concentration should be maintained above 12 mg/L by additions of K-Mag.

- Sodium concentration also may sometimes be too low. In ponds with less than 500 mg/L Na, application of sodium chloride would seem prudent.

- Research should be conducted to determine the possibility of maintaining Ca and TA concentration above 30 mg/L and 150 mg/L, respectively.

Table 1. Farm means ± standard deviations (SD) and coefficients of variation (CV) for survival and production in ponds at Greene Prairie Aquafarm between 2001 and 2014.

	Survival		Production	
Year	Mean ± SD (%)	CV (%)	Mean ± SD (kg/ha)	CV (%)
2001	19.2 ± 11.3	58.9	632 ± 361	57.1
2002	61.9 ± 24.3	39.3	4,248 ± 1,437	33.8
2003	47.0 ± 14.8	31.5	3,215 ± 1,220	37.9
2004	74.5 ± 15.8	21.2	3,610 ± 1,213	33.6
2005	55.4 ± 22.8	41.2	2,809 ± 1,277	45.5
2006	28.6 ± 17.1	59.8	1,630 ± 887	54.4
2007	40.8 ± 25.3	62.0	3,327 ± 2,024	60.8
2008	47.6 ± 13.9	29.2	3,479 ± 1,548	44.5
2009	71.4 ± 24.2	33.9	4,582 ± 2,360	51.5
2010	48.8 ± 19.9	40.8	3,916 ± 1,704	43.5
2011	51.7 ± 15.9	30.8	4,006 ± 1,084	27.0
2012	60.1 ± 9.5	15.8	4,524 ± 1,378	30.5
2013	74.1 ± 23.2	31.3	5,129 ± 1,343	26.2
2014	44.2 ± 18.3	41.4	3,475 ± 1,271	36.6
Grand mean	51.8 ± 16.1	31.1	3,470 ± 1,182	34.1

Table 2. Pond means ± standard deviations (SD) and coefficients of variation (CV) for shrimp survival and production in 16 ponds at Greene Prairie Aquafarm between 2001 and 2014.

Pond	Mean ± SD (% / kg/ha)	CV (%)	Pond	Mean ± SD (% / kg/ha)	CV (%)
		Survival			
N-1	54.1 ± 16.3	30.1	S-1	69.6 ± 18.7	26.9
N-2	37.3 ± 23.0	61.7	S-2	46.6 ± 23.5	50.4
N-3	41.4 ± 20.3	49.0	S-3	55.7 ± 13.6	24.4
N-4	57.6 ± 21.9	38.0	S-4	48.7 ± 28.7	58.9
N-5	57.3 ± 27.4	47.8	S-5	71.5 ± 42.6	59.6
N-6	66.1 ± 20.2	30.6	S-6	49.1 ± 18.5	37.8
N-7	54.8 ± 21.8	39.7	S-7	50.2 ± 24.5	48.8
N-8	59.4 ± 22.1	37.2	S-8	52.4 ± 25.9	49.4
			Average	54.5 ± 23.1	42.3
		Production			
N-1	4,039 ± 1,614	40.0	S-1	3,822 ± 1,310	34.3
N-2	2,297 ± 1,128	49.1	S-2	3,306 ± 1,692	51.1
N-3	2,859 ± 1,566	54.8	S-3	4,831 ± 2,915	60.3
N-4	4,383 ± 1,966	44.9	S-4	2,911 ± 1,720	59.1
N-5	3,803 ± 1,944	49.8	S-5	3,791 ± 1,647	43.4
N-6	4,090 ± 1,609	39.3	S-6	3,396 ± 1,596	47.0
N-7	3,122 ± 1,446	46.3	S-7	3,267 ± 1,799	55.1
N-8	3,636 ± 1,166	32.1	S-8	3,542 ± 1,887	53.3
			Average	3,578 ± 1,687	47.1

Table 3. Grand means, standard deviations (SD), and ranges for water chemistry data in ponds at Greene Prairie Aquafarm in 2013 and 2014.*

	Mean ± SD	CV (%)	Range
Salinity (g/L)			
2013	1.5 ± 0.3	17.7	1.1 - 1.8
2014	1.6 ± 0.5	30.1	1.0 - 2.5
Sodium (mg/L)			
2013	502 ± 91	18.1	378 - 637
2014	576 ± 169	29.4	370 - 863
Potassium (mg/L)			
2013	35.8 ± 7.5	20.9	26.7 - 58.2
2014	37.5 ± 8.8	23.6	27.8 -63.3
Sodium/potassium			
2013	14.6 ± 3.6	25.0	7.2 - 21.0
2014	15.4 ± 3.4	22.0	10.3 - 21.5
Calcium (mg/L)			
2013	30.8 ± 4.1	13.4	25.6 - 41.6
2014	31.4 ± 4.3	13.6	23.0 - 39.5
Magnesium (mg/L)			
2013	12.5 ± 3.1	24.7	9.2 - 22.5
2014	11.2 ± 5.1	45.9	6.3 - 29.5
Alkalinity (mg/L)			
2013	138 ± 24	17.4	84 – 178
2014	133 ± 19	14.3	95 – 170

*Differences in mean concentrations of measured variables were not statistically significant at $P \leq 0.05$ as determined by t-tests.

Table 4. Averages ± standard deviations (mg/L) and coefficients of variation (CV) for water quality variables measured weekly in inland shrimp ponds at the Dickie Odom Farm (DOF) and Forkland Springs Farm (FSF) near Forkland, Alabama in 2014.

	DOF		FSF	
Variable	Mean ± SD	CV (%)	Mean ± SD	CV (%)
Salinity (g/L)*	5.1 ± 0.5	9.6	3.7 ± 0.8	21.3
Sodium (mg/L)*	1,597 ± 125	7.8	1,233 ± 251	20.4
Potassium (mg/L)	64.5 ± 5.2	8.0	58.0 ± 16.8	29.0
Sodium/potassium	24.9 ± 1.3	5.4	23.0 ± 6.0	26.0
Calcium (mg/L)*	103.7 ± 12.3	11.9	80.0 ± 17.3	21.4
Magnesium (mg/L)*	25.9 ± 1.5	6.0	16.6 ± 3.7	22.0
Total alkalinity (mg/L)	76.8 ± 14.8	19.3	76.8 ± 5.2	6.8

* Grand means differed between farms ($P<0.05$) as determined by Tukey's test that included water quality grand means for Greene Prairie Aquafarm.

