

**Water Quality in Inland Ponds for Low-Salinity Culture of Pacific White Shrimp**  
*Litopenaeus vannamei*

by

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## Abstract

Four investigations were performed in inland, low-salinity shrimp farms in Blackland Prairie Region in western Alabama. Three of these investigations were related to performance of shrimp responses to variation of water quality variables in ponds. The fourth investigation was performed to address the concern of environmental impacts as a result of utilization of saline water for aquaculture purpose in inland region.

Common water quality variables in nine ponds exhibited wide variation in concentration among ponds and overtime. Shrimp performance varied significantly among ponds as follows: survival, 16 to 128%; production, 928 to 5,950 kg/ha; feed conversion ratio, 1.18 to 2.89. None of the water quality variables were at the lethal concentrations but few variables were occasionally outside optimum ranges for shrimp production and may have stressed shrimp. Survival and production were positively correlated ( $P < 0.05$ ) with increasing concentration of methyl orange alkalinity, total alkalinity, and calcium hardness. Production was negatively correlated with higher pH and temperature but these correlations may have resulted from lower temperature and pH during final days of the crop in ponds harvested in October rather than from actual effect of temperature and pH on growth. Variables that were correlated with shrimp survival and growth or outside optimal ranges deserved to be further investigated to ascertain whether or not they are causal and harmful.

Concentrations of 18 trace elements tended to be greater than concentration in normal seawater except for molybdenum, boron, and silicon. Concentrations of most trace elements varied greatly among ponds and sampling dates. The analytical method used in this investigation measured total concentrations of trace elements, includes free ionic forms, hydrolysis products, ion pairs, and coordination compounds (chelated forms), but only the free ionic forms that are toxic to aquatic organisms. Although concentrations of iron, aluminum, zinc, and selenium were occasionally higher than typical, safe concentration limits for continuous exposure to aquatic life, it is doubtful that free ionic concentrations in these waters were great enough to harm shrimp. Positive correlations ( $P < 0.05$ ) between shrimp survival and production, and increasing concentrations of zinc, cobalt, and iron should be further investigated to ascertain if additions of these elements to ponds might improve shrimp performance.

Monthly, mean water temperature among eight ponds differed by  $3.40^{\circ}\text{C}$  in May and  $2.83^{\circ}\text{C}$  in September, but there was less than  $1^{\circ}\text{C}$  difference among pond in June, July, and August. Differences in water temperature among ponds were not related to pond water surface:volume ratio. The differences in water temperature among ponds probably were related mainly to variation in plankton density. The negative correlations ( $P < 0.05$ ) between water temperature and shrimp production and survival are thought to have resulted from variation in crop duration and therefore are not causal. However, differences in water temperature among ponds in May and September were great enough to have possibly influenced shrimp survival and production.

Investigations of effluent volume and pollutant loads of an inland, low-salinity shrimp farm were conducted in 2008 and 2010 and the draining effluent at harvest of

both years were 50.4% and 57.9 % of impounded water, respectively. Farm effluent caused a notable increase in concentrations of all variables other than pH at sampling station downstream of the farm outfall. But only chloride concentration exceeded 230 mg/L— upper standard for freshwater streams set by the Alabama Department of Environmental Management. Average pollutant discharge per tonne of shrimp was 444.9 kg total suspended solids, 51.3 kg 5-d biochemical oxygen demand, 15.2 kg total nitrogen, 1.33 kg total phosphorus, and 4,402 kg chloride. Concentrations of turbidity, settleable solids, total suspended solids, total phosphorus and possibly other variables in effluent could be lessened by installation of a settling basin to remove medium and coarse solids before final discharge. Release of less water from the farm during harvest, and improvement in feed conversion ratio also would lessen pollutant loads per tonne of shrimp.

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## I. Introduction

The shrimp aquaculture industry has experienced remarkable growth in the past two decades. Products from shrimp aquaculture account for more than 40 % of world shrimp production. The global shrimp production from aquaculture has increased from 700,000 metric tonnes in 1997 to about 4.6 million tonnes in 2006 (Food and Agriculture Organization of the United Nations 2009) . In the past, two major marine shrimp species were commercially cultured in two different hemispheres — the black tiger shrimp *Penaeus monodon* and the Pacific white shrimp *Litopenaeus vannamei* were cultured in the eastern and western hemispheres of the globe, respectively. However, the two species from the two different hemispheres had at least one thing in common, they were cultivated in brackish or seawater of 15 to 30 ppt salinity in ponds situated in near-coastal areas. The culture of marine shrimp in low-salinity water in inland areas has become common in many regions of the world including: Australia, China, Ecuador, India, Thailand, the United States, and other countries (Boyd and Thunjai 2003). The most common marine shrimp species that has been cultivated in low-salinity water throughout the world has been *L. vannamei*. In Thailand, low-salinity cultivation of marine shrimp often is performed by transporting hyper-saline water at salinity of 80 to 200 ppt or sometime even more from coastal seawater evaporation ponds to inland ponds and mixing with freshwater in ponds to obtain the desirable salinity of 0.5 to 5 ppt (Boyd et al. 2002, Limsuwan et al. 2002). In the United States, culture of marine shrimp in inland

area has become popular, and currently, it is conducted in several states including Alabama, Arizona, Arkansas, Florida, Illinois, Indiana, and Texas (Samocha et al. 1998, Samocha et al. 2002, Saoud et al. 2003). In Alabama, the inland cultivation of Pacific white shrimp is possible through use of the ancient saltwater deposited in underground aquifers in the Blackland Prairie region. The commercial, inland cultivation of Pacific white shrimp in Alabama started about a decade ago in the west-central region of the state. With four active producers and a culture area of more than 30 ha, these farms produce around 135,000 kg shrimp production each year with a total farm gate value of more than 1 million US dollars (Whitis 2007).

In the past decade, much progress has been made in inland, low-salinity culture of marine shrimp in Alabama. However, further research is still necessary to increase its potential and achievement. Comprehension of the influence of each particular water quality variable, chemical and physical, on the performance of shrimp is key to success in shrimp culture. In addition, there is a growing global concern of the possible negative environmental consequences of aquaculture on the surrounding environment. Thus, further studies on water quality in ponds at inland shrimp farms are needed both for improving production and for protecting environment. The Blackland Prairie region of Alabama is economically depressed, and any information that will encourage the expansion and profitability of inland shrimp farming in that region would be highly beneficial.

## II. Literature Review

### Water sources for inland, low-salinity shrimp culture

In some countries, for example Thailand, the source of briny water for inland, low-salinity cultivation of shrimp is mainly seawater evaporation ponds (Boyd et al. 2002, Limsuwan et al. 2002). This water typically has salinity of 80 to 200 ppt, and in some cases even more. High-salinity water is trucked to the inland culture sites and mixed with freshwater in ponds to obtain desirable salinities. In several other countries, the primarily source of saline water for inland, low-salinity marine shrimp culture is groundwater from saline aquifers (Boyd and Thunjai 2003). Saline groundwater used for filling inland, low-salinity shrimp pond in Alabama has salinity of about 2 to 10 ppt. Water from underground aquifer from the Blackland Prairie region in Alabama is lower in magnesium and potassium levels in comparison to seawater of the same strength (Boyd and Thunjai 2003). Potassium and magnesium have been reported to be necessary for shrimp, because they play particular roles in physiological processes (Boyd and Thunjai 2003). There is evidence that low concentrations of potassium and magnesium in culture water of shrimp in inland, low-salinity ponds in Alabama lessen performances of Pacific white shrimp (Roy et al. 2010). The ion deficiency problem is mitigated by applying potassium and magnesium fertilizers; muriate of potash (potassium chloride), and Kmag (potassium magnesium sulfate) to elevate concentrations to 40 mg/L and 20 mg/L, respectively (McNevin et al. 2004).

In Alabama, the supplies of water from drilled wells are not adequate to allow water exchange when a farmer feels that one or more water quality variables are out of range as have been practiced in shrimp aquaculture in near-coastal areas. In contrast to shrimp aquaculture in other parts of the world, inland shrimp producers in Alabama store as much water as possible in their ponds for the next crop.

#### Pacific white shrimp and water quality variables

Pacific white shrimp *Litopenaeus vannamei* is a marine species that can tolerate wide range of salinities from 0 to 45 ppt (Araneda et al. 2008, Bray et al. 1994, McGraw and Scarpa 2002, Menz and Blake 1980). However, the best success in cultivation of this species has been documented in waters 0.5 to 28.3 ppt salinity (Samocha et al. 1998). This species can grow and survive at low-salinity as long as there are sufficient concentrations of major ions (McGraw and Scarpa 2002). Pacific white shrimp widely cultured for more than a decade in several countries in Asia and the America as well as Australia. In Alabama, *L. vannamei* has been cultured in inland, low-salinity ponds since 1999 (Whitis 2007).

Both intrinsic and extrinsic factors play roles in biological activities of many crustacean species (Achituv and Cook 1984, Emmerson 1985, Hagerman and Weber 1981, Schmidt-Nielsen 1983, Spanopoulos-Hernandez et al. 2005). With the exception of organically bound elements carbon, hydrogen, nitrogen, and oxygen, there are several other elements that are considered to be essential for animal life — the macroelements and microelements. The macroelements include; the cationic group — calcium, magnesium, potassium, and sodium; and the anionic group — phosphorus,

chlorine, and sulfur. The trace or microelements include; cobalt, chromium, copper, fluorine, iodine, iron, manganese, molybdenum, nickel, selenium, silicon, tin, zinc, and vanadium (Food and Agriculture Organization of the United Nations 1987).

Macro and microelements have several significant-biological functions in living organisms, for instances serving as constituents of skeletal and soft tissue structures, necessary for transmission of nerve impulses and muscle contraction, maintenance of osmotic pressure, roles in the regulation of pH of blood and other fluids in body, and are vital for function of many enzymes, hormones, vitamins, and respiratory pigments. For example, they serve as cofactors in metabolism, catalysts, and enzyme activators (Food and Agriculture Organization of the United Nations 1987).

Magnesium serves as a cofactor for several enzymes important for the metabolism of proteins, carbohydrates, and lipids (Davis and Gatlin 1996). Magnesium is necessary for forming specific enzyme complexes that necessary for osmotic and ionic regulation in acclimation of shrimp to lower salinity water. Magnesium is also necessary for neuromuscular transmission and skeletal tissue metabolism (Cheng et al. 2005). In water with an imbalance of ions, shrimp are stressed (Auburn University and USDA-Natural Resources Conservation Service). In inland, low-salinity culture systems, water often contains a lower concentration of some ions than would be expected in full strength seawater diluted to the same salinity. In Alabama, low concentrations of magnesium and potassium in culture water affect shrimp growth performance. Stress and mortality of *L. vannamei* is reduced when the potassium ion concentration increases from 2 mg/L to 25 mg/L (Castillo-Soriano et al. 2010). Concentrations of magnesium and potassium in natural saline groundwater in west Alabama are, approximately, 4.6 mg/L and 6.2 mg/L,



respectively (McNevin et al. 2004). These concentrations are not sufficient for shrimp and addition of these ions is needed. To mitigate the problem, producers add magnesium and potassium to ponds through fertilizers to provide concentrations to about 20 mg/L and 40 mg/L, respectively. Addition of fertilizers resulted in significantly increases in production and survival rate (McNevin et al. 2004).

Solubility of most metals in water is decreased as pH and salinity increase (Boyd 2000). Water alkalinity in pond is typically influenced by pond soil and can be predicted from soil pH (Boyd and Munsiri 1997). Inland shrimp farms in Alabama are located in an area where soil contains free carbonate (Hajek et al. 1975) and, as a result, pond waters have moderate to high alkalinity and pH level of about 7.5 to 8.

Phytoplankton are the base of the food web, responsible for primary productivity in natural water bodies (Wetzel 2001). One simple approach used for assessing primary productivity in water is measuring chlorophyll *a* concentration. Phytoplankton productivity is usually limited by phosphates in water and availability of phosphorus in pond water is usually limited by rapid uptake of this nutrient by pond bottom soils (Boyd and Munsiri 1996). High concentrations of chlorophyll *a* have been observed in zero-water exchange, intensive culture of *P. vannamei* and chlorophyll *a* values were negatively correlated with calcium and zinc concentrations (Castillo-Soriano et al. 2010). Trace elements also are essential factors for growth of phytoplankton. There are several studies that report trace element limitations of phytoplankton productivity in some freshwater lakes (see review by Wetzel 2001). For example, phytoplankton photosynthesis increased following addition of trace element into high elevation lakes in California (Goldman and Wetzel 1963). Different trace elements play different roles in

phytoplankton physiology. Molybdenum will be used as an example to show the importance of trace elements. This trace element plays significant roles in the formation of nitrate reductase enzyme and in nitrogen fixation. Molybdenum is essential for the growth of higher plants and has been demonstrated to be essential for *Chlorella* and *Scenedesmus*; it is assumed to be essential for other algae. Some inland waters have low levels of molybdenum and, as a result, limits their productivity (Goldman 1960).

Water salinity plays significant roles in osmotic and ionic regulations of marine shrimp and other marine-aquatic animals. *Litopenaeus vannamei* has the strong ability to regulate osmotic strength in different saline media. *Litopenaeus vannamei* juveniles cope with wide variations of water salinity (Wickins 1976) by regulating ion concentrations in their haemolymph in respond to external salinity variations. To obtain the maximum production in culturing *L. vannamei*, a shrimp farm needs to maintain cultured water to the optimum salinity. This species hyper-regulates between salinities of 10 and 20 ppt and hypo-regulates between 20 to 40 ppt (Buckle et al. 2006). Alvarez et al. (2004) reported that activity of the  $\text{Na}^+/\text{K}^+$ -ATPase enzyme is higher at low salinities. Smaller shrimp suffered osmotic stress and consumed more oxygen at high salinity. In contrast, larger shrimp suffered much stress at low salinity (Bett and Vinatea 2009). Acclimation and salinity of water during stocking of Pacific white shrimp postlarvae is positively correlated to the survival and growth of post larvae (Alvarez et al. 2004, Saoud et al. 2003) but not survival rate during growout (Alvarez et al. 2004). Atwood et al. (2003) and Sowers et al. (2005) reported that Pacific white shrimp did not grow as efficiently in seawater diluted with freshwater supplemented with a salt mixture as in regular seawater, but osmotic dysfunction was not detected. Sowers et al. (2006) reported higher growth of

this species in low-salinity water of 2 ppt than in seawater. However, the influence of water salinity on shrimp growth is not well documented (Bett and Vinatea 2009, Bray et al. 1994, Samocha et al. 1998).

Dissolved oxygen (DO) is one of the most important factors in aquaculture ponds, especially in semi-intensive and intensive culture systems, because it is essential in respiration, and its availability determines the capacity of aquaculture ponds (Boyd 1990). A DO concentration of 5 mg/L or more is the ideal level for aquatic life. However, the exact concentration of dissolved oxygen varied from pond to pond because of variations of chemical, biological, and ecological characteristics. Dissolved oxygen consumption rate of *P. vannamei* is influenced by interaction of temperature, salinity, and shrimp size. Oxygen consumption of *P. vannamei* is higher when water salinity is below 25 ppt at a temperature lower than 20°C, while oxygen consumption seems to be more stable at lower salinities — 15 to 25 ppt and at temperature of 25 to 30°C (Bett and Vinatea 2009). McGraw et al. (2001) reported increasing yield of *P. vannamei* and *P. stylirotris* when minimum dissolved oxygen in water increased from 15% to 65% of saturation.

Ammonia is the main nitrogenous excretory product of most aquatic animals including crustaceans (Forster and Goldstein 1969) and it is the product of the ammonification of organic matter in culture systems (Chen et al. 1990). Ammonia is highly toxic to marine species (Armstrong et al. 1978). Un-ionized ammonia is highly lipid soluble with the capability to diffuse across the cell membrane (Chen and Kou 1993) and can be detrimental to shrimp at high concentrations. Ammonia exists in solution primarily as ionized ammonium ( $\text{NH}_4^+$ ) ion and un-ionized ammonia ( $\text{NH}_3$ )

molecule. The proportion of ionized and un-ionized ammonia is highly pH-dependent (Armstrong et al. 1978).

Temperature is one of the most important and most changeable physical factors affecting living organisms. Temperature can affect reproductive systems, growth and development, morphological systems, and the respiratory system. Respiration of *L. stylirostris* (Spanopoulos-Hernandez et al. 2005) and *P. chinensis* (Chen and Nan 1993) increase with increasing water temperature. Lower water temperature seems to reduce the hyperosmoregulatory ability of juvenile Pacific white shrimp (Bett and Vinatea 2009).

#### Effluents from shrimp culture and environment

Human activities often have negative environmental impacts and aquaculture is no exception. The rapid development of inland shrimp farming has led to concerns over sustainability of resources and potential, negative environment impacts associated with the activity. The major concerns are over salination and nutrient pollution of natural waters, and soil salination resulting from farm effluents. Effluents from shrimp aquaculture activities released during harvest could possibly deteriorate their adjacent environment. Excess nutrient and waste loads released to estuaries may possibly lead to eutrophication and harmful algal blooms, excess toxic chemicals and other toxins, changes in community structure and biodiversity, and introducing diseases and exotic materials into receiving water. However, the severity of impacts due to effluents from shrimp aquaculture differs from place to place and from time to time, and they can be minimized or prevented through use of good management practices.

Small to moderate amounts of water from inland shrimp ponds in west Alabama can be lost to its environment through seepage and overflow during or after raining as well as through drainage during harvest (Boyd et al. 2006). The effluents have potential to salinate freshwater streams to concentrations that are greater than recommended by the United States Environmental Protection Agency (Benoit 1988). Seepage from ponds also can salinate shallow, freshwater aquifers near farms (Boyd et al. 2006).

In inland, low-salinity culture of Pacific white shrimp in west Alabama, producers try to conserve water as much as possible for the next growout year, nevertheless part of water is released to adjacent freshwater streams. Boyd et al. (2006) reported that the majority of salts input into ponds of these shrimp farms was loss to receiving water through discharged water, whereas a small percentage of the salt input remained in pond water or was adsorbed by pond bottom soils. The salts discharged in effluents resulted in increasing salinity of the receiving water (Boyd et al. 2006, Pine and Boyd 2010). Likewise, inland shrimp farms in Thailand lose the majority of salts to their environment through seepage and harvest effluent (Braaten and Flaherty 2001).

In the United States, an aquaculture facility is classified as a concentrated aquatic animal production (CAAP) facility if it is discharging 30 days or more annually and producing more than 45,455 harvest weight kilograms annually. A CAAP facility is subjected to the United States Environmental Protection Agency (USEPA) aquaculture effluent rule (Federal Register 2004) and is requires a National Pollutant Discharge Elimination System (NPDES) permit.

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### III. Effects of Major Water Quality Variables on Shrimp Production in Inland, Low-Salinity Ponds in Alabama

#### Abstract

Common water quality variables in nine inland, low-salinity shrimp ponds in Alabama exhibited wide variation in concentrations among ponds and over time. Shrimp performance also varied considerably among ponds in 2008 as follows: survival, 16 to 128%; production, 928 to 5,950 kg/ha; feed conversion ratio (FCR), 1.18 to 2.89. None of the measured water quality variables were at concentrations high enough to be lethal to shrimp, but a few variables such as, water temperature, dissolved oxygen, carbon dioxide, total ammonia nitrogen, calcium, and magnesium were occasionally outside optimum ranges for shrimp production and may have stressed shrimp. Survival and production were positively correlated ( $P < 0.05$ ) with increasing concentrations of methyl orange alkalinity, total alkalinity, and calcium hardness. Negative correlations between production and higher pH and water temperature may have resulted from lower water temperature and pH during final days of the crop in ponds harvested in October rather than from an actual effect of temperature and pH on growth. Nevertheless, those variables that were outside optimal ranges or correlated with shrimp survival or growth should be further investigated to ascertain whether or not excursions outside optimum ranges are harmful and to determine if observed correlations are causal.

## Introduction

Low concentrations of potassium and magnesium in saline, groundwater used in inland, low-salinity shrimp ponds in Alabama lessen survival and production of the culture species *L. vannamei* (Roy et al. 2010). Potassium and magnesium fertilizer applications are used to counteract this problem (McNevin et al. 2004, Boyd et al. 2007), but producers still complain of high variation among ponds in shrimp survival and production. For example, in 17 ponds on one farm, the manager reported that survival ranged from 11.9 to 114.6% and harvest yields were 1,110 to 7,247 kg/ha in 2007. Some of these variations were attributed to inaccuracy in counting postlarvae at stocking and from differential survival of newly-stocked postlarvae resulting from acclimation and handling. However, because few disease problems were noted during grow-out, all ponds were heavily aerated, and potassium and magnesium concentrations were augmented, the manager feels strongly that imbalances in water quality variables other than low concentrations of potassium, magnesium, or dissolved oxygen negatively affected shrimp performance in some ponds.

An investigation of the influence of a wide range of water quality variables on shrimp production in ponds on this farm was initiated. This report concerned the influence of major water quality variables on shrimp production.

## Materials and Methods

The shrimp farm is located in the Blackland Prairie region of Alabama about 5 km north of Forkland on state Highway 43 in southeastern Greene County. There are 17

ponds of which nine were selected for the study. These ponds were chosen because the manager had observed problems with low survival, slow growth, or both during the previous crop. Ponds were stocked between 23 April and 7 June 2008 with about 16 to 30 postlarvae/m<sup>2</sup> (Table 1). A pelleted, 35% crude protein-content feed was offered to shrimp twice daily, and mechanical aeration was applied at 8.5 to 13.0 hp/ha mostly at night. Ponds were harvested in September and October 2008, and data on feed use and shrimp production were obtained from the farm manager.

Water quality was monitored at 2-wk intervals. Because of the 3-h driving time to reach the farm, samples and measurements were taken between 1000 and 1400 h. Water temperature, dissolved oxygen concentration, pH, conductivity, total dissolved solids, and salinity were measured *in situ* (surface and 10 cm above bottom) using a YSI 556 MPS Multiprobe system (Yellow Springs Instrument Company, Yellow Springs, Ohio, USA), and Secchi disk visibility was determined.

In each pond, a water sample from approximately 10 cm above the bottom was collected from a pier in water of 1.0 to 1.25m deep by aid of a bottom-water sampling bottle described by Boyd and Tucker (1992). Bottom water samples and samples of surface water from each pond were immediately analyzed for carbon dioxide and hydrogen sulfide using a Hach Model CA-23 Carbon Dioxide Test Kit and a Hach Model HS-WR Hydrogen Sulfide Test Kit (Hach Company, Loveland, Colorado, USA).

Another water sample was collected from a depth of 0.5 m in each pond, put into a 2-L plastic bottle, placed on ice in an insulated chest, and transported to Auburn University for analyses. Methods described by Eaton et al. (2005) were used for chlorophyll *a* (membrane filtration, acetone extraction, and spectroscopy), calcium,

magnesium and total hardness (versenate titration), phenolphthalein, methyl orange, and total alkalinity (acidimetry), nitrite (diazotization procedure), and turbidity (nephelometry). Total ammonia nitrogen was determined by the salicylate method (Bower and Holm-Hansen 1980), and nitrate was measured by the szechrome NAS reagent method (Van Rijn 1993). Aliquots of water samples were digested in alkaline persulfate solution as described by Eaton et al. (2005). This digestion converted all phosphorus and nitrogen in the unfiltered samples to phosphate and nitrate, respectively. Phosphate concentrations in digestates were measured by the ascorbic acid procedure (Eaton et al. 2005), and nitrate was determined by the ultraviolet spectrophotometric screening method (Eaton et al. 2005, Gross et al.1999) to provide concentrations of total phosphorus and total nitrogen.

Averages and standard deviations for water quality data were calculated as follows: (1) each pond across all sampling dates (pond means); (2) all ponds for each sampling date (farm means); (3) all ponds and all sampling dates (grand means). The relationships between feed inputs to ponds (X variables) and pond means for water quality variables and between pond means for water quality variables (X variables) and shrimp survival and production were analyzed by regression using SigmaPlot statistical software version 10.0 (Aspire Software International, Ashburn, Virginia, USA).

## Results and Discussion

### Water quality

Ponds were constructed in soils of similar properties and filled with water from a single source; nevertheless, most water quality variables exhibited high variation which



will be illustrated using a variable that varied greatly, total ammonia nitrogen, and one that was less variable, total hardness. On 26 August, concentrations of total ammonia nitrogen and total hardness ranged from 0.0 to 0.962 mg/L and from 127.2 to 209.6 mg/L, respectively among the nine ponds. The farm means for this date were  $0.204 \pm 0.328$  mg/L for total ammonia nitrogen and  $162.4 \pm 33.2$  mg/L for total alkalinity. Over the 10 sampling dates, farm means for total ammonia nitrogen were 0.0 to 0.376 mg/L, and those for total hardness were 152.1 to 210.5 mg/L. The coefficients of variation for the farm means were 0.0 to 225.6% for total ammonia nitrogen, but from 16.1 to 25.0% for total hardness.

When data for all sampling dates were averaged across each pond (pond means), total ammonia nitrogen varied from  $0.060 \pm 0.075$  mg/L in Pond N-4 to  $0.430 \pm 0.168$  mg/L in Pond N-8; total hardness ranged from  $126.8 \pm 10.4$  mg/L in Pond N-4 to  $201.2 \pm 14.8$  mg/L in Pond S-3. Coefficients of variation for pond means were from 108.0 to 193.6% for total ammonia nitrogen and 6.4 to 20.0% for total hardness.

Considering all 79 measurements (ponds + dates), ranges were 0.0 to 2.057 mg/L for total ammonia nitrogen and 102.4 to 241.4 mg/L for total hardness. These values had coefficients of variation of 185.4% and 22.0%, respectively. The distribution of the values was highly skewed to the left for total ammonia nitrogen, but values were more normally distributed for total hardness (Fig. 1). These observations and reference to other variables measured in the study suggest that variables controlled mainly by biological activity (carbon dioxide, dissolved oxygen, turbidity, chlorophyll *a*, Secchi disk visibility, and nitrogen and phosphorus fractions) are much more variable than variables controlled primarily by chemical and physical process (water temperature, pH,

salinity, conductivity, alkalinity, hardness, calcium, magnesium, and total dissolved solids).

The data in this dissertation will be limited to farm means (average of all ponds on each date) and grand means (average of all ponds across all dates).

Farm means for conductivity did not differ appreciably between surface and bottom water, but as a result of dilution by heavy rainfall in mid August, conductivity was greater during the first half as compared to the second half of the grow-out period (Fig. 2). Individual measurements of conductivity in bottom water ranged from 4,538 to 7,578  $\mu\text{S}/\text{cm}$  and had a grand mean of 5,880  $\mu\text{S}/\text{cm}$  (Table 2). Total dissolved solids and salinity also were measured; grand means were 3,570 mg/L and 2.94 ppt, respectively (Table 2). There was a high correlation between conductivity measurements and those for total dissolved solids and salinity (Fig. 3). Changes in concentrations of total dissolved solids and salinity over time were not presented, because they followed the same trend as those presented for conductivity (Fig. 2).

The culture species, *L. vannamei*, can tolerate salinities of 0.5 to 45 ppt (Bray et al. 1994, Menz and Blake 1980), and recent studies suggest that this species can possibly be cultured at salinity below 0.5 ppt (Araneda et al. 2008, Cuvin-Aralar et al. 2009). However, most successful shrimp culture appears to have been done at salinities above 2 ppt (Roy et al. 2010). Ponds of the present study always had over 2 ppt salinity, and salinity averaged 2.94 ppt (Table 2; Fig. 3), suggesting that waters in all ponds contained an adequate quantity of dissolved salts for production of the culture species.

Mechanical aeration of ponds mixed pond water, and there was little difference in farm means between surface and bottom water temperature (Fig. 2; Table 2). The grand

mean for water temperature was 28.82°C with individual values ranging from 20.48 to 33.96°C. The highest farm means for water temperatures were recorded in June, and values steadily decreased as the study progressed (Fig. 2). The lowest temperatures were below optimum for culture of *L. vannamei* (Lester and Pante 1992), but these temperatures were recorded only in ponds harvested in late October. Although low water temperature probably did not negatively impact shrimp growth during the grow-out period, low temperature on both ends of the grow-out period determines the length of the growing season for *L. vannamei* in the southeastern United States (Green and Popham 2008). The maximum water temperatures were no greater than those that have been observed in aquaculture ponds in tropical areas (Boyd and Tucker 1998).

Farm means for pH tended to be slightly greater in surface water than in bottom water (Fig. 2). This is typical in pond aquaculture, because removal of carbon dioxide by phytoplankton in surface water for use in photosynthesis causes pH to rise during daylight hours (Boyd and Tucker 1998). Shrimp spend most of the time on the bottom, and the range in water pH in bottom water based on all individual measurements was 7.27 to 9.37 (Table 2); pH above 9 was observed in 11 samples from eight ponds. The minimum pH of 7.27 would not be harmful to the culture species. Although mortality will not occur until pH rises to 10 or more, negative, sub-lethal effects may occur if pH rises above 9, and high pH results in a greater proportion of un-ionized ammonia that can be toxic to shrimp (Boyd and Tucker 1998).

Farm means for dissolved oxygen concentration usually were 2 to 5 mg/L greater in surface water than in bottom water (Fig. 2) as would be expected because of greater photosynthetic rate in surface water. Dissolved oxygen concentration in bottom water –

was always measured near midday – had a grand mean of 10.81 mg/L (Table 2). A minimum value of 2.44 mg/L was recorded in an individual pond, and on three occasions, individual ponds had a concentration below 5 mg/L. Occasional occurrence of low dissolved oxygen concentrations in bottom water near midday suggests that low dissolved oxygen concentration may be common in late evening and early morning. Most warmwater aquaculture species will not die until the dissolved oxygen concentration falls below 1.5 to 2 mg/L and remains at this level for several hours. However, exposure to dissolved oxygen levels below 3 or 4 mg/L for a shorter time may cause stress leading to greater susceptibility to disease, poor appetite, and slow growth (Boyd and Tucker 1998). A study by McGraw et al. (2001) revealed that survival and production of shrimp improved in ponds as minimum, average daily oxygen concentrations increased from 1.1 mg/L to 4.6 mg/L. The greatest improvement was noted when ponds with minimum concentration of 1.1 mg/L were compared with ponds where the minimum concentration was 2.8 mg/L.

Carbon dioxide concentration (Fig. 2; Table 2) is difficult to assess because it also was measured near midday. On some sampling dates, ponds had no free carbon dioxide as a result of phytoplankton photosynthesis, but in those that did, bottom water contained a greater carbon dioxide concentration than surface water because of greater photosynthetic rate in surface water. Although the grand mean for carbon dioxide was only 4.66 mg/L in bottom waters, a peak individual concentration of 33.93 mg/L was measured, and concentrations above 10 mg/L were recorded on 14 occasions. It is likely that high carbon dioxide concentrations are common during late evening and early morning. Carbon dioxide is not highly toxic to aquaculture species, but when dissolved

oxygen concentration is low, high carbon dioxide concentration interferes with absorption of oxygen from the water by culture species (Boyd and Tucker 1998).

Farm means for total alkalinity concentration increased from near 120 mg/L in mid June to a maximum of about 180 mg/L in early October (Fig. 4). The grand mean for total alkalinity concentration was 148.8 mg/L (Table 2) – about 25 mg/L higher than the alkalinity of average seawater (Goldberg 1963). The minimum alkalinity concentration of 71.5 mg/L observed in this study is slightly lower than the recommended minimum concentration for shrimp culture of 75 mg/L (Boyd et al. 2002). However, only one sample had alkalinity below 75 mg/L, and alkalinity was less than 100 mg/L in only five samples. Because samples were taken about midday when pH was often above 8.3 because of photosynthesis, a fraction of the alkalinity in most samples resulted from carbonate (phenolphthalein alkalinity) (Fig. 4). The difference in total alkalinity and methyl orange alkalinity in Fig. 4 represents phenolphthalein alkalinity.

Farm means for total hardness increased from near 150 mg/L to about 200 mg/L during the study (Fig. 4). Individual concentrations of this variable ranged from 102 to 241 mg/L and the grand mean was 163.5 mg/L (Table 2). Total hardness concentration was much less than that found in normal seawater ( $\approx$  6,000 mg/L), and it was less than half of the total hardness of about 500 mg/L that could be expected in seawater diluted to the average salinity of the study ponds. Calcium hardness made up about 60% of the total hardness (Fig. 4) – magnesium hardness is the difference in total hardness and calcium hardness.

Concentrations of magnesium and calcium, the cations primarily responsible for total hardness, were estimated from magnesium hardness and calcium hardness

concentrations: magnesium hardness  $\div 4.12 = \text{Mg}^{2+}$ ; calcium hardness  $\div 2.5 = \text{Ca}^{2+}$  (Boyd 2000). Farm means for magnesium and calcium concentration ranged from 12 to 40 mg/L, respectively, in mid June to 18 and 50 mg/L, respectively, in late October. The minimum concentrations of major cations needed for successful culture of shrimp and other marine animals in low-salinity water is not known. Boyd et al. (2002) suggested that the seawater equivalent concentration – the concentration of an ion expected in normal seawater diluted to the salinity of the culture system water – should be an acceptable concentration, and Atwood et al. (2003) demonstrated that ionic proportions in diluted seawater are ideal for survival and growth of *L. vannamei* in low-salinity water. The grand mean for salinity of the study ponds equals to a seawater equivalent concentration of 31.5 mg/L for calcium and 106.5 mg/L for magnesium. Calcium concentration as low as 21.3 mg/L was recorded, and calcium was below the seawater equivalent concentration in 15 samples. The minimum magnesium concentration was 4.2 mg/L, and all samples were below the seawater equivalent concentration.

Potassium concentrations were not measured in this study, because a previous study showed that the farm manager added enough potassium fertilizer to ponds to maintain potassium concentration above the seawater equivalent concentration (Boyd et al. 2007, McNevin et al. 2004). These previous studies also showed that sodium always exceeded the seawater equivalent concentration, and therefore, sodium was also not measured.

Total ammonia nitrogen concentration was relatively low in the ponds (Fig. 5) despite the large inputs of feed. Surface waters had relatively high pH that favors loss of un-ionized ammonia ( $\text{NH}_3$ ) to the air through diffusion (Gross et al. 1999, Weiler 1979).

The high rate of mechanical aeration used in ponds splashed water into the air, a process that also favors ammonia loss by diffusion to the air. Ammonia nitrogen is rapidly absorbed by phytoplankton for use in protein synthesis (Tucker et al. 1984). Moreover, ammonia is oxidized to nitrate by nitrifying bacteria (Boyd and Tucker 1998). The highest recorded total ammonia nitrogen concentration in an individual pond was 2.06 mg/L (Table 2), and on only three occasions did concentrations exceeded 1 mg/L.

In laboratory toxicity tests, the 48-h LC50 of total ammonia nitrogen to *L. vannamei* was 39.7 mg/L for 47-day-old shrimp exposed at 29°C, pH 7.9, and 10 ppt salinity (Schuler et al. 2010). This LC50 value equated to 2.09 mg/L un-ionized ammonia – the toxic form. However, the safe concentration for continuous exposure of aquaculture species to un-ionized ammonia is probably about 0.05 times the 48-h LC50 (Boyd 2000). Based on the findings of Schuler et al. (2010), shrimp should not be exposed to more than 0.1 mg/L un-ionized ammonia for long periods.

In the present study, water temperature and pH sometimes exceeded 30°C and 9.0, respectively. Under such conditions, more than 45% of total ammonia nitrogen would be in un-ionized form. Total ammonia nitrogen concentrations could have temporarily exceeded, on several occasions, the limit considered safe for continuous exposure, and these events – though not lethal – might have stressed the shrimp.

Nitrite-nitrogen was occasionally detected in pond waters (Fig. 5), and the maximum recorded concentration in an individual pond was 1.08 mg/L. The 48-h LC50 of nitrite was found to be 154 mg/L in laboratory toxicity tests (Schuler et al. 2010). Using an application factor of 0.05 (Boyd 2000), the minimum safe concentration for long-term exposure would be 7.7 mg/L – considerably more than the maximum

concentration found in the ponds. Nitrite toxicity does not seem to be a reasonable explanation for the variation in shrimp survival and production in Alabama ponds.

Nitrate-nitrogen was seldom detectable in pond waters (Fig. 5). This was an unexpected observation, because aquaculture ponds usually have nitrate-nitrogen concentrations of 0.1 to 0.3 mg/L (Boyd and Tucker 1998). The major source of nitrate is oxidation of ammonia nitrogen, and ponds had low concentration of ammonia nitrogen – possibly, this is the reason for the lack of nitrate. Of course, nitrate could have been reduced to nitrogen gas by denitrifying bacteria in anaerobic sediments (Hargreaves 1998).

Hydrogen sulfide, a substance that can be toxic at low concentrations to fish and shrimp (Boyd and Tucker 1998), was not detected in water from any of the ponds (Fig. 5).

Farm means for total nitrogen concentration steadily increased from about 5 mg/L in mid June to around 7 mg/L in late October (Fig. 5), and the grand mean was 5.86 mg/L (Table 2). Contrary to total nitrogen, pond means for total phosphorus remained relatively constant, fluctuating from about 0.27 to 0.45 mg/L (Fig. 5), and the grand mean for total phosphorus was 0.36 mg/L (Table 2).

Ponds usually had dense phytoplankton blooms as evident from high chlorophyll *a* concentration (Fig. 6); farm means steadily increased from about 160 µg/L in mid June to around 575 µg/L in early October. The chlorophyll *a* concentration then declined with onset of lower temperatures (Fig. 6). The grand mean for chlorophyll *a* was 464 µg/L (Table 2).



Secchi disk visibility decreased as turbidity increased during the study (Fig. 6), and considering all measurements, the two variables were strongly correlated ( $r = 0.871$ ;  $P < 0.01$ ) (Fig. 7). Both Secchi disk visibility and turbidity also were highly correlated with chlorophyll *a* concentration ( $P < 0.01$ ), but Secchi disk visibility would be a better predictor of chlorophyll *a* concentration than would turbidity (Fig. 7). Suspended particles that cause turbidity and lessen Secchi disk visibility in aerated ponds originate from two main sources – phytoplankton and sediment particles suspended by aerator-induced turbulence (Thomforde and Boyd 1991). However, it is not clear why Secchi disk visibility was a better predictor of chlorophyll *a* concentration.

The largest external input of inorganic nutrients and organic matter to ponds was feed, and feed input varied from 2,684 kg/ha to 9,558 kg/ha in the nine ponds. The average concentrations of some water quality variables were correlated ( $P < 0.05$ ) with feed input (Table 2).

In summary, the water quality data suggest that several variables, water temperature, dissolved oxygen, carbon dioxide, total ammonia nitrogen, total alkalinity, calcium, and magnesium were at times outside optimum limits for shrimp production. Although these variables were never at toxic levels, but they may have stressed shrimp, and this could have negatively influence survival, feeding activity, growth rate, and production.

### Shrimp production

Shrimp production data varied among the nine ponds (Table 3); survival and production ranged from 16% to 128%, and production had a range of 928 kg/ha to 5,950

kg/ha. Because of the difficulty of counting tiny, postlarval shrimp, it is not unusual for counting errors to result in survival over 100%. Low survival rates may also be caused by errors in estimating the number of postlarvae stocked. Nevertheless, production was highly correlated with percentage survival ( $r = 0.912$ ;  $P < 0.01$ ) (Fig. 8). Ponds were stocked during a short period (Table 1), but harvest was spread over a longer time (Table 3). This resulted in variation in the length of the grow-out period, but there was not a correlation between crop duration and production (Fig. 8).

#### Correlations between production data and water quality

The pond means (variables for each pond averaged across all dates) for water quality variables were used as X variables, and correlation analysis was conducted using both shrimp survival and shrimp production in each pond as Y variables (Table 4). Survival was positively correlated with increasing concentrations of total alkalinity, methyl orange alkalinity, calcium hardness, and total nitrogen, while it was negatively correlated with increasing water temperature, pH, and Secchi disk visibility. Production was positively correlated with greater total alkalinity and methyl orange alkalinity, calcium hardness, and total nitrogen, but negatively correlated with higher water temperature and pH.

Correlation of production with total nitrogen is not likely causal because ponds with high production had high survival and feed input, and total nitrogen concentration increased with greater feed input (Table 2). The negative correlation between Secchi disk visibility and survival also is not thought to be causal. Ponds with high survival had greater feed input, and more nutrients reached the water to stimulate phytoplankton

growth (decrease Secchi disk visibility). Thus, low Secchi disk visibility would not be the cause of high survival, but rather, the result of it.

Neither survival nor production was correlated with magnesium concentration. This suggests that the application of magnesium fertilizer to ponds provided sufficient magnesium to meet shrimp requirements albeit magnesium concentrations in the water were consistently below the seawater equivalent concentration. The positive correlation between calcium hardness and both survival and production is interesting, because calcium ion has not been considered as a possible limitation in the ponds. It was noted earlier that the calcium concentration was sometimes lower than the seawater equivalent concentration.

Survival and production each were positively correlated with total alkalinity and methyl orange alkalinity. However, calcium hardness tended to increase with increases in both total alkalinity and methyl orange alkalinity. Thus, if a causal relationship exists for this group of variables, it is likely for calcium.

Multiple linear regression (MLR) was used to investigate the relationship between water quality variables (independent variables) and shrimp production, feed conversion ratio, and survival rate (dependent variables). The MLR modeling method was performed by the SAS 9.1.2 statistical analysis software (SAS Institute, 2008). After obtained various equations with all independent and dependent variables mentioned above, the best equation is selected based on the highest multiple coefficient of determination ( $r^2$ ), lowest standard deviation (SD), and F-ratio value. The best model derived by the application of MLR method is:

$$\text{Production} = -9455.37044 + 71.63166 (\text{MetAlk}) + 33.43784 (\text{CaH})$$

The equation provides  $r^2 = 0.9826$  meaning that 98% of total variance for the estimation of production is explained by the MLR model for independent variables, methyl orange alkalinity (MetAlk) and calcium hardness (CaH), measured was statistically significant ( $P < 0.01$ ) in estimating dependent variable, shrimp production in inland, low-salinity pond.

Negative correlations between water temperature and pH and shrimp survival and production also merit further consideration. However, it is possible that both may be related to cooler water temperature and lower daytime pH because of declining rates of photosynthesis during the final few weeks of culture in ponds of long crop duration that are harvested after mid September.

Findings of this study did not identify any specific water quality conditions that were clearly out of balance and negatively affecting shrimp survival and production. However, the findings suggest that the farm should implement a daily monitoring program for early morning dissolved oxygen concentration to ascertain if the amount of aeration is sufficient to prevent dissolved oxygen concentrations below 3 mg/L. Also, a weekly or twice weekly monitoring effort for total ammonia nitrogen concentration and pH could provide data for ascertaining if un-ionized ammonia nitrogen concentration is often great enough to stress shrimp.

The correlation between calcium hardness and both survival and growth suggest that increasing calcium concentration might improve shrimp performance. This hypothesis could be tested by applying calcium sulfate to a few ponds and comparing shrimp performance to that in other untreated ponds.

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Table 1. Pond information and data on stocking, aeration, and feed application in nine ponds on an inland, low-salinity shrimp farm in Alabama.

Pond	Area (ha)	Average Depth (m)	Stocking data		Aeration (hp/ha)	Feed applied (kg/ha)
			Date	PL/m <sup>2</sup>		
N2	0.57	1.64	7 Jun	26.95	8.8	4,805
N4	1.21	1.62	23 Apr	16.08	10.7	4,623
N6	1.50	1.60	22 May	25.02	9.7	5,779
N7	2.02	2.07	22 May	19.25	13.0	5,821
N8	1.54	1.76	22 May	22.34	9.7	6,525
S1	1.17	1.26	21 May	27.94	8.5	4,490
S3	1.01	1.29	21 May	29.70	12.9	4,682
S6	1.42	1.63	22 May	25.00	9.2	2,684
S8	1.94	1.57	21 May	27.89	11.9	9,558

Table 2. Grand means and standard deviations for water quality variables measured at 2-wk intervals for nine ponds on an inland, low-salinity shrimp farm in Alabama.

Correlation coefficients (r) between total feed input and water quality variables also are given.

Variable	Mean	Min	Max	r
Total ammonia nitrogen (mg/L)	0.191±0.353	0.00	2.057	0.486
Nitrite-nitrogen (mg/L)	0.033±0.142	0.00	1.098	-
Nitrate-nitrogen (mg/L)	0±0.004	0.00	0.037	-
Total phosphorus (mg/L)	0.36±0.12	0.12	0.66	0.687*
Total nitrogen (mg/L)	5.85±2.24	2.32	14.91	0.593
Hydrogen sulfide (mg/L)	0.00±0.00	0.00	0.00	-
Carbon dioxide at the surface (mg/L)	2.05±5.79	0.00	30.50	0.870*
Carbon dioxide at the bottom (mg/L)	4.66±8.34	0.00	35.25	0.901*
Dissolved oxygen at the surface (mg/L)	13.48±4.28	5.40	26.20	0.138
Dissolved oxygen at the bottom (mg/L)	10.81±4.28	2.40	26.00	0.077
pH at the surface	8.56±0.51	7.50	9.80	-0.883*
pH at the bottom	8.33±0.48	7.30	9.40	-0.924*
Temperature at the surface (°C)	29.43±3.39	20.51	35.80	-0.893*
Temperature at the bottom (°C)	28.82±3.03	20.48	33.96	-0.809*
Salinity (ppt)	2.94±0.31	2.15	3.84	0.164
Conductivity at the surface (µS/cm)	5,948±733	4,551	7,740	0.061
Conductivity at the bottom (µS/cm)	5,880±704	4,538	7,578	0.061
Phenolphthalein alkalinity (mg/L as	6.32±5.38	0.00	24.42	-0.629
Methyl orange alkalinity (mg/L as	137.5±30.9	59.90	209.5	0.761
Total alkalinity (mg/L as CaCO <sub>3</sub> )	143.8±28.8	71.40	212.2	0.756*
Total hardness (mg/L as CaCO <sub>3</sub> )	163.5±36.0	102.4	241.3	0.615
Calcium hardness (mg/L as CaCO <sub>3</sub> )	96.1±26.6	53.20	157.1	0.787*
Magnesium hardness (mg/L as CaCO <sub>3</sub> )	67.4±18.8	17.10	120.3	0.573
Secchi disk visibility (cm)	24.8±9.8	12	60	-0.697*
Turbidity (NTU)	56.5±35.1	12.80	208	0.726*
Chlorophyll <i>a</i> (µg/L)	463±302	29	1,467	0.551
Total dissolved solids (mg/L)	3,570±358	2,670	4,567	0.170

\* Significant at P < 0.05

Table 3. Harvest data and production performance in nine ponds on an inland, low-salinity shrimp farm in Alabama.

Pond	Harvest data		Crop duration (days)	Survival	FCR	
	Date	Shrimp/m <sup>2</sup> (kg/ha)				
N2	7 Oct	17.59	4,066	122	65	1.18
N4	29 Sep	10.42	2,781	159	65	1.66
N6	21 Oct	15.31	3,142	152	61	1.84
N7	23 Oct	24.54	4,406	154	128	1.32
N8	26 Oct	23.38	4,656	157	105	1.40
S1	1 Oct	11.65	2,506	133	42	1.79
S3	6 Oct	10.16	2,453	138	34	1.91
S6	19 Sep	4.01	928	120	16	2.89
S8	29 Oct	33.50	5,950	161	120	1.61

Table 4. Correlation coefficients among water quality variables and shrimp survival and production in nine ponds at an inland, low-salinity shrimp farm in Alabama.

X-Variable	Y-Variable	
	Survival	Production
Conductivity	0.114	0.104
Water temperature	-0.782*	-0.779*
pH	-0.834*	-0.879*
Total alkalinity	0.754*	0.832*
Methyl orange alkalinity	0.814*	0.897*
Phenolphthalein alkalinity	-0.496	-0.473
Total hardness	0.564	0.587
Calcium hardness	0.671*	0.692*
Magnesium hardness	0.192	0.200
Total ammonia nitrogen	0.574	0.469
Total nitrogen	0.622	0.667*
Total phosphorus	0.377	0.435
Chlorophyll <i>a</i>	0.579	-0.437
Secchi disk visibility	-0.701*	-0.555
Turbidity	0.192	0.089

\* Significant at  $P < 0.05$

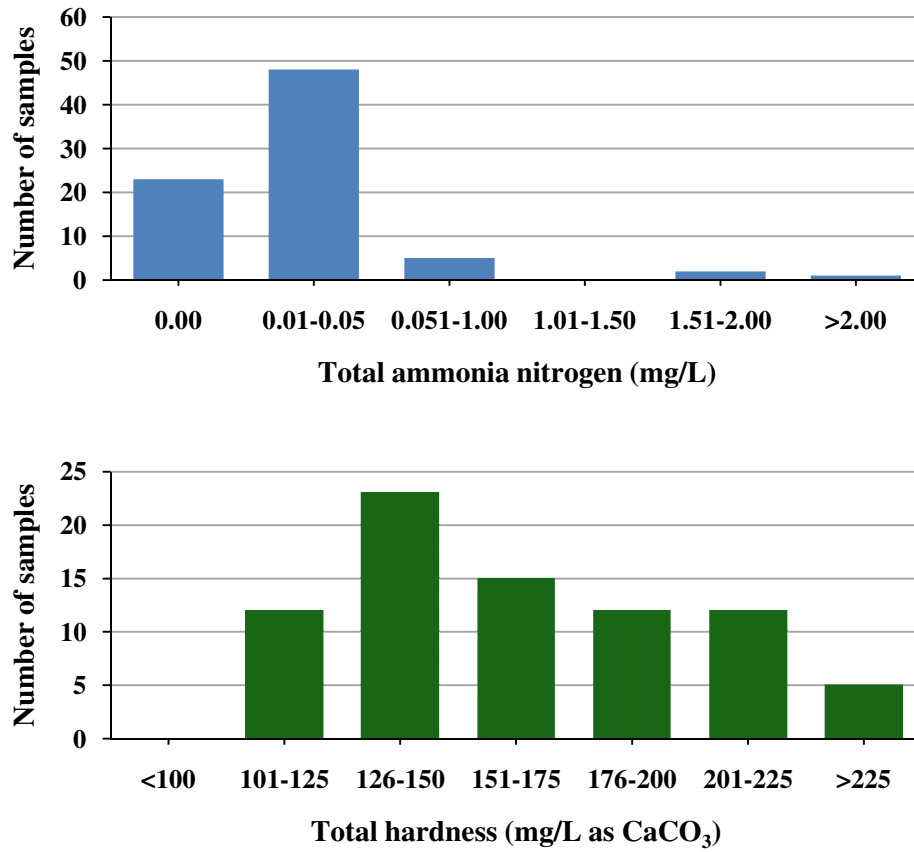


Fig. 1. Distribution of concentrations of total ammonia nitrogen and total hardness measured in nine ponds at 2-wk intervals at an inland, low-salinity, shrimp farm in Alabama.

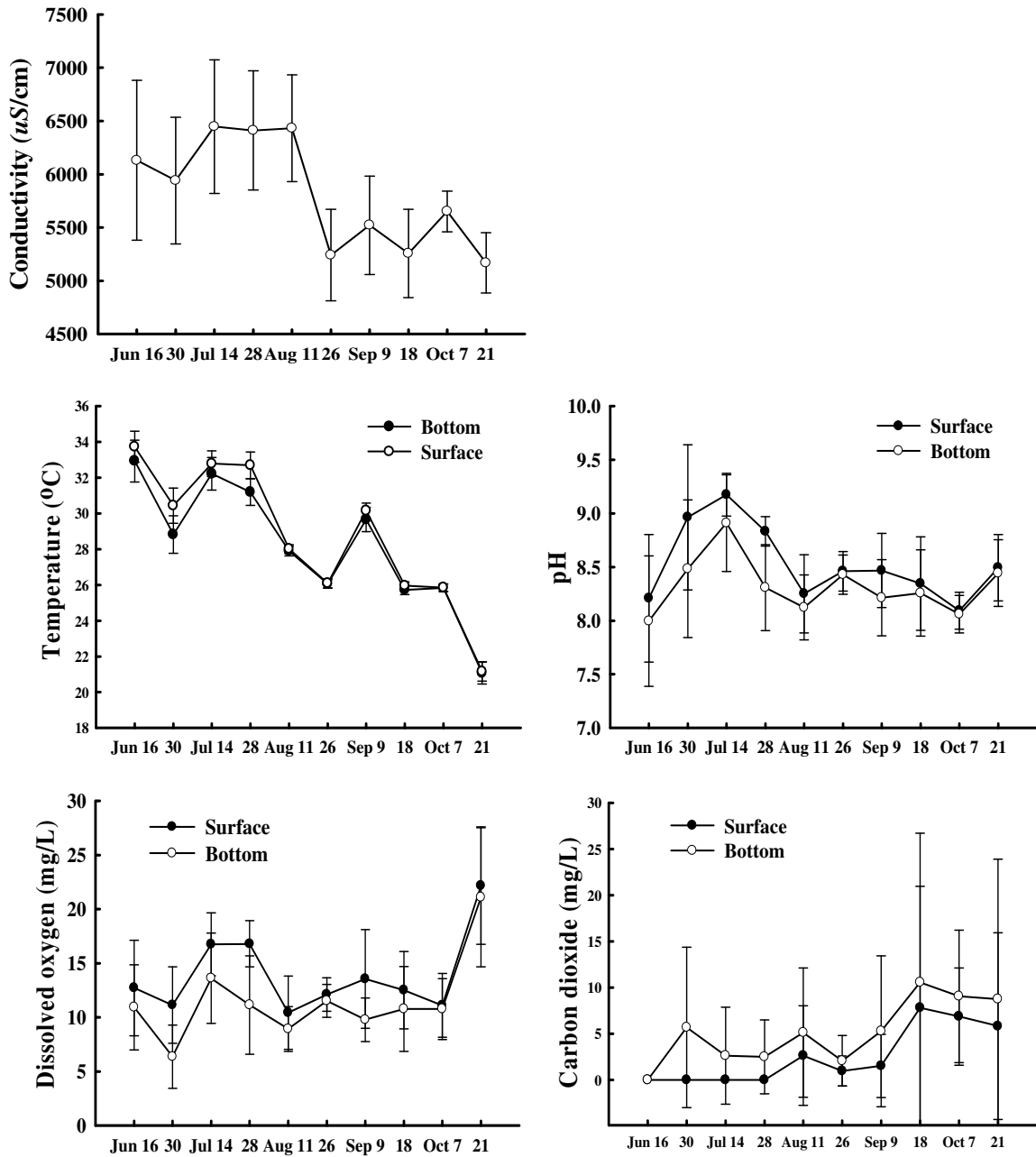


Fig. 2. Farm means (all ponds averaged by date) and standard deviations for conductivity, water temperature, pH, dissolved oxygen, and carbon dioxide in nine ponds at an inland, low-salinity shrimp farm in Alabama.

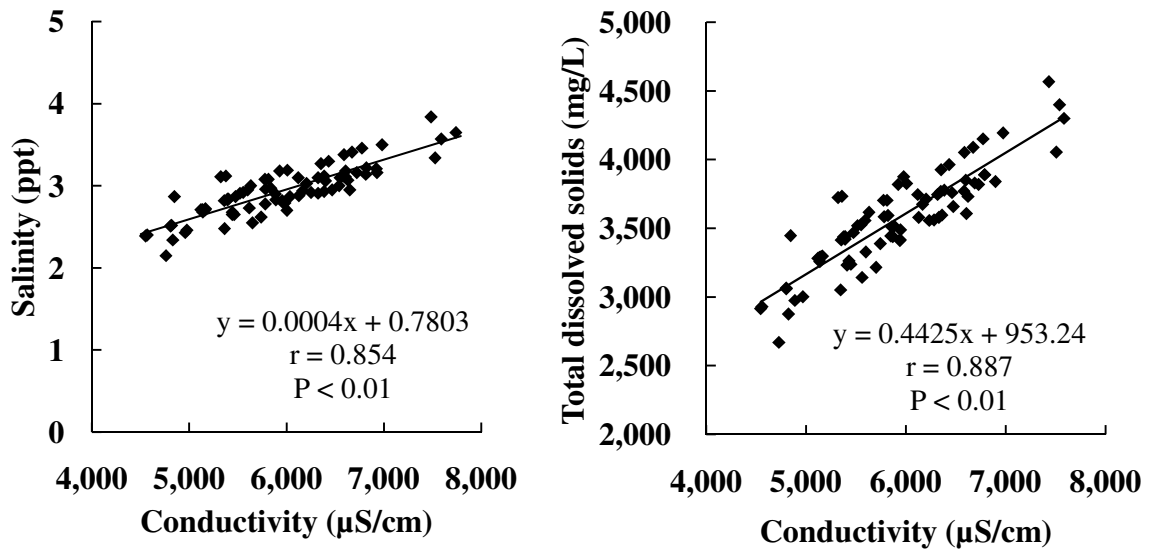


Fig. 3. Correlations between conductivity and salinity and total dissolved solids in waters of ponds at an inland, low-salinity shrimp farm in Alabama.

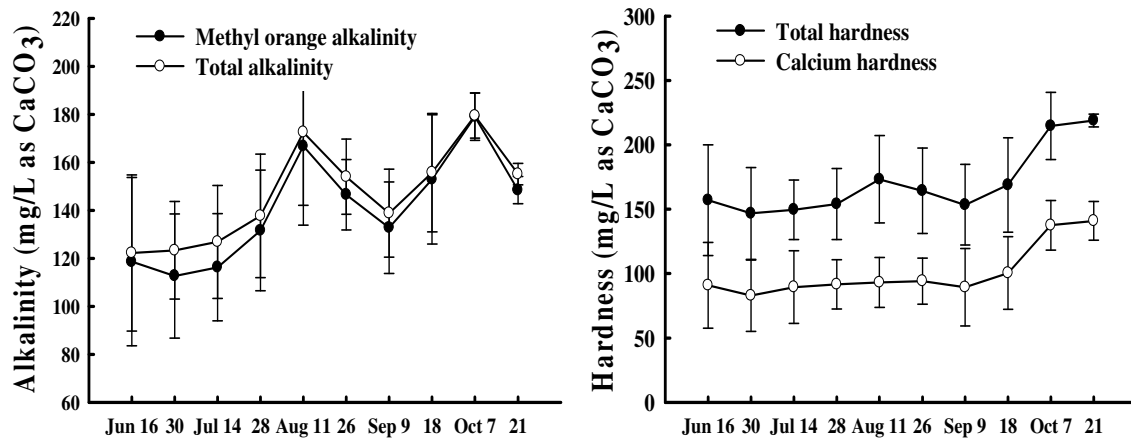


Fig. 4. Farm means (all ponds averaged by date) and standard deviations for total alkalinity and methyl orange alkalinity and total hardness and calcium hardness in nine ponds at an inland, low-salinity shrimp farm in Alabama.



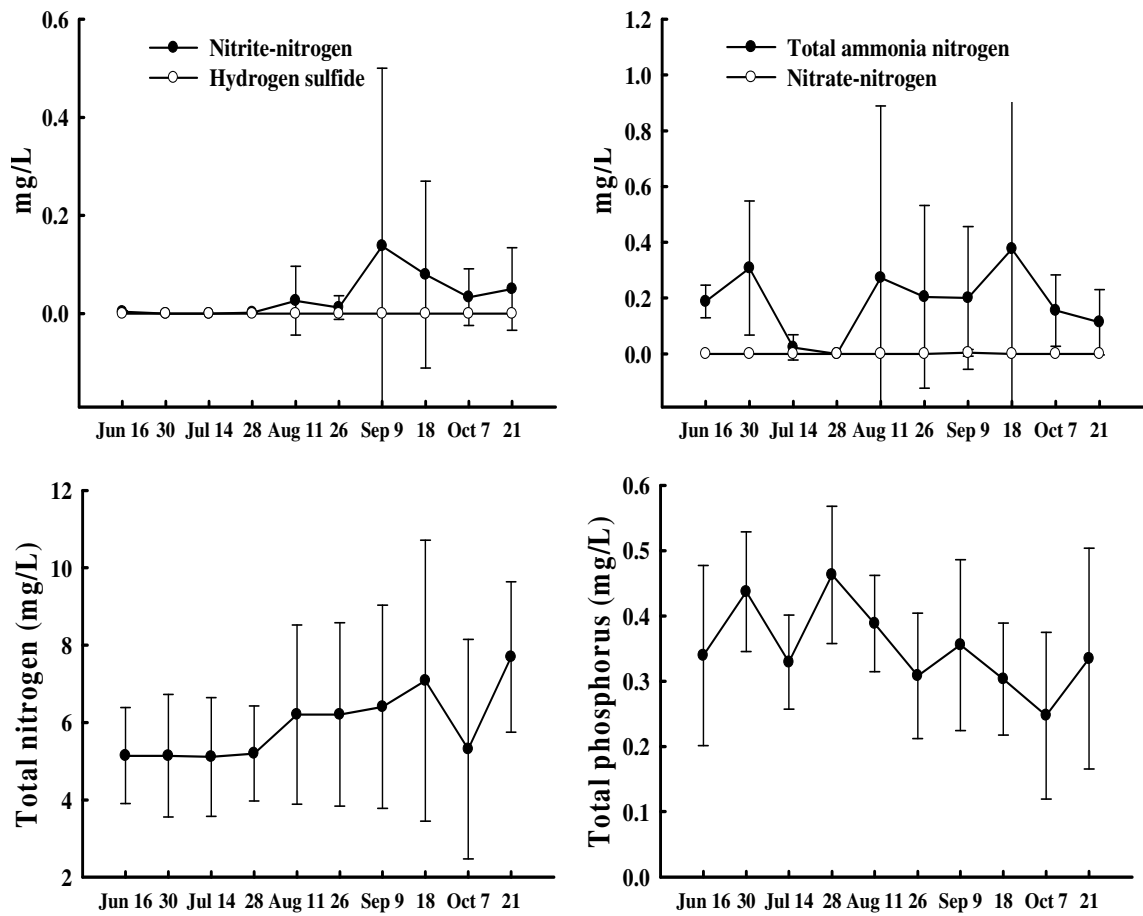


Fig. 5. Farm means (all ponds averaged by date) and standard deviations for hydrogen sulfide, nitrite-nitrogen, total ammonia nitrogen, nitrate-nitrogen, total nitrogen, and total phosphorus in nine ponds at an inland, low-salinity shrimp farm in Alabama.

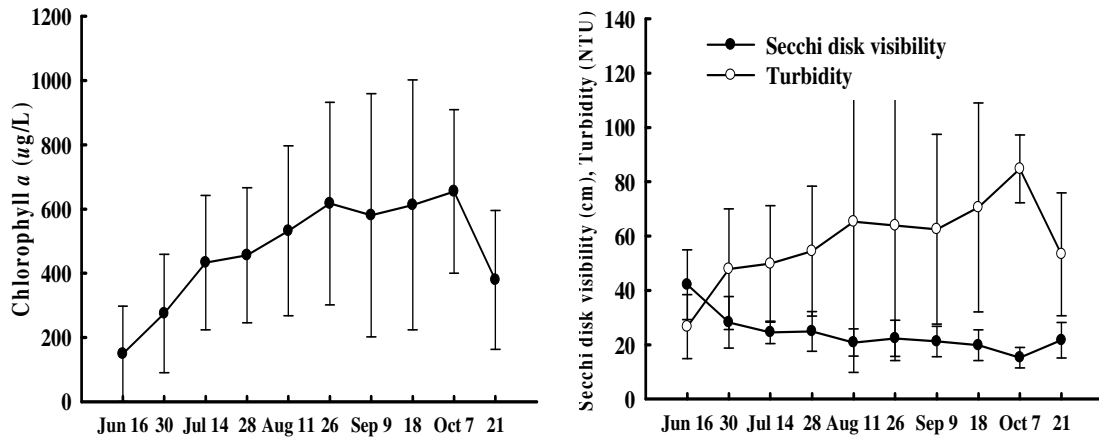


Figure 6. Farm means (all ponds averaged by date) and standard deviations for Secchi disk visibility, turbidity, and chlorophyll *a* in waters of nine ponds on an inland, low-salinity shrimp farm in Alabama.

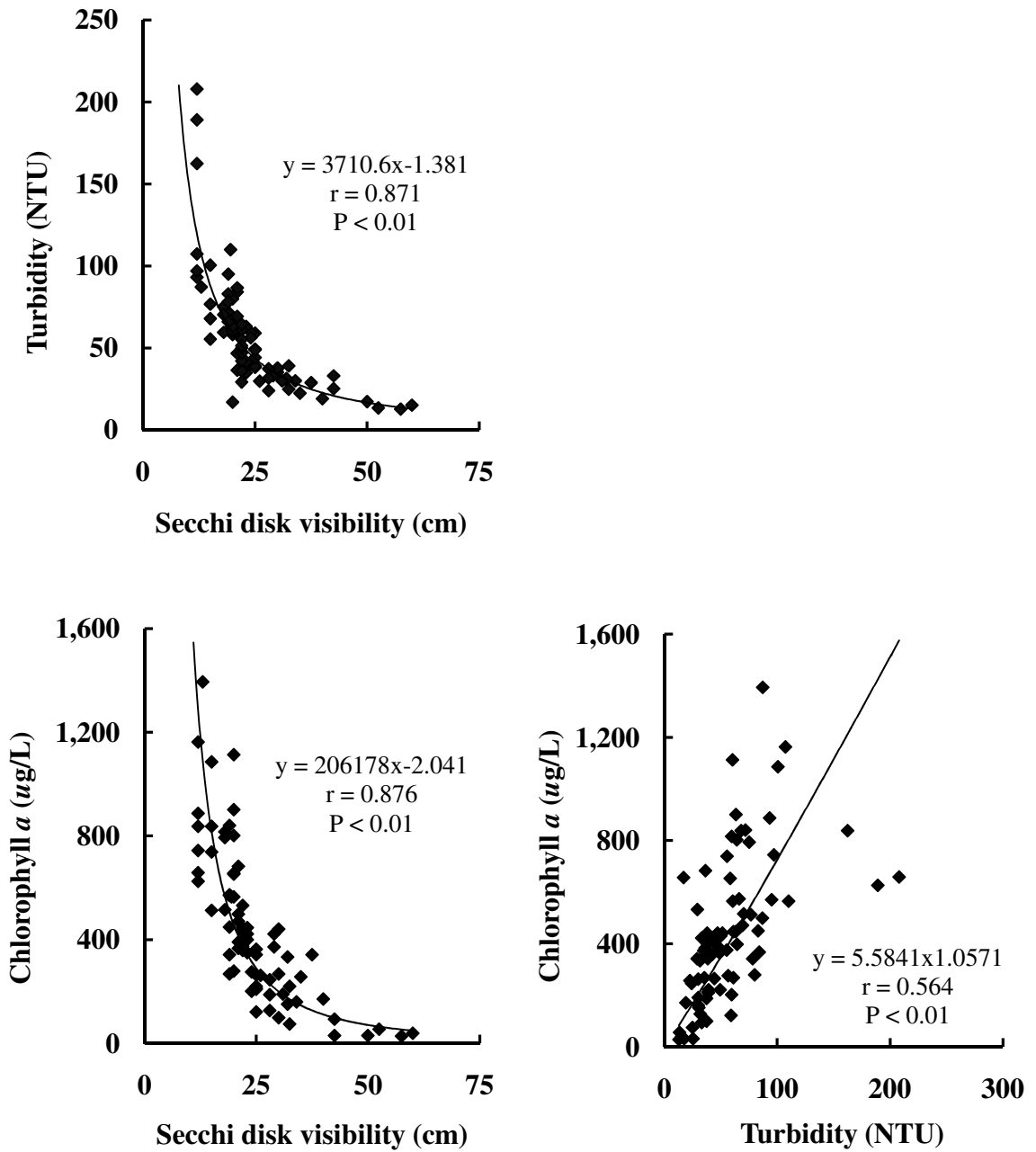


Fig. 7. Regression lines and correlation coefficients (r) for relationships among Secchi disk visibility, turbidity, and chlorophyll *a* data collected from nine ponds over the grow-out season at an inland, low-salinity shrimp farm in Alabama.

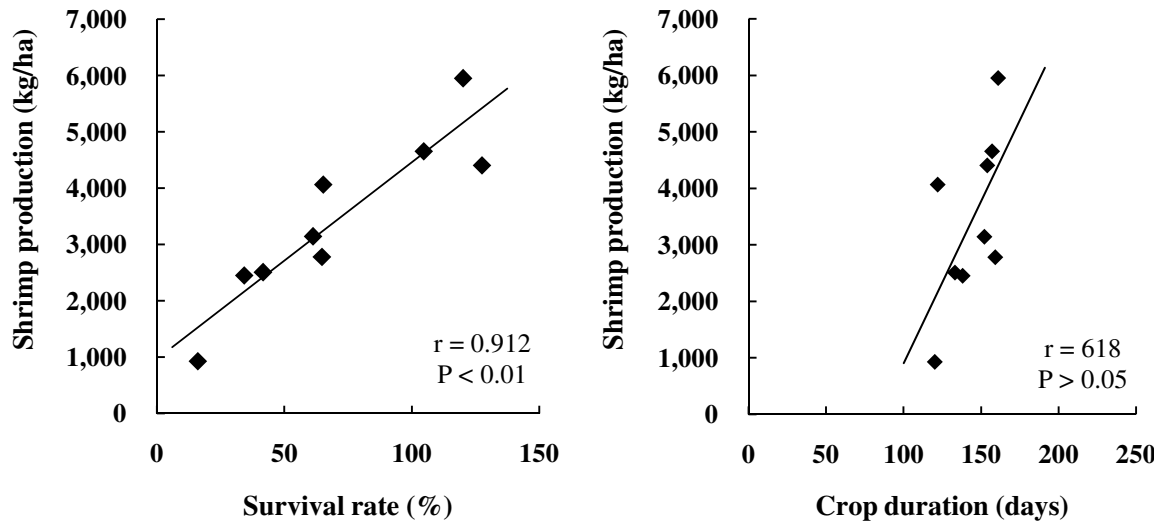


Fig. 8. Correlation between survival and crop duration and production of shrimp at an inland, low-salinity shrimp farm in Alabama.

#### IV. Trace Elements in Waters of Inland, Low-Salinity Shrimp Ponds in Alabama

##### Abstract

Concentrations of 18 trace elements in waters of ten inland, low-salinity shrimp ponds in Alabama tended to be greater than concentrations found in normal seawater – molybdenum, boron and silicon were exceptions. Concentrations of most trace elements varied greatly among ponds on individual sampling dates, and averages based on all sampling dates in individual ponds also varied considerably. The analytical method used, digestion of water samples in nitric acid followed by inductive coupled plasma spectrophotometry, measured total concentrations of trace elements. However, only the free ionic forms of most trace elements are toxic to aquatic life, and hydrolysis products, ion pairs, and coordination compounds (chelated forms) of most trace elements contributed greatly to total concentration. Although measured concentrations of iron, aluminum, zinc, and selenium were occasionally higher than typical, safe concentration limits for continuous exposure to aquatic life, it is doubtful that free ionic concentrations of these trace elements were great enough to harm shrimp. Positive correlations ( $P < 0.05$ ) between shrimp survival and production, and increasing concentrations of zinc, cobalt, and iron should be investigated further to ascertain if additions of these elements to ponds might improve shrimp performance.

## Introduction

Groundwater of 2 to 10 ppt salinity from wells is used in ponds for inland culture for Pacific white shrimp *L. vannamei* in Alabama. Shrimp survival and production in this water is limited by low concentrations of potassium and possibly of magnesium (Davis et al. 2005, Roy et al. 2010). Applications of potassium and magnesium fertilizers are used to overcome these deficiencies and allow survival rates and production levels similar to those obtained in coastal shrimp ponds filled with seawater or brackish water (Boyd et al. 2007, McNevin et al. 2004). Nevertheless, producers still complain about variation in survival and production among ponds.

A study of commonly measured water quality variables in inland, low-salinity shrimp ponds in Alabama conducted in 2008 revealed that several variables occasionally were outside optimum concentration ranges. There also were correlations between both survival and growth of shrimp and total alkalinity, calcium, water temperature, and pH that deserve further investigation. However, trace element concentrations were not considered in the earlier study, and the present study was undertaken to assess possible effects of trace elements on shrimp in inland, low-salinity ponds.

## Materials and Methods

Ten ponds in which farm managers observed low survival, low production, or both in previous crops were selected for study. Ponds are located on three shrimp farms in a fairly small area (6-km radius) of southeastern Greene County in the Blackland Prairie region of Alabama. Farms will be designated as A (seven ponds), B (two ponds), and C (one pond). Ponds were built in clayey, slightly alkaline soils typical of the Blackland Prairie region (Hajek et al. 1975) and filled from wells from the same saline-water aquifer.

Reliable information on ponds and their management were available only for Farm A (Table 1). The seven ponds ranged from 0.57 to 1.94 ha in water surface area, and they had average depths of 1.16 to 1.49 m. Postlarval shrimp (PL) were stocked in May 2009 at rates of 15.0 to 28.2 PL/m<sup>2</sup>. A pelleted, 35% crude protein feed was applied twice daily, and total feed input varied from 4,967 to 8,688 kg/ha. Aeration rates in ponds were 8.5 to 11.8 hp/ha and aerators were operated mainly at night. Ponds at Farm B were of comparable size to those at Farm A, but the pond at Farm C was about 10-ha in area. Ponds at Farms B and C had aeration rates similar to those used at Farm A, and stocking rates and feed inputs also were thought to be comparable.

Surface water samples were collected from ponds at 2-wk intervals between 6 May and 6 October 2009. The samples were placed in 100-mL, plastic bottles preserved with 50% nitric acid (2.0 mL/100 mL), and transported to Auburn University for analysis. Samples were digested by a slight modification of Method EPA 200.8 (Revised 5.4) (United States Environmental Protection Agency 1994). A 50-mL aliquot of each acidified sample was transferred to a 125-mL Erlenmeyer flask, and the sample was

heated on a hot plate until about 5-mL remained. Following filtration, the sample was quantitatively transferred to a 50-mL volumetric flask and made to volume with glass-distilled water. The solution was analyzed by inductive coupled plasma spectrometry (SpectroCiros<sup>CCD</sup>, SPECTRO Analytical Instruments, Inc., Mahwah, New Jersey, USA) for aluminum (Al), arsenic (As), barium (Ba), boron (B), cadmium (Cd), chromium (Cr), cobalt (Co), copper (Cu), lead (Pb), iron (Fe), manganese (Mn), molybdenum (Mo), nickel (Ni), selenium (Se), silicon (Si), titanium (Ti), zinc (Zn), and zirconium (Zr).

Shrimp samples from three ponds and feed samples were obtained at Farm A. The samples were dried at 60°C in a mechanical convection oven and pulverized with a mortar and pestle. Aliquots of samples were ashed in a muffle furnace for 8 h at 450°C, and ash was dissolved in 1.0 N nitric acid as described by Boyd and Teichert-Coddington (1995). Resulting solutions were passed through Whatman Number 42, acid-washed, filter papers, and analyzed for trace elements by inductive coupled plasma spectrophotometry.

Production data were obtained following harvest of ponds, but survival percentage and crop duration were available only for farm A. Data on feed input and composition were used to estimate the amount of each trace element added to each pond in feed. Trace element concentrations were averaged across all sampling dates for each pond. Correlation coefficients were determined for trace element inputs in feed (X variable) and mean pond water concentrations of trace elements. Correlation coefficients also were obtained for mean concentrations of trace elements (X variable) and shrimp survival in ponds at Farm A and shrimp production for all ponds. Statistical analyses



were conducted by aid of SigmaPlot statistical software version 10.0 (Aspire Software International, Ashburn, Virginia, USA).

## Results and Discussion

Variation in concentrations of trace elements among ponds on each sampling date exhibited variation similar to that shown for Pond N-8 at Farm A (Fig. 1) for six essential elements, boron, iron, copper, zinc, molybdenum and cobalt, and two non-essential ones, lead and nickel. The range in average concentrations of trace elements for sampling dates also was wide; for example, zinc concentration averaged between  $14.8 \pm 2.7 \mu\text{g/L}$  on 6 May to  $41.8 \pm 14.3 \mu\text{g/L}$  on 14 July, while molybdenum concentration averaged from  $1.22 \pm 0.92 \mu\text{g/L}$  on 14 July to  $4.00 \pm 2.45 \mu\text{g/L}$  on 28 July. Coefficients of variation ranged 7.5 to 34.2% for zinc and from 46.6 to 100% for molybdenum. As shown in Fig. 1 for Pond N-8, no trends of increase or decrease in concentrations of trace elements with respect to time were noted in individual ponds.

A similar degree of variation was observed when concentrations for all sampling dates were averaged for each pond. For example, zinc concentrations ranged from  $17.4 \pm 2.6$  to  $25.3 \pm 10.0 \mu\text{g/L}$  and molybdenum concentration varied from  $0.74 \pm 0.80$  to  $4.18 \pm 2.32 \mu\text{g/L}$  in individual ponds.

Standard deviations, coefficients of variation, and ranges for grand means of individual variables are presented (Table 2). The coefficients of variation allow a comparison of the degree of variability of different trace elements; they ranged from 27.9% for boron to 400% for zirconium. Ten of the 18 variables had coefficients of variation above 100%. The ponds are all built in similar soil and filled with water from

the same aquifer. One of the ponds at Farm B was previously used for channel catfish *Ictalurus punctatus* culture and had been treated repeatedly in the past with copper sulfate to control blue-green algae responsible for off-flavor (Tucker 1996, McNevin and Boyd 2004). The high, average concentration of copper in this pond, 34.2  $\mu\text{g/L}$ , as compared to those of other ponds, 1.9 to 6.7  $\mu\text{g/L}$ , was no doubt the result of previous copper additions. Otherwise, the main input of trace elements to ponds besides the water source was feed. However, there were no correlations ( $P > 0.05$ ) between the amounts of feed applied to ponds at Farm A and the concentrations of trace elements (Table 2).

Average concentrations of trace elements – other than molybdenum, boron, and silicon – in waters of the inland shrimp ponds were generally higher than concentrations of these elements in normal seawater (Goldberg 1963). Concentrations of most trace elements were within typical ranges reported for freshwater lakes, ponds, and streams (Arce and Boyd 1980, Boyd and Tucker 1998, Hem 1970, Hutchinson 1957). This difference probably reflects the influence of prolonged contact of saline groundwater and pond water with soil and other geological formations. Moreover, it suggests that inland, low-salinity shrimp ponds have higher concentrations of most trace elements than coastal ponds filled with water of marine origin.

Free ionic forms of most trace elements are not highly soluble in water even if they are relatively abundant in geological material with which water has been in contact (Boyd 2000, Hem 1970). Concentration of ionic iron, manganese, copper, zinc, cobalt, and aluminum are particularly low in waters with pH above 5 or 6 (Boyd 2000). The measured concentration of a trace element, however, usually is several fold greater than expected at equilibrium between its mineral form(s) in geological material and its free,

ionic form in natural water. This results because common analytical procedures such as the one used in this study measure soluble hydrolysis products such as  $\text{Fe}(\text{OH})_2^+$ ,  $\text{Fe}(\text{OH})_4^-$ ,  $\text{Al}(\text{OH})_2^+$ , and  $\text{Al}(\text{OH})_4^-$ , etc., ion pairs such as  $\text{CuCO}_3^\circ$  and  $\text{ZnCl}_2^\circ$ , etc., and coordination compounds formed through chelation of positively charged metal ions by dissolved organic matter (Schindler 1967; Boyd 2000). Moreover, iron and aluminum contained on colloidal clay particles is detected by the analytical method used in this study.

The toxicity of most trace elements to aquatic organisms is related to concentrations of their free, ionic forms (Boyd 2000, Pagenkoff 1978). Because of this, it is difficult to predict toxicity of trace elements to shrimp and other organisms directly from concentrations measured by normal analytical procedures. Moreover, most data on toxicity of trace elements were developed for a few, selected species of freshwater fish (Boyd and Tucker 1998), and almost no information related specifically to shrimp and particularly to *L. vannamei* could be found. However, it is likely that the sensitivity of farm-reared shrimp to trace elements is similar to that of common species of freshwater fish (Boyd and Tucker 1998).

Assuming the worst case scenario of measured concentrations of trace elements being equal to free ionic concentrations, aluminum, iron, selenium, and zinc concentrations in some samples exceeded safe concentrations reported for aquatic life (Table 3). However, as already discussed, there is little free aluminum ion in pond water unless pH is below 6, and pond waters in this study typically had pH of 7 to 9. Aluminum toxicity can be accounted as a factor influencing shrimp survival and production. Iron exceeded the safe concentration limit in 13 samples, but again, at the pH

of the ponds, most of the iron was in hydrolysis products, ion pairs, and coordination compounds that are not toxic. Zinc was 1 to 12  $\mu\text{g/L}$  above the reported safe limit of 50  $\mu\text{g/L}$  (Table 3) in three samples, and not thought to have been toxic. Selenium, however, exceeded the safe limit of 10  $\mu\text{g/L}$  in 21 samples that had from 11 to 22  $\mu\text{g/L}$ . Thus, selenium is the only trace element that possibly had a negative influence on shrimp.

Complete data on shrimp production were available only from ponds at Farm A (Table 4). Ponds had production of 3,005 to 5,425 kg/ha, survival rates of 32.6 to 95.2% and feed conversion ratios (FCRs) of 1.28 to 2.37. Production in the two ponds at Farm B was 2,989 and 3,297 kg/ha. The pond at Farm C had a production of 3,363 kg/ha.

The influence of trace element concentrations on shrimp was further assessed by correlation analysis (Table 5). There were no negative correlations ( $P > 0.05$ ) between concentrations of trace elements in pond water and percentage survival or production of shrimp. This supports the conclusion above that concentrations of trace elements – including selenium – were not high enough to harm shrimp.

Several elements — iron, manganese, copper, zinc, cobalt, and possibly others — are essential for shrimp. Except for copper, feed contained a greater amount of each trace element than was contained in shrimp at harvest. Nevertheless, there was no correlation ( $P > 0.05$ ) between copper in water and shrimp survival. There was a positive correlation ( $P < 0.05$ ) between concentrations of zinc and cobalt in the water and shrimp survival (Table 3). The positive correlation between lead concentration in water and shrimp survival is probably not causal, because lead is not an essential element for animals (Pais and Jones 1997).

Shrimp production was positively correlated ( $P < 0.05$ ) with cobalt, iron, and zinc concentrations in the water, and FCR was negatively correlated — became less — with greater concentrations of lead and zinc. There is no reason to believe that lead would be beneficial to FCR, but zinc is an essential element.

Findings of this study suggest that trace element concentrations in waters of inland shrimp ponds in Alabama do not reach toxic levels. However, correlations found between survival, production, and FCR and concentrations of cobalt, iron, and zinc deserve further study to ascertain whether or not they are causal. Feed contained more cobalt, iron, and zinc than present in shrimp. Thus, assuming that the correlations of these three elements with shrimp performance are causal, the reason might be because of an increase in the quantity, nutritional quality, or both of natural food organisms that shrimp eat in addition to manufactured feed. This possibility could be investigated by comparing production results in ponds fertilized with cobalt, iron, and zinc compounds with those obtained in ponds untreated with trace elements.

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Table 1. Data on pond area and depth, stocking date and postlarval (PL) density, aeration rate, and amount feed applied for seven ponds at Farm A, an inland, low-salinity shrimp farm in Alabama.

Pond	Area (ha)	Average Depth (m)	Stocking data		Aeration Rate (hp/ha)	Feed Applied (kg/ha)
			Date	PLs/m <sup>2</sup>		
N-2	0.57	1.64	May 7, 2009	28.2	8.8	7,158
N-4	1.21	1.62	May 7, 2009	26.0	10.7	8,212
N-6	1.50	1.60	May 7, 2009	27.0	8.7	6,947
N-8	1.54	1.76	Apr 28, 2009	23.9	9.8	6,329
S-1	1.17	1.26	May 7, 2009	15.0	8.5	4,967
S-6	1.42	1.63	Apr 24, 2009	20.9	9.2	5,724
S-8	1.94	1.57	Apr 24, 2009	22.6	11.8	8,688

Table 2. Dry weight concentrations of trace elements in shrimp and feed, grand means, standard deviations (SD), ranges and coefficients of variation (CV) for trace element concentrations for waters of ten ponds at inland, low-salinity shrimp farms in Alabama. Correlation coefficients (r) between feed inputs of trace elements (X variable) and concentrations of these elements in waters of seven ponds on one farm also are shown.

Element	Shrimp (mg/kg)	Feed (mg/kg)	Pond water			r
			Grand Mean ± SD ( µg/L)	Range ( µg/L)	CV (%)	
Aluminum	244	261	704 ± 732	137-3,007	104.0	0.302
Arsenic	1.3	ND <sup>1</sup>	2.5 ± 3.01	ND-13.0	120.4	---
Barium	45	7.6	145 ± 133	20-710	91.7	-0.389
Boron	50	7.4	687 ± 192	248-1,063	27.9	-0.463
Cadmium	ND	0.6	ND	---	---	---
Chromium	1.2	1.2	2.1 ± 2.6	ND-15	123.8	0.352
Cobalt	0.2	30.6	1.4 ± 2.8	ND-17	200.0	0.290
Copper	62	10.6	5.9 ± 13.0	1.9-77	220.3	0.264
Lead	ND	ND	2.3 ± 3.5	1.1-18	152.2	-0.257
Iron	145	242	479 ± 596	1.5-3,060	124.4	0.316
Manganese	31	87	24 ± 73	ND-380	304.2	-0.017
Molybdenum	ND	1.6	2.2 ± 1.9	ND-10	86.4	-0.521
Nickel	1.4	4.1	4.2 ± 2.6	ND-14	61.9	-0.205
Selenium	1.0	ND	5.4 ± 5.8	ND-22	107.4	-0.173
Silicon	ND	13.8	1,037 ± 1,214	97-5,501	117.1	0.383
Titanium	7.3	ND	7.2 ± 6.0	ND-34	83.3	0.271
Zinc	50	185	21.5 ± 8.7	11-62	40.5	0.045
Zirconium	ND	ND	0.5 ± 2.0	ND-16	400.0	-0.645

<sup>1</sup>ND = not detectable (< 0.1 µg/L).

Table 3. Summary of safe concentrations of trace elements to aquatic life for long-term exposure. Developed from data presented by Australian and New Zealand Council on Environmental Protection (1992), Boyd and Tucker (1998), and United States Environmental Protection Agency (1976).

Element	Maximum safe concentration (µg/L)	Comments
Aluminum	100 to 200	For pH < 6, but a higher concentration can be tolerated at greater pH.
Arsenic	50	
Barium	1,000	
Boron	NF <sup>1</sup>	Ocean water has 4,500 µg/L — much more than found in study ponds.
Cadmium	5	
Chromium	100	
Copper	Alkalinity (mg/L) × 2.5	The maximum safe concentration would be about 175 µg/L in study ponds.
Lead	60	
Iron	1,000	
Manganese	1,000	
Molybdenum	75	
Nickel	50	
Selenium	10	
Silicon	NF <sup>1</sup>	Ocean water has 3,000µg/L. Only one sample in the study ponds exceeds this level.
Titanium	NF <sup>1</sup>	Not considered highly toxic.
Zinc	50	
Zirconium	NF <sup>1</sup>	Not considered highly toxic.

<sup>1</sup>NF = not found in literature.

Table 4. Harvest data, crop duration, survival, production, and feed conversion ratio (FCR) for seven ponds at Farm A, an inland, low-salinity shrimp farm in Alabama.

Pond	Harvest data		Crop duration	Production		
	Date	Shrimp/m <sup>2</sup>	(Days)	Survival	(kg/ha)	FCR
N-2	Oct 15, 2009	9.2	161	32.9	3,005	2.38
N-4	Oct 1, 2009	18.4	147	70.4	4,794	1.71
N-6	Sep 23, 2009	25.7	139	95.4	5,425	1.28
N-8	Oct 27, 2009	11.5	182	47.9	3,565	1.78
S-1	Sep 28, 2009	12.6	144	84.0	3,722	1.33
S-6	Sep 9, 2009	14.3	138	68.3	3,361	1.70
S-8	Oct 22, 2009	19.2	181	84.7	4,767	1.82

Table 5. Correlation coefficients between trace element concentrations in water (X variable) and shrimp survival, production, and feed conversion ratio (FCR) in inland shrimp ponds in Alabama. Asterisks by entries denote significance at  $P < 0.05$ .

Element	Water vs Survival <sup>1</sup>	Water vs Production <sup>2</sup>	Water vs FCR <sup>1</sup>
Aluminum	0.327	0.308	-0.200
Arsenic	-0.239	0.604	-0.582
Barium	-0.318	-0.473	-0.126
Boron	-0.228	0.114	0.358
Chromium	0.288	0.554	-0.045
Cobalt	0.844*	0.755*	-0.551
Copper	0.063	-0.373	-0.141
Lead	0.852*	0.370	-0.854*
Iron	0.481	0.711*	-0.361
Manganese	0.530	0.377	-0.538
Molybdenum	-0.283	0.045	0.055
Nickel	0.318	0.228	-0.513
Selenium	-0.114	-0.475	0.179
Silicon	0.352	0.524	-0.182
Titanium	0.152	0.469	-0.089
Zinc	0.902*	0.673*	-0.802*
Zirconium	0.293	0.055	-0.451

<sup>1</sup>d<sub>f</sub> = 5.

<sup>2</sup>d<sub>f</sub> = 8.

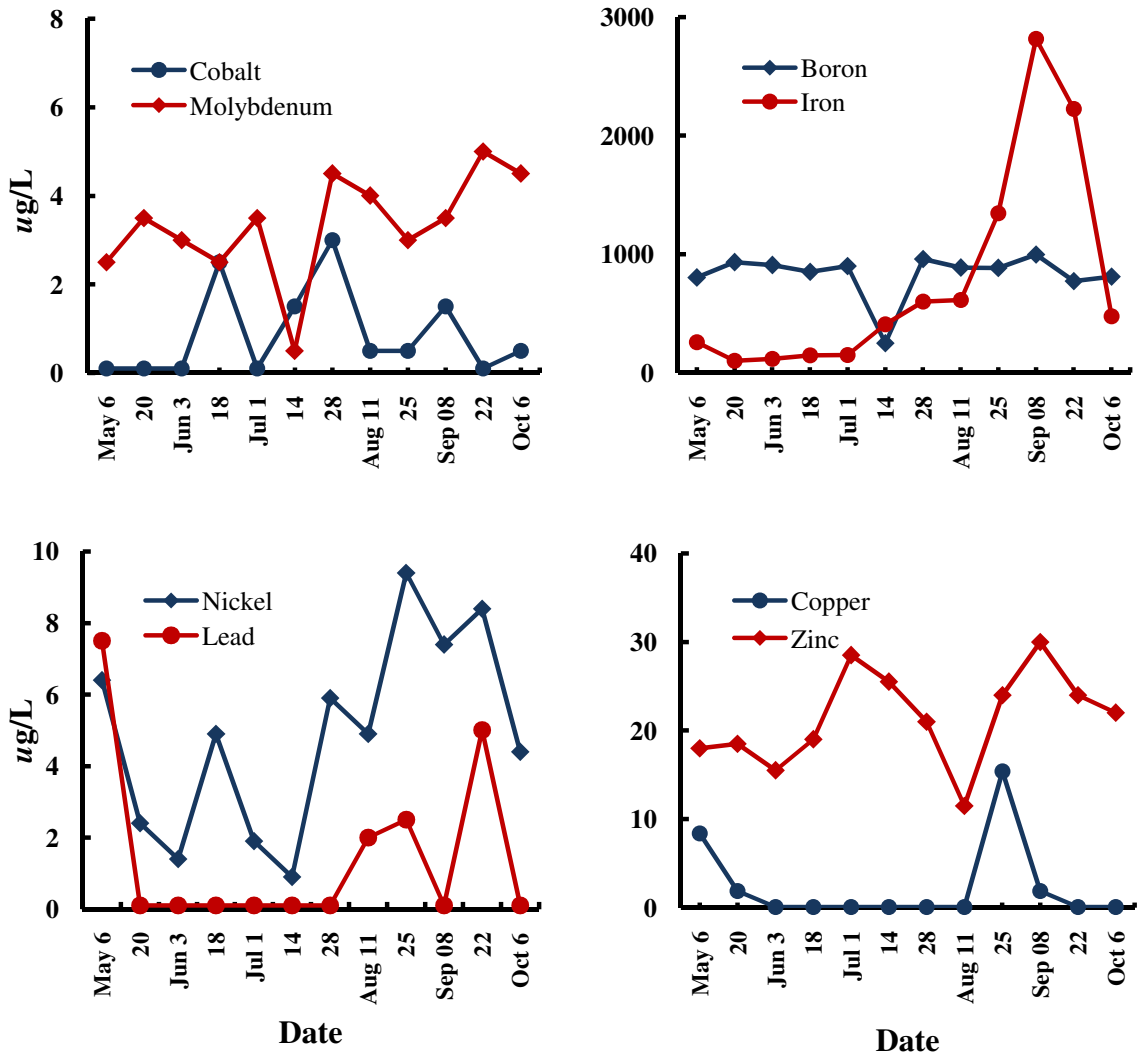


Fig. 1. Variation in concentrations of eight trace elements; cobalt, molybdenum, boron, iron, nickel, lead, copper, and zinc on each sampling date for pond N-8 at Farm A.

## V. Water Temperature in Inland, Low-Salinity Shrimp Ponds in Alabama

### Abstract

Average, daily water temperature in low-salinity, inland shrimp ponds in Alabama was estimated closely by averaging minimum and maximum, daily water temperatures. There was a high correlation ( $P < 0.01$ ) between mean, daily air and water temperatures; pond water usually averaged 3 to 4°C warmer than the air. Monthly, mean water temperatures among eight ponds differed by 3.40°C in May and by 2.83°C in September, but there was less than 1°C difference among ponds in June, July, and August. Differences in temperature among ponds were not related to the pond water surface:volume ratio, but in July and September there was a negative correlation ( $P < 0.05$ ) with increasing aeration rate. However, differences in water temperature among ponds probably were related mainly to variation in plankton density among ponds (a variable not measured in the study). The negative correlations ( $P < 0.05$ ) between average water temperature over the entire culture period in each pond and both shrimp survival and production are thought to have resulted from variation in crop duration and therefore are not causal. Nevertheless, differences in water temperature among ponds in May and September were great enough to have possibly influenced shrimp survival and production.

## Introduction

Water temperature is an important variable in aquaculture; it influences growth rates of culture species as well as those of all other living things — both desirable and undesirable — in production systems (Stickney 2000). Temperature also controls rates of physical processes and chemical reactions that impact environmental quality in culture systems (Boyd and Tucker 1998, Stumm and Morgan 1996). Much is written about temperature preferences of individual species and of the effects of temperature on the culture milieu. Aquaculture species are selected for a particular location based upon their temperature tolerances (Stickney 2000), the timing of stocking and harvesting is scheduled to avoid temperature limitations on survival and growth (Green and Popham 2008, Stickney 2000), and temperature influences water quality management decisions (Boyd and Tucker 1998).

Variation in temperature patterns among years has a major influence on fish and shrimp production in ponds (Boyd and Pine 2006), but little attention has been given to daily, monthly or seasonal variations in temperature among culture units that could possibly result from differences in depth, water quality conditions, and management inputs. Heat is gained and lost through pond water surfaces, and the amount of heat that can be stored in a pond is a function of volume (Yoo and Boyd 1994). In static ponds, heat gains and losses through inflows and outflows of water are minor, and the surface area:volume ratio might be an important factor influencing heat balance and water temperature.

Suspended organic particles absorb heat, and these particles increase the rate of light quenching in water. Thus, plankton blooms increase heat absorption in surface



water, while restricting the penetration of solar energy into deeper water (Idso and Foster 1974). Finally, mechanical aeration of aquaculture ponds increases the rate of vertical mixing of the water column to lessen thermal gradients, and the splashing action of aerators encourages evaporation and heat loss from ponds (Boyd and Pine 2006).

The purpose of this study was to monitor water temperature in eight ponds on a shrimp farm to ascertain if differences in temperature could be detected among ponds of different surface area : volume ratios and mechanical aeration rates. In addition, the possible contribution of water temperature to variation in shrimp survival and production among ponds was investigated.

### Materials and Methods

The shrimp farm for this study is located in southeastern Greene County, Alabama on state Highway 43 about 5 km north of Forkland. This farm has 17 ponds, eight of which were selected for this study (Table 1). The ponds were selected to provide a range in water surface:volume ratio; they varied in water surface area from 0.57 ha to 2.02 ha and from 1.16 m to 1.77 m in average depth. The ponds are filled with water of about 4 ppt salinity from wells extended into a saline water aquifer. Water exchange is not practiced on the farm, and storage volume to capture rainfall is provided by maintaining water levels 10 to 15 cm below the intake of overflow pipes (Boyd 1982, Cathcart et al. 1999). In previous years, water has seldom been observed to overflow from ponds.

Seven ponds were stocked on 11 May 2010, and the other one was stocked on 25 May 2010 with 11.8 to 31.3 postlarvae/m<sup>2</sup> (Table 1). Aerators were installed at 8.5 to

12.8 hp/ha and operated mostly at night (Table 1). A pelleted feed containing 35% crude protein was applied twice daily, and amounts used were recorded. Shrimp were harvested between 8 September and 20 October 2010.

A Model 64K HOBO Pendant Temperature Data Logger (HOBOware<sup>®</sup>, Janesville, Wisconsin, USA) was attached to the top of a concrete block (20 cm tall) and placed at 1 m depth in each study pond on 12 May 2010. A Model HOBO Temp/External air temperature data logger was mounted in the shade at a height of 3 m under an open tractor shed — roof but no sides — located within 700 m of the most distant pond. The temperature loggers were installed one day after the first ponds were stocked and left in ponds until harvest. The recorders were programmed to monitor temperature at 2-h intervals. At the end of the study, data from temperature loggers were downloaded into a lap-top computer using software provided by the manufacturer; HOBOware Pro and BoxCar Pro 4.3 for water temperature data logger and air temperature data logger, respectively.

Mean, daily air and water temperatures were calculated two ways: by averaging all 2-h reading each day; by averaging the daily maximum and minimum temperature. Monthly and culture period means (estimated from stocking to harvest) for each pond and the grand mean for all ponds were based on average of daily temperature data.

## Results and Discussion

The range in daily, water temperature was fairly small as illustrated for Pond N-7 (Fig. 1). The difference in daily minimum and daily maximum temperatures averaged  $1.48 \pm 1.82^{\circ}\text{C}$ , and ranged from  $0.19^{\circ}\text{C}$  on 12 May to  $2.77^{\circ}\text{C}$  on 8 October. The

relatively small differences between daily minimum and maximum water temperatures were the result of water mixing by mechanical aeration.

The average, daily water temperature in ponds, calculated by either averaging all daily values or by averaging daily minimum and maximum values (Fig. 2), were nearly always slightly higher than average, daily air temperature as also illustrated with data from Pond N-7. There was a high correlation between mean, daily water temperatures estimated by the two different methods of averaging (Fig. 3), and it was decided to work with the daily averages based on minimum and maximum values. There also was an excellent correlation ( $P < 0.01$ ) between average, daily air and water temperatures for Pond N-7 (Fig. 3).

Average, daily water temperature in Pond N-7 (Fig. 2) was 25°C or more from stocking on 11 May [ordinal day (OD) 131] until 29 September (OD 272) – a period of 141 d. Water temperature was 30°C or greater for 101 d – 6 June (OD 157) to 24 September (OD 267). Between 23 July (OD 204) and 13 August (OD 225), water temperature often averaged between 34 and 35°C. Average, daily water temperature diminished below 25°C after 29 September, and it fell to about 19°C on the day of harvest (20 October, OD 293).

The range in average, daily water temperature among ponds was fairly large in May and June, but it was less later in the grow-out period as illustrated with data for one day in each month from May to September: 12 May, 23.63 to 27.03 °C; 12 June, 30.35 to 32.20°C; 12 July, 32.10 to 32.61°C; 12 August, 33.20 to 34.23°C; 12 September, 30.76 to 31.07°C. Monthly averages for water temperature differed by 2.06°C among ponds in

May, by 0.51 to 0.68°C during the period June through August, and by 2.83°C in September.

Culture period means for water temperature in the eight ponds ranged from 29.66 to 31.56°C, and they were as follows : N-2, 31.56°C; N-4 31.07°C; N-5, 30.39°C; N-6, 30.01°C; N-7, 29.66°C; N-8, 30.7°C; S-1, 30.06°C; and S-3, 30.97°C. The grand mean was 30.51°C with a standard deviation of  $\pm 0.637^\circ\text{C}$ . These differences also were not correlated ( $P > 0.05$ ) with either aeration rate or pond surface area:volume ratio (Fig. 4), but the correlations possibly were affected by the influence of low water temperatures in ponds harvested after OD 272 (29 September) on culture period means.

Monthly averages for water temperatures in ponds were not correlated with pond surface water area:volume ratio either. Differential heat absorption by waters caused by differences in turbidity, and particularly phytoplankton abundance, likely caused most of the variation in water temperature among ponds, but it was not possible to obtain estimates of either turbidity or phytoplankton abundance in this study. There were, however, negative correlations between monthly water temperature and aeration rate for July ( $r = -0.667$ ;  $P < 0.05$ ) and September ( $r = -0.923$ ; ( $P < 0.01$ ).

Data on shrimp performance provided by the farm manager after harvest varied as follows: production, 2,267 to 6,516 kg/ha; crop duration, 106 to 162 days; survival 25.5 to 79.3%; FCR, 1.38 to 4.11 (Table 3). The manager overestimated survival rate in pond N-8 and applied excessive feed resulting in one high FCR.

Production and survival appeared to decline as average culture period water temperature increased (Fig. 5), but only the correlation for production was significant. There was no correlation ( $P > 0.05$ ) of water temperature with FCR even if the pond with

FCR = 4.11 (Pond N-8) is omitted (Fig. 5). The data are complicated by two facts: water temperature was negatively correlated with crop duration (Fig. 6) as a result of longer crops extending into the cooler fall days, by chance or intention, ponds with smaller production were harvested first. Nevertheless, variation in daily and monthly, mean water temperatures among ponds were as much as 3.40°C and 2.44°C, respectively (in May) and 2.83°C and 2.72°C, respectively (in September). Shrimp, like other aquaculture species, probably have a  $Q_{10}$  for growth of around 2 (Boyd and Tucker 1998), and in May and September, the differences in water temperature among ponds probably influenced growth rate. During June, July, and August, the small differences in water temperatures among ponds probably did not have much effect on growth. Frequent estimates of growth rate – a variable not available for ponds in this study – would be needed to verify that monthly or shorter differences in water temperature among ponds influence shrimp production.

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Table 1. Pond areas and depths, stocking dates, aeration rates, and amounts of feed applied to eight study ponds at an inland, low-salinity shrimp farm in Alabama.

Pond	Area (m <sup>2</sup> )	Average Depth (m)	Stocking data		Aeration rate (hp/ha)	Feed applied (kg/ha)
			Date	PLs/m <sup>2</sup>		
N-2	5,666	1.32	25 May	31.3	8.8	3,603
N-4	12,141	1.43	11 May	22.7	10.7	4,432
N-5	13,335	1.22	11 May	29.2	9.7	8,939
N-6	14,973	1.34	11 May	29.9	8.7	10,454
N-7	20,234	1.77	11 May	29.0	11.4	11,152
N-8	15,378	1.43	11 May	28.9	9.8	9,323
S-1	11,736	1.16	11 May	29.6	8.5	8,438
S-3	10,117	1.19	11 May	11.8	12.8	4,108



Table 2. Production, crop duration, survival rates, and feed conversion ratio (FCR) for eight ponds at an inland, low-salinity shrimp farm in Alabama.

Pond	Harvest date	Production (kg/ha)	Crop duration (days)	Survival (%)	FCR
N-2	8 Sep	2,613	106	25.6	1.38
N-4	15 Sep	2,993	127	44.9	1.48
N-5	3 Oct	5,055	145	57.1	1.77
N-6	18 Oct	6,516	160	79.2	1.60
N-7	20 Oct	4,914	162	61.9	2.27
N-8	11 Oct	2,267	153	29.8	4.11
S-1	14 Oct	6,071	156	73.8	1.39
S-3	28 Sep	2,607	140	58.5	1.58

Table 3. Monthly, average water temperature for eight ponds at an inland, low-salinity shrimp farm in Alabama. The letter H designated that ponds were harvested in September and could not be used in the average.

Month	N-2	N-4	N-5	N-6	N-7	N-8	S-1	S-3
May	26.68	27.81	26.30	27.93	27.15	28.74	28.25	28.05
June	31.77	32.08	31.84	32.07	31.62	32.20	31.63	31.81
July	32.10	31.88	32.10	32.36	32.13	32.09	32.39	31.99
August	32.05	31.89	31.77	32.36	32.27	31.99	32.02	32.12
September	H	H	28.58	29.49	26.30	29.02	29.13	H

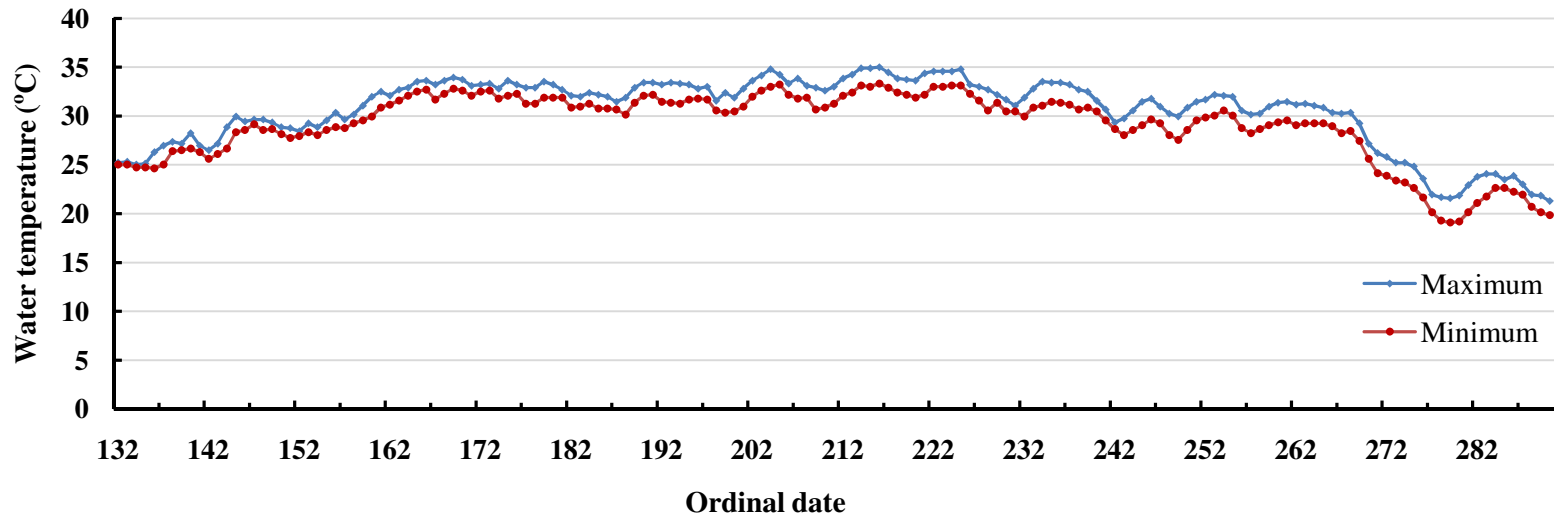


Fig. 1. Daily minimum and maximum water temperature in Pond N-7 at an inland, low-salinity shrimp farm in Alabama.

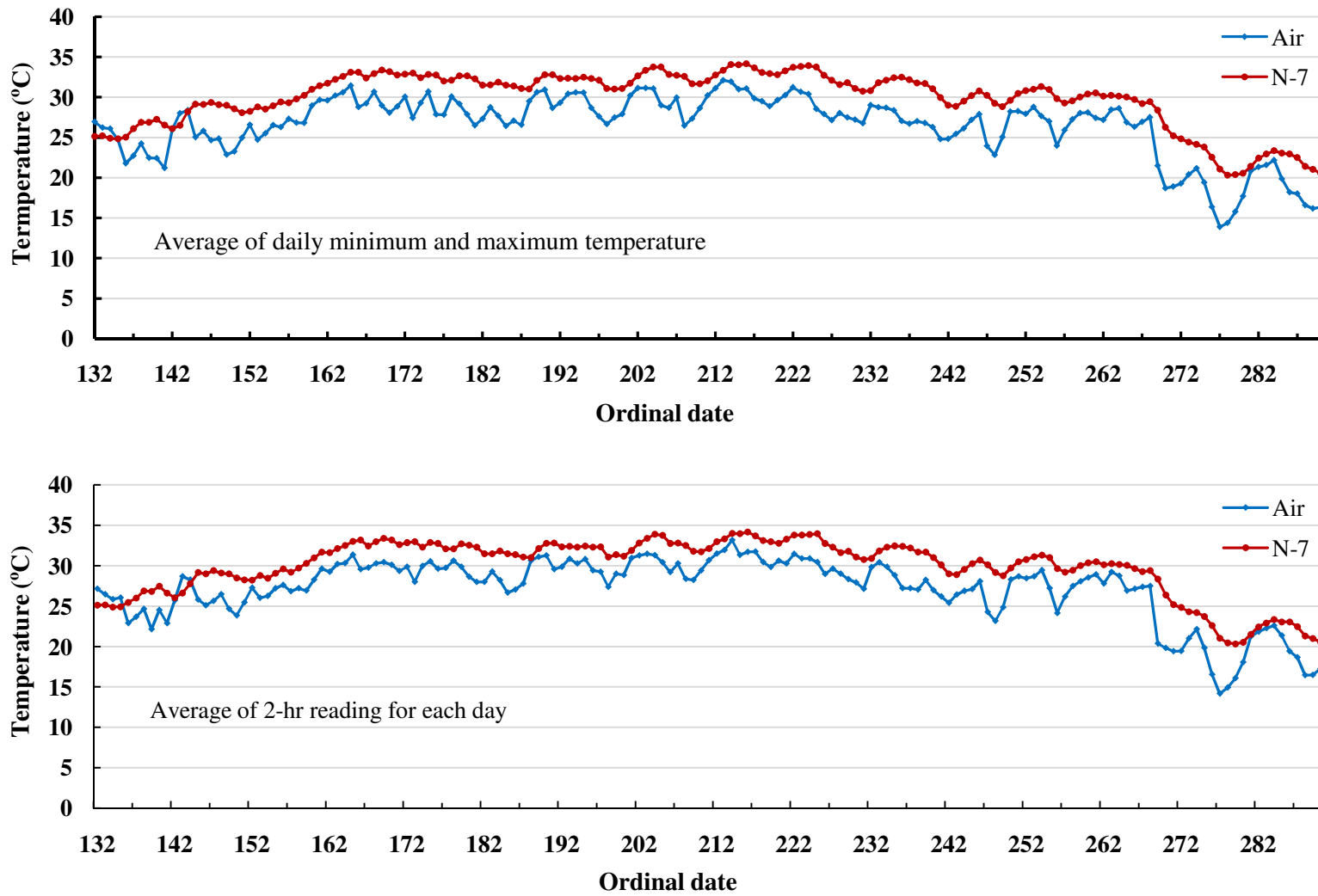


Fig. 2. Mean daily, air temperatures and water temperatures in Pond N-7 at an inland, low-salinity shrimp farm in Alabama.

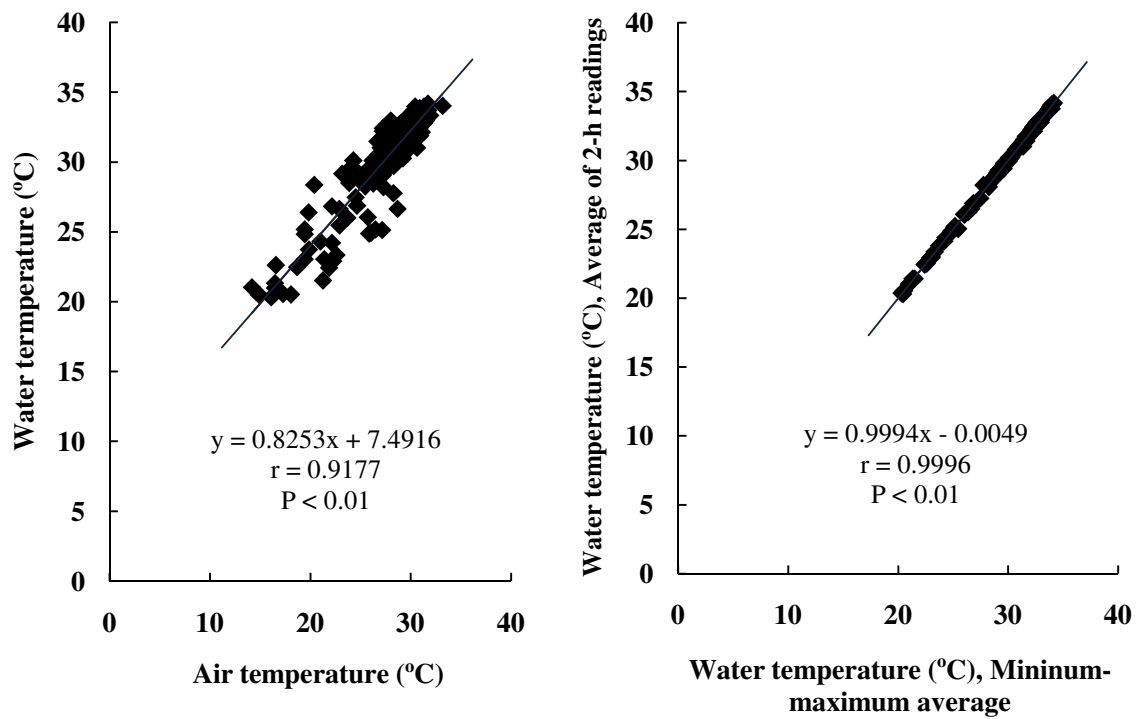


Fig. 3. Left: Relationship between average, daily air temperatures and water temperatures in Pond N-7 at an inland, low-salinity shrimp farm in Alabama. Right: Relationship between average, daily water temperatures averaged by two methods: average of 2-h reading and minimum-maximum temperature averaging.

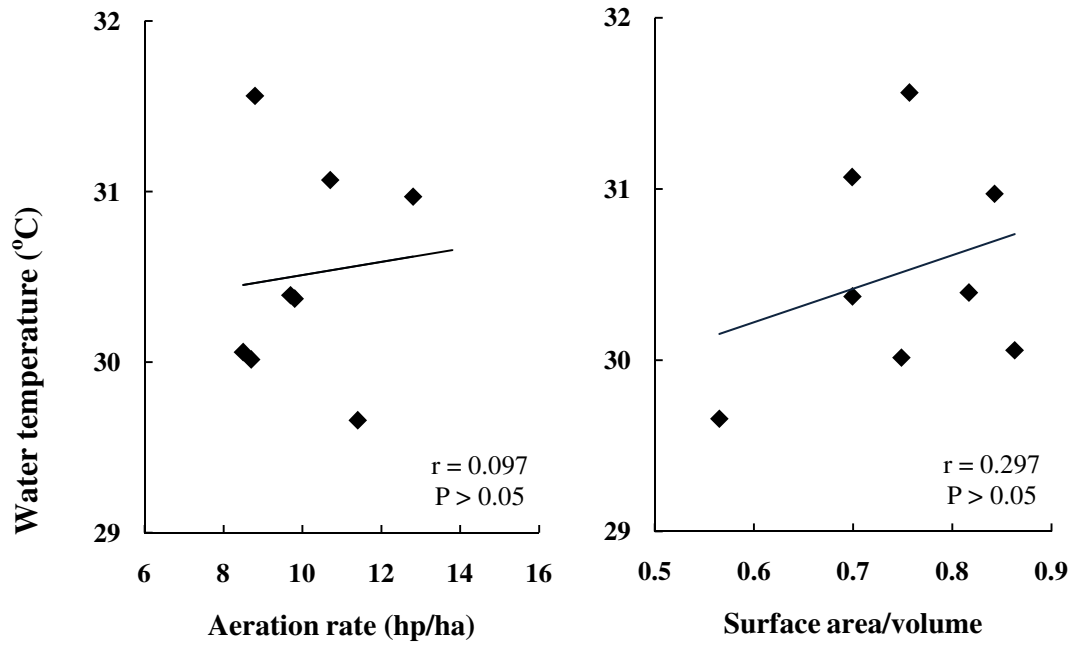


Fig. 4. Relationship between aeration rate and pond surface area/volume ratio to average, and average, crop-period water temperatures in eight ponds at an inland, low-salinity shrimp farm in Alabama.

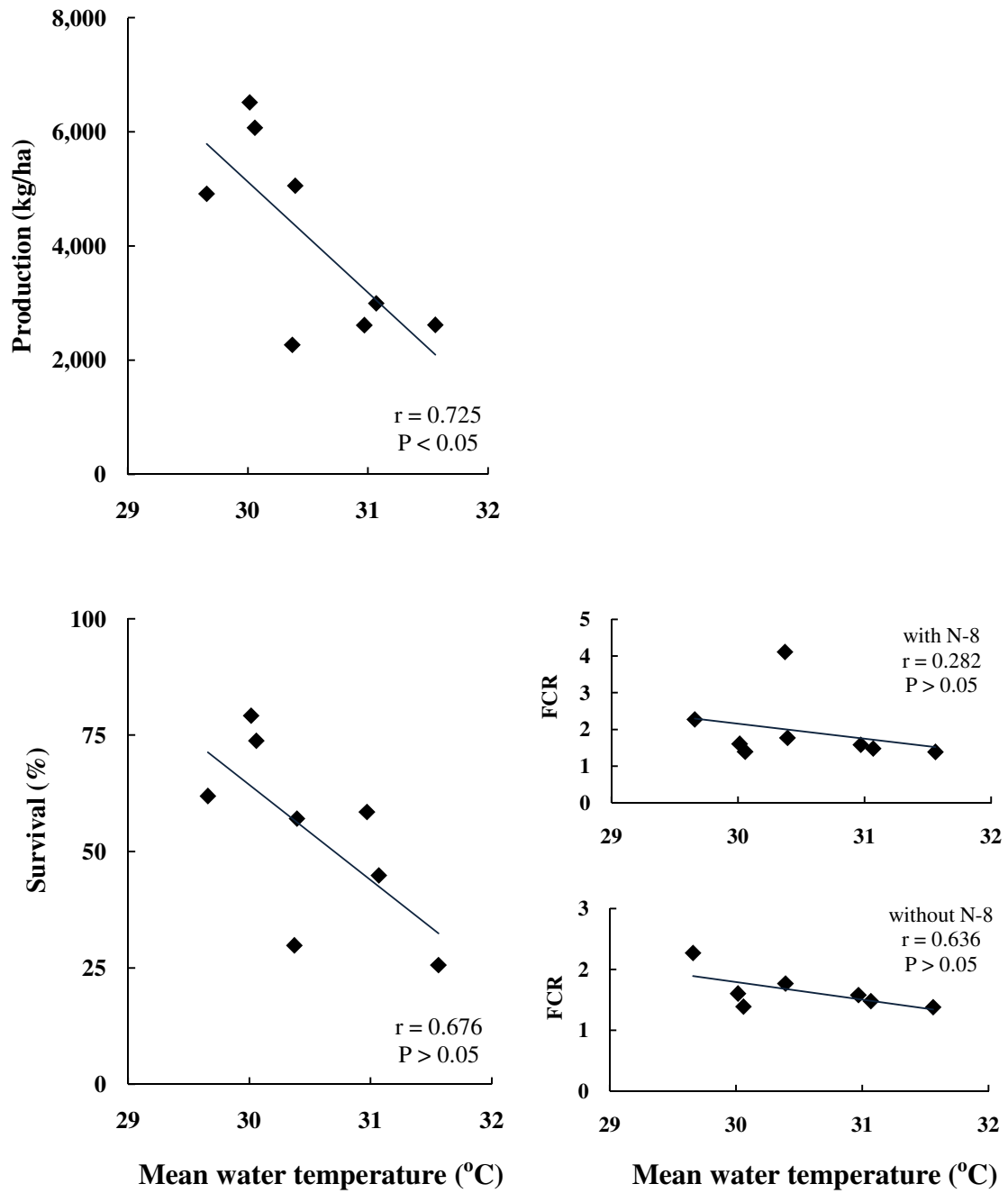


Fig. 5. Relationships between average, crop-period water temperature and shrimp production variables in eight ponds at an inland, low-salinity shrimp farm in Alabama.

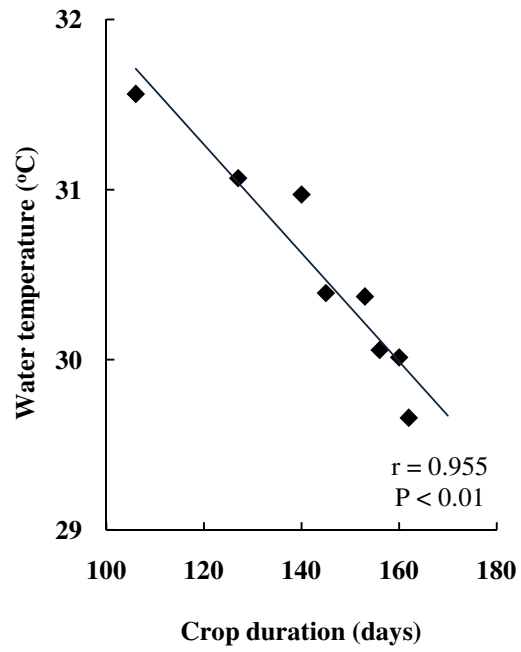


Fig. 6. Relationship of crop duration to average, crop-period temperature in eight ponds at an inland, low-salinity shrimp farm in Alabama.



## VI. Effluent Volume and Pollutant Loads at an Inland, Low-Salinity Shrimp Farm in Alabama

### Abstract

An inland, low-salinity shrimp farm in Alabama did not release effluent during the grow-out period, but draining effluent at harvest included 50.4% and 57.9% of impounded water in 2008 and 2010, respectively. Maximum concentrations of water quality values in draining effluent were: total suspended solids, 627 mg/L; settleable solids, 11 ml/L; turbidity, 448 NTU; total phosphorus, 1.15 mg/L; total nitrogen, 11.5 mg/L; 5-d biochemical oxygen demand, 33.5 mg/L; chloride, 2,450 mg/L; and pH, 8.1. Weighted concentrations of these variables were considerably lower – except for pH which was about the same. Farm effluent caused a marked increase in concentrations of all variables other than pH at a sampling station about 500 m downstream of the farm outfall. But only chloride concentration exceeded concentrations allowed in Alabama streams classified for fish and wildlife propagation. Average pollutant discharge per tonne of shrimp was 444.9 kg total suspended solids, 51.3 kg 5-d biochemical oxygen demand, 15.2 kg total nitrogen, 1.33 kg total phosphorus, and 4,402 kg chloride. Concentrations of turbidity, settleable solids, total suspended solids, total phosphorus and possibly other variables in effluent could be lessened by installation of a settling basin to remove medium and coarse solids before final discharge. Release of less water from the

farm during harvest, and improvement in feed conversion ratio – which was 1.52 in 2008 and 1.86 in 2010 – also would lessen pollutant loads per tonne of shrimp.

## Introduction

Inland culture of marine shrimp in low-salinity water is a new method of shrimp production that is increasing in importance (Roy et al. 2010). In Alabama, low-salinity water (2 to 10 ppt) for inland shrimp culture is pumped from a saline aquifer. This water has low concentrations of potassium and magnesium, and it must be treated with potassium and magnesium fertilizers to counteract cation imbalances that negatively affect shrimp survival and growth (Boyd et al. 2007, McNevin et al. 2004, Pine and Boyd 2010). Water exchange is not used, and farmers attempt to reduce discharge during harvest in order to lessen pumping costs to refill pond and to conserve potassium and magnesium applied in fertilizers.

The major environmental concern about inland shrimp farming has been possible salination of streams, aquifers, and soils by discharge from ponds (Boyd et al. 2006, Braaten and Flaherty 2001, Pine and Boyd 2010, Roy et al. 2010). In other types of pond aquaculture, there is concern about the discharge of nutrients, organic matter, and suspended solids that could cause eutrophication and sedimentation in receiving water bodies. This issue has not been raised for inland shrimp farms, apparently because the potential for salination is obvious and efforts to reduce salt discharge also would lessen release of other pollutants.

The purpose of this study was to evaluate effluent volume and pollutant loads at a low-salinity, inland shrimp farm in Alabama.

## Materials and Methods

### Farm and management

The study was conducted during two annual grow-out cycles – 2008 and 2010 – at a shrimp farm in southeastern Greene County, Alabama about 5 km north of Forkland on state Highway 43. This farm has 17 ponds (Fig. 1) ranging from 0.49 to 2.02 ha in “full-pond” water surface area for a total of 21.77 ha. Average full depths vary from 1.26 m to 2.07 m (average = 1.58 m). The total water volume for the farm is 340,079 m<sup>3</sup>.

Ponds were completely filled with saline ground water (~ 4 ppt) from two wells in March and April each year. Fertilizer grade potassium chloride or muriate of potash (~50% K) and potassium magnesium sulfate or KMag<sup>®</sup> (Mosaic Company, Plymouth, Minnesota, USA) (17.8% K and 10.5% Mg) were applied to ponds to raise potassium concentration to 50 mg/L and magnesium concentration to 30 mg/L as is common practice in Alabama inland shrimp culture (Boyd et al. 2007, McNevin et al. 2004, Pine and Boyd 2010). Pacific white shrimp *L. vannamei* from Harlingen shrimp farm, Los Fresnos, Texas and from Shrimp Improvement Systems, Islamorada, Florida were stocked at an average density of 24 postlarvae/m<sup>2</sup> between late April and early June in 2008 and with an average 27 postlarvae/m<sup>2</sup> between late April and late May in 2010. Shrimp were fed twice daily with a commercial, pelleted feed containing 35 % crude protein. One or two, 10-hp electric, paddlewheel aerators were installed in each pond and one, 3-hp Aire-O<sub>2</sub><sup>®</sup> aerator was also installed in 10 ponds. The average, aerator power rate was 10.9 hp/ha (range of 6.2 to 23.7 hp/ha). Aerators were operated mostly during nighttime.

Water levels in ponds were 2 to 10 cm below tops of overflow pipes when shrimp were stocked, and water was pumped into ponds as needed to replace seepage and evaporation and maintain water levels 10 to 15 cm below tops of drains. This water-level management technique is called the “drop-fill” method, and it provides storage capacity for rainfall thereby preventing overflow after normal rainfall events (Boyd 1982, Cathcart et al. 1999). Water was not observed to overflow from ponds during the production period either year.

Shrimp were harvested during September and October both years. At harvest, water was discharged from ponds into Needham creek (Fig. 1), or it was pumped into adjacent ponds for reuse.

#### Water volume

Ponds have embankments with 2:1 (horizontal to vertical) side slopes around the area in which water is stored. Bottoms slope towards drains to facilitate draining at harvest, but until water levels drop about 1.2 m below tops of overflow pipes, water surfaces are above toes of embankments even on the shallow-most sides of ponds. The manager agreed to manage draining so that at completion ponds would either be completely empty or have water levels not more than 1.0 m below tops of overflow pipes.

The vertical distance of the water level below the top of the overflow pipe was measured in each pond when drawdown for harvest was initiated. On the day after the final pond was harvested, empty ponds were identified and vertical distances of water levels below tops of the overflow pipes were measured in the other ponds. The volume of water discharged for individual ponds was calculated as follows:

Ponds empty after harvest:

$$VD = [H - \Delta h]_b [A - (2L \times \Delta h)]_b$$

Ponds with water after harvest:

$$VD = [[H - \Delta h][A - (2L \times \Delta h)]_b - [[H - \Delta h][A - (2L \times \Delta h)]_a]$$

where VD = volume of discharge (m<sup>3</sup>); H = average full pond depth (m); Δh = distance from water level to top of overflow pipe (m); A = full pond water surface area (m<sup>2</sup>); L = shoreline length at full pond water level (m), a and b = after and before draining for harvest, respectively.

Discharge volumes of all ponds were summed to estimate total farm discharge.

#### Sampling and analyses

Water samples were collected from four selected ponds during drawdown for harvest both years. Samples were collected before initiating drawdown, and when drawdown was estimated to be 50%, 75%, 95%, and 99% complete. Water samples were put into 2-L plastic bottles, stored in insulated ice chests, and transported to Auburn University for analyses. Analyses were made according to methodology recommended by Eaton et al. (2005) as follows: pH (glass electrode); settleable solids (Imhoff cone); total suspended solids (TSS, glass fiber filtration followed by gravimetry); turbidity (nephelometer); biochemical oxygen demand (standard, 5-d test, BOD<sub>5</sub>); chloride (diphenylcarbazone method). Samples for total nitrogen and total phosphorus were subjected to persulfate digestion (Eaton et al. 2005), and nitrate and orthophosphate in digestates were measured by the ultraviolet spectrophotometric screening method (Eaton

et al. 2005, Gross et al.1999) and ascorbic acid methods, respectively (Gross and Boyd 1998 ).

The weighted average for each water quality variable was calculated using the following equation:

$$X_w = [(X_{100} + X_{50})/2] 0.5 + [(X_{50} + X_{25})/2] 0.25 + [(X_{25} + X_5)/2] 0.2 + [(X_5 + X_0)/2] 0.05$$

where  $X_w$  = weighted average for variable X ( $g/m^3$ ); subscripts 0 to100 = stage of drawdown when water sample collected, e.g., 25% of water remained in pond when sample  $X_{25}$  was taken; decimal fractions = proportion of water discharged between two sampling times.

The discharge of pollutants per tonne of shrimp production each year was calculated as follows:

$$PL_x = \frac{(X_w)(V_D)(10^{-3})}{S}$$

where  $PL_x$  = pollution load of variable X (kg/tonne shrimp);  $X_w$  = weighted average for variable X ( $g/m^3$ );  $V_D$  = farm discharge ( $m^3$ );  $10^{-3}$  = kg/g; S = farm shrimp production (tonne).

Three feed samples and three shrimp samples were collected, dried by lyophilization and pulverized to 40-mesh. Nitrogen concentration was measured with a Leco CHN analyzer (Leco Corporation, Algonquin, Illinois, USA). Aliquots of samples were digested in perchloric acid (Boyd and Teichert-Coddington 1995b), and phosphorus concentration was measured with a SpectroCiros<sup>CCD</sup>, induced coupled plasma

spectrophotometry (SPECTRO Analytical Instruments, Inc., Mahwah, New Jersey, USA).

## Results

Total discharge from the farm was 154,826 m<sup>3</sup> in 2008 and 174,931 m<sup>3</sup> in 2010. The total volume of water on the farm at the beginning of harvest was 306,987 m<sup>3</sup> and 302,360 m<sup>3</sup> in 2008 and 2010, respectively. Thus, a greater percentage of pond water was discharged for harvesting ponds in 2010 (57.9%) than in 2008 (50.4%).

During draining for harvest, concentrations of pH and chloride remained fairly constant during drawdown, but concentrations of other variables tended to increase as drawdown progressed and especially in the final 25% of the effluent as illustrated for data collected in 2008 (Fig. 2). This pattern of change in concentrations of water quality variables in effluent is typically observed when ponds are drained for harvest (Boyd 1978, Schwartz and Boyd 1994b, Teichert-Coddington et al. 1999). The data were used to estimate weighted averages for concentrations of variables in draining effluent (Table 1) that were similar for both years. The effluent caused concentrations of all water quality variables other than pH in the receiving stream to increase downstream of the farm outfall (Table 2).

Feed use and shrimp production on the farm were 118,132 kg and 77,809 kg, respectively, in 2008 and 154,201 kg and 82,697 kg, respectively, in 2010. Thus, the farm feed conversion ratio (FCR) was 1.52 in 2008 and 1.86 in 2010.



The nitrogen and phosphorus concentrations in shrimp were 2.75% and 0.25% of live weight, respectively. The air dry feed contained 6.08% nitrogen and 1.2% phosphorus.

## Discussion

The United States Environmental Protection Agency (USEPA) aquaculture rule (Federal Register 2004) defined warmwater concentrated aquatic animal production (CAAP) facilities as ponds, raceways, or other production units that produce over 45,455 harvest kilograms per year but excluded facilities that discharge less than 30 d/yr except for excess runoff. The farm is a CAAP facility because it produced over 45,455 kg of shrimp each year, and because ponds were drained over 44-d period in 2008 and a 46-d period in 2010, resulting in more than 30-d of discharge both years. The EPA rule requires that CAAP facilities obtain a National Pollutant Discharge Elimination System (NPDES) permit. But, the rule does not impose effluent limitation guidelines. Rather, it recommends the use of best management practices to lessen the volume and pollutional strength of effluent. Thus, there are no specific aquaculture effluent standards against which to compare the effluent quality from the inland shrimp farm.

Water quality standards of the Alabama stream classification system for stream classified for fish and wildlife propagation limits turbidity to 75 NTU, prohibits dissolved oxygen concentration  $> 5$  mg/L, and requires pH to be between 6 and 8.5 (Boyd 2000). Shrimp pond effluent usually had greater turbidity than allowed by the stream standard, but effluent pH was within an acceptable range. Dissolved oxygen concentrations were not measured in the samples, but the shrimp farm monitored dissolved oxygen in the

ponds during drawdown and reported that it did not fall below 5 mg/L. The BOD<sub>5</sub> and TSS limits typically allowed in NPDES permits for municipalities and industries range from 20 to 30 mg/L (Boyd and Gross 1999, Carter 1984). The shrimp pond effluent often exceeded these limits.

The Alabama Department of Environmental Management adopted the recommendation of Benoit (1988) that effluent should not cause in-stream chloride concentration to rise above 230 mg/L. The shrimp farm effluent was much higher than the in-stream chloride standard. Standards for nitrogen and phosphorus seldom are included in NPDES permits in the southeastern United States (Schwartz and Boyd 1994a). Although shrimp pond effluent exceeded limits for some variables typically included in NPDES permits, they were relatively dilute with respect to effluents from industrial and municipal sources. For example, according to Tchobanoglous et al. (2003), untreated municipal sewage contains 20 to 70 mg/L total nitrogen, 4 to 12 mg/L total phosphorus, 110 to 350 mg/L BOD<sub>5</sub>, and 120 to 400 mg/L TSS.

The input of nitrogen in feed was 8,798 kg in 2008 and 9,374 kg in 2010; the corresponding input of phosphorus was 1,736 kg in 2008 and 1,850 kg in 2010. Shrimp harvest removed 2,140 kg nitrogen and 194 kg phosphorus in 2008 and 2,274 kg nitrogen and 207 kg phosphorus in 2010. Thus, the loads of nitrogen and phosphorus to the ponds were 6,658 kg nitrogen and 1,542 kg phosphorus in 2008 and 7,104 kg nitrogen and 1,643 kg phosphorus in 2010. However, the effluent loads of nitrogen and phosphorus (Table 1) were much lower. Of the nitrogen applied in feed and not recovered in shrimp, effluent contained only 16.2% in 2008 and 18.5% in 2010; corresponding values for phosphorus were 5.0% in 2008 and 7.3% in 2010. Thus, the ponds were quite effective

in removing nitrogen and phosphorus. Nitrogen was lost through ammonia volatilization, denitrification, and in organic matter that settled to pond bottoms (Gross et al. 1999, 2000). The main loss of phosphorus was precipitation from the pond water as calcium phosphate and adsorption by bottom soils (Gross et al. 1998).

The amounts of selected pollutants discharged per tonne of shrimp produced (Table 2) – except for BOD<sub>5</sub> – were fairly similar both years. The BOD<sub>5</sub> in aquaculture ponds results primarily from phytoplankton (Boyd and Gross 1999), and phytoplankton density must have been greater in 2010 than in 2008. The large discharge of chloride per tonne of shrimp is in agreement with findings of Boyd et al. (2006), and it is of particular concern, because ponds discharge into a freshwater stream.

The high chloride concentration at the upstream site in the creek (Table 3) apparently was the result of drought conditions during 2008. The period 2003 to 2006 had more rainfall, and chloride concentration at the upstream site was below 100 mg/L during that 4-year period (Boyd et al. 2006). Nevertheless, during shrimp harvest in 2008, chloride concentration at the station downstream of the farm outfall was much greater than the concentration at the upstream station. Of course, data collected during the years with more rainfall (Boyd et al. 2006) also revealed that chloride concentration increased in the stream below the farm outfall during the period of shrimp harvest and exceeded the in stream standard of 230 mg/L.

Concentrations of total suspended solids, turbidity, total phosphorus, total nitrogen, and BOD<sub>5</sub> also were elevated at the downstream station relatively to the upstream station. However, the concentrations of these variables in the stream were not

in excess of concentrations allowed in Alabama streams classified for fish and wildlife propagation (Boyd 2000).

Suspended solids concentration and turbidity in effluent could be lessened by sedimentation. Ideally, a sedimentation basin should be constructed in the ditch that conveys water from the farm to the creek. Alternatively, it might be possible to schedule pond draining so that one of the lower ponds is harvested first and this pond could then serve as a sedimentation basin. There are two problems related to the alternative: the water would have to be pumped from the lower pond to ponds at higher elevation at considerable expense; the lower pond would gradually filled with sediment making it less useful for shrimp production.

Sedimentation also would reduce the total phosphorus concentration in effluent, because much of the phosphorus in pond water is associated with suspended soil particles (Boyd 2000). However, sedimentation would not have much effect on the concentrations of total nitrogen, BOD<sub>5</sub>, or chloride. The chloride concentration seems to be the variable of most concern with respect to water quality in the stream. The practice that would be of most benefit in protecting water quality in the receiving stream would be to discharge less water at harvest. This should be possible by transferring more water to adjacent ponds and storing it rather than releasing it during harvest. Of course, more emphasis should be devoted to feed management, because the FCRs of 1.52 in 2008 and 1.86 in 2010 are higher than often achieved in *P. vannamei* culture (Shrimp Aquaculture Dialogue 2010). Reducing the FCR would lessen the amount of nitrogen and phosphorus discharged per tonne of shrimp produced.

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Table 1. Weighted means ( $\bar{x}$ )  $\pm$  standard errors (SE) and for concentrations of selected water quality variables in draining effluent from four ponds on an inland, low-salinity shrimp farm in Alabama in 2008 and 2010.

Variable	2008		2010	
	$\bar{x} \pm \text{SE}$	Range	$\bar{x} \pm \text{SE}$	Range
pH	7.75 $\pm$ 0.24	7.02 - 8.32	7.58 $\pm$ 0.08	7.32 - 7.81
Settleable solids (mg/L)	3.00 $\pm$ 3.04	0.05 - 25.00	4.47 $\pm$ 3.15	0.01 - 18.50
Total suspended solids (mg/L)	207 $\pm$ 32	40 - 672	225 $\pm$ 68	56 - 943
Turbidity (NTU)	174 $\pm$ 94	27 - 788	232 $\pm$ 101	46 - 655
BOD <sub>5</sub> (mg/L)	19.5 $\pm$ 4.3	8.4 - 54.8	29.9 $\pm$ 8.1	11.3 - 79.9
Total nitrogen (mg/L)	6.95 $\pm$ 0.86	4.41 - 14.70	7.52 $\pm$ 1.02	4.06 - 16.28
Total phosphorus (mg/L)	0.50 $\pm$ 0.09	0.23 - 1.68	0.69 $\pm$ 0.09	0.26 - 1.55
Chloride (mg/L)	2,279 $\pm$ 73	1,905 - 2,393	2,022 $\pm$ 144	1,255 - 2,481



Table 2. Total quantities of selected pollutants discharged and the amount of each pollutant per tonne of shrimp production in 2008 and 2010 at an inland, low-salinity shrimp farm in Alabama.

Variable	2008		2010	
	(kg)	(kg/tonne shrimp)	(kg)	(kg/tonne shrimp)
Total suspended solids	32,049	411.9	39,359	476.0
BOD <sub>5</sub>	3,019	38.8	5,230	63.3
Total nitrogen	1,076	13.8	1,315	15.9
Total phosphorus	77.4	0.99	120.7	1.46
Chloride	352,848	4,535	353,710	4,278

Table 3. Concentrations of water quality variables in a creek upstream of an inland, low-salinity shrimp farm contrasted with downstream concentrations of the variables during draining of ponds for harvest in 2008.

Variable	Upstream	Downstream	
	18 Sep	18 Sep	7 Oct
pH	7.16	7.18	7.18
Total suspended solids (mg/L)	50.00	55.00	70.00
Turbidity (NTU)	20.60	34.90	66.20
BOD <sub>5</sub> (mg/L)	6.09	10.12	21.04
Total nitrogen (mg/L)	1.29	3.97	5.90
Total phosphorus (mg/L)	0.12	0.24	0.30
Chloride (mg/L)	150	2,124	1,672

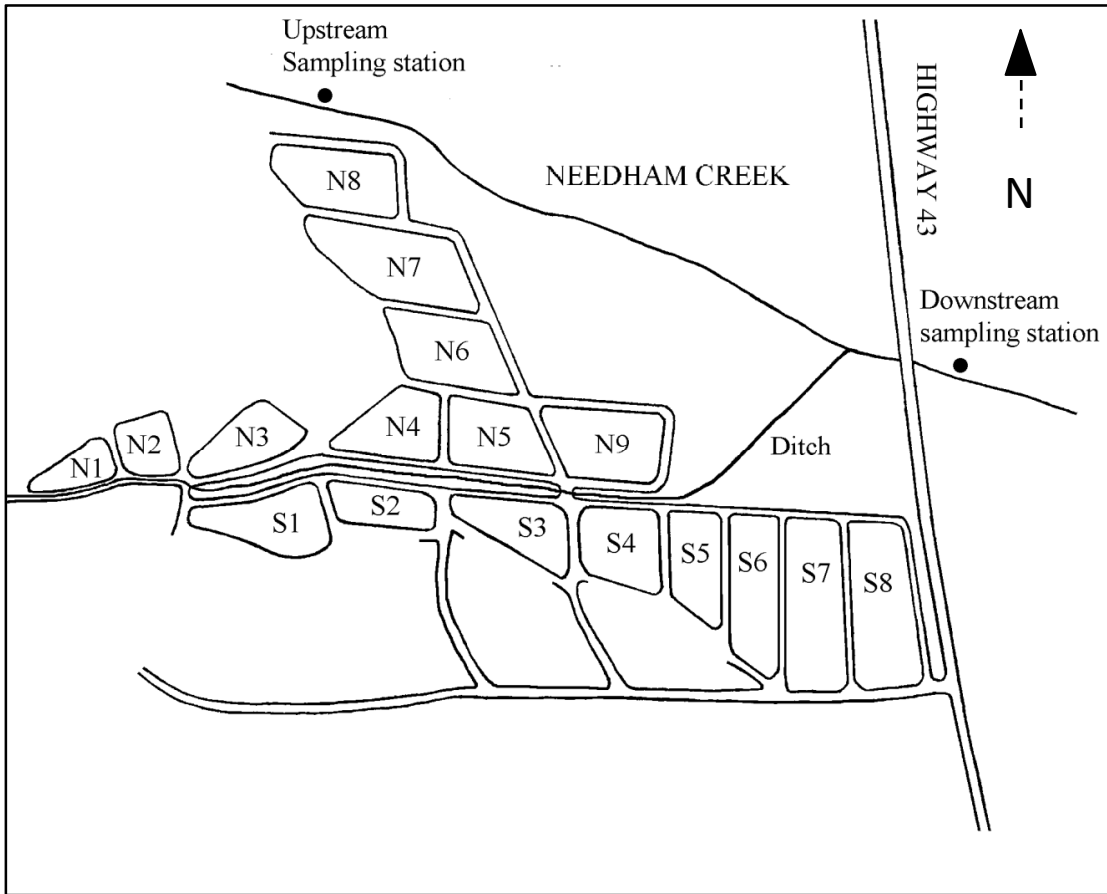


Fig. 1. Map of the inland, low-salinity shrimp farm in Alabama where the study was conducted.

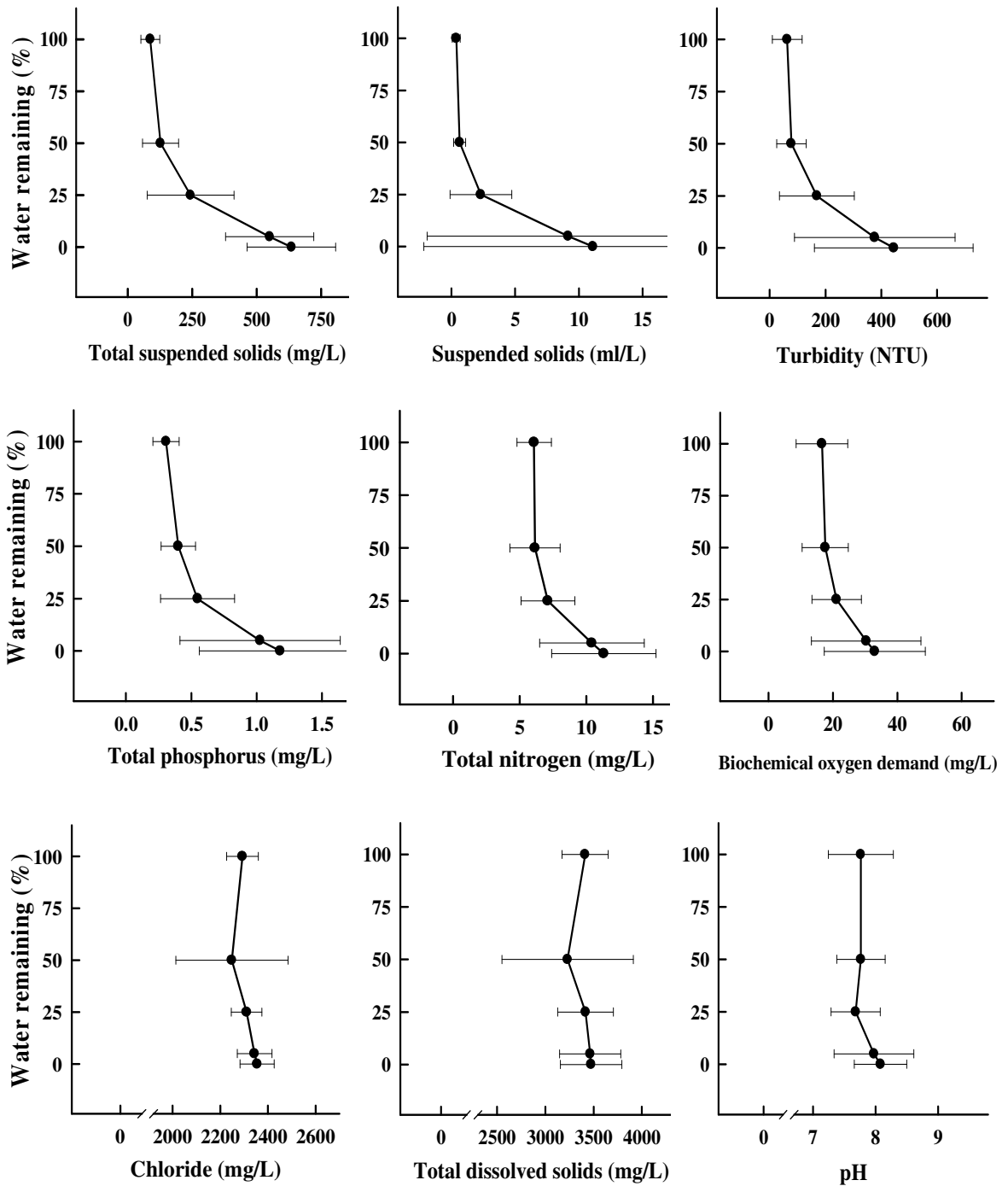


Fig. 2. Mean concentrations and standard deviations for water quality variables measured at different stages of pond water level drawdown for harvest at an inland, low-salinity shrimp farm in Alabama.