EFFECT OF SODIUM NITRATE TREATMENT ON WATER AND SEDIMENT QUALITY IN LABORATORY AND POND STUDIES

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EFFECT OF SODIUM NITRATE TREATMENT ON WATER AND SEDIMENT

QUALITY IN LABORATORY AND POND STUDIES

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VITA

Suwanit Chainark, son of Suchai and Lianteng Chainark, was bone on June 11, 1970, at Phangnga, Thailand. He graduated from Thongmapround Vitaya School in 1985. He received Advanced Certificate from Tinsulanon Songkhla Fisheries College, Songkhla in March 1991. He entered Rajamangala Institute of Technology at Bangpra, Chonburi and earned Bachelor of Science Degree in Fisheries with Second-Class Honors in March 1993. He continued his study by enrolling in the graduate school, Kasetsart University and received Master of Science in Aquaculture in April 1997. Following his graduation, he was appointed to the staff of the Faculty of Agricultural Technology, Phuket Rajabhat University, Phuket. In 2004, he was admitted to the graduate school, Auburn University, to study in Ph.D. Program at Department of Fisheries and Allied Aquacultures, College of Agriculture funded by Thai Government. He graduated from Auburn University in December 2008 and returned to work in the same position in Thailand. He married Pitchaya Chainark, daughter of Senior Captain Boonchun Chinupravat and Chomchuen Chinupravat.

DISSERTATION ABSTRACT

EFFECT OF SODIUM NITRATE TREATMENT ON WATER AND SEDIMENT QUALITY IN LABORATORY AND POND STUDIES

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Sodium nitrate ($NaNO_3$) has been widely used in shrimp aquaculture ponds as a fertilizer, a water quality enhancer and a bottom soil oxidant. This study was conducted to determine whether or not treatment with sodium nitrate can improve water quality, bottom soil condition, phytoplankton abundance and community structure and fish yield in freshwater channel catfish ponds. The study consisted of a pond study and a laboratory study. In the pond study, sodium nitrate was applied at 2 mg/L NO_3^--N at 2-week intervals to rectangular ponds of 400-m^2 water surface area stocked with 400 channel catfish *Ictalurus punctatus* fingerings and 10 grass carp *Ctenopharyngodon idella*. Water quality, phytoplankton communities, sediment condition and fish production were compared between triplicate treatment and control ponds. The results showed that catfish production and survival rate did not differ ($P>0.1$) between treated and control ponds. There were higher mean concentrations of nitrate nitrogen, total nitrogen, soluble reactive phosphorus, total phosphorus, turbidity and chlorophyll *a* in sodium nitrate-treated ponds than in control ponds ($P<0.1$). Transparency was greater in control ponds ($P<0.1$). The pH and concentration of total alkalinity, ammonia nitrogen, and dissolved oxygen were not different between treated and control ponds ($P>0.1$). There were also no differences in pH and organic matter concentration of sediment ($P>0.1$) between control and treated ponds. However, application of sodium nitrate caused a decline in redox potential between the beginning and the end of grow-out period in sediment ($P<0.1$), and upon draining, sediment in treated ponds was lighter colored than that of control ponds. This suggests that nitrate treatment enhanced oxidation at the sediment surface.

In the laboratory study, sodium nitrate was further investigated to determine if it would influence redox potential, denitrification rate, and the rate of organic matter decomposition when added to sediment. Results revealed no differences ($P>0.05$) in redox potential and organic matter concentration in sediment treated with 0 to 32 mg/kg of NO_3^--N. There was no increase in denitrification ($P>0.05$) in sediment to which nitrate was applied at 0 to 10 mg/L to the water. Dissolved oxygen declined at similar rates in water samples held in BOD bottles and treated with 0 to 8 mg/L NO_3^--N. Nitrate and ammonium also were compared as nitrogen source for phytoplankton. Uptake rate of ammonium by green algae was greater than that of nitrate. However, diatom and blue-green algae communities appeared to use both forms of nitrogen.

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INTRODUCTION

Overfishing and deteriorating aquatic environments are mainly responsible for the stagnation in natural aquatic animal production. Aquaculture has become a significant industry of many nations, and it will dramatically increase its role in the future.

The world population is increasing and more food, including seafood, will be needed in the future. The population presently is just over 6 billion, and is expected to reach over 8 billion by 2050. FAO (2008) reported that the total fishery production in 2006 was 159.9 million tons, of which 66.7 million tons from aquaculture practices. That accounts for about 41.7 % of total fishery production. World capture fisheries production was 93.2 million tons in 2006. It has been near this amount each year since the late 1980s, and it is not expected to increase in the future. Aquaculture can prevent a shortage of fisheries products in the market, and it can prevent natural fisheries from being over exploited. Some countries, such as the United States, import large amounts of fisheries products (Boyd and Tucker, 1998), and aquaculture is an important source of imports into these countries. However, domestic aquaculture may be an important source of some species even in countries that rely heavily on imported fisheries products.

There are several types of aquaculture production systems. However, pond aquaculture, particularly in earthen ponds is one of the most popular systems throughout the world (Steeby and Avery, 2003). Success in pond aquaculture depends on several aspects such as having suitable sites, adequate water supplies, good soil properties, and using good management practices (Egna and Boyd, 1997).

Channel catfish *Ictalurus punctatus* culture has become the United States' largest and most important aquaculture industry. One of the most frequent causes of poor survival and growth of channel catfish in ponds is impaired water quality. Dense phytoplankton blooms that develop in ponds can lead to low dissolved oxygen concentrations. These blooms often are dominated by species of blue-green algae that may produce odorous compounds that impart off-flavor to fish. Some blue-green algae also may be toxic to fish. High ammonia concentrations in ponds are stressful to fish. Toxic microbial metabolites such as nitrite and hydrogen sulfide may diffuse from bottom soils into the water column to stress or kill culture animals.

Pond effluents contain high concentration of nutrients, organic matter, and suspended solids, and they create environmental problem in receiving water. These problems can be minimized by adopting management practices presented by Boyd and Tucker (1998) and Tucker and Hargreaves (2004).

Marine shrimp producers encounter many of the same water quality problems faced by channel catfish producers. In Central and South America, many shrimp producers make periodic application of sodium nitrate to ponds as a general water and bottom soil quality enhancers. Although this practice is not supported by a large body of literature, there are theoretical reasons may if might be effective (Boyd and Tucker, 1998).

The aim of this study was to evaluate the possible benefits of sodium nitrate in improving phytoplankton abundance and composition, as a water quality enhancer and as a bottom soil oxidant in freshwater ponds for channel catfish.

LITERATURE REVIEW

Channel catfish farming

The channel catfish *Ictalurus punctatus* the only spotted North America catfish with a deeply forked tail, is the most commercially cultured catfish species in the United States (Tucker and Hargreaves, 2004). Channel catfish farming is essentially an industry of the southeastern United States. Catfish are grown largely in four states: Mississippi, Alabama, Arkansas, and Louisiana. NASS (2008) reported that these four states accounted for 94 % of U.S. total sales of 445 million dollars in 2007 with production of about 258,000 metric tons.

Channel catfish are native to flowing water environments within North America, including the United States, southern Canada and northern Mexico. Channel catfish can be reared in ponds, cages, circular tanks or linear raceways. Pond, monoculture dominates in the U.S. (Stickney, 2008). Most farm-raised channel catfish are cultured in ponds constructed with earthen levees. Average pond size is 8.1 ha; however, smaller pond sizes between 2 ha and 4 ha are preferable because they are easier to management (Chapman, 2008). Dietary requirements of channel catfish are based on differences in age, size, water temperature and natural food availability in the pond. Recommended dietary levels of crude protein in manufactured feed vary from 25 to 36 %, based primarily on quality of the dietary protein and other components of the feed. In general, fingerings are fed from 2% to 5 % of their body weight per day, and large fish receive 1% to 2% of their weight per day. Stocking rate of fingerings in grow-out ponds ranges from 12,000 fish to 24,000 fish per hectare. The average yield of a fed and aerated pond is around 4,000 kg of catfish per hectare of water; by multiple harvesting yield of 5,000 to 6,000 kg/hectare or more can be obtained (Chapman, 2008).

Nitrogen, phosphorus and carbon

Nitrogen is considered an important element in pond aquaculture because it is a major component of plants and animals and influences productivity (Hargreaves, 1998). Nitrogen in fish ponds originates from different sources, such as the water supply, uneaten feed, fixation by some bacteria and algae, excretion by fish and from decomposition of dead plants and animals. In a study by Gross et al. (2000), feed accounted for 87.9% of N input to catfish ponds, loss of nitrogen occurred from pond water by fish harvest (31.5%); denitrification (17.4%); ammonia volatilization (12.5%); accumulation in pond bottom soil (22.6%).

According to Boyd and Tucker (1995, 1998) the organic matter, nitrogen, and phosphorus added in feed, 17.1%, 28.3%, and 29.4%, respectively, were removed in fish and 3.1%, 28.5%, and 7.0%, respectively, were discharged in effluent. The remainder, 79.8% of organic matter, 43.2% of nitrogen, and 63.6% of phosphorus was assimilated by the pond, converted to atmospheric gases, or stored in the bottom soil.

Bottom soil

Bottom soil condition probably is as important as water quality in aquaculture ponds. Although bottom soil plays an important ecological role in fish ponds (Masuda and Boyd, 1994a), much less attention has been given to bottom soil condition than to water quality. Bottom soil functions in storing organic matter and nutrients. It also can release many substances from storage. Organic and inorganic particles settle from the water column onto pond soil. A variety of plants, animals and microorganisms live in pond soil. There is an exchange between soil particles and the above water by adsorption-desorption, ion exchange, precipitation-dissolution, and filtration (Boyd, 1995a). Chemical, physical, and biological processes occurring in pond soil affect water quality, and many water quality problems originate in pond soils (Boyd, 1990). The upper 5- cm layer of soil is more biologically and chemically active than deeper layers, and exchange substances between soil and water occur in this layer (Boyd et al., 1994). Movement of reduced substances in pore water of soil sediment in the overlaying water can be prevented by the oxidized layer at soil-water interface. Maintaining oxidized soil-water interface can reduce the likelihood of enough hydrogen sulfide and nitrite to cause toxicity from diffusing into the pond water (Masuda and Boyd, 1994a, Boyd, 1997). There is a large input of organic matter to the bottom of channel catfish ponds and other aquaculture ponds in form of uneaten feed, feces, dead plants and animals. The organic fraction of the inputs may decompose readily to lower redox potential at the soil-water interface allowing reduced substances to enter the pond water to harm aquatic animals (Boyd, 1997). To prevent this problem, sodium nitrate has been introduced into shrimp ponds as alternative electron acceptor when oxygen is used up. Therefore, by applying sodium nitrate to ponds, it might be possible to poise the redox potential in pond water and bottom soil at a level above which iron and manganese oxide and hydroxide and sulfate are reduced (Boyd, 1995b).

Phytoplankton

Dynamics of changes in phytoplankton abundance and species composition has been attributed to seasonal changes in light, temperature, nutrient and competitive advantages for limiting factor that some taxa have over others (Zimba et al., 2001). Seasonal changes affecting the quantity and quality of phytoplankton in catfish ponds were studied by Tucker and Lloyd (1984). Chlorophyll *a* concentrations were generally highest in summer (averaging >200 µg/L), but the highest individual chlorophyll *a* value recorded (910 µg/L) occurred in the winter during a bloom of *Dictyosphaerium pulchellum*. On the average, green algae (Chlorophyta) and euglenoids (Euglenophyta) represented relatively constant proportions of the phytoplankton community seasonally (about 35 and 10%, respectively). In the summer and fall, blue-green algae (Cyanophyta) became abundant. Diatoms were relatively abundant at all times and constituted the majority of the community in the winter and spring. Cyanobacteria, well known as off-flavor producers in channel catfish ponds, are often dominant numerically in summer (Tucker and Lloyd, 1984, Zimba et al., 2001). Cyanobacteria exhibit features that give them a competitive advantage over other species. They can increase or decrease the volume of gas vacuoles to alter their buoyancy so that they maintain the depth position for optimum light availability. They also can modify their photo-pigment composition to reduce photo-oxidative damage, and enhance efficiency of conversion of light to chemical energy by increasing specific pigment pools (Zimba et al., 2001, Zimba et al., 1999). Moreover, blue-green algae can produce and excrete compounds toxic to other species of algae (Boyd and Tucker, 1998), some species of blue-green algae also produce and excrete odorous compounds that cause off-flavor in flesh when absorbed by fish. The most common off-flavor compounds are geosmin and methylisoborneol (MIB) (Boyd and Tucker, 1998). The advantage to blue-green algae of producing the odorous compounds is not known, but these compounds cause serious problems in channel catfish farming. Fish that are strongly off-flavor are not acceptable in the market.

Daniels and Boyd (1993) found that the combination of nitrate and silicate fertilization favored diatom production. Burford and Pearson (1998) studied the effect of different nitrogen sources on phytoplankton composition in aquaculture ponds. They compared phytoplankton species dominance in shrimp ponds treated with urea and sodium nitrate fertilizer at equivalent nitrogen concentration. Their results showed that there was no different in phytoplankton species to both fertilizers. Thus, there is conflicting evidence about the benefit of nitrate fertilization in increasing diatom abundance even in brackishwater ponds.

Sodium nitrate

Sodium nitrate ($NaNO_3$) or “Chilean saltpeter”, consists of white, hydroscopic crystals. It has a vapor pressure at 20 ˚C; melting point at 307 ˚C and boiling point at 380 ˚C (Young, 2002). Sodium nitrate is a strong oxidizing agent. It reacts violently with flammables, combustibles, many organic compounds and other reducing agents such as granulated or powered aluminum, magnesium, and other metals (Young, 2002).

Sodium nitrate application has been suggested in aquaculture ponds with several environmental and economical benefits such as its suitability as nitrogen source, and that it does not produce acidity by nitrification as an ammonium fertilizer (Boyd, 1997, 1995b). Conversely, adding nitrate to seawater may increase pH because of its dissolution process in seawater (Burford and Pearson, 1998). It is not toxic to fish and shrimp when used at moderate levels. The 96 h, LC 50 of nitrate on channel catfish fingerings is between 1,355-1,423 mg NO_3^--N/L depending on temperature (Colt and Tchobanoglous, 1976). The main toxic action of nitrate is the result of the conversion of oxygen-carrying pigment to forms that are incapable of carrying oxygen (Camargo *et al.*, 2005). It does not exert an oxygen demand. Sodium nitrate is a natural product manufactured by extracting it from deposits of the mineral caliche; thus, its production is not a fuel – intensive synthetic process such as used to produce ammonia by the reduction of atmospheric nitrogen or to synthesize urea from ammonia (Boyd, 1997, 1995b).