Table 5. Normal, monthly rainfall and monthly rainfall (in centimeters) for 2013 and 2014 at Demopolis, Alabama.

Month	Normal	2013	2014
J	13.8	19.6	4.0
F	13.6	26.1	13.1
M	12.8	10.0	17.7
A	11.3	11.9	23.2
M	10.5	7.4	6.4
J	10.5	19.3	6.8
J	11.9	16.0	6.5
A	11.7	8.6	12.0
S	9.4	4.4	2.0
O	9.0	8.3	4.3
N	11.7	11.6	6.5
D	12.2	29.2	13.1
Year	138.3	172.4	115.6

http://www.usclimatedata.com/climate/demopolis/alabama/united-states/usa10155/2013/5.

Table 6. Simple, linear coefficients of determination among water chemistry variables and shrimp survival and production at Greene Prairie Aquafarm near Forkland, Alabama.

	Y-variable			
	Survival		Production	
X-variable	2013	2014	2013	2014
Salinity	0.00041	0.07404	0.03876	0.21726*
Sodium	0.00054	0.06939	0.02367	0.20576*
Potassium	0.20115*	0.00394	0.19497*	0.07976
Na/K ratio	-0.06444	0.07678	-0.01398	0.10348
Total alkalinity	0.15964	0.05212	0.29116*	0.20073*
Calcium	0.02654	0.23477*	0.00144	0.1634
Magnesium	0.06652	0.09321	0.14854	0.10558

*Significant correlation at P<0.05.

Table 7. Variables with variance inflation values (VIF) below 7.5, coefficients, and probabilities for ordinary least squares regression analysis.

	Survival			Production		
Variable	Coefficient	P-value	VIF	Coefficient	P-value	VIF
			Alkalinity and cations			
Sodium	0.03	0.52	2.4	4.1	0.24	2.4
Potassium	-0.04	0.64	2.1	-16.4	0.76	2.1
Alkalinity	0.60	0.02	1.9	39.5	0.02	1.9
Calcium	2.96	0.01	1.2	153.7	0.04	1.2
Magnesium	-0.29	0.79	1.5	-28.2	0.86	1.5
			Ratios			
Sodium/potassium	0.60	0.78	1.2	137.8	0.38	1.2
Calcium/potassium	70.9	0.25	6.3	2,983.2	0.43	6.3
Calcium/magnesium	3.30	0.69	2.3	323.7	0.54	2.3
Alkalinity/calcium	0.30	0.14	7.2	24.9	0.12	7.2

Table 8. Salinity and concentrations of alkalinity and cations in well water and pond water at the Greene Prairie Aquafarm near Forkland, Alabama. Source: McNevin et al. (2004).

Variable	Well water	Pond water[1]	SEC pond water
Salinity (g/L)	3.7	2.6	---
Sodium (mg/L)	1,402	971	791
Potassium (mg/L)	11.6	6.2	28.6
Sodium/potassium	121	157	---
Calcium (mg/L)	118	60	28.6
Magnesium (mg/L)	5.5	4.6	102
Total alkalinity (mg/L)	273	120	---

[1] Ponds were filled from the well and water allowed to stand for 2-3 wk. Water had not been treated with potassium or magnesium amendments.

Table 9. Percentage deviations in the seawater equivalent concentration (SEC) for cations and the Na/K ratio of normal seawater in pond waters from inland, low-salinity shrimp farms near Forkland, Alabama.

	GAF[1]			
Variable	2013	2014	DOF[1]	FSF[1]
Sodium	+9.8	+18.3	+2.9	+9.5
Potassium	+117.0	+113.1	+16.4	+42.5
Calcium	+77.0	+69.7	+73.2	+88.7
Magnesium	-368.8	-458.9	-689.5	-773.5
Na/K	-89.0	-72.2	-14.5	-20.0

[1] GAF = Green Prairie Aquafarm; DOF = Dickie Odom Farm; FSF = Forkland Springs Farm.

Table 10. Averages and minimum concentrations of water quality variables in ponds at Greene Prairie Aquafarm with the best survival and production during 2013 and 2014.

	2013						2014					
	S-4		S-6		N-5		S-6		S-8		N-8	
Variable	Av.	Min.	Av.	Min.	Av.	Min.	Av.	Min.	Av.	Min.	Av.	Min.
Production (kg/ha)	6,518		6,267		6,550		4,886.9		5,008.1		4,594.3	
Survival (%)	104		101		104		60.8		65.4		63.4	
Salinity (g/L)	1.3	1.0	1.3	1.2	1.7	1.5	2.2	1.6	1.3	1.0	2.4	1.8
Na (mg/L)	417.3	230.0	414.9	260.0	589.3	280.0	750.0	170.0	458.8	200.0	850.0	250.0
K (mg/L)	58.2	51.0	44.2	39.0	36.5	33.0	34.7	28.0	33.8	28.0	45.5	40.0
TA (mg/L)	146.8	108.0	147.7	118.0	178.3	146.0	161.5	124.0	120.5	48.7	144.1	81.5
Ca (mg/L)	29.6	18.0	34.8	18.8	28.3	10.0	27.7	9.6	33.3	13.6	29.5	4.8
Mg (mg/L)	15.8	12.1	11.8	10.2	16.1	12.6	8.7	5.6	8.7	5.1	10.6	6.6

