Investigations of Ammonia Nitrogen in Aquaculture：the Methodology, Concentrations, Removal, and Pond Fertilization

by

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ABSTRACT

Four investigations were performed related to ammonia nitrogen in aquaculture.

The first investigation compared the precision and accuracy of (1) Nessler, (2) phenate, (3) salicylate, and (4) ammonia electrode procedures for total ammonia nitrogen (TAN) concentration determination in waters of aquaculture. Salicylate method was selected as a standard method for its high precision and accuracy. Addition of Rochelle salt increased the precision and accuracy for TAN analyses by the Nessler method. TAN analyses by the phenate I and the salicylate methods were not different in freshwater. The salicylate kit method is a suitable alternative to the standard salicylate method, while the Nessler kit is not. Electrodes for sensing NH_3 and NH_4^+ were less precise or accurate in most cases.

The second investigation assessed TANconcentration in 31ictalurid catfishponds on six farms, in the Blackland Prairie region of Alabama (USA). Concentrations of TAN were measured 26 times (weekly June through September and less frequently other months) between May 2013 and May 2014. The farm average, annual TAN concentrations were 1.05-1.78 mg L^{-1} at five farms and 4.17 mg L^{-1} at the other. Nearly half of the TAN concentrations was< 1 mg L^{-1}, the majority was<5 mg L^{-1}, but some ranged from 5 to 15 mg L^{-1}.Analysis of the literature on ammonia toxicity to channel catfish suggested that the no-observed-effect level (NOEL) is around 1.0 mg L^{-1} NH_3-N in ponds with pH of 7.5 and above where NH_3-N concentration fluctuates greatly because of daily change in temperature and especially pH.The findings reveal that TAN concentrations often are at chronically toxic levels for ictalurid catfish in Alabama ponds. There usually is no practical emergency treatment for reducing

NH$_3$-N (or TAN) concentration in ponds exceeding the NOEL. Use of good management practices is recommended to avoid excessively high TAN concentrations.

The third investigation studied the effectiveness of two processed samples of New Zealand mordenite for possible use in removing total ammonia nitrogen (TAN) in aquaculture application.The percentage reduction in TAN concentration in 100-mL solutions held on a rotating shaker increased linearly with greater mordenite application rate, while the amount of TAN removed per gram of mordenite (adsorptive efficiency) declined. Ammonia removal and adsorptive efficiency decreased with increasing salinity up to 30 g L^{-1}. Although mordenite is capable of reducing TAN concentration in water of laboratory tests, it probably is not highly effective for this purpose in aquaculture ponds.

The fourth investigation studied the bluegill yield in response to nitrogen and phosphorus versus phosphorus-only fertilization in ponds at different times since sediment removal. The experiment was conducted in 40-yr-old research ponds at the Auburn University E. W. Shell Fisheries Center from which sediment had been removed 2 to 9yr earlier to restore bottoms nearly to their original soil composition. Bluegill production was uncorrelated with time since sediment removal in ponds treated only with phosphate fertilizer.Soluble reactive phosphorus and total ammonia concentration were correlated with time since sediment removal(R^2 = 0.312 and R^2 = 0.514, respectively, P < 0.05).Results suggest pond managers might omit nitrogen fertilizer if phytoplankton blooms do not waneafter only 2 yr of fertilization with nitrogen and phosphorus.

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I. INTRODUCTION

Aquaculture, accounts for nearly half of world's food fish production, is one of the fastest growing animal food sectors with its development, extension, and intensification in almost every possible region of the world. Global food fish aquaculture production volume increased at an average rate of 6.2 % per year between 2000 and 2012, more than doubled from 32.4 million tonnes to 66.6 million tonnes. Global capture fishery although with a production volume of 91.3 million tonnes in 2013, is considered to be stagnant.Many stocks of the most productive species in world marine capture fishery are fully fished or overfished. Therefore, with the global population growing by another 2 billion by 2050, aquaculture still needs to reach 80 million tonnes to maintain the current level of per capita consumption according to FAO's estimates.