Since sodium nitrate is highly soluble and quickly dissolves in water; it would not be expected to accumulate at the soil surface if broadcast over ponds (Boyd, 1997). Nitrate generated by nitrification or added to ponds as an amendment will enter one of several biological pathways. Plants and microbes may absorb nitrate and reduce it to ammonia for amino acid synthesis in cells. When dissolved oxygen concentration is low, nitrate may function as a terminal electron acceptor by denitrifying bacteria during the oxidation of organic matter. Denitrification results in the reduction of nitrate to NO, N_2O and N_2 or NH_3 and diffuse to the atmosphere (Hargreaves, 1998, Boyd and Tucker, 1998). Nitrate may penetrate deeper into the sediment than oxygen and create a larger pool of ferric iron than could be obtained by oxygen (Hansen et al., 2003). As a result, many theoretical advantages of sodium nitrate application, Seo and Boyd (2001) compared three different bottom soil management approaches; (1) dry-till treatment; (2) dry-till with sodium nitrate to maintain a high level of redox potential at the soil-water interface; (3) control (no drying, tilling or sodium nitrate application) on water quality in channel catfish *Ictalurus punctatus* ponds. The results revealed that treatment ponds, dry, tilled bottom soil and dry, tilled bottom soil with sodium nitrate, had lower concentration of soluble reactive phosphorus, nitrate-nitrogen, total ammonia-nitrogen, total suspended and turbidity, and higher values of pH, Secchi disk visibility, total alkalinity, total hardness and calcium hardness ($P<0.01$) as compared to control ponds. Ponds of the dry-till treatment had lower concentration of total nitrogen and total phosphorus than control ponds. Concentration of dissolved oxygen and chemical oxygen demand did not differ among treatments. Organic carbon, total phosphorus and soil pH also did not differ among treatments. These findings suggest that water quality improvement can be achieved by drying and tilling between crops. Applying sodium nitrate to dry, tilled pond bottom neither increased the extent of water quality improvement nor enhanced the ability of bottom soil to remove phosphorus from the water (Seo and Boyd, 2001). However, Yosoff et al. (2003) and Boyd et al. (1994) suggested that sodium nitrate is an oxidizing agent and may contribute to control the release of phosphorus and ammonia from pond sediment by maintaining oxidizing conditions at soil-water interface.

Pavek (1998) reported that nitrate-nitrogen, dissolved oxygen, pH, total ammonia-nitrogen and chlorophyll *a* were significantly higher in catfish ponds treated with sodium nitrate at dose of 5 to 10 mg/L NO_3^--N than in control ponds. Whereas, redox potential at the soil-water interface, temperature and soluble reactive phosphorus were unaffected by the treatment, fish production was higher in control ponds. A study of sodium nitrate application on water and sediment quality was carried out in black tiger prawn *Penaeus monodon* ponds by the Department of Fisheries, Ministry of Agriculture and Cooperatives, Thailand (unpublished data). Four ponds served as control ponds and four were treated with the product Nutrilake R (SQM, Santiago, Chile) which consisted primarily of sodium nitrate. Pond bottom soil were treated at 188 kg/hectare during pond preparation. During grow-out, ponds were treated with Nutrilake at dose of 1.34 mg/L for the first 4 weeks, and weekly a 0.89 mg/L, thereafter. The results of study showed that the treatment ponds and the control ponds had similar water quality except the concentrations of nitrate-nitrogen and total phosphorus in the first month of culture were higher in the Nutrilake treatment than in the controls. During the rest of the crop, there were no appreciable differences in water quality. Soil quality variables were also similar in treatment and control ponds. The Nutrilake treated ponds had slightly higher pH and less organic carbon, total nitrogen, BOD_5 and phosphate in water samples than did the control ponds, but the differences were not significant ($P>0.05$). Regarding shrimp production, the pond of the Nutrilake treatment had 8% greater shrimp survival than the control ponds. In addition, shrimp production in the treatments was 414 kg/pond higher than the controls. However, there were no statistical differences in shrimp production ($P>0.05$).

Effect of nitrate on oxygen

Certain bacteria are able to respire nitrate in the presence of oxygen. According to Carter et al. (1995) twenty-two strains from three genera (*Pseudomonas*, *Aeromonas*, and *Moraxella*) collected from three soils and a fresh water sediment showed significant rate of nitrate respiration in the presence of oxygen. They suggested that the corespiration of nitrate and oxygen may make a significant contribution to the flux of nitrate to nitrite in the environment.

Effect of nitrate on denitrification

Denitrification is an anaerobic process in which nitrate is reduced to nitrogen gas. It is commonly conducted by heterotrophic, facultative anaerobic bacteria of genera, such as *Pseudomonas, Bacillus, Micrococcus*, and *Achromobacter.* However, some sulfide oxidizing bacteria such as *Thiobacillus denitrificans* cause denitrification. During denitrification, bacteria use nitrate or other oxidized forms of nitrogen as terminal electron acceptor in respiration instead of oxygen and nitrate ions are converted to gaseous forms of nitrogen and lost to the atmosphere as suggested by the following pathway (Boyd and Tucker, 1998, Brady, 2002).

$$NO_3 \rightarrow NO_2 \rightarrow N_2O_2 \rightarrow N_2O \rightarrow N_2$$

Denitrification rate varies with temperature, pH, abundance of denitrifying bacteria, concentration of NO_3^--N, organic carbon and dissolved oxygen (Hargreaves, 1995). Denitrification proceeds fastest at temperature between 25 and 35˚C and the optimum pH is between 6 to 8 (Boyd and Tucker, 1998). In most ponds, there is little direct input of NO_3^--N and NO_3^--N for denitrification is derived mainly from NO_3^--N produced in nitrification (Gross et al., 2000).

Effect of nitrate on redox potential

Oxidation and reduction reactions are important in pond aquaculture because many biological processes that influence water quality, soil condition and aquatic animal yield are biologically mediated oxidations and reductions. The oxidation-reduction potential or redox potential is an index of the degree of oxidation and reduction in a chemical system (Boyd, 1995a). Some common oxidation-reduction reactions in aquatic soils are listed in Table 1. Oxygen is the energetically preferred terminal electron acceptor for the oxidation of organic substances. However, when oxygen concentration becomes limiting (~ 0.1 to 0.2 mg l^{-1} or E_h < 220 mV), heterotrophic, facultative anaerobes shift to nitrate as the terminal electron acceptor. The energy yield from the oxidation of organic carbon (e.g., glucose) by oxygen (^G = - 686 kcal $mole^{-1}$) is only slightly greater than for oxidation by nitrate (^G = - 649 kcal $mole^{-1}$) (Hargreaves, 1998). Measurement of *E*h is particular useful as it reveals the source of oxygen used in the mineralization of the sediment organic matter and reduction of sulphates to the potential toxic sulfides taking place at a redox potential below about -200 mV (Hussenot and Martin, 1995). Dissolved inorganic substances such as nitrate, manganic-manganese, ferric ion, sulfate and carbon dioxide may be used as alternative electron acceptors by microorganisms. The shift to other electron acceptors is sequential, that is, depletion of an electron acceptor is required before another electron acceptor is utilized. These shifts occur and cover a progressively declining redox potential (Masuda and Boyd, 1994a).

Table 1. Standard electrode potential (^oE) for oxidation-reduction reactions in pond soils.

Reaction[a]	^oE (V)
$O2$ (aq) + $4H^+ = 2H_2O$	+1.27
$2NO_3^- + 12H^+ + 10e^- =$	+1.24
MnO_2 (8) + $4H^+ + 2e^- = Mn^{2-} + 2H_2O$	+1.23
$NO_2^- + 8H^+ + 6e^- = NH_4^+ + 2H_2O$	+0.89
$NO_3^- + 10H^+ + 8e^- = NH_4^+ + 3H_2O$	+0.88
$NO_3^- + 2H^+ + 2e^- = NO_2^- + H_2O$	+0.85
$Fe^{3+} + e^- = Fe^{2-}$	+0.77
$SO_4^{2-} + 10H^+ + 8e- = H_2S$ (g) + $4H_2O$	+0.31
$CO_2 + 8H^+ + 8e^- = CH_4$ (g) + $2H_2O$	+0.17

[a] In equations, aq = aqueous; s = solid; g=gas (Boyd, 1997)

The redox system in the pond bottom is made of both organic components and reduced inorganic species, however, the driving force to the development of high oxygen demand and anoxic conditions is the organic matter (Avnimelech et al., 2004). Maintaining a high redox potential should prevent phosphorus, sulfides, nitrite and other toxic metabolites from entering the pond water above the bottom soil (Seo and Boyd, 2001, Boyd, 1997).

Effect of nitrate on pH

According to previous studies carried out by Pavek (1998) and the Thailand Department of Fisheries (unpublished data), addition of sodium nitrate in aquaculture pond tended to increase the pH slightly. Also, when denitrification rate increased in pond water, it resulted in significantly higher morning pH values (Pavek, 1998). This observation resulted because denitrification results in the removal hydrogen ion for use in reducing nitrate, hyponitrite ($N_2O_2H_2$), hydroxylamine (NH_2OH), or nitrous oxide (N_2O). These reactions (Boyd, 1990) are presented below:

$$2HNO_3 + 4H^+ \longrightarrow 2HNO_2 + 2H_2O$$

$$2HNO_2 + 4H^+ \longrightarrow N_2O_2H_2 + 2H_2O$$

$$N_2O_2H_2 + NH^+ \longrightarrow 2HH_2OH$$

$$2NH_2OH + 4H \longrightarrow 2NH_3 + 2H_2O$$

$$N_2O_2H_2 + 2H^+ \longrightarrow N_2 + 2H_2O$$

$$N_2O_2H_2 \longrightarrow N_2O + H_2O$$

$$N_2O + 2H^+ \longrightarrow N_2 + H_2O$$

Effect of nitrate on organic carbon

The main sources of organic carbon in aquaculture ponds are planktonic algae, invertebrates, uneaten feed, manure and feces of aquatic animals (Boyd, 1995a). High concentration of organic matter in bottom soil can cause various problems to the pond environment by increasing oxygen demand and the presence of reduced chemical compounds such as Fe^{2+}, Mn^{2+}, NO_2^-, H_2S and CH_4 that harm aquatic animal when they diffuse into the overlying water (Ayub et al., 1993).

Fish and shrimp farmers often think that organic matter concentration increases at a rapid rate in pond bottoms. They are willing to try sodium nitrate or other treatments that might lower organic matter concentrations. The literature does not support the opinion of farmers. Steeby (2004) noted that organic carbon concentration in sediment of catfish ponds that has been in continuous catfish production from 14 days to 21 years ranged from 0.76 to 3.43% of dry matter. Boyd et al. (1994) reported that mean carbon concentration of sediments sampled from 358 freshwater fish ponds and 346 brackishwater shrimp ponds were almost identical. Most soils contained less than 5% carbon and about one-half of samples contained less than 2.5% carbon. An average of 1.9 % carbon was obtained for analyses of bottom soil samples from 35 tilapia ponds aging between 3 to 39 years in Thailand (Thunjai et al., 2004). Bottom soil organic carbon concentration was 1.2% for 42 catfish ponds, 1.07% for 40 freshwater prawn ponds and 2.08% for carp ponds in Thailand (Wudtisin and Boyd, 2006). The average organic carbon concentration from 58 catfish ponds in West-Central Alabama was 1.02% (Silapajarn et al., 2004). These observations confirm the statement by Steeby et al (2004) that accumulation of organic matter in soils of aquaculture ponds does not increase greatly over time. However, there was a modest increase from less than 1% soil organic matter to 2 to 3 % soil organic matter during the first 2 to 3 year of new ponds production (Munsiri et al., 1995)

Nitrogen uptake by phytoplankton

Ammonium is used more efficiently than nitrate in protein synthesis; it requires 401 to 454 µmol photons to synthesize 1 mg protein from ammonia whereas nitrate requires 598 to 651 µmol photons [Raven (1984) as cited in Buford and Pearson (1998)]. It also is taken up faster and has a lower saturation constant than nitrate [Dortch (1990) as cited in Buford and Pearson (1998)]. Both characteristics are accentuated by low light intensity and high nitrogen availability. The growth rate of phytoplankton on nitrate may equal or exceed that obtained on ammonium in low light. Low nitrogen availability may increase the preference for ammonia uptake, although the growth rate of plankton is as rapid as or faster than that on ammonia (Burford and Pearson, 1998). Nitrate concentrations are usually less than 0.1 mg NO_3^--N/L during the summer. Nutrient availability is a major factor affecting phytoplankton abundance and community structure. Ponds with low to moderate densities of phytoplankton brought about by moderate nutrient loading rates tend to have green algae, euglenophytes, and diatoms. Excessively dense blue-green algae often become dominant in nutrient-enriched ponds (Boyd and Tucker, 1998). In summer, elevated concentrations of both ammonia-nitrogen (0.5 to 2.0 mg/L) and total phosphorus (0.1 to 0.3 mg/L) are common in channel catfish ponds, but nitrate concentrations usually are less than 0.1 mg/L NO_3^--N in summer. This has led some observers to speculate that application of sodium nitrate might reduce the abundance of blue-green algae in channel catfish ponds.

MATERIALS AND METHODS

This study consisted of a pond study in which water quality, phytoplankton communities, sediment condition and fish production were compared between replicated treatment and control ponds. A laboratory study also was conducted to further investigate issues not revealed in the pond study.

Pond Study

Ponds and sodium nitrate treatment

Ponds used in this research are located on the Auburn University E. W. Shell (EWS) Fisheries Center, Department of Fisheries and Allied Aquacultures, Auburn, Alabama. Six, rectangular ponds of 400- m^2 water surface area and with average and maximum depths of about 0.8 and 1.5 m, respectively, were used in this study. Ponds were assigned randomly to receive the treatments or serve as control: Ponds E-37, E-45 and E-46 were treated with sodium nitrate, and Ponds E-38, E-47 and E-48 served as un-treated controls. Nitrate-treated ponds received doses of sodium nitrate fertilizer at 2 mg/L NO_3^--N at 2-week intervals. The appropriate amount of sodium nitrate for each pond was weighed and put into a plastic bucket with pond water and stirred until the sodium nitrate dissolved. The solution was then disseminated over the pond surface.

Pond management

Ponds were stocked on 15 April 2005 at the rate of 400 channel catfish fingerings averaging 42 g and 10 grass carp *Ctenopharyngodon idella* averaging 150 g per pond. Fish were fed daily with a pelleted, commercial catfish feed containing 28% crude protein at 2% of body weight. Amount of feed offered per day was adjusted every 2-weeks for weight gain. Aeration was applied with 0.5-hp floating, electric aerator when dissolved oxygen (DO) concentration was expected to fall below 3 mg/L over night. Ponds were drained and fish were harvested on 28 October 2005 after 200 days of culture. The number of catfish in each pond was counted and total weight of catfish was recorded.

Water analyses

The dissolved oxygen concentration was measured frequently as a routine management technique with a Yellow Spring Instrument company Model 57 polarographic oxygen meter.

Water samples were collected in the morning at 2-week interval using a 90-cm water column sampler (Boyd and Tucker, 1992). The samples were analyzed as indicated below:

Water pH - Electrometric method (Franson and Eaton, 2005) with an Orion pH meter Model 230 and glass electrode.

Total ammonia-nitrogen - Persulfate digestion and finish by ultraviolet screening method (Gross and Boyd, 1998).

Nitrate-nitrogen - Szechrome NAS reagent method (Polysciences, Inc., Technical Data Sheet 239).

Total phosphorus - Persulfate digestion with ascorbic acid finish (Gross and Boyd, 1998).

Soluble reactive phosphorus - Ascorbic acid method (Franson and Eaton, 2005).

Total alkalinity - Titration to methyl orange endpoint with standard sulfuric acid (Franson and Eaton, 2005).

Chlorophyll *a* - Acetone-methanol extraction as described by Pechar (1987).

Turbidity - Orbeco-Hellige Turbidimeter Model 965-10A and Secchi disk visibility.

Algal identification and enumeration

A 50-mL of water from each pond was put in a graduated, plastic, conical centrifuge tube when water samples were colleced for chemical analyses. These samples were preserved with 0.15 mL of Lugol's solution (Boyd and Tucker, 1992). Phytoplankters (colonies, filamantens, trichomes, or single cell) were counted using a Sedgwick-Rafter counting cell (50 mm X 20 mm X 1 mm deep or 1,000 mm^3) under an inverted compound microscope using an ocular fitted with the Whipple ocular grid and the 10 X ocular. The grid is about 1 mm on a side, delimiting an area of about 1 mm^2 (Hasle and Sournia, 1978, Boyd and Tucker, 1992, Franson and Eaton, 2005). Algae were identified to genus according to Whitford and Schumacher (1984).

Bottom soil analyses

Sediment samples collected at the beginning of the experiment before starting sodium nitrate treatment and at the end before draining pond for harvest of fish. Each time, samples were collected to depth of 5-cm from five places in each pond using a diameter plastic core sample tube as described by Wudtisin and Boyd (2006). Redox potential of the samples was determined at once using a Orion Model 230 meter with combination ORP electrode. The samples were then dried at 60 ˚C in a mechanical convection oven, pulverized and analyzed for organic carbon and pH (Boyd and Tucker, 1992).

Catfish data

The following data were recorded related to fish production: date fish stocked; number fish stocked; average weight fish stocked; number fish harvested; weight distribution of sample of fish from each pond harvested; total weight of fish harvested; total amount of feed applied to each pond; feed conversion ratio.

Laboratory Study

The laboratory study was separated into five experiments.

Nitrate as oxygen source

Oxygen demand of sediment. - Sediment samples were taken from Pond B-2 at the EWS Fisheries Center. A 2-g layer of sediment was then put into bottoms of BOD bottles, and bottles were filled carefully with clear tap water containing the following nitrate concentration, 0, 0.5, 1, 2, 4 and 8 mg/L NO_3^--N. Each concentration was replicated 5 times. Samples were incubated in the dark at 25 ˚C and dissolved oxygen concentration was measured with a polarographic oxygen meter (YSI Model 57 and YSI 5905 BOD Probe) at 0 hr, 12 hr and every day until oxygen was depleted.

Oxygen demand of plankton. - This trial was carried out in the same way as the previous trial, however, water from Pond E-24 at the EWS Fisheries Center containing a moderate plankton bloom was used instead of tap water and no sediment was added to the BOD bottles.

Denitrification

Sediment was taken from Pond F-5 on the EWS Fisheries Center. Exactly 2.0-g of fresh sediment were put into BOD bottles containing pond water to which 0, 1, 5, and 10 mg/L NO_3^--N had been added. The three samples of each concentration were removed to measure nitrate concentration remaining after 1, 2, 4, 6, 8, 12, 16, 20, 24 and 28 days.

Redox potential

Sediment samples were collected from Ponds B-2, B-3 and B-4 at the EWS Fisheries Center. These ponds were being used to raise channel catfish at the time and were thought to contain relatively large amount of fresh organic matter. Soil samples were put in to shallow pans (5-cm depth of sediment with 5-cm layer of water above) and mixed with enough crushed fish feed to give a feed concentration of 0.1 %. Sodium nitrate fertilizer was added into sediments to acquire the following concentrations: 0, 1, 2, 4, 8, 16 and 32 mg/kg NO_3^--N. Pans were held at room temperature, and pH and redox potential were measured with a Orion Model 230 pH/Redox meter using a combination glass electrode and a combination ORP electrode, respectively. The measurement were made at five places in each pan after 1, 2, 4, 8, 12, 16, 20, 24, 28 and 32 days.

Organic matter decomposition

Sediment samples from Ponds B-2, B-3 and B-4 also were placed in pans and treated with sodium nitrate to give concentrations of 0, 1, 2, 4, 8, 16 and 32 mg/kg NO_3^--N. Samples were removed immediately after treatment (0 day) and after 1, 2, 4 and 8 weeks. These samples were oven-dried at 60 ˚C in a mechanical convection oven and crushed to pass a 0.85-mm screen. Organic matter concentration was determined using the Walkley Black potassium dichromate-sulfuric acid oxidation method (Nelson and Sommers, 1996, Boyd, 1995a)

Uptake of nitrate and ammonium by phytoplankton

The four samples of pond water containing different densities of plankton were obtained from Pond M-16, F-24, N-1, and H-41 at the EWS Fisheries Center. The samples were analyzed for chlorophyll *a* concentration (Pechar, 1987) to provide an estimate of plankton abundance. There were three treatments of this experiment: addition of 0.5 mg/L NH_4^+-N; addition of 0.5 mg/L NO_3^--N; addition of a mixture of 0.5 mg/L of both nutrients. The study was conducted in 1-liter flasks of pond water using five replications per treatment. The flasks were exposed to sunlight for 12 hrs on clear days, and concentrations of nitrate-nitrogen and ammonia-nitrogen remaining after 12 hours were measured.

Data Analysis

Catfish, water quality, bottom soil and phytoplankton data were analyzed by t-test with a probability level of 0.1. All laboratory trial data were tested with the analysis of variance followed by Tukey's Studentized Range (HSD) for identifying differences among treatment means. The probability used for rejection of null hypothesis was set at 0.05 for laboratory studies. All data were analyzed with the SAS 9.1.2 statistical analysis software (SAS Institute, 2004).

RESULTS AND DISCUSSION

Pond Study

Water quality

The concentrations of each water quality variable in nitrate-treated ponds and in control ponds were averaged across the three replications and standard errors of the mean ($s_{\bar{x}}$) computed. The data were plotted for each variable (Figs. 1 to 11). In addition, grand means for each water quality variable were determined in treatment and control ponds, means were tested for significance, and the results presented in Table 1. Each variable is discussed below.

Mean values for pH varied between 7.5 and 9.0 (Fig. 1). The control ponds had two peaks in pH near 9 – one in late May and the other in late June. Except for these two peaks, pH was between 7.6 and 7.9 on all sampling dates in control ponds. The pH of nitrate-treated pond waters increased to near 8.5 by late May and then declined slightly until early August. There were two dates, one in mid August and one in mid September, when pH reached 8.5 and 8.8, respectively, to exceed the pH of the control ponds ($P<0.1$). Although pH was greater in nitrate-treated ponds on two sampling dates, the grand mean for pH was not different between nitrate-treated and control ponds (Table 2).

These pH values are similar to those typically encountered in channel catfish production experiments at the EWS Fisheries Center (Boyd, 1990).

The pH of pond water increases during the daytime in response to removal of carbon dioxide for use by phytoplankton in photosynthesis, and it declines at night when photosynthesis stops but respiration continues (Boyd and Tucker, 1998). Daytime peaks in pH observed in this study were related to increases in photosynthetic rate by phytoplankton rather than from sodium nitrate additions.

The total alkalinity concentration increased from near 30 mg/L at the beginning of the experiment to around 50 mg/L in both nitrate-treated and control ponds (Fig. 2). An increase in alkalinity in channel catfish ponds at the EWS Fisheries Center often occurs between spring and fall (Boyd, 1974, Boyd and Tucker, 1998). This increase is thought to result from the alkaline reaction of denitrification and from concentration of ions by evaporation. During the period July through September, total alkalinity concentration tended to be higher in treated ponds than in control ponds, but neither means for individual sampling dates (Fig. 2) or grand means (Table 2) differed ($P>0.1$).

One rationale for applying sodium nitrate to aquaculture ponds is to oxidize bottom soils (Boyd, 1995a). Nitrate is used by denitrifying bacteria, and as long as nitrate is present at the sediment surface and in sediment pore water, the redox potential should remain poised at a level conducive to denitrification. The denitrification reaction produces hydroxide and thereby contributes to alkalinity. Much of the nitrate applied to the ponds obviously was denitrified because the nitrate-nitrogen concentration never reached 2 mg/L (Fig. 3), but the total amount of sodium nitrate applied was equivalent to a concentration of 32 mg/L of nitrate-nitrogen. Nevertheless, periodic application of sodium nitrate to ponds in this study did not result in a greater concentration of total alkalinity than found in control ponds.

Nighttime aeration of ponds was effective in avoiding excessively low dissolved oxygen concentration (Fig. 4). Early morning concentrations of dissolved oxygen in both treated and control ponds usually were above 6 mg/L. Only on the last sampling date in the treated ponds did average dissolved oxygen concentration fall slightly below 4 mg/L. Most commercial channel catfish producers strive to avoid early morning dissolved oxygen concentrations below 3 to 4 mg/L (Boyd and Tucker, 1998). Dissolved oxygen concentrations tended to decline as the study progressed and fish standing crop and feeding rate increased. There was no clear difference in dissolved oxygen concentration as a result of nitrate treatment. There were differences ($P<0.1$) between control and treatment ponds on five dates (Fig. 4) – concentrations were higher in treated ponds on two of these dates. The grand means for dissolved oxygen concentration were 6.84 mg/L in control ponds and 6.85 mg/L in treated ponds ($P>0.1$) (Table 2).

Total ammonia-nitrogen concentration in nitrate-treated ponds averaged below 0.1 mg/L until September and then increased to about 0.2 mg/L during September and early October (Fig. 5). In the control ponds, total ammonia-nitrogen concentrations were about 0.1 mg/L until July when they increased to more than 0.3 mg/L. Concentrations in controls then declined to values numerically less than those of the treated ponds. No differences in total ammonia-nitrogen were observed for individual sampling dates (Fig. 5) or for grand means (Table 2).

The main source of ammonia was excretion by fish. Ammonia is lost from the water by diffusion to the atmosphere, uptake by phytoplankton, and oxidation to nitrate by nitrifying bacteria (Tucker and Hargreaves, 2004). Nevertheless, concentrations of total ammonia-nitrogen up to 2 mg/L sometimes are observed in ponds on the EWS Fisheries Center (Boyd, 1990). There are two likely reasons for the low concentrations of ammonia-nitrogen found in this study. Ponds were stocked at a modest rate, and feed was applied conservatively. This reduced nitrogen input and favored effective conversion of nitrogen in feed to nitrogen in fish. Mechanical aeration was used at night, and low concentrations of dissolved oxygen did not occur. The oxidation of ammonia to nitrate by nitrifying bacteria is more effective in ponds where dissolved oxygen is not low (Boyd and Tucker, 1998).

As expected, mean nitrate-nitrogen concentrations were numerically greater in treated ponds than in control ponds on several dates (Fig. 3). However, there was much variability in nitrate-nitrogen concentrations, and on only one date did sodium nitrate-treated ponds contain more nitrate-nitrogen ($P<0.1$) than control ponds. The grand mean for nitrate-nitrogen was higher ($P<0.1$) in the treated ponds than in the control ponds (Table 2). The greatest nitrate-nitrogen concentrations in treated ponds were observed in October. A maximum, average concentration of slightly above 1 mg/L was achieved on the last sampling date. The sodium nitrate-treatment rate was 2 mg/L nitrate-nitrogen at 2-week intervals. Concentrations were measured in the ponds 1 week after application. Nitrate is not absorbed by soil or sediment (Brady, 2002), and water discharge did not occur through the drain pipe during the study. This suggests that the loss of nitrate resulted primarily from phytoplankton uptake or denitrification.

Total nitrogen concentration followed a trend of change similar to nitrate-nitrogen concentration. It increased to a peak in control ponds during July and August with maximum average concentration of 1.4 mg/L (Fig 6). There was a trend of increasing total nitrogen concentration from the beginning to end of the study in the treated ponds, and on the last five sampling dates, total nitrogen was at higher concentration ($P<0.1$) in the treated ponds than in the control ponds (Fig. 6). This pattern probably resulted from the continuing input of sodium nitrate to treated ponds. The grand mean for total nitrogen was greater ($P<0.1$) in treated ponds than in the controls.

Soluble reactive phosphorus concentrations (Fig. 7) and total phosphorus concentrations (Fig. 8) were higher ($P<0.1$) on several dates, and the grand means for these variables were greater ($P<0.1$) in treated ponds than in control ponds (Table 2). This was unexpected, for Masuda and Boyd (1994b) suggested that application of sodium nitrate to ponds should increase the redox potential of sediment, lessen the solubility of iron phosphate in the sediment, and result in a lower soluble reactive phosphorus concentration in the water. This unexpected finding cannot be explained from data available in this study. Both treatment ponds and control ponds received similar quantities of feed, and phosphorus fertilizers were not applied to any of the ponds. Water for initially filling ponds and for maintaining water levels in them was from the same source.

All ponds were somewhat turbid with sediment particles suspended by erosion within ponds caused by aerator-generated water currents. Aerators of less than 0.5 hp are not available at the EWS Fisheries Center and the ponds used in this study were only 0.04 ha in area. The aeration rate was quite high – 12.5 hp/ha. Nevertheless, the turbidity was greater ($P<0.1$) in treated ponds than in control ponds on five sampling dates (Fig. 9), and the grand mean for turbidity also was higher ($P<0.1$) in the treated ponds (Table 2).

Treatments and control ponds were aerated at the same rate, and the greater turbidity in treatment ponds is thought to have resulted from greater plankton biomass in them. The converse was true for Secchi disk visibility (Fig. 10; Table 2), because increasing turbidity causes the Secchi disk visibility to decline. The treated ponds had especially high turbidity and low Secchi disk visibility during late summer and fall.

Chlorophyll *a* concentration often is used as an indicator of phytoplankton biomass (Franson and Eaton, 2005). Chlorophyll *a* concentrations (Fig. 11) were higher in treated ponds ($P<0.1$) on four sampling dates between mid July and November and numerically higher on several other dates. The grand mean for chlorophyll *a* concentration also was higher ($P<0.1$) in treated ponds. This finding confirms that there was greater phytoplankton biomass in treated ponds, and this likely is the reason for the greater turbidity and a lower Secchi disk visibility. The greater concentration of phosphorus in treated ponds favors phytoplankton growth. Nitrate also is a plant nutrient, and greater concentrations of this nutrient in the treated ponds might have stimulated phytoplankton growth.

Previous studies at the EWS Fisheries Center have revealed that organic nitrogen accumulates in bottom of ponds, and mineralization of organic matter provides enough nitrogen to support abundant phytoplankton growth in older ponds (Swingle et al. 1963). However, many ponds, including the ones in this study, were recently renovated with the removal of sediment. A study by Yuvanatemiya and Boyd (2006) revealed that sediment removal greatly reduced the concentration of labile organic matter in pond bottoms, and Boyd et al. (2008) reported a large response to nitrogen fertilization in renovated ponds.

Nitrate applied to the treatment ponds may have stimulated phytoplankton growth beyond that possible from nitrogen entering ponds from feeding waste and other sources.

Phytoplankton communities

Cells, colonies, or filaments of each phytoplankton species were counted. The results of the counts were reported as the number of phytoplankton individuals, i.e., cells, colonies, or filaments per milliliter. The abundance of phytoplankton individuals did not differ between treatments on individual dates (P>0.1), but the number of individuals was numerically larger in treated ponds than in control ponds on nine or 14 sampling dates (Fig. 12). The grand means for total abundance of phytoplankton were 266, 916 individuals/mL in control ponds and 331,845 individuals/mL in treated ponds (Fig. 13). However, variation in phytoplankton abundance was great and the grand means did not differ (P>0.1).

This observation does not necessarily conflict with the conclusion based on chlorophyll *a* concentration that there was greater phytoplankton biomass in nitrate-treated ponds than in control ponds. Phytoplankton species vary greatly in size (Wetzel, 2001, Boyd and Tucker, 1998). Two species may be present in the phytoplankton community in equal abundance, but the larger of the two species would constitute the greater biomass. Phytoplankton cells tend to contain an amount of chlorophyll *a* that increases in direct proportion with their biomass (Boyd, 1990). For this reason, chlorophyll *a* concentration is considered more reliable than phytoplankton abundance as a way of assessing phytoplankton biomass.

Secchi disk visibility and turbidity frequently are used to roughly estimate the relative abundance of phytoplankton in aquaculture ponds (Almazan and Boyd, 1978). However, suspended soil particles and non-living organic matter also contribute to turbidity and influence Secchi disk visibility (Boyd and Tucker, 1998). Thus, chlorophyll *a* concentration also is considered a better index of phytoplankton biomass than Secchi disk visibility or turbidity.

The phytoplankton community (Fig. 14) in control ponds consisted largely of genera of Cyanophyta (blue-green algae), or as some prefer to call them, cyanobacteria. The abundance of blue-green algae was similar in sodium nitrate-treated and control ponds, but the treated ponds contained more ($P<0.1$) Chlorophyta (green algae). The percentage of blue-green algae in phytoplankton communities was about 75% in treated ponds and roughly 92% in control. Diatoms were not common in either treated or control ponds as indicated by the low abundance of Chrysophyta (Fig. 14).

The most abundant blue-green algae in control and treated ponds were species of *Microcystis*, *Oscillatoria*, and *Anabaena* (Figs. 15 and 16). According to Jüttner and Watson (2007), all three of these genera have been associated with production of the odorous compounds geosmin (trans-1, 10-dimethyl-trans-9-decalol) and MIB (methyl-isoborneol). These compounds are excreted into the water by algae and absorbed by fish to impart an off-flavor in fish flesh (Tucker, 2000, 1996, Tucker and van der Ploeg, 1999). Off-flavor makes fish less acceptable in the market and is a serious problem in channel catfish culture (Tucker, 2007, Hanson and Schrader, 2006).

Three other genera of blue-green algae were common. The genus *Trichodesmium* was found in both sodium nitrate-treated ponds and control ponds; this genus has not been associated with production of odorous compounds. Treated ponds contained *Raphidiophis*, another genus not associated with production of odorous compounds, while *Lyngbya*, a genus containing species capable of producing odorous compounds, was present in the control ponds.

Complete lists of all planktonic algal genera found in control and sodium nitrate-treated ponds are provided in Table 3. A total of 27 genera were found in treated ponds, and 28 genera were observed in the control ponds. A total of 42 samples each were collected from treatment and control ponds (14 sampling dates × three replications). Only *Microcystis* was found in every sample from treatment and control ponds. The genera *Gleocystis*, *Coelastrum*, and *Oscillatoria* were found in at least 20 samples from both control and treatment ponds. *Melosira* also was common occurring in 11 samples from treated ponds and 17 samples from control ponds. The desmid, *Pediastrum*, occurred in 20 samples from treatment ponds, but only four samples from control ponds. It was the only genus that appeared to be favored by sodium nitrate treatment. Although *Anthrodesmus*, *Closterium*, and *Lyngbya* were found only in control ponds, and *Selenastrum* and *Euglena* were found only in control ponds, these occurrences were only in one to three samples and not considered indicative of a treatment effect.

Phytoplankton communities in which several genera (or species) are rather common are considered to have a greater diversity than communities in which most individuals are of one or a few genera. Highly diverse phytoplankton communities are considerably more stable than less diverse ones and are favored by aquaculturists (Boyd and Tucker, 1998). Margalef (1958) presented a simple equation for estimating diversity as follows:

$$H = \frac{S-1}{\ln N}$$

Where H = the Margalef diversity index; S = the number of species (or other taxonomic group) of phytoplankton; N = the total number of phytoplankton individuals.

Diversity indices (Table 4) ranged from 0.17 on 17 July to 1.34 on 11 May in sodium nitrate-treated ponds, and from 0.37 on 19 July to 1.30 on 11 May in control ponds (Table 3). The average diversity index was 0.69 in control ponds and 0.71 in treated ponds. There were no differences between treated and control ponds with respect to average diversity on any sampling date or for the grand means ($P>0.1$).

The analysis of the algal count data suggests that treatment with sodium nitrate did not cause changes in phytoplankton composition. Although there was a slightly lower percentage of blue-green algae in treated ponds, nitrate stimulated phytoplankton growth and the abundance of blue-green algae was equal to that of the treated ponds. Sodium nitrate treatment does not seem to be a promising means of lessening the abundance of phytoplankton species responsible for off-flavor. At least in these freshwater ponds, nitrate treatment did not increase the abundance of diatoms. However, an earlier study by Daniels and Boyd (1993) showed that nitrate fertilization stimulated diatom abundance in brackishwater ponds. Similar results also were achieved in farm trials with sodium nitrate fertilization of marine shrimp ponds in Thailand (Thailand Department of Fisheries, unpublished report).

Sediment

The results of sediment analyses made before stocking ponds with fish and at the end of the culture period (Table 5) revealed that the changes in sediment organic matter concentration and pH during the production period did not differ (P>0.1) between the treatment and control ponds. The redox potential, however, exhibited a greater decline during the crop in the control ponds than in the treated ponds. The higher pH in the sediment of treated ponds is most likely the result of increased denitrification caused by the addition of nitrate.

When drained for harvest, the newly exposed bottoms of the nitrate-treated ponds were a lighter color than those of the control ponds. Reduced sediment has a darker color than oxidized sediment (Boyd, 1995a). Thus, the visual evidence supports the redox potential data in Table 4 and provides additional evidence that sodium nitrate application enhanced the oxidation of pond bottoms.

Fish production

Feed input to the ponds was almost identical, but fish survival was numerically greater in the nitrate-treated ponds than in the control ponds (Table 6). The numerically greater production and better feed conversion ratio (FCR) in the treated ponds resulted primarily from greater survival of fish in treated ponds than in control ponds. Had survival in the control been equal to that of the treatment and the fish been of the same weight, average production in the control ponds would have been similar to that of the treatment ponds.

Fish production was equivalent to 3,388 kg/ha in the control ponds and 3,860 kg/ha in the treated ponds. The corresponding FCR values were 1.48 and 1.25, respectively. However, neither production nor FCR differed (P>0.1) between control and treatment ponds. The production in this experiment was lower than that normally achieved by channel catfish farmers, because fewer fish were stocked (10,000 kg/ha as compared to 12,500 to 15,000 kg/ha) and feed was applied conservatively. The FCR was excellent in both control and treatment ponds, when compared to FCRs of 2.0 to 3.0 typically achieved by farmers (Greg Whitis, personal communications). Boyd et al. (2000) made a survey of 25 channel catfish ponds in Alabama and reported an average FCR of 1.88 with a range of 1.25 to 2.5.

Assessment of sodium nitrate treatment

The results of this study indicate that sodium nitrate treatment of channel catfish ponds did not lead to significant improvement in bottom soil or water quality. The treatment did not cause a greater total alkalinity concentration, a higher concentration of dissolved oxygen, a reduction in the abundance of blue-green algae, or an increased diversity of the phytoplankton community. Sodium nitrate treatment did result in a slightly higher concentration of both soluble reactive and total phosphorus, a greater availability of nitrogen for plants, and a slightly higher phytoplankton biomass. The increase in phosphorus cannot be explained, but the most likely source of increased nitrogen was the sodium nitrate treatment. An increase in phytoplankton biomass in aquaculture ponds with feeding is not necessarily a desirable outcome, because it may lead to greater nighttime depletion of dissolved oxygen concentration.

With respect to bottom soil, nitrate treatment did result in less decline in redox potential between the beginning and end of the grow-out period. This effect also was obvious at pond draining, for freshly-drained pond bottoms of nitrate-treated ponds had a lighter color than those in control ponds. A similar observation was made by Drs. Noel Morrissey and Craig Lawrence when nitrate-treated ponds for yabbie culture (*Cherax destructor*) in Western Australia were compared with control ponds (Boyd, 1995a).

The increase in redox potential at the soil-water interface is considered desirable for it lessens the opportunity for reduced organic and inorganic substances from diffusion from anaerobic zones in the pond bottom into the pond water.

There was no significant improvement in fish production variables as a result of sodium nitrate treatment. Thus, the few differences in water quality, phytoplankton biomass, and soil chemistry observed in this study did not have an effect on fish survival and growth.

Two earlier pond studies of sodium nitrate treatment, one in channel catfish ponds (Pavek, 1998) and the other in marine shrimp ponds in Thailand (Thailand Department of Fisheries, unpublished report) produced results similar to this study. Few differences in pond water or sediment quality were observed and production was not better in treated ponds than in control ponds. Nevertheless, at harvest, lighter-colored sediment was observed in both studies suggesting that nitrate treatment oxidized the sediment-water interface. This effect alone might make sodium nitrate treatment beneficial in highly intensive aquaculture ponds. The effect might be especially beneficial in intensive marine shrimp ponds where high sulfate concentrations in the water are normal and favor hydrogen sulfide production in anaerobic zones. Hydrogen sulfide is very toxic to shrimp and fish (Boyd and Tucker, 1998). However, at moderate stocking and feeding rates, there does not appear to be any significant benefit of treatment freshwater ponds for channel catfish production with sodium nitrate.

Laboratory Study

Dissolved oxygen and nitrate depletion trials

Several concentrations of nitrate-nitrogen were added to BOD bottles containing clear, dechlorinated tap water and 2-g pond sediment. The dissolved oxygen concentration declined rapidly for 6 days in both control and treated bottles as illustrated for 0 and 8 mg/L nitrate-nitrogen treatments (Fig. 17). The average, daily depletion of dissolved oxygen concentration over the first 6 days of incubation was as follows: 0 mg/L NO_3^--N, 0.775 mg/L; 0.5 mg/L NO_3^--N, 0.770 mg/L; 1 mg/L NO_3^--N, 0.790 mg/L; 2 mg/L NO_3^--N, 0.780 mg/L; 4 mg/L NO_3^--N, 0.795 mg/L; 8 mg/L NO_3^--N, 0.785 mg/L. Thus, the addition of nitrate to the sediment-water systems in the BOD bottles did not have a measurable effect on the dissolved oxygen concentration.

The experiment was repeated in BOD bottles containing pond water with a moderate plankton bloom. Again, there was little difference among nitrate-nitrogen additions and dissolved oxygen concentration as illustrated in Fig. 18 for 0 and 8 mg/L NO_3-N treatments. The decline in dissolved oxygen concentration was more rapid than in the trial with tap water (Fig. 17). The average, daily rates for dissolved oxygen decline over the first 6 days were as follows: 0 mg/L NO_3^--N, 1.49 mg/L; 0.5 mg/L NO_3^--N, 1.45 mg/L; 1 mg/L NO_3^--N, 1.44 mg/L; 2 mg/L NO_3^--N, 1.44 mg/L; 4 mg/L NO_3^--N, 1.50 mg/L; 8 mg/L NO_3^--N, 1.55 mg/L. These findings reveal that there was no decline in the rate of dissolved oxygen disappearance from the water in the BOD bottles as a result of nitrate addition.

The dissolved oxygen depletion trials were repeated using more bottles so that nitrate-nitrogen concentrations could be measured at intervals. The results (Table 7) show that nitrate-nitrogen concentrations did not decline during the trials. This observation suggests that denitrification did not occur in BOD bottles.

The dissolved oxygen depletion studies revealed that nitrate treatment did not reduce the rate of dissolved oxygen decline in aerobic systems of water or sediment. The studies also cast doubt on the assumption that adding nitrate will spare dissolved oxygen in ponds by stimulating denitrification (Boyd, 1995b). As long as there is dissolved oxygen in the water above the sediment, the sediment-water interface will be aerobic and denitrification will not occur. Nitrate in water might diffuse or move with infiltrating waters into deeper layers of sediment to stimulate denitrification. However, denitrifying bacteria do not use molecular oxygen, so this action would not spare dissolved oxygen. The dissolved oxygen would be used by non-chemotrophic bacteria in processes unrelated to denitrification.

Redox potential and pH

In this study, the redox potential and pH were measured in wet sediment covered with a thin layer of water. The redox potential was initially lower in sediment samples treated with greater amounts of nitrate-nitrogen (Fig. 19). However, by day 3, redox potential was similar at all nitrate-nitrogen rates. The redox potential tended to increase in all treatments and control over time reaching peak values after about 3 weeks as illustrated in Fig. 20. The final redox potential values ranged from 126 mv in the 32 mg/kg treatment to 196 mv in the 4 mg/kg treatment, but none of the means differed ($P>0.05$).

The pH tended to fluctuate between 6.6 and 6.9 over time in all treatments and control. The average initial pH in all treatments was 6.87 while the final average pH was 6.79. The pH, nevertheless, tended to increase with increasing nitrate-nitrogen rate as illustrated for days 1 and 32 (Fig. 21). This rise in pH with respect to sodium nitrate addition likely resulted from the alkaline nature of the denitrification process.

The initial decline in redox potential caused by increasing the nitrate application dose was not expected. It has been suggested many times that nitrate addition will poise the redox potential at the level conducive to denitrification (Boyd, 1995b). However, the added nitrogen apparently stimulated the decomposition of organic matter by non-denitrifying bacteria causing the redox potential to fall before the denitrification process began.

It was extremely difficult to make redox potential measurements in sediment in the shallow trays. The redox probe is fairly large, and it takes at least 2 or 3 minutes to reach a stable reading. Thus, when it is inserted into the sediment, oxygenated water from above the sediment entered the space opened into the sediment around the circumference of the probe. The introduction of the oxygenated water likely caused the redox potential to increase.

Organic matter decomposition

Contrary to common belief, the organic matter in pond sediment tends to be fairly stable (Boyd, 1995a) and it decomposes slowly. The Walkley-Black procedure used for measuring sediment organic carbon is not highly precise (Boyd, 1995a). Thus, it is not surprising that the soil incubation study (Table 8) did not reveal a significant reduction in soil organic carbon concentration over an 8-week period. Moreover, nitrate application would only stimulate decomposition of organic matter by denitrifying bacteria. It could be that the overall rate of sediment organic carbon mineralization is unrelated to the amount of nitrate present.

Nitrogen uptake by phytoplankton

The lowest abundance of phytoplankton (5 mg/m^3 chlorophyll *a*) consisted primarily of unidentified diatom species. These algae absorbed nitrate-nitrogen better than ammonia-nitrogen when provided in individual solutions. However, they actually absorbed ammonia-nitrogen better than nitrate-nitrogen from a mixed solution, but the total uptake of nitrogen from the mixed solution was less than for the nitrate solution (Fig. 22).

The next lowest abundance of phytoplankton was in a sample with 34 mg/m^3 of chlorophyll *a* (Fig. 22). This sample contained a mixture of green algae. These algae absorbed ammonia-nitrogen better than nitrate-nitrogen when provided single sources of the two nutrients. They did not absorb nitrate from the mixed solution, but absorbed about the same amount of ammonia-nitrogen as from the solution containing only ammonia-nitrogen.

The two samples with the greatest abundance of phytoplankton contained primarily a single species of *Anabaena* (a blue-green algae). The uptake of ammonia - nitrogen was roughly twice that of nitrate-nitrogen in the sample with 242 mg/m^3 of chlorophyll *a* (Fig. 22). The uptake of nitrogen in ammonium and nitrate were approximately in the same proportion in the mixture as in the individual solutions. In the sample with 812 mg/m^3 chlorophyll *a*, the uptake of nitrogen in the two forms was nearly equal from individual solutions and in the same proportion in the mixture.

The results suggest that the community of green algae used ammonium in preference to nitrate. However, diatom and blue-green algae communities appeared to use both forms.

Assessment of laboratory study

The laboratory studies did not reveal any appreciable effect of sodium nitrate treatment on the depletion rate of dissolved oxygen in sediment-water systems. Treatment with this substance did not have an influence on redox potential, pH, or organic carbon concentration in sediment samples.

Several authors have assumed that sodium nitrate treatment increased sediment redox potential (Ripl, 1976, Boyd, 1995b, Avnimelech and Zohar, 1986). It also seems logical that nitrate additions would stimulate denitrification, a process in which hydroxyl ion is produced in proportion to the amount of nitrate reduced, resulting in a greater sediment pH. There have not been studies to verify these assumptions, and laboratory results reported here do not support them. However, the findings of the pond study lend some credence to the assumption that nitrate additions will increase redox potential and pH of sediment.

INVE Technologies provided the results from a laboratory study in which sodium nitrate treatment of sediment from shrimp ponds greatly accelerated the decline in sediment chemical oxygen demand concentration during a 4-week incubation period (Boyd et al., 2007). In the sediment incubation trial of the present study, sodium nitrate treatment did not accelerate the decline in sediment organic carbon concentration.

Table 2. Grand means ± standard errors of water quality variables in channel catfish ponds treated with sodium nitrate and in control ponds.

Water quality parameters	Control	Nitrate
pH	7.98 ±0.08	8.00 ±0.09
Alkalinity (mg/L)	51 ±2	53 ±3
Turbidity (NTU)	112 ±10[a]	175 ±15[b]
Soluble reactive phosphorus (mg/L)	0.022 ±0.001[a]	0.041 ±0.004[b]
Total phosphorus (mg/L)	0.12 ±0.01[a]	0.20 ±0.02[b]
Ammonia-nitrogen (mg/L)	0.106 ±0.027	0.073 ±0.016
Nitrate-nitrogen (mg/L)	0.133 ±0.024[a]	0.299 ±0.07[b]
Total nitrogen (mg/L)	0.90 ±0.06[a]	1.30 ±0.11[b]
Secchi disk visibility (cm)	25 ±2[b]	19 ±2[a]
Dissolved oxygen (mg/L)	6.84 ±0.19	6.85 ±0.29
Chlorophyll *a* (mg/m3)	27 ±3[a]	75 ±18[b]

Means in horizontal represented by the different superscript letters were statistical difference at the 0.1 probability level.

Table 3. The number of samples containing different genera of algae found in sodium-nitrate treated ponds and control ponds. There were three replications and 14 sampling dates for a trial of 42 samples from both treatment and control.

Phylum	Genus	Nitrate	Control
Chlorophyta	*Chlamydomonas*	4	1
	Pandorina	7	4
	Gloeocystis	24	20
	Sphaerocystis	2	4
	Pediastrum	20	4
	Coelastrum	26	20
	Ankistrodesmus	5	2
	Chlorella	8	5
	Kirchneriella	1	2
	Oocystis	2	1
	Schroederia	2	1
	Selenastrum	3	-
	Actinastrum	1	2
	Crucigenia	4	4
	Scenedesmus	5	4
	Arthrodesmus	-	1
	Closterium	4	3
	Cosmarium	-	1
	Staurastrum	4	4
Euglenophyta	*Euglena*	1	-
	Trachelomonas	3	8
Pyrrhophyta	*Ceratium*	2	2
Cryptophyta	*Cryptomonas*	3	3
Chrysophyta	*Melosira*	11	17
	Synedra	1	2
Cyanophyta	*Microcystis*	42	42
	Lyngbya	-	1
	Oscillatoria	22	32
	Trichodesmium	1	1
	Anabaena	7	11

Table 4. Margalef diversity indices (H) of genera of phytoplankton found in channel catfish ponds treated with sodium nitrate and in control ponds. Differences between mean did not differ at the 0.1 probability level.

	H	
Dates	Treated ponds	Control ponds
13-Apr-05	0.69	1.07
11-May-05	1.34	1.30
23-May-05	0.71	0.63
6-Jun-05	0.87	0.80
21-Jun-05	0.74	0.49
5-Jul-05	0.90	0.61
19-Jul-05	0.17	0.37
2-Aug-05	0.69	0.53
16-Aug-05	0.72	0.60
30-Aug-05	0.66	0.47
13-Sep-05	0.72	0.53
27-Sep-05	0.71	0.71
11-Oct-05	0.65	0.77
26-Oct-05	0.38	0.72
Average	0.71	0.69

Table 5. Means ± standard errors of organic carbon concentration, redox potential and pH of sediment in ponds treated with sodium nitrate and in control ponds before stocking (beginning) and before harvest (end).

Variables	Control			Nitrate		
	Beginning	End	Δ	Beginning	End	Δ
Organic carbon (%)	0.93±0.13	1±0.06	0.07	0.82±0.02	1.06±0.07	0.23
Redox potential (mV)	107±45	-149±6	-2.57^{b}	32±16	-113±27	-1.45^{a}
pH	6.33±0.08	6.47±0.12	0.14	6.43±0.06	6.59±0.11	0.16

The different superscript letters between Δ of control and sodium nitrate treatment indicate statistical difference at the 0.1 probability level.

Table 6. Means ± standard errors of channel catfish production, amount of feed used, survival rate, and feed conversion ratio from ponds treated with sodium nitrate and in control ponds.

Variables	Control	Nitrate
Initial fingering weight (kg/ha)	418 ±0.2	420 ±0.2
Feed used (kg/ha)	5,014 ±4.5	4825 ±6
Survival rate (%)	79 ±4	86 ±3
Catfish production (kg/ha)	3,388 ±9.5	3,860 ±7.7
Feed conversion ratio	1.48 ±0.13	1.25 ±0.13

Means did not differ between control and treated ponds at the 0.1 probability level.

Table 7. Mean nitrate-nitrogen concentrations (mg/L) remaining in BOD bottles that contained 2 g of sediment and different, initial nitrate-nitrogen concentrations.

Days	Nitrate-nitrogen concentrations (mg/L)			
	0	1	5	10
1	0.0 [d]	0.9 [c]	5.4 [b]	10.5 [a]
2	0.0 [d]	1.0 [c]	5.9 [b]	11.0 [a]
4	0.0 [d]	1.1 [c]	6.3 [b]	11.7 [a]
6	0.0 [d]	1.2 [c]	6.5 [b]	11.6 [a]
8	0.0 [d]	1.3 [c]	7.1 [b]	12.2 [a]
12	0.1 [d]	1.7 [c]	7.5 [b]	12.6 [a]
16	0.1 [d]	1.7 [c]	7.5 [b]	13.0 [a]
20	0.0 [d]	1.8 [c]	7.9 [b]	13.6 [a]
24	0.3 [d]	2.0 [c]	7.9 [b]	13.2 [a]
28	0.4 [d]	1.8 [c]	7.2 [b]	12.4 [a]

Means in horizontal represented by the different superscript letters were statistical difference at the 0.05 probability level.

Table 8. Mean organic carbon concentration (%) in sediment treated with different nitrate-nitrogen concentrations for different time periods.

Weeks	Nitrate-nitrogen concentrations (mg/L)						
	0	1	2	4	8	16	32
0	1.02	1.06	1.03	1.05	1.06	1.09	1.05
1	1.04	1.03	0.97	1.04	1.05	1.08	1.05
2	1.06	1.07	1.07	1.09	1.09	1.08	1.10
4	0.99	1.00	1.00	1.02	1.00	1.01	1.00
8	1.05	1.00	1.03	1.01	1.03	1.01	1.01

Means did not differ between nitrate-nitrogen concentration and times at the 0.05 probability level.

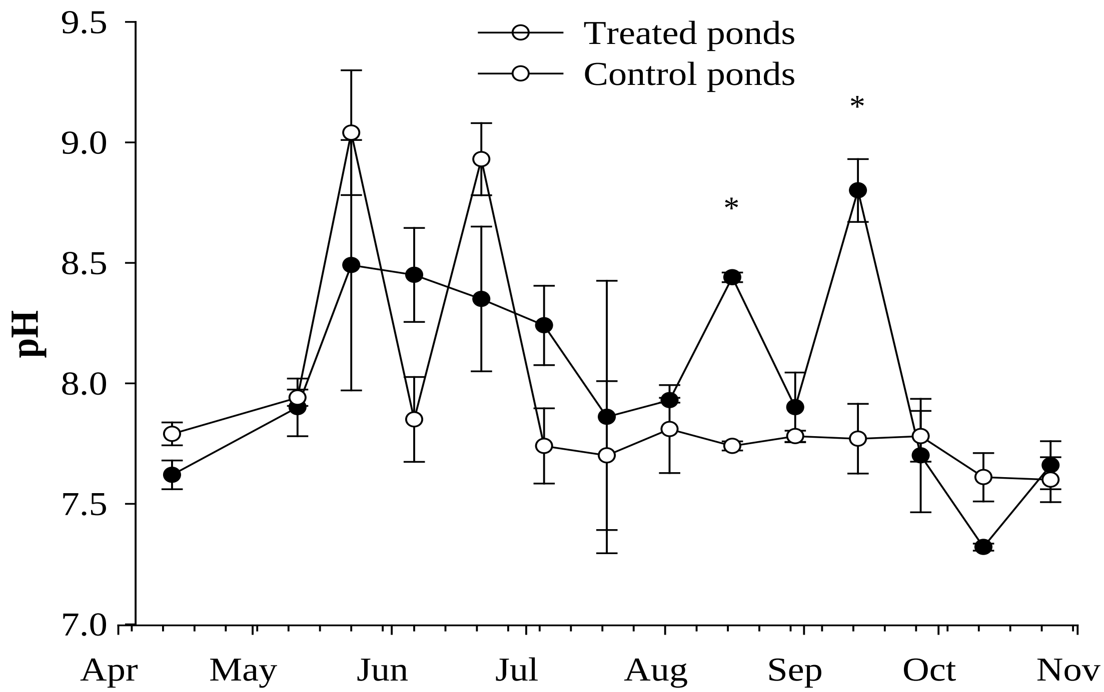

Figure 1. Mean pH and standard errors in channel catfish ponds treated with sodium nitrate and in control ponds. The asterisks denote statistical difference at the 0.1 probability level.

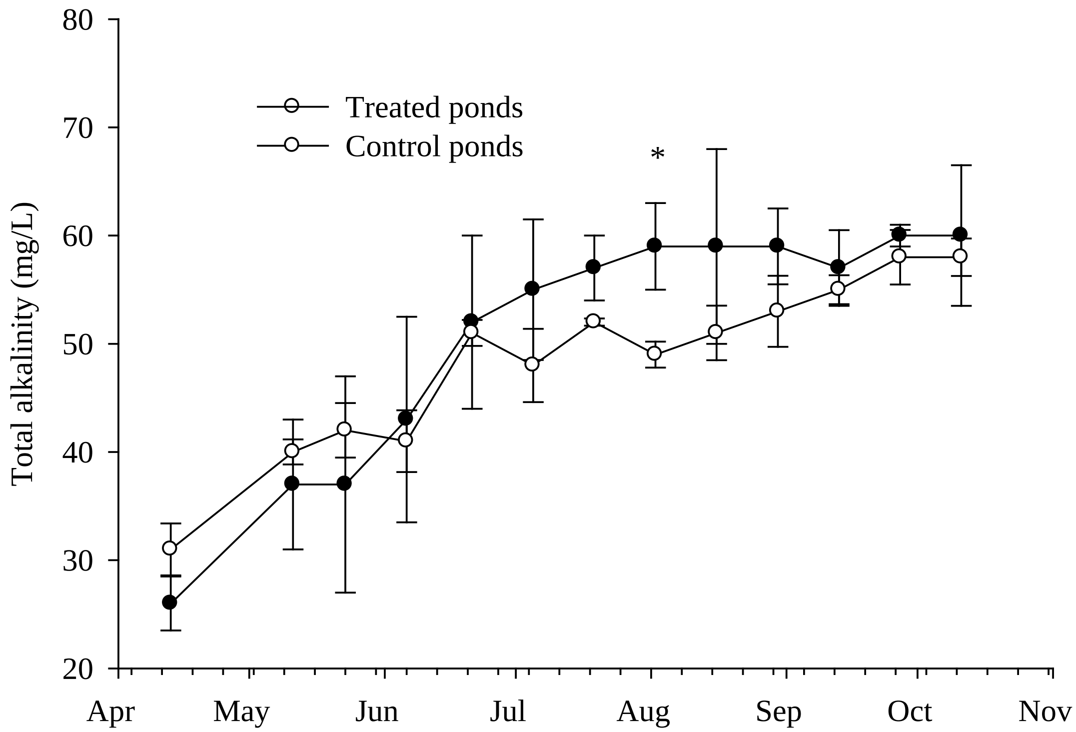

Figure 2. Mean total alkalinity concentrations and standard errors in channel catfish ponds treated with sodium nitrate and in control ponds. The asterisk denotes statistical difference at the 0.1 probability level.

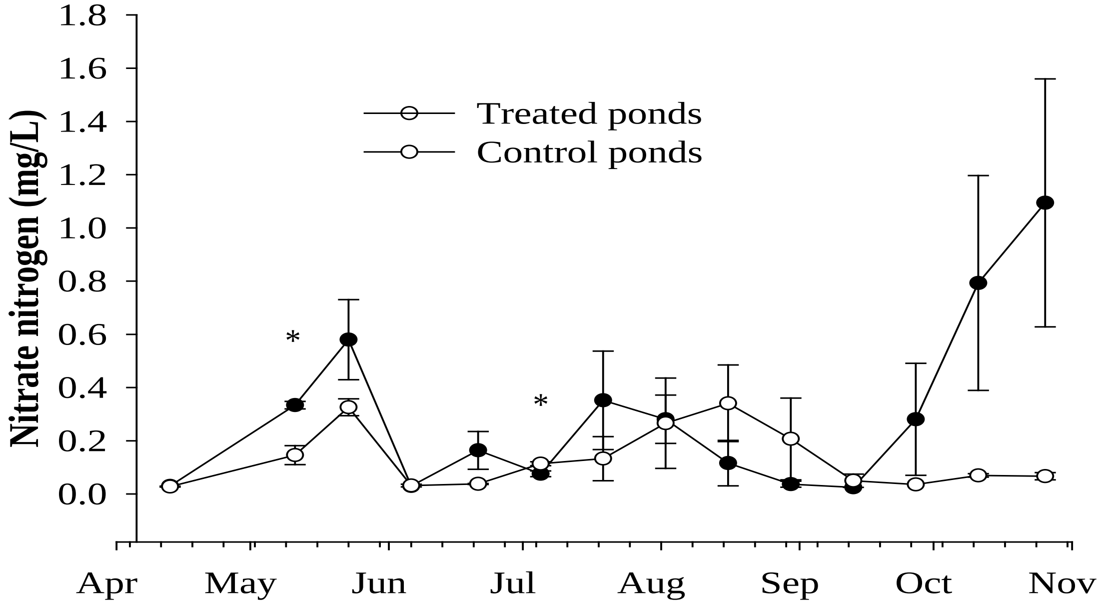

Figure 3. Mean nitrate nitrogen concentrations and standard errors in channel catfish ponds treated with sodium nitrate and in control ponds. The asterisks denote statistical difference at the 0.1 probability level.

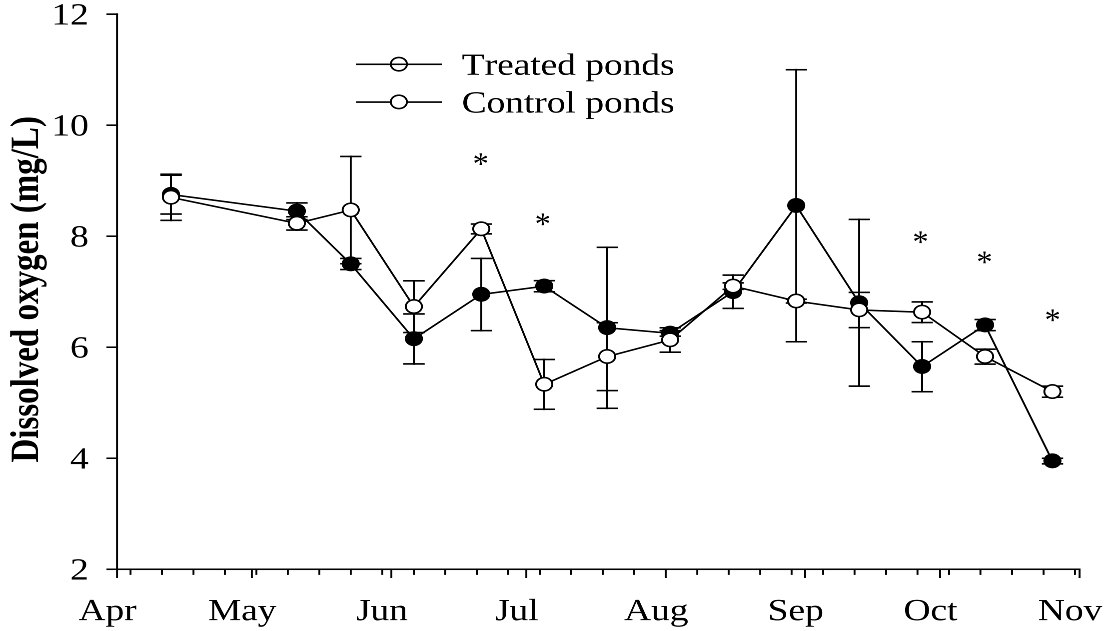

Figure 4. Mean dissolved oxygen concentrations and standard errors in channel catfish ponds treated with sodium nitrate and in control ponds. The asterisks denote statistical difference at the 0.1 probability level.

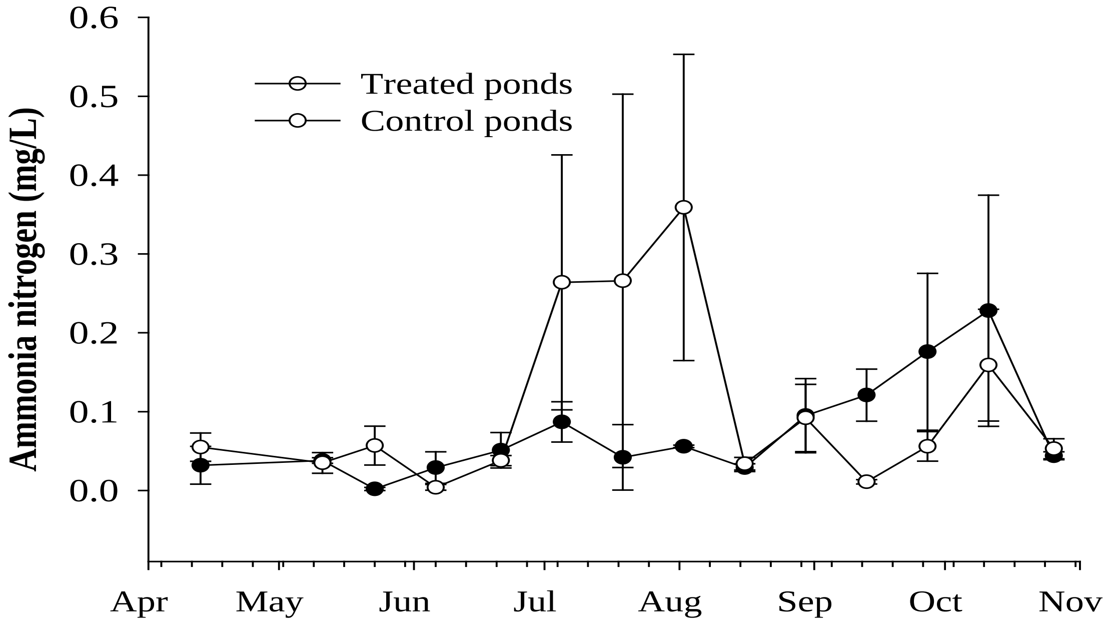

Figure 5. Mean ammonia nitrogen concentrations and standard errors in channel catfish ponds treated with sodium nitrate and in control ponds.

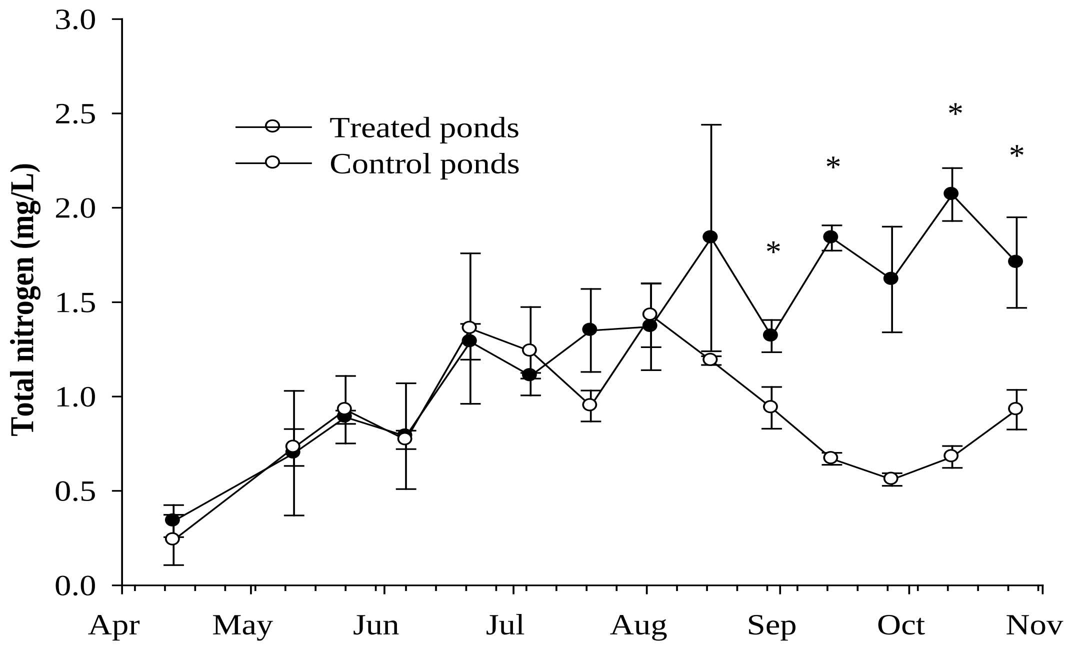

Figure 6. Mean total nitrogen concentrations and standard errors in channel catfish ponds treated with sodium nitrate and in control ponds. The asterisks denote statistical difference at the 0.1 probability level.

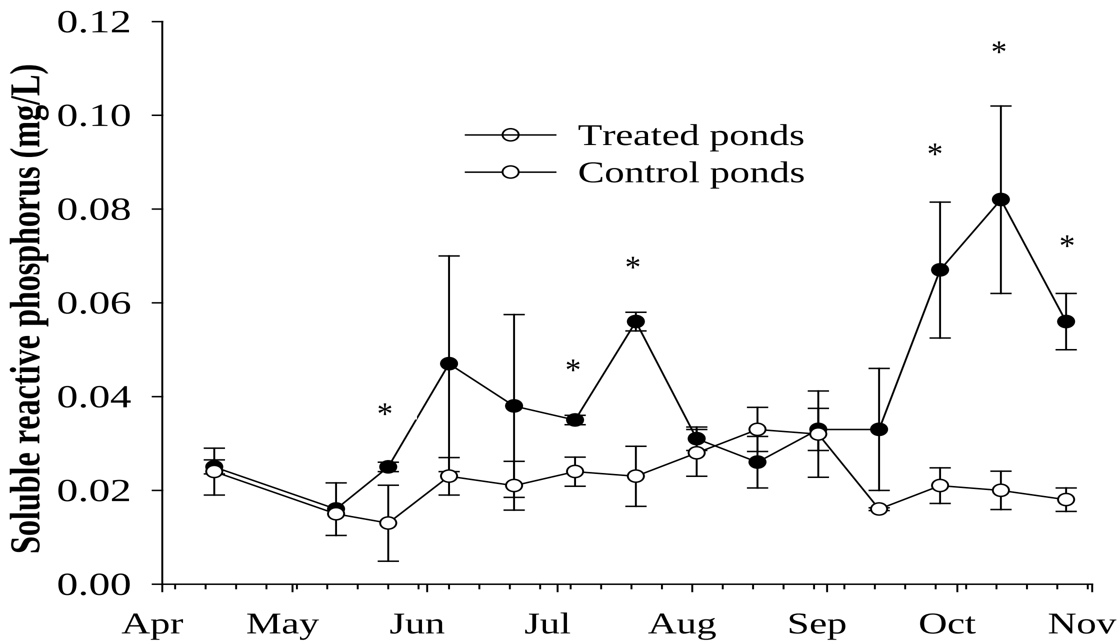

Figure 7. Mean soluble reactive phosphorus concentrations and standard errors in channel catfish ponds treated with sodium nitrate and in control ponds. The asterisks denote statistical difference at the 0.1 probability level.

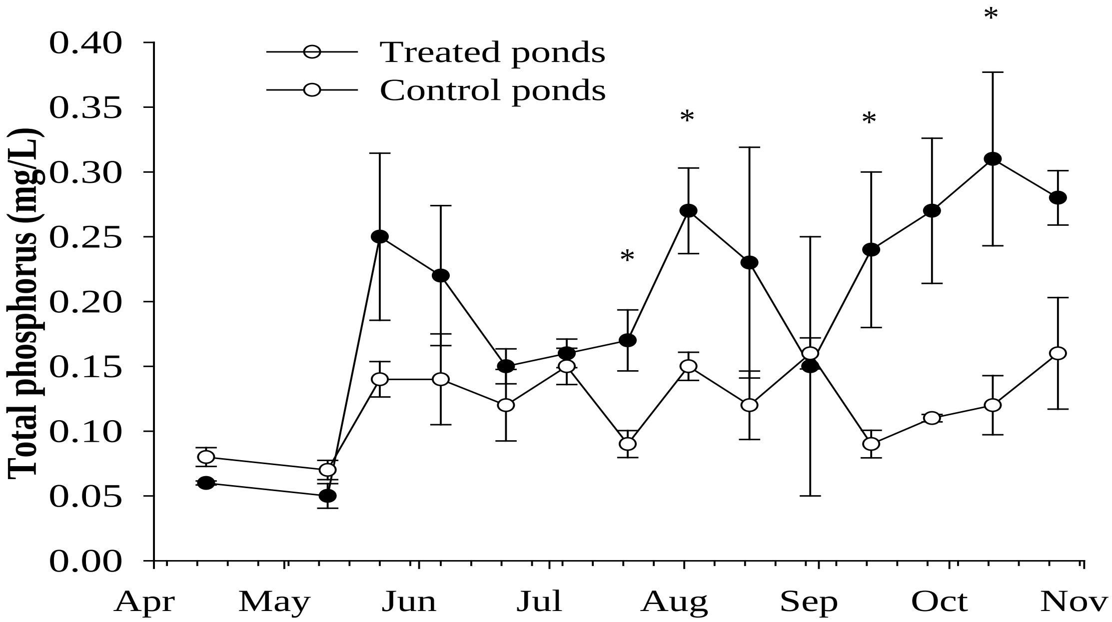

Figure 8. Mean total phosphorus concentrations and standard errors in channel catfish ponds treated with sodium nitrate and in control ponds. The asterisks denote statistical difference at the 0.1 probability level.

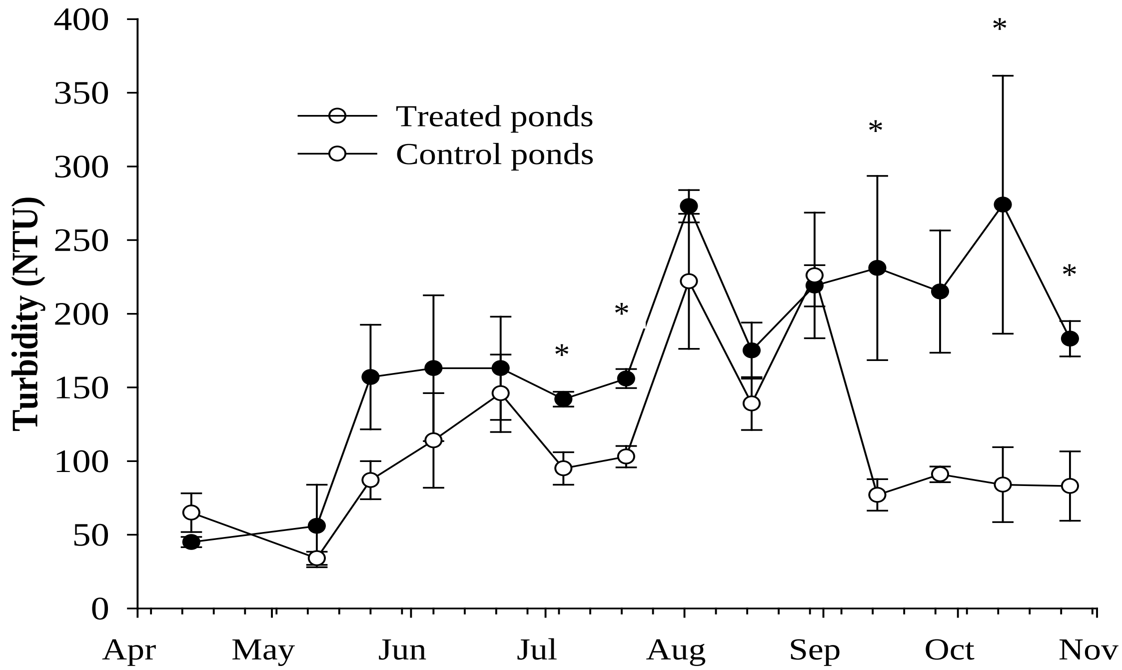

Figure 9. Mean turbidity concentrations and standard errors in channel catfish ponds treated with sodium nitrate and in control ponds. The asterisks denote statistical difference at the 0.1 probability level.

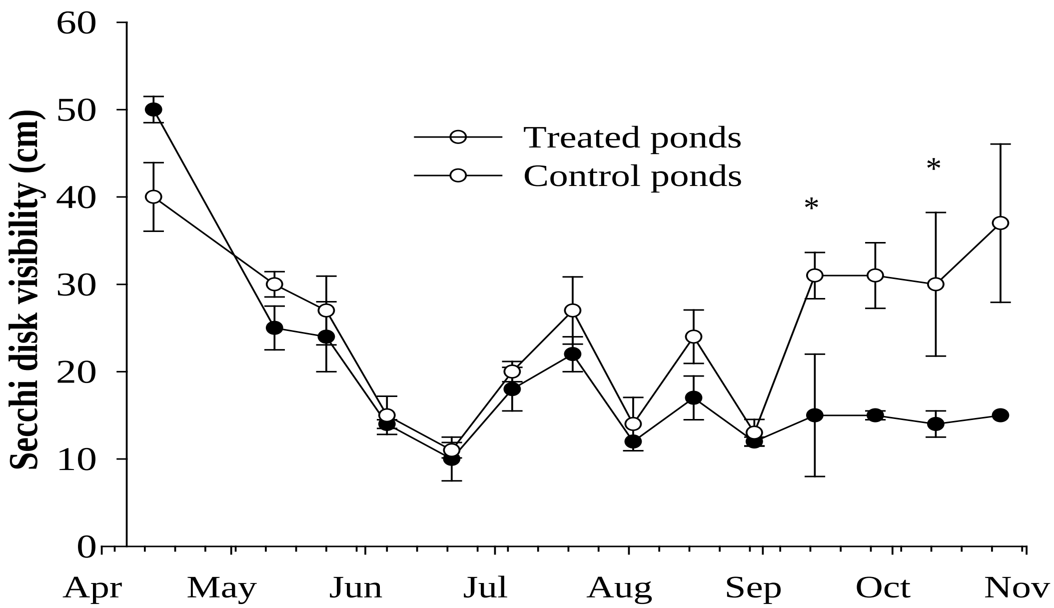

Figure 10. Mean secchi disk visibility and standard errors in channel catfish ponds treated with sodium nitrate and in control ponds. The asterisks denotes statistical difference at the 0.1 probability level.

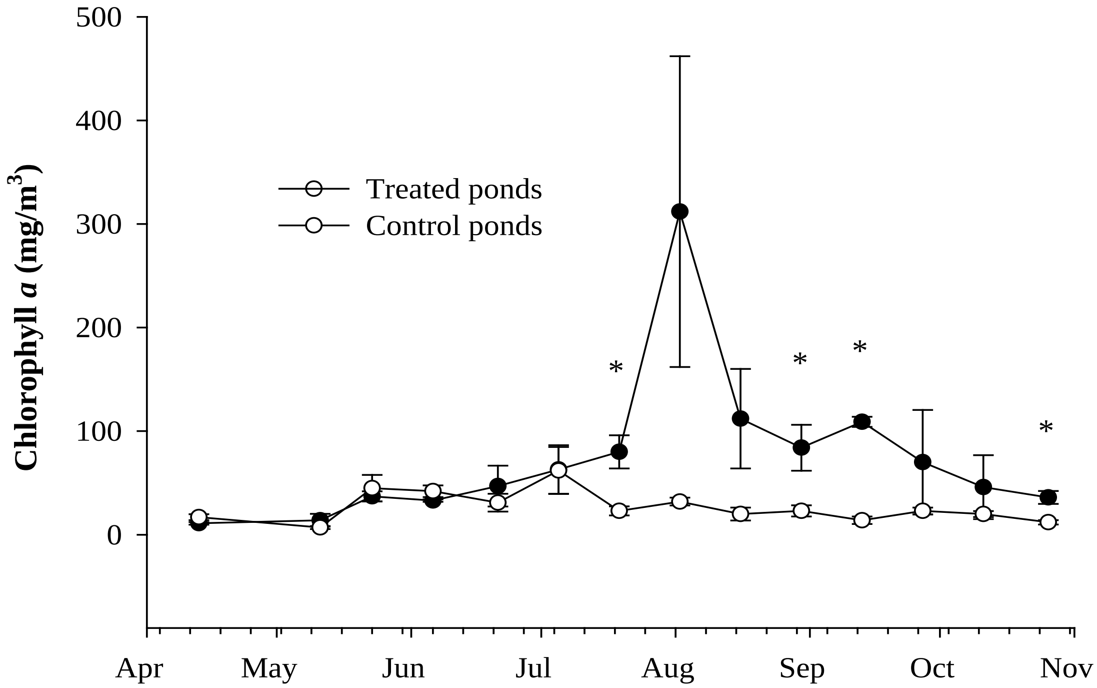

Figure 11. Mean chlorophyll *a* concentrations and standard errors in channel catfish ponds treated with sodium nitrate and in control ponds. The asterisks denote statistical difference at the 0.1 probability level.

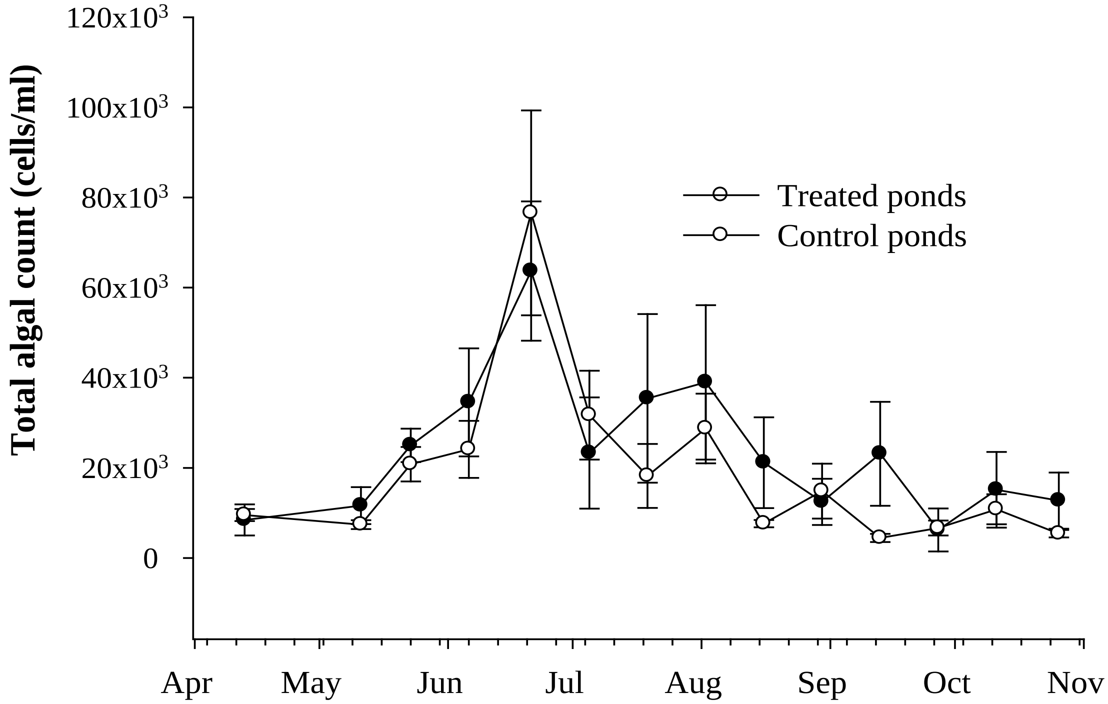

Figure 12. Mean total abundance of phytoplankton individuals in sodium nitrate-treated and control ponds during the period of study. Means did not differ statistically between treated and control ponds (P>0.1).

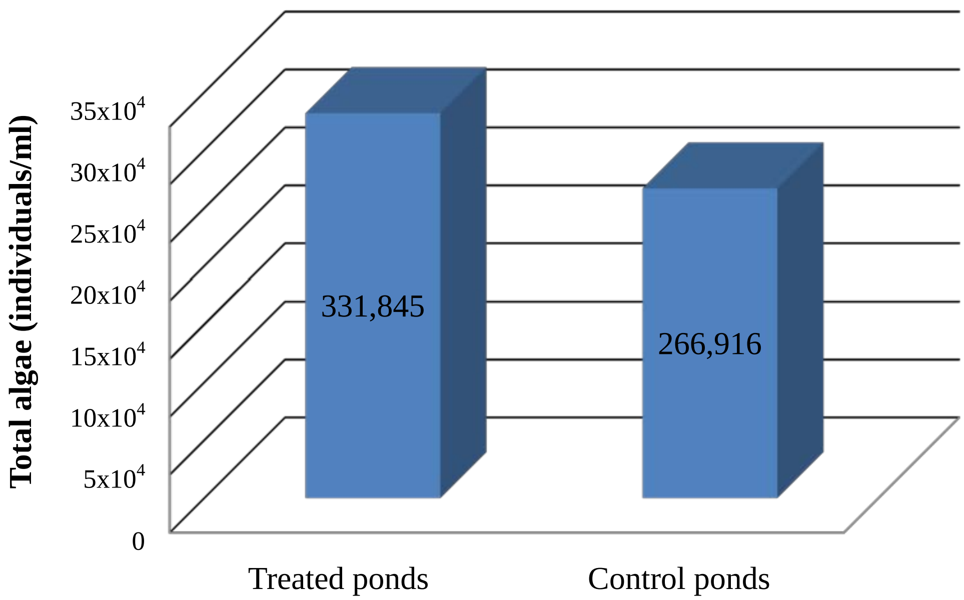

Figure 13. Grand mean total algae abundance in channel catfish ponds treated with sodium nitrate and in control ponds. There was no statistically significant difference between treated and control ponds (P>0.1).

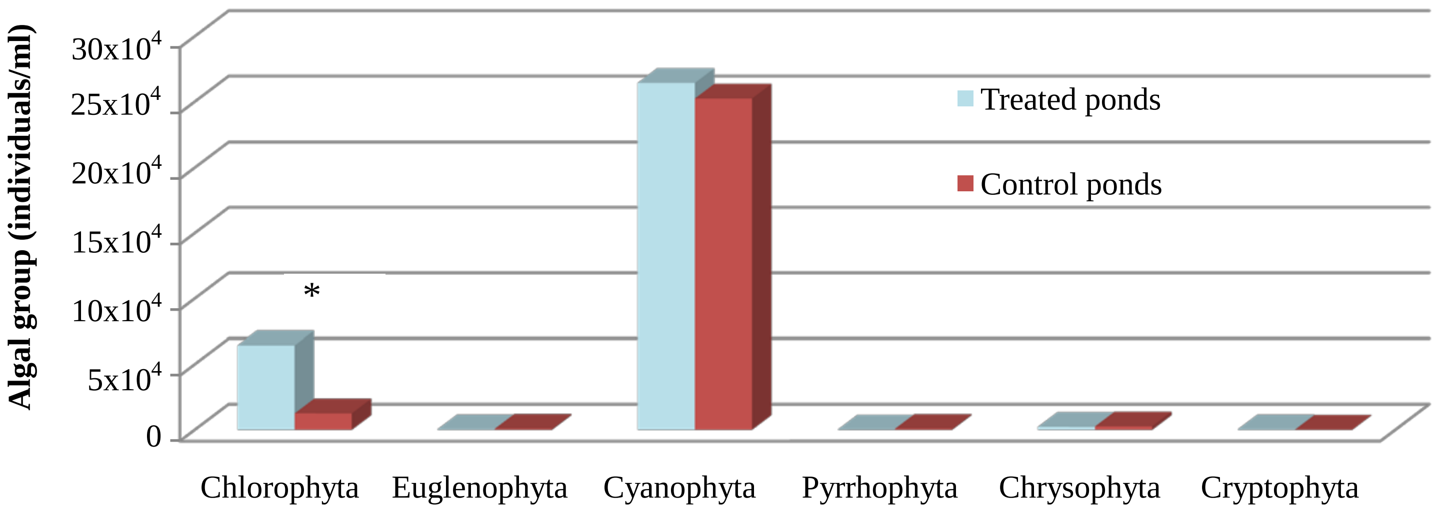

Figure 14. Grand mean abundance of major algal taxons in sodium nitrate-treated and control ponds during the study. The asterisk denotes statistical difference at the 0.1 probability level.

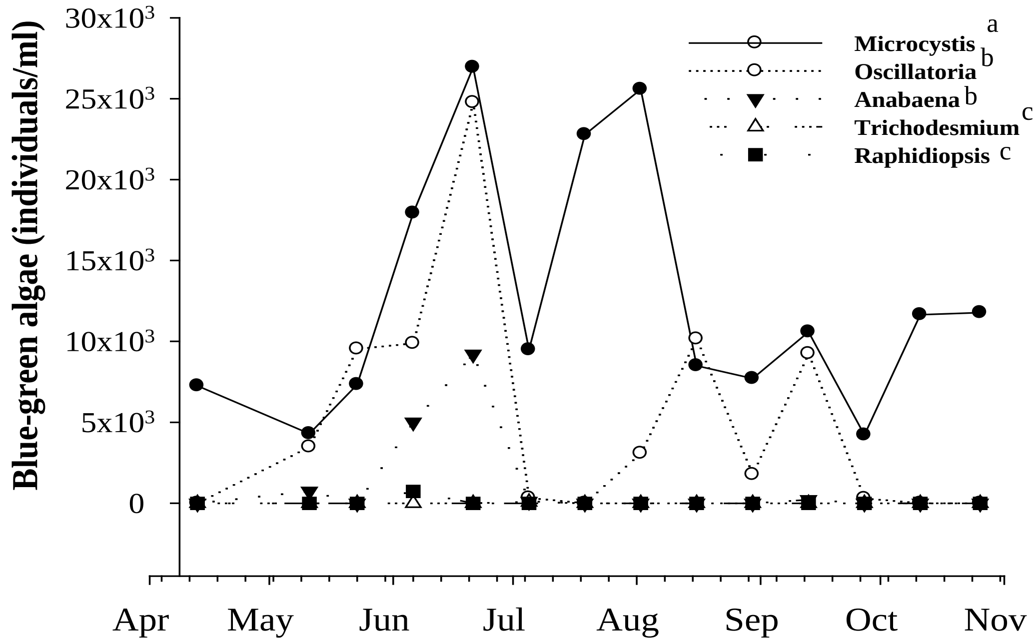

Figure 15. Mean abundance of genera of blue-green algae found in sodium nitrate-treated ponds during the period of study. The different superscript letters indicate statistical difference among grand mean for abundance of genera of blue-green algae at the 0.1 probability level.

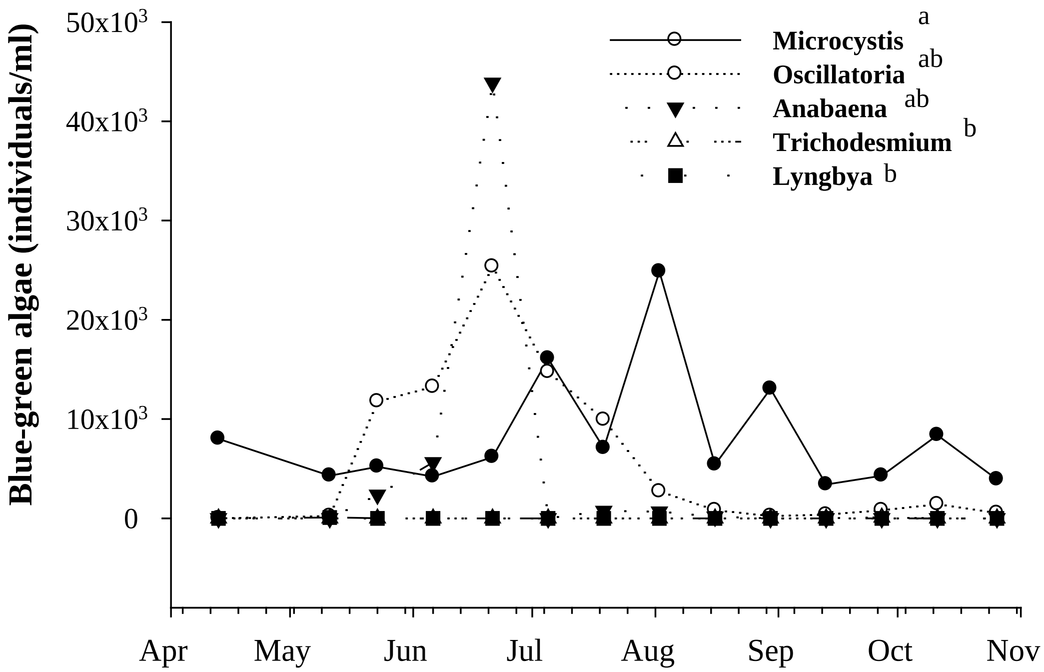

Figure 16. Mean abundance of genera of blue-green algae found in control ponds during the period of study. The different superscript letters indicate statistical difference among grand mean for abundance of genera of blue-green algae at the 0.1 probability level.

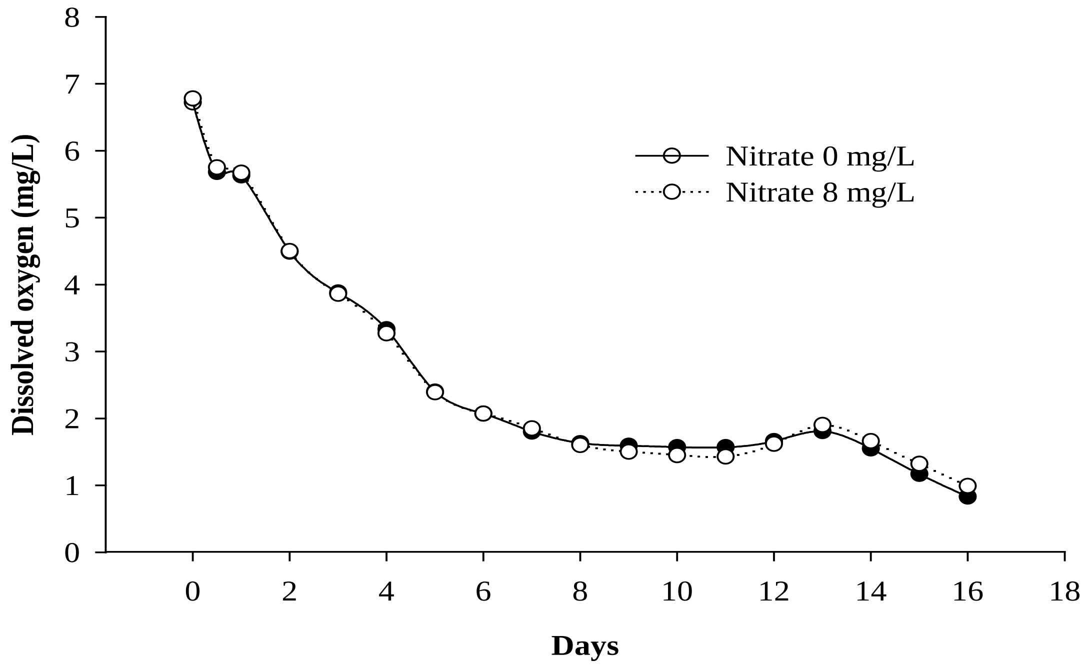

Figure 17. Mean dissolved oxygen concentrations in tap water containing 0 and 8 mg/L of nitrate-nitrogen for different incubation periods in BOD bottles to which a 2-g layer of sediment had been added.

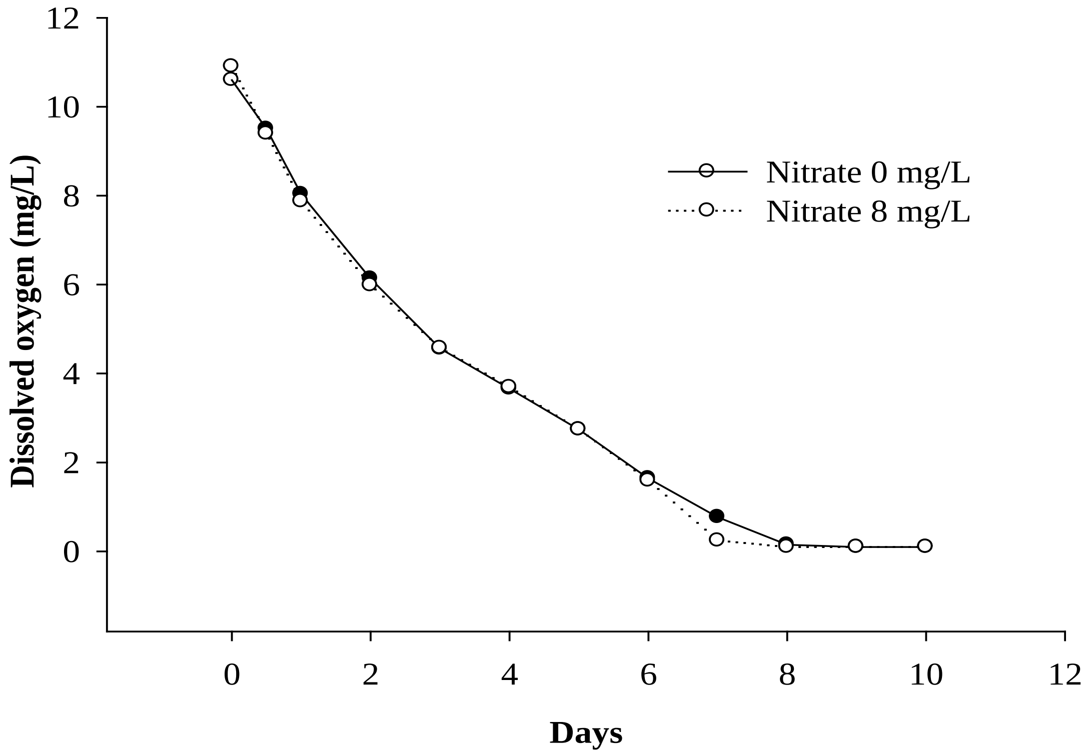

Figure 18. Mean dissolved oxygen concentrations in pond water containing a moderate plankton bloom at 0 and 8 mg/L of nitrate-nitrogen in BOD bottles for different incubation periods.

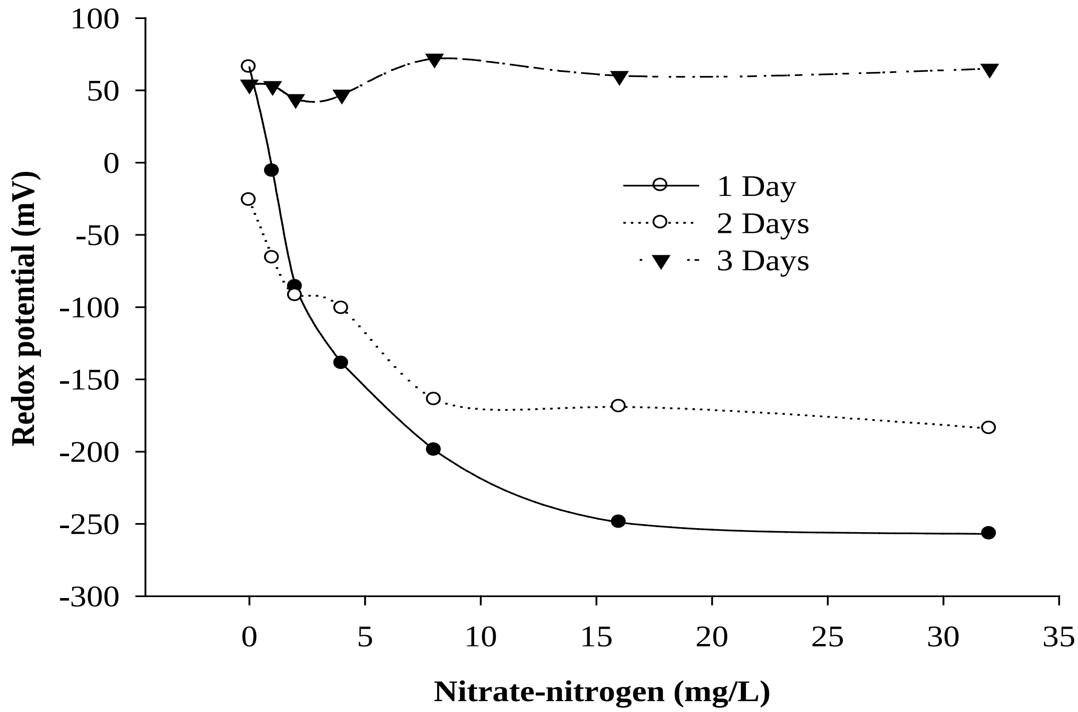

Figure. 19. Mean redox potential in laboratory water-sediment systems at 1, 2, and 3 days at different concentrations of nitrate-nitrogen.

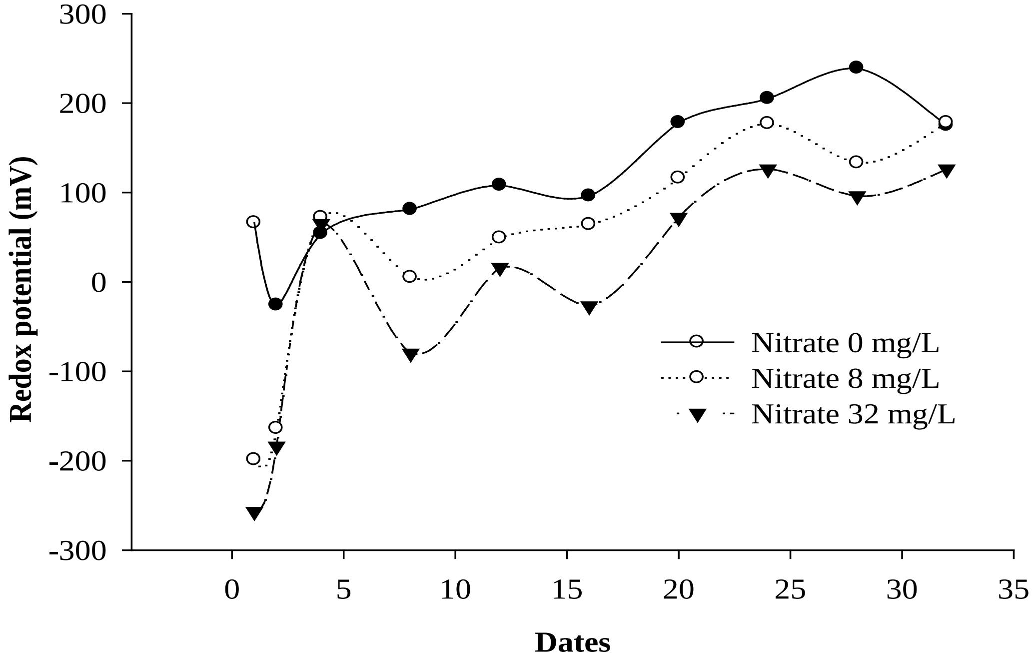

Figure 20. Mean redox potential in laboratory water-sediment systems following addition of 0, 8, and 32 mg/L of nitrate-nitrogen.

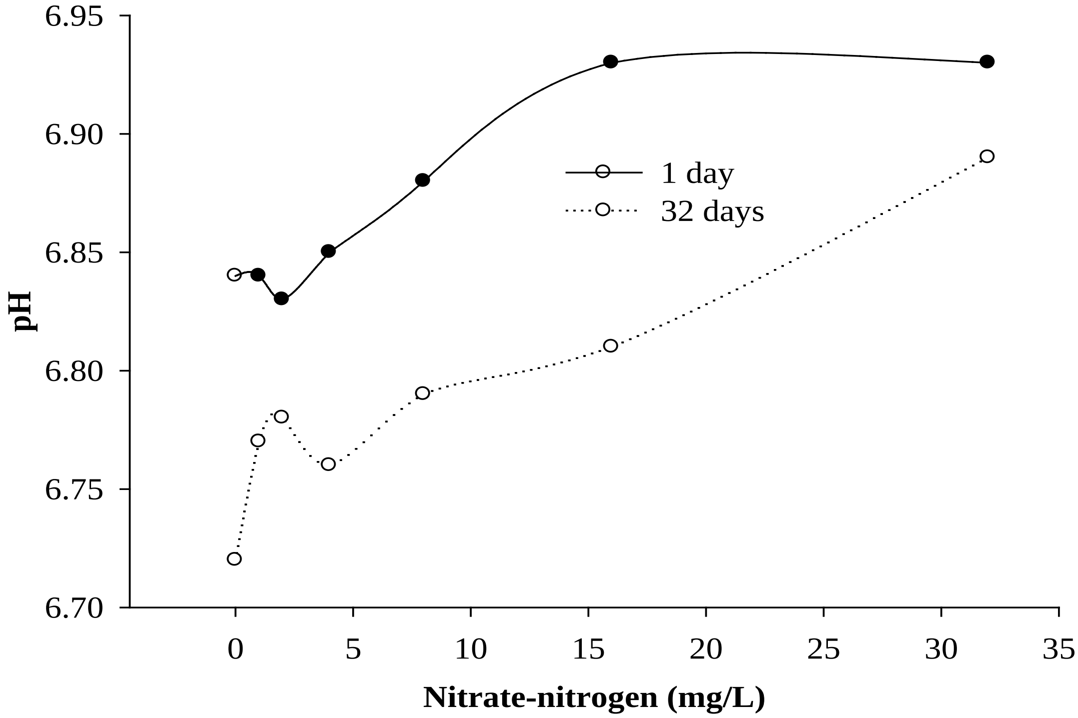

Figure 21. Mean pH in laboratory water-sediment systems at 1 and 32 days after treatment with different concentrations of nitrate-nitrogen.

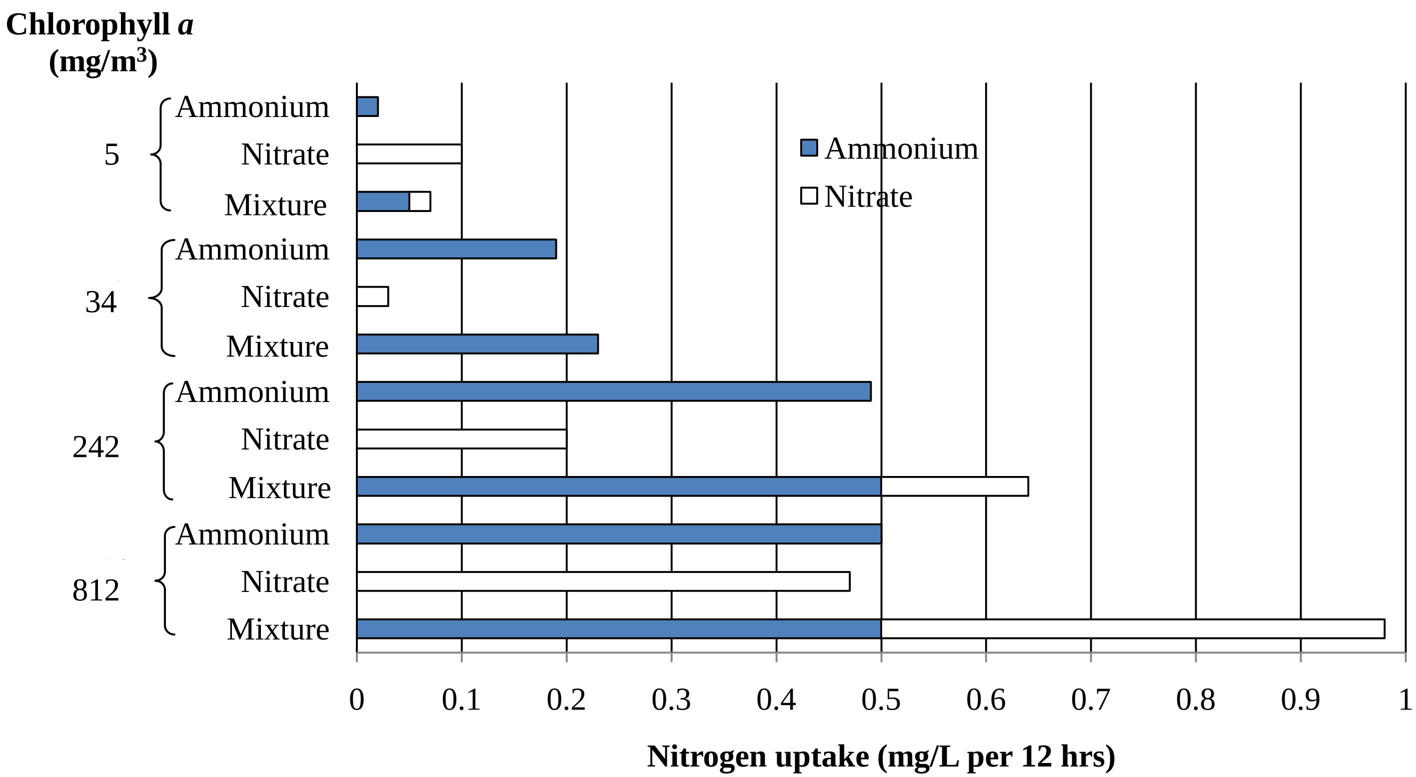

Figure 22. Rate of nitrogen uptake from solution containing 0.5 mg/L ammonium-nitrogen, 0.5 mg/L nitrate-nitrogen, or 0.5 mg/L ammonium-nitrogen + 0.5 mg/L nitrate-nitrogen (mixture) during 12 hr exposure in natural daylight.

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