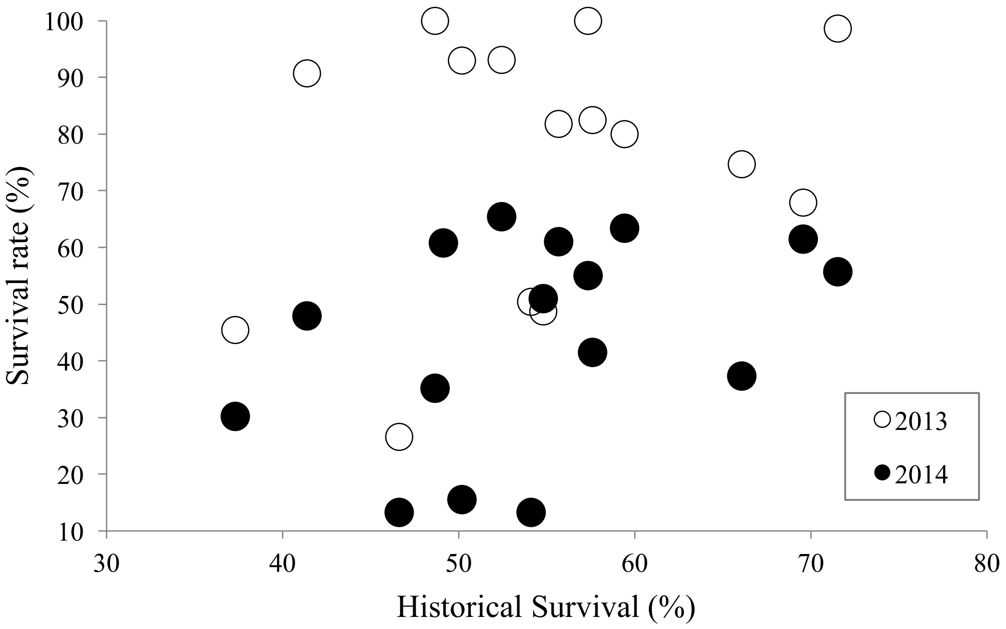
Table 11. Calcium carbonate saturation pH (pH_{sat} of Langelier, 1936) for waters in ponds of Greene Prairie Aquafarm.

	pH_{sat}	
Condition for calculation	2013	2014
Farm average (TDS, alkalinity, and hardness)		
25°C	7.58	7.60
30°C	7.48	7.50
35°C	7.39	7.41
Individual ponds (at 30°C)		
Minimum TDS	7.55	7.70
Maximum TDS	7.50	7.48
Minimum alkalinity	7.53	7.30
Maximum alkalinity	7.45	7.64
Minimum calcium	7.58	7.68
Maximum calcium	7.48	7.55

Fig. 1. Greene Prairie Aqua Farm (GAF), Alabama, USA

Fig. 2. Dickie Odom Farm (DOF), Alabama, USA

Fig. 3. Forkland Springs Farm (FSF), Alabama, USA



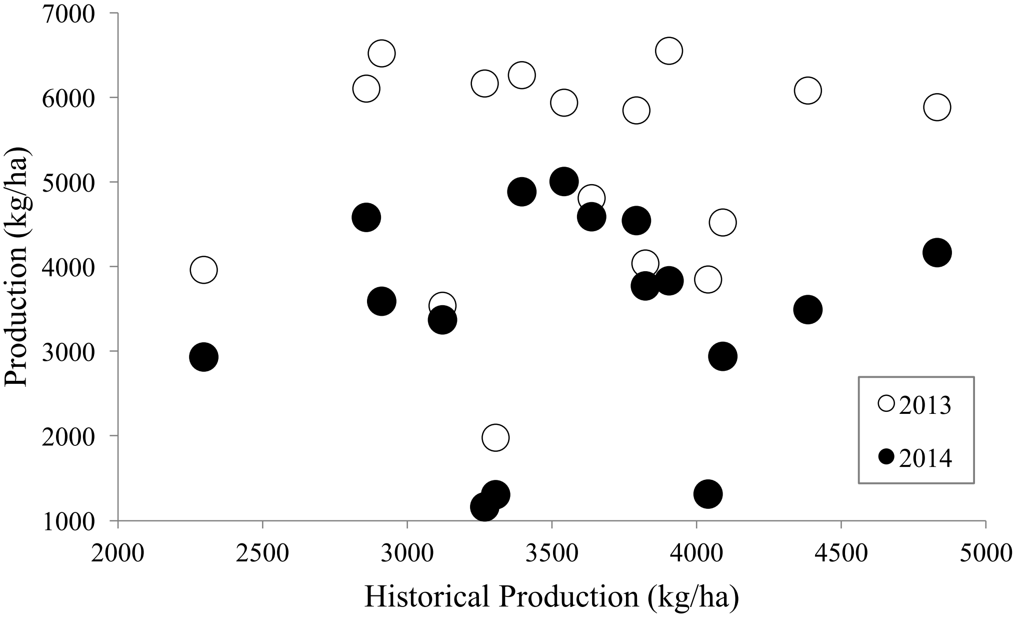
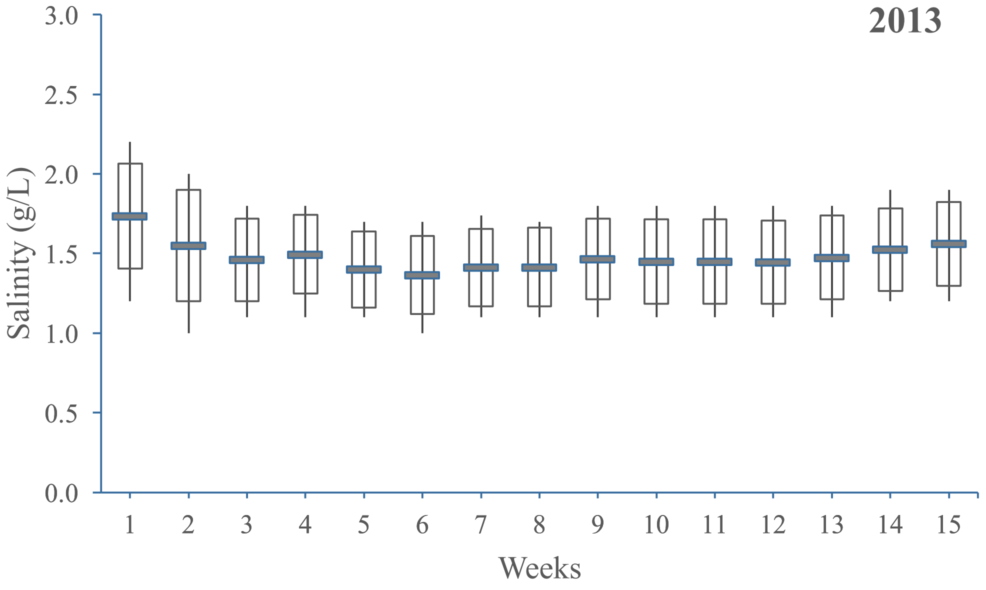


Fig. 4. Plots of historical survival and production of individual ponds at Greene Prairie Aquafarm versus survival and production in 2013 and 2014.



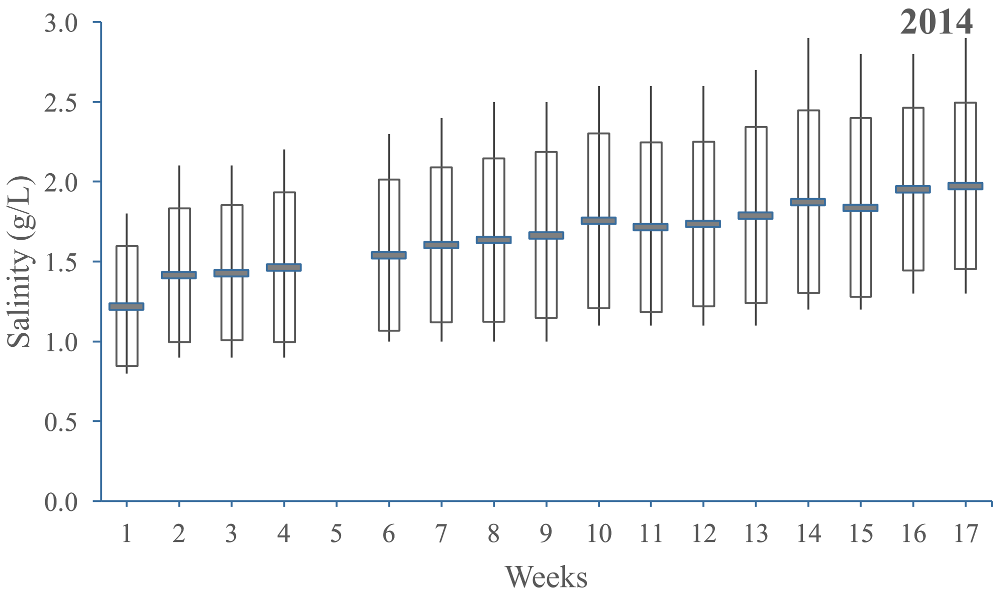
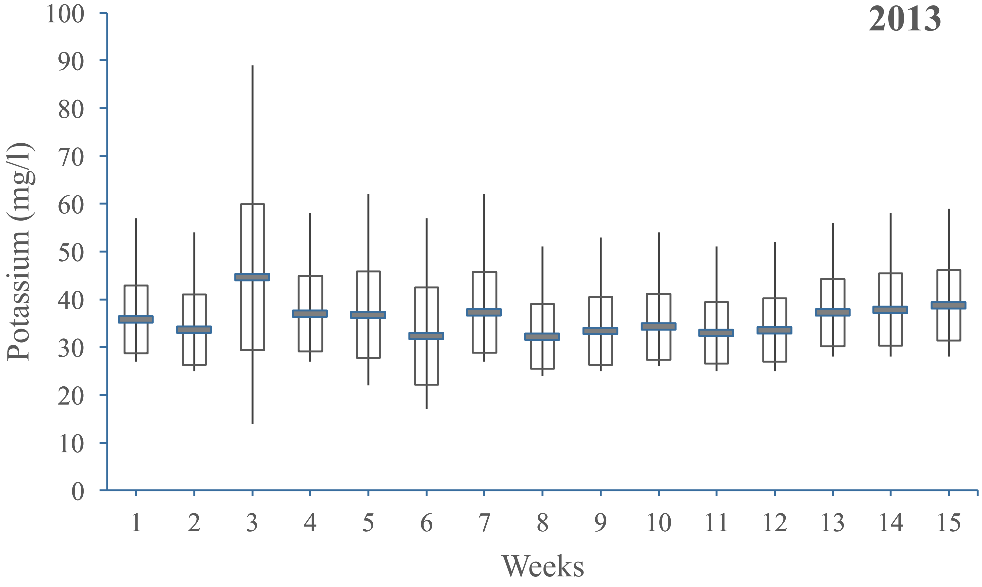


Fig. 5. Mean, minimum, and maximum salinity at weekly intervals in ponds at Greene Prairie Aquafarm (2013 and 2014).



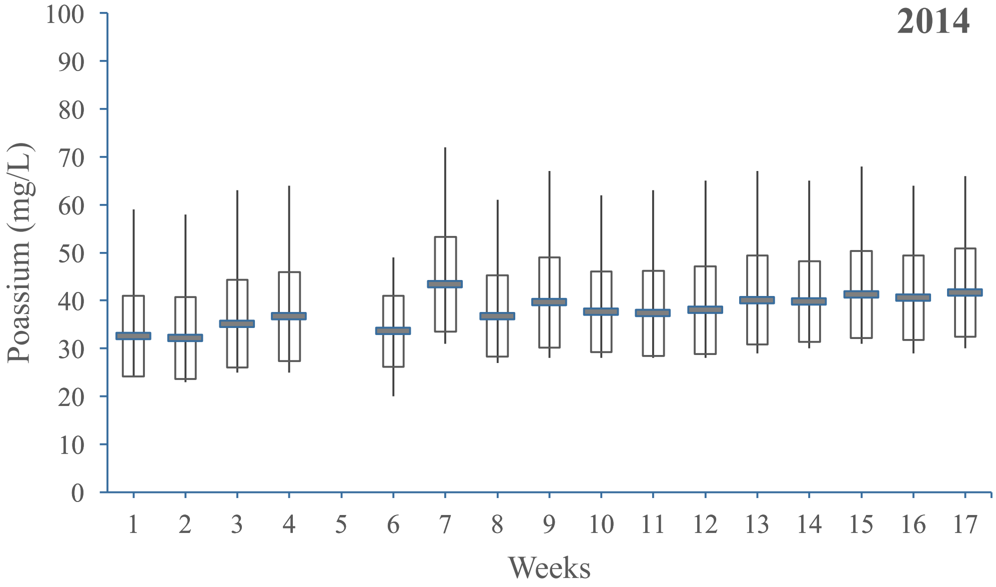


Fig. 6. Mean, minimum, and maximum concentrations of potassium at weekly intervals in ponds at Greene Prairie Aquafarm (2013 and 2014).

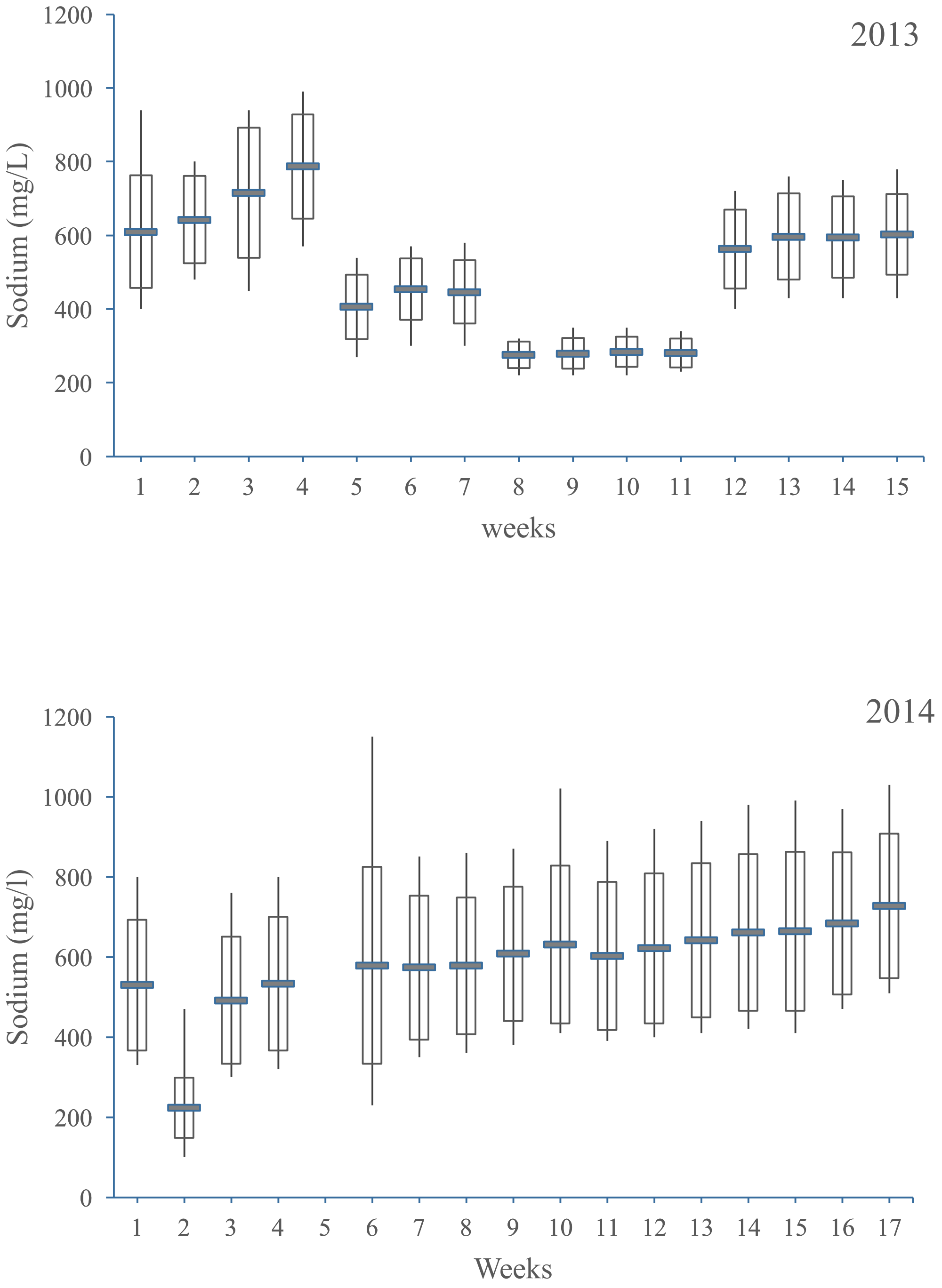
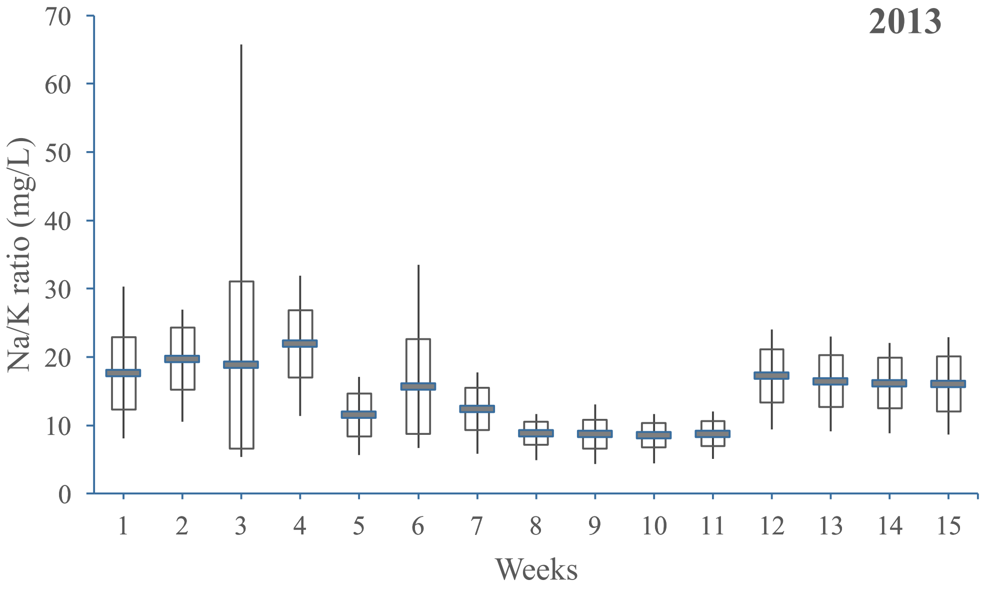


Fig. 7. Mean, minimum, and maximum concentrations of sodium at weekly intervals in ponds at Greene Prairie Aquafarm (2013 and 2014).



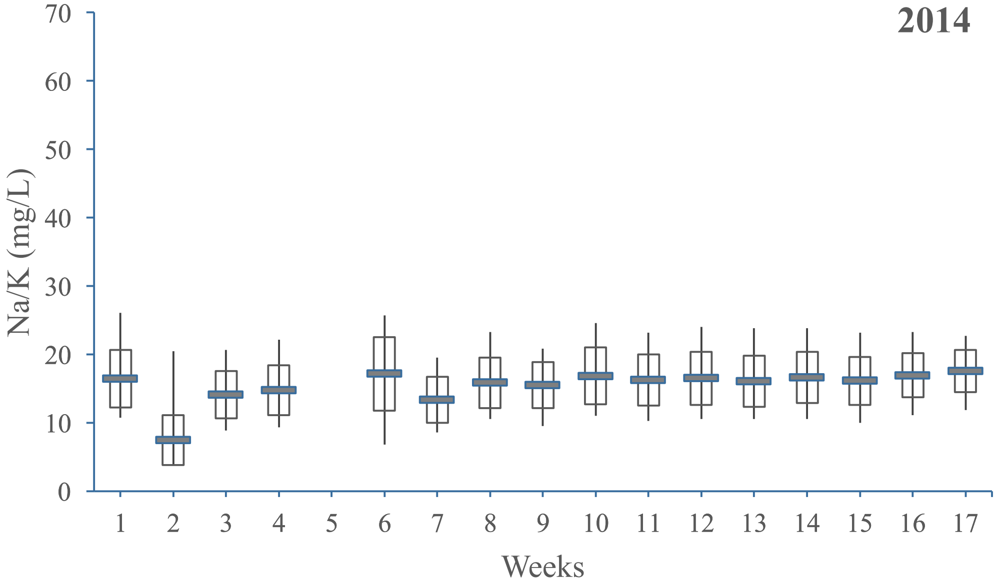
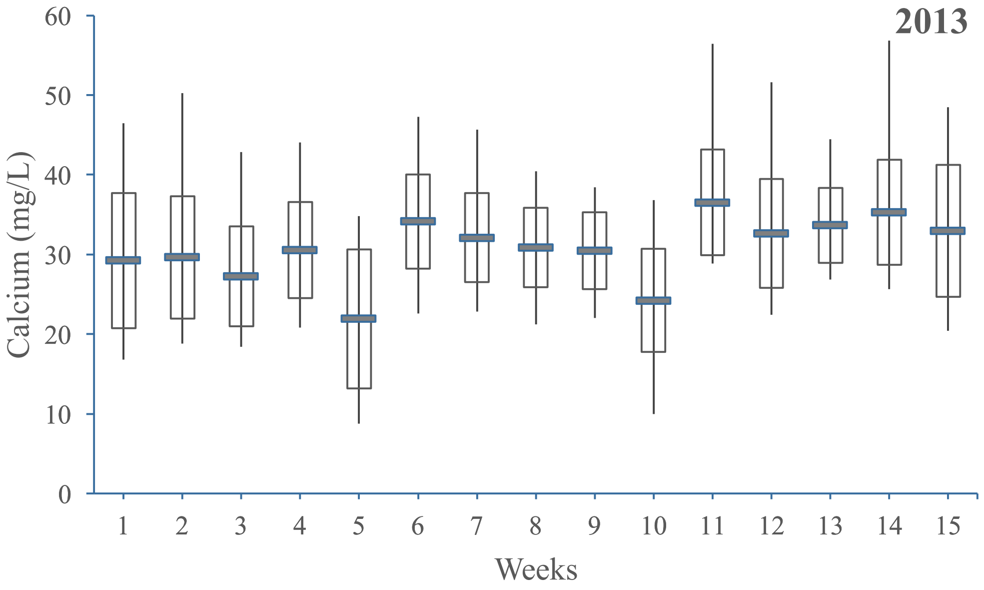


Fig. 8. Mean, minimum, and maximum sodium/potassium (Na/K) ratios at weekly intervals in ponds at Greene Prairie Aquafarm (2014 and 2014).



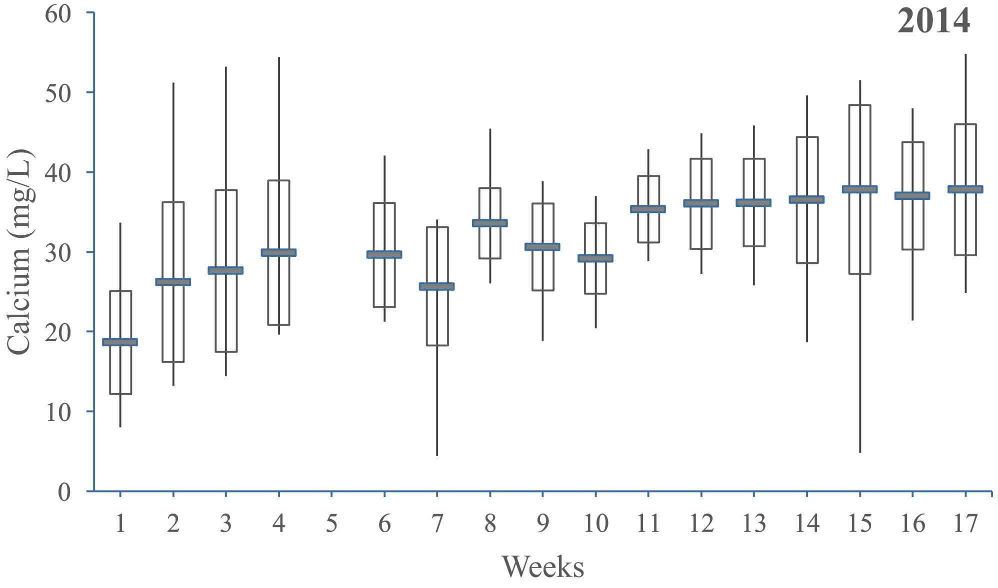
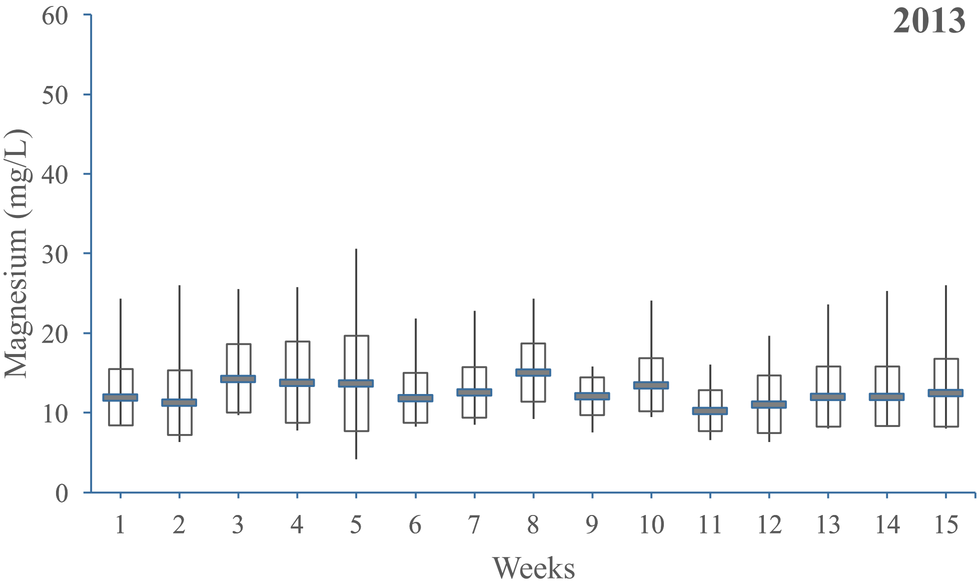


Fig. 9. Mean, minimum, and maximum concentrations of calcium at weekly intervals in ponds at Greene Prairie Aquafarm (2013 and 2014).



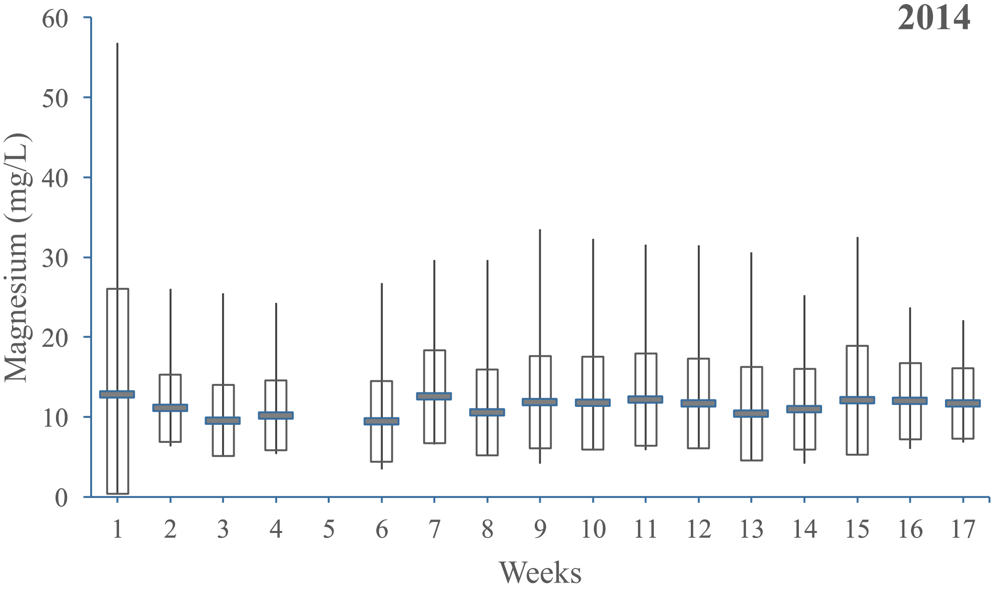
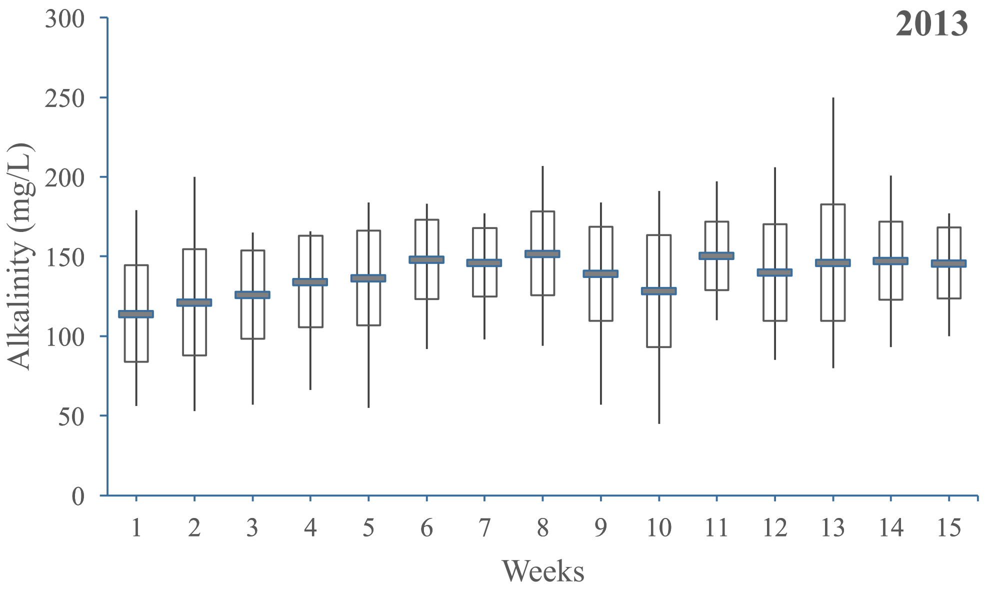


Fig. 10. Mean, minimum, and maximum concentrations of magnesium at weekly intervals in ponds at Greene Prairie Aquafarm (2013 and 2014).



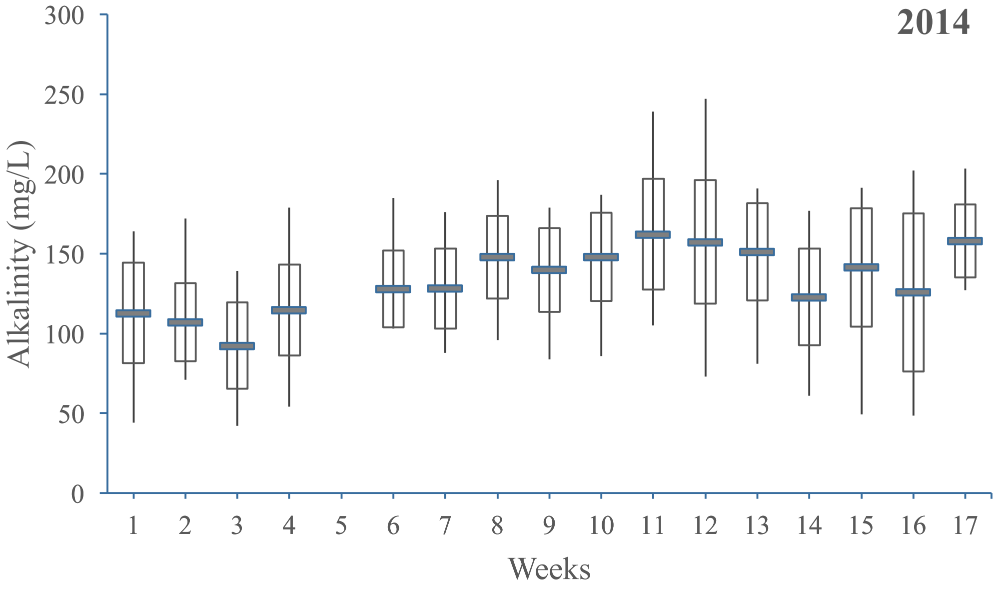
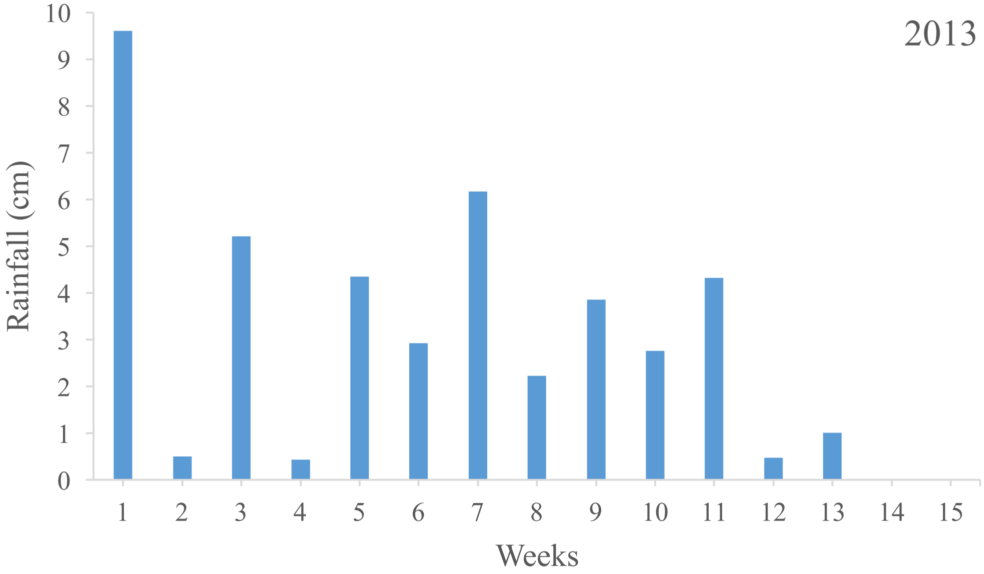


Fig. 11. Mean, minimum, and maximum concentrations of alkalinity at weekly intervals in ponds at Greene Prairie Aquafarm (2013 and 2014).



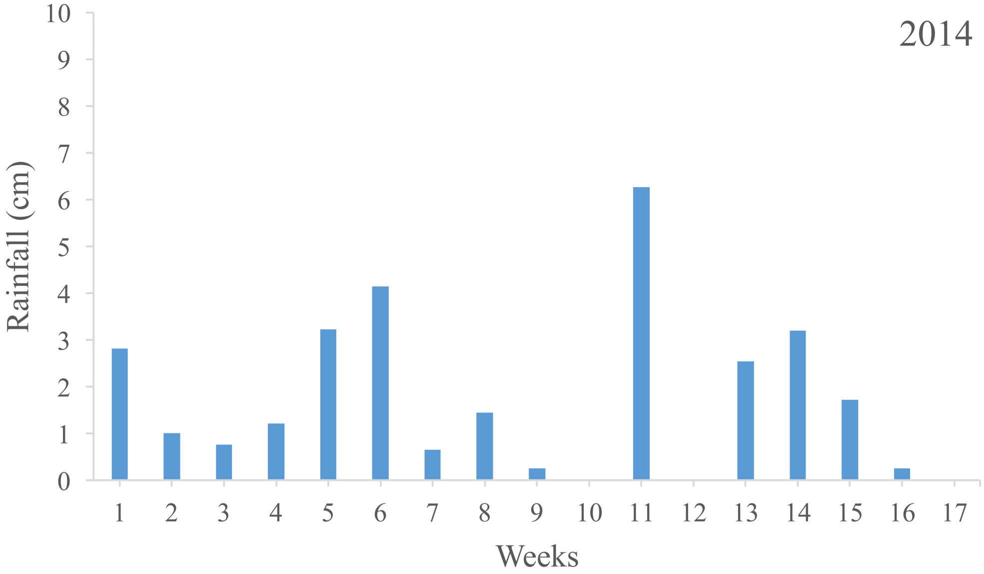


Fig. 12. Amounts of rainfall per week beginning 1 week before ionic measurements began and each week until termination of the effort at Greene Prairie Aquafarm in 2013 and 2014.

Fig. 13. Map showing survival and production at Green Prairie Aquafarm, developed under ArcGIS.

Fig. 14. Map showing Potassium and calcium concentration at Green Prairie Aquafarm, developed under ArcGIS.

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