The use of increasingly intensive grow-out is a major reason for the increasing production in aquaculture. Maintaining good water quality is of big concern in intensive aquaculture systems. Ammonia nitrogen, resulting from high feeding rates in semi-intensive and intensive systems, is one of the most important water quality variables that affect fish behavior and health. It is the nextmost important factor after low dissolved oxygen in limiting the amount offish that can be produced in a culture system. High concentrations of ammonia nitrogen can be stressful or even lethal to fish.

Reliable measurement of TAN concentration is very important for farmers to manage ponds and other culture systems or for scientists doing research on

ammonia nitrogen dynamics and effects in aquaculture systems. There are four basic methods for measuring total ammonia nitrogen (TAN) in water: Nesslers; phenate; salicylate; ammonia electrode. The Nesslers method may be conducted with and without use of Rochelle salt solution, and there are two types of ammonia electrodes – one senses NH_3 and the other senses NH_4^+. Nesslers and salicylate methods are the basis for ammonia test kits used by some farmers. The first investigation (Chapter III) evaluates the precision and accuracy of different possible ways of measuring TAN concentrations at different aquaculture facilities in Alabama. The goal is to reveal the most suitable method for saline water and freshwaterand provide recommendations on the methods that should be used for determining TAN concentrations in water samples.

Channel catfish *Ictaluruspunctatus*is one of important culture species in Southeastern United States with steadily increasing production for more than two decades. The high densities of culture species and high feeding rates result in higher than desirable TAN concentrations. Some farmers even reported high TAN concentration of 10 mg L^{-1} with ammonia kits in catfish ponds. The second investigation was conducted to determine the range and fluctuation in TAN concentrations in Alabama commercial catfish ponds on a one-year basis and to assess the possible risk of ammonia toxicity to catfish.

Ammonia nitrogen can be removed from water through air stripping, absorption, ion exchange, and biological methods. Zeolite, used as an ion exchange medium, is widely applied to ponds in Asia to remove ammonia nitrogen. However, there is no research findings support this practice. The third investigation was conducted in a laboratory trial to evaluate the ammonia nitrogen removal efficiency of two New Zealand zeolites – mordenite under different conditions of water quality and to ascertain if the two New Zealand mordenite has potential to lower TAN concentrations of aquaculture systems.

Excess load of nitrogen accompanied with phosphorus in effluent is the primary cause for eutrophication. Avoiding overuse of nitrogen fertilizer such as ammonia nitrate，ammonia phosphate, and urea in aquaculture is one possible way to reduce the potential of eutrophication. Nitrogen fertilizer has reported to be of little benefit in promoting some culture species production in ponds with a 15-yr history of complete fertilization. The process of nitrification of blue-green algae and nitrogen minimization of organic matter accumulated from pond bottoms provide sufficient nitrogen source in older ponds. In the fourth investigation, seventeen sportfish ponds that had been fertilized for 2 to 9 years since renovation were selected to evaluate the response to nitrogen plus phosphorus fertilization and phosphorus-only fertilization.The goal was to verify whether nitrogen fertilization in older ponds was needed. Results suggest that nitrogen fertilization of sportfish ponds possibly can be ceased after only 2 yr of fertilization with nitrogen and phosphorus.

II. REVIEW OF LITERATURE

Ammonia nitrogen is a nutrient in aquaculture

Nitrogen is an essential nutrient for all creatures in the world to grow and live. It is a component of vital organic compounds such as amino acids, protein and DNA in organisms. Nitrogen gas (N_2), although accounts for 78% of the air by volume, cannot be directly absorbed as nutrition by organisms. In aquatic environment, ammonia nitrogen is one of important N forms that can be used by phytoplankton, algae, plants, heterotrophic bacteriaand nitrifying bacteria (Montoya and Velasco,2000).

The ammonia nitrogen in aquaculture systems primarily comes from fertilizers such as urea, ammonia phosphate and ammonia nitrate, fish via their waste and organic matter decomposition (Boyd, 2001). Application of nitrogen fertilization promotes primary productivity therefore enhances the production of the fish depend on the development of autotrophic food webs (Boyd et al., 2008). In feed-based aquaculture, about 20–40% of nitrogen in feed is recovered in harvest biomass. The remaining nitrogen can be regarded as N excretion to N flow in aquaculture, which consists of 62% of dissolved N and 13% of particulate N (Folke and Kautsky, 1989). Mineralization of organic matter from pond bottom is another important source of ammonia. Hargreaves (1997) developed a mechanistic model to simulate annual variation of ammonia concentration in commercial catfish ponds in the southeastern United States. The model estimated that 25 to 33% of the ammonia in water came from the sediment. Ammonia can also be added to ponds in process of nitrogen fixation by heterocystos cyanobacteria. Acosta-Nassar et al. (1994) reported a 10% of N input by

nitrogen fixation in a tropical freshwater fish pond.

Generally, phytoplankton is considered to uptake most of ammonia nitrogen from water. Phytoplankton prefers ammonia to nitrate as inorganic N source because nitrate assimilation and incorporation is more energy-consuming (Hargreaves, 1998). Ammonia concentrations are inversely related to phytoplankton density, increasing dramatically during phytoplankton die-off and declining as phytoplankton increase (Krom et al., 1989; Tucker et al., 1984). Another principle sink for ammonia is nitrification. Nitrification rate might exceed phytoplankton uptake during cooler months (Hargreaves, 1997). Ammonia nitrogen could also be removed from aquaculture systems through volatilization. Lorenzen et al. (1997) estimated a 30% and 8% of added N were lost through volatilization from an intensive shrimp pond and semi-intensive shrimp pond, respectively. NH_4^+ could be weakly absorbed to the clay minerals or organic matter through ion exchange in the sediment. Acosta-Nassaret al. (1994) found that roughly 2% of the added N in a freshwater fish pond was absorbed in the sediment.

Ammonia nitrogen is a toxin in culture system

Ammonia nitrogen occurs in water as un-ionized ammonia (NH_3) and ammonium ion (NH_4^+)：

$$NH_3 + H_2O = NH_4^+ + OH^- \qquad\qquad K_b = 10^{-4.74} \qquad (1)$$

The ratio NH_3:NH_4^+ increases with greater pH as obvious from Eq. 1. Moreover, examination of K_b of Eq. 1 for different temperatures (Bates and Pinching, 1949) shows that the NH_3:NH_4^+ ratio also increases with rising temperature. The concentrations of each of the two forms can be calculated with Eq. 1 using the measured pH and the appropriate K_b for the observed water temperature. However, convenient tables for estimating the percentage of TAN present as NH_3-N at different pHs and water temperatures are available (Trussell, 1972;

Emerson et al., 1975), and even more convenient NH_3-N calculators are available on-line – an excellent one can be found at http://www.hbuehrer.ch/Rechner/Ammonia.html.

Biological membranes are more permeable to NH_3 than to NH_4^+, and ammonia toxicity is attributed primarily to NH_3. Nevertheless, high NH_4^+ concentration in the water interferes with the outward movement of ammonia through the gills (Liew et al., 2013). Thus, NH_4^+ has some degree of toxicity, but much less than that of NH_3. NH_3 may cause toxicity to nitrifying bacteria –*Nitrosomonas*spp. and *Nitrobacter*spp. bacteria so that the nitrification process is inhibited (Russo 1985). The increased accumulation of ammonia or nitrite from the bacterial inhibition in the aquatic environment intensifies the toxicity to aquatic animals and beneficial bacteria (Russo 1985).

Ammonia toxicity is usually reported as 96-hr LC50 – the lethal concentration of ammonia (as NH_3-N) that required to kill half of the tested population in 96 hours. The tolerance of ammonia toxicity varies among different species. According to Boyd's summarization (2013), for example, the 96-hr LC50 for rainbow trout is between 0.32 to 0.93 mg L^{-1}; cutthroat trout, 0.50 – 0.80 mg L^{-1}; freshwater prawns, 2.00 – 2.50 mg L^{-1}; school prawns, 1.39 mg L^{-1}; southern white shrimp, 0.69 – 1.20 mg L^{-1}; pacific white shrimp, 1.20 – 2.95mg L^{-1}. In realty, producers are concerned over the sub-lethal effects of ammonia more than the LC50. Much research of chronic exposure of ammonia on culture species was conducted. Colt and Tchobanoglous (1978) showed the growth of juvenile channel catfish was reduced by 50% at 0.517 mg L^{-1} NH_3-N and no growth occurred at 0.967 mg L^{-1}during a 31 day growth trial when fish were exposed to concentrations of NH_3-N ranging from 48 to 989 μgL^{-1}. Foss et al. (2004) showed that the growth of juvenile Atlantic cod, *Gadusmorhua*, was significantly reduce at NH_3-N concentration of 0.17 mg L^{-1} during a 96 days at 13 ℃ when fish were exposed to concentrations of NH_3-N ranging from 0.0005 to 0.17mgL^{-1}.

The sub-lethal or lethal effects of ammonia in sensitive fish that are reported include gill damage,blood oxygen-carrying capacity reduction, lack and depletion of adenosine triphosphate (ATP) in the brain, and the liver and kidneys malfunction (Arillo et al.1981; Camargo and Alonso 2006; Lang et al. 1987; Russo 1985; Tomasso et al. 1980).

Several strategies for fish to avoid ammonia toxicity were found. Many fish have the capacity to detoxify ammonia to glutamine when exposedto elevated environmental ammonia. Increased concentration of brain glutamine also found in common carp *Cyprinuscarpio* (Dabrowskaand Wlasow, 1986), the mudskippers, *Periophthalmodonschlosseri*and *Boleophthalmusboddaerti* (Peng et al., 1998) when exposed to ammonia. Some fish can convert ammonia to the less toxic urea, an more energy-consuming process (Atkinson and Bourke,1987) found only in inland vertebrates and marine fish using urea as an osmotic filler. However, Saha and Ratha (1987) found the air-breathing freshwater fish, *Heteropneustesfossilis,* increasedurea synthesis when exposed to air. Randall et al. (1989) reported that the Lake Magadi tilapia converted ammonia to only urea to survive the very alkaline environment in Lake Magadi. Fish can also reduce ammonia production as a strategy to reduce ammonia toxicity. Wilson et al. (1998) reported ammonia excretion and production were inhibited in rainbow trout during severe alkaline exposure. Fish can also reduce ammonia toxicity through ammonia volatilization (Tsui et al., 2002) and active excretion of ammonium ion (Randall et al., 1999).

Ammonia nitrogen is a pollutant when discharged to the environment

The development of aquaculture has resulted in many adverse impacts on environment and nature sources including destruction of sensitive aquatic ecosystems, excess use of chemicals, invasion of foreign species, landscape modification, introduction of aquatic animal disease and water pollution (Dierberg and Kiattisimukul, 1996; Goldburg and Triplett,

1997; Naylor et al., 1998, 2000). Water pollution through aquaculture discharge is received the most complaint and attracted official attention worldwide.

Discharges from aquaculture contain organic matter, nutrients such as nitrogen (TAN and nitrate) and phosphorus, and solids (Crippsand Bergheim, 2000; Bergheim and Brinker, 2003). Ammonia nitrogen is an important form of total nitrogen in aquaculture waste. The total nitrogen produced within flow-through system is mainly discharged in the form of ammonia nitrogen (Bergheim and Âsgård, 1996).It has generally been considered that the dominant dissolved form of N in shrimp ponds was ammonia nitrogen (Lorenzen et al., 1997). Ammonia is regarded as one of the most important pollutants in the aquatic environment for its highly toxic nature discussed above and its ubiquity in surface water systems (Russo 1985). Many effluents need to be treated extensively in order to keep the ammonia levels in surface water from being unacceptably high (Rand, 1995; USEPA, 2009).

Excess ammonia in effluent could accelerate eutrophication in receiving water bodies, resulting in dissolved oxygen depletion and fish toxicity (Du et al., 2005). Alabaster (1982) reported cage aquaculture increased the load of N, P, and organicmatter and enriched water and underlying sediment. Tucker and Lloyd (1985) showed that effluent especially total nitrogen and ammonia and chemical oxygen demand from channel catfish ponds was an important point source of pollution. Similarity, Zieman et al. (1992) reported the increase of total ammonia in the effluent from Hawaiian aquaculture facilities (freshwater fish, freshwater prawn, marine fish, and marine shrimp ponds).Guo and Li (2003) showed that mass-input of exogenousnutrients might cause negative effects on water quality in areas from the cage to a distance of 50 moutwards.Herbeck et al. (2013) reported the input of aquaculture effluents rich in dissolved inorganic and organic matter from ponds at the NE coast of Hainai caused eutrophic conditions in the nearby coastal waters.

The national criteria for ammonia in fresh water in USEPA (2013) recommends an criterion acute concentration of 17 mg L^{-1} total ammonia nitrogen and a criterion chronic concentration of 1.9 mg L^{-1} total ammonia nitrogen at pH of 7 and temperature of 20 ℃. The Global Aquaculture Alliance (GAA) effluent standard for TAN is 5 mg L^{-1} as an initial standard and 3 mg L^{-1} as a target standard (Boyd, 2001; Tucker and Hargreaves, 2009). This standard is based on intensive shrimp culture, where TAN concentration is very high–often exceed 5 mg L^{-1} (Boyd, 2001).

Ammonia nitrogen can be removed by many techniques including earthen treatment ponds or reservoirs, bio-filtration, periphytontreatment technique and bio-flocs technology. Many studies on ammonia removal in aquaculture have been conducted. Ng et al. (1996) removed 82% of ammonia in aquaculture influent by using a bench-scale fluidised bed bioreactor when the reactor was loaded with ammonia-nitrogen (NH_4-N)between 0.201 and 0.397 g $m^{-2}day^{-1}$. Shan and Obbard (2001) maintained concentration of TAN less than 0.5 mg L^{-1} under a fed-batch condition of 3.2 mg TAN L^{-1} per day by using pellet immobilization of indigenous nitrifying bacteria. Lyssenko and Wheaton (2006) removed ammonia by trickling and submerged-upflowbiofilters for intensive recirculating aquaculture. Gendel andLahav (2013) recommended a new method comprising ion exchange and electrochemical regeneration to remove ammonia from fresh-water recirculated aquaculture systems.

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III. AN ASSESSMENT OF NESSLER, PHENATE, SALICYLATE, AND ION SELECTIVEELECTRODE PROCEDURES FOR DETERMINATION OF TOTALAMMONIANITROGE IN AQUACULTURE

ABSTRACT

An assessment was conducted to compare the precision and accuracy of Nessler, phenate, salicylate, and ammonia electrode procedures for total ammonia nitrogen (TAN) concentration determination in waters of aquaculture. Salicylate method was selected as a standard method for its high precision and accuracy. In replicate analyses of water samples for precision estimate, Nessler method gave higher mean concentration of TAN than other methods and it is not recommended for use for its high coefficient of variance. Results from Nessler's with Rochelle salt method were more accurate than Nessler without Rochelle salt method. Electrodes for sensing NH_3 and NH_4^+usually gave higher mean concentration of TAN than salicylate method. NH_4^+ electrode, although with high accuracy, was strongly affected by sodium and potassium and not recommended to use especially in waters of high salinity. The finding also suggested that the salicylate kit by using YSI photometer also was a preferable alternative to salicylate method while Nessler kit not. TAN concentrations measured in 27 water samples by all methods were highly correlated (R^2= 0.919 to 0.996) with salicylate method. All of the slopes except phenate I and II method were different from 1.0 (P<0.05); all of the intercepts except phenate I and salicylate kit were different from 0.0 (P< 0.05).

INTRODUCTION

Feed-based aquaculture is becoming increasingly intensive, because efficiency is improved by concentrating culture animals in a small volume of water to facilitate feeding, mechanical aeration, use of water quality amendments, and harvest. It is not unusual for intensive ponds to have standing crops of 8,000 to 10,000 kg ha^{-1} and daily feeding rates up over 100 kg ha^{-1} as encountered in ictalurid catfish farming in the United States (Boyd and Tucker, 2014). Mechanical aeration is effective in avoiding excessively low dissolved oxygen concentration in intensive ponds, but high concentration of total ammonia nitrogen (TAN) commonly occurs. For example, intensive ictalurid catfish ponds in Alabama (United States) typically had TAN concentrations above 2 mg L^{-1}, and concentrations above 10 mg L^{-1} were measured in some ponds (Zhou and Boyd, 2015).

Analytical methods for ammonia nitrogen in water measure TAN that consists of un-ionized ammonia (NH_3) and ammonium (NH_4^+) in a pH-and temperature- dependent equilibrium, un-ionized ammonia contributes primarily to ammonia toxicity, but high NH_4^+ concentration has some degree of toxicity because it interferes with the outward movement of ammonia through the gills (Liew et al., 2013). The proportion of NH_3 increases with rising pH and temperature (Trussell, 1972), and the NH_3 concentration must be estimated from the TAN concentration. Tables of factors for calculating NH_3 from TAN, pH, and water temperature are available (Trussell, 1972; Emerson et al., 1975; Boyd and Tucker, 2014) and on-line ammonia calculators such as the one found at http://www.hbuehrer.ch/Rechner/Ammonia.html may be used. Total ammonia nitrogen concentrations in culture systems are sometimes great enough to stress culture animals but seldom high enough to cause direct mortality (Boyd and Tucker, 2014; Zhou and Boyd, 2015).

The threat of ammonia stress increases in intensive aquaculture andthere isneed to monitor TAN concentration in culture systems. Severalmethods of measuring TAN concentration are used in aquaculture to include standard laboratory colorimeter procedures (Nessler, phenate, and salicylate methods), either NH_3 or NH_4^+ sensing electrodes (Eaton et al., 2005),and test kits that rely on either the Nesslertechnique or the salicylate methods. Elevated calcium and magnesium (hardness) concentration and salinity can interfere with the determination of TAN concentration (Eaton et al., 2005). Aquaculture is conducted in freshwater, estuarine water, and seawater, and there is increasing production of marine shrimp in low salinity (1 to 10 g L^{-1}) inland waters (Roy et al., 2010). The salicylate method for TAN is commonly used for seawater, but a recent study (Le and Boyd, 2012) revealed that this method also gave highly satisfactory results for water ranging from salinities of 0.1 to 24 g L^{-1}. Thus, the purpose of the present study was to compare the salicylate method to other methods of determining TAN concentration in freshwater of different hardness and in low-salinity inland water.

BACKGROUND ON ANALYTICAL METHODS

Nessler's reagent – named after Julius Nessler, the German chemist who first made this reagent in 1856 – is a solution consisting of mercury (II) iodide and potassium iodide in highly alkaline solution. It will react to form a yellow color in proportion to TAN concentration that may be assessed colorimetrically (Krug et al. 1979; Leonard 1963; Remy 1956). The basic reaction is

$$2\ K_2HgI_4 + NH_3 + 3\ KOH \longrightarrow O{\overset{\displaystyle Hg}{\underset{\displaystyle Hg}{\diagup\kern-0.5em\diagdown}}}NH_2I + 7\ KI + 2\ H_2O \qquad (1)$$
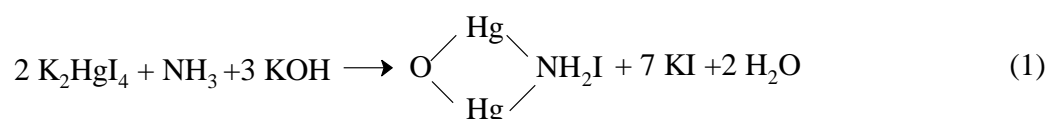
Nessler's reagent raises the sample pH causing precipitation of calcium and magnesium (hardness cations) as hydroxides creating turbidity that interferes with colorimetric measurement of the yellow color – especially in harder waters. Distillation of a water sample at high pH results in a distillate containing the ammoniacal nitrogen of the sample free of hardness cations. But, this method is time consuming and tedious. The more common means of removing interference by hardness cations is to treat samples with alkaline zinc sulfate solution followed by filtration. Rochelle salt [potassium sodium tartrate ($KNaC_4H_4O_6 \cdot 4H_2O$)] solution is then added to remove residual hardness cations that might react with Nessler's reagent.

The phenate (or phenol) method is based on the Bethelot reaction in which ammonia reacts with phenol and hypochlorite under alkaline condition. Ammonia is converted to monochloramime at pH 9.7 – 11.5, which reacts with phenol in the presence of hydrochlorine to form blue-colored indophenol in proportion to the ammonical nitrogen concentration in the sample (Searle, 1984), the reactions are:

$$2 \quad \text{—O}^- + NH_3 + 3\,ClO^- \longrightarrow {}^-O\text{—}\quad\text{—}N\text{=}\quad\text{=}O + 2\,H_2O + OH^- + 3\,Cl^- \quad (2)$$

phenol   indophenol (blue)
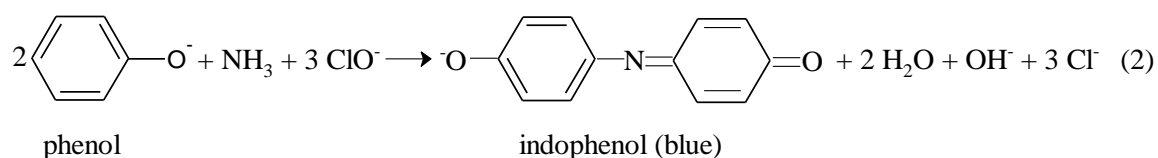
Sodium nitroprusside is generally used to catalyze indophenol reaction and intensity the blue color (Lubochinsky and Zaltal, 1954; Mann, 1963). A citrate buffer often is added to stabilize pH and prevent precipitation of hydroxides (Solarzano, 1969; Hampson, 1977; Verdouw et al., 1978). Rochelle salt solution with manganese sulfate catalyst sometimes added to samples before applying the other reagents to lessen the inference of calcium hydroxide precipitation in hard waters(Boyd and Tucker, 1992).

The salicylate method actually is a modification of the phenate method in which sodium salicylate is substituted for phenol. This modification eliminates production of ortho-

chlorophenol that is toxic and highly volatile (Lammering and Burbank, 1960; Roberts et al., 1977; Verdouwet al., 1978). In the salicylate method, monochloramime formed by the reaction of ammonia and hydrochlorine reacts with salicylate to form blue-green colored 5-aminosalicylate in proportion to the amount of ammoniacal nitrogen presented as follows:

$$\text{[salicylate structure]} -OH + NH_3 + ClO^- \longrightarrow H_2N- \text{[aminosalicylate structure]} -OH + H_2O + Cl^- \qquad (3)$$
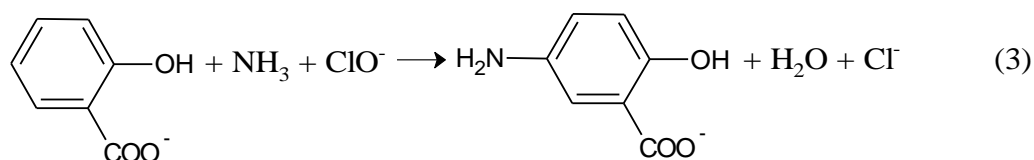
Sodium nitroferricyanide acts as a catalyst to intensify the color of 5-aminosalicylate.

An ion-selective electrode (ISE) – often called a specific ion electrode – responds to the concentration of a particular ion or gas in solution. The result is a potential difference that can be measured by a voltmeter (the voltmeter usually is the function of a pH meter). The voltage, in accordance with the Nernst equation, depends upon the common logarithm of the activity of the ammonium ion or gaseous ammonia (NH_3) in solution.

There are two types of electrodes for sensing TAN concentration. One senses NH_3 using a hydrophilic gas-permeable membrane to separate the sample from a solution of ammonium chloride within the electrode (internal solution). A strong base such as lithium hydroxide added to the sample converts essentially all $NH_4^+$to NH_3. Ammonia diffuses through the membrane until the partial pressure of ammonia is equal on both sides. The pH change in the internal solution is sensed by the electrode and is proportional to the partial pressure (or concentration) of NH_3in the sample.

The NH_4^+ sensing electrode has a polyvinylchloride (PVC) membrane containing an ammonium-carrier. The water sample is acidified to lower the pH and convert essentially all NH_3 to NH_4^+. The electrode potential of the sample relative to the reference electrode of the NH_4^+ sensing probes is proportional to the NH_4^+ activity (or concentration) in the sample.

Field kits for measuring TAN concentration in freshwater rely on the Nessler method. Kits for brackishwater and seawater typically rely on the salicylate method.

MATERIALS AND METHODS

The TAN procedures evaluated in this study, the concentration ranges for the methods without sample dilution, and references for analytical protocol are given in Table 1. The water analysis kits, NH_3 and NH_4^+ sensing electrodes, the prepackaged dry reagents and solutions for these procedures were purchased new. Most reagents for other procedures were prepared from ASC-grade laboratory reagents and high-quality, ammonia-free distilled water. The TAN standards were prepared from ammonium chloride. The absorbances of samples – except in analyses made by test kits – were measured (1-cm path length) with an Aquamate Spectrophotometer (Thermo Fisher Scientific, Atlanta, GA USA). A VWRsymphony model B20PI Benchtop meter (VWR Scientific Products, West Chester, PA, USA) was used to measure the electrical potentials of the NH_3 and NH_4^+ sensing electrodes.

The water samples were obtained from three locations. Freshwater samples of low total dissolved solids concentration (specific conductance of 60 to 120 μmho cm^{-1}, calcium of 5 to 15 mg L^{-1}, and magnesium of 2 to 4 mg L^{-1}) were collected from ponds at the Auburn University E.W. Shell Fisheries Center (SFC) 5 km N of Auburn, Alabama. Freshwater samples of medium to high total dissolved solids concentration (specific conductance of 200 to 5000 μmho cm^{-1}, calcium of 40 to 80 mg L^{-1}, and magnesium of 8 to 12 mg L^{-1}) were taken from commercial ictalurid catfish ponds in Dallas, Greene, and Hale counties of the Blackland Prairie region of west-central Alabama. Samples of saline water (2 to 6 g L^{-1} of salinity) were collected from ponds for inland culture of marine shrimp located the vicinity of Forkland and Boligee in Greene County, Alabama.

The study was conducted in two stages. In the first stage, the standard Nessler, phenate, and salicylate methods and the Nessler kit were evaluated. The second stage

involved evaluating the method that had the best precision and accuracy in the first stage, the NH_3 and NH_4^+ sensing electrodes, and the salicylate kit.

Accuracy and precision estimates were made from measurement of low, medium, and high TAN concentrations. Samples for this task were screened for TAN concentration by the salicylate method that previously had been proven highly reliable (Le and Boyd, 2012). The TAN concentrations in the sample had the following ranges: low (0.03 – 0.25 mg L^{-1}); medium (0.3 – 0.7 mg L^{-1}); high (0.8 – 2.0 mg L^{-1}). These ranges were chosen so as not to exceed the upper limit of sensitivity of some of the methods.

Accuracy of each method was assessed by spike-recovery (Boyd and Tucker, 1992). Ten replicates of a water sample of each concentration category were analyzed for TAN concentration by a particular TAN method. The aliquots were then spiked with 0.30 mg L^{-1} TAN, mixed thoroughly, and analyzed for TAN concentration. The percentage recovery of the spike – an estimate of accuracy – was calculated as follows:

$$\text{Spike recovery (\%)} = \frac{F}{I + S} \times 100$$

where I = initial TAN concentration; S = TAN concentration increased by spike; F = final TAN concentration.

The variation in percentage recovery by a given method for a particular sample was assessed by the relative standard deviation (coefficient of variability) of the replicates. The results also were compared with the salicylate methods for difference in percentage recovery by Tukey'sStudentized Range (HSD) test and for precision by the F-test for homogeneity of variance.

Precision estimates were based on the coefficients of variation for 10 replicate analyses at each of the three concentration categories by each method. The salicylate method was used as a standard for F-test comparisons of heterogeneity of variances with other

methods. The means of the replicate analyzes were compared for differences by the Tukey'sStudentized Range (HSD) test.

Twenty-seven freshwater samples including both low and high concentration of hardness and ranging from 0.04 to 1.5 mg L^{-1} in TAN concentration (as determined by the salicylate method) were analyzed by all methods. Results for each method (Y variable) were regressed versus the results of the salicylate method. Slopes and intercepts of the regression lines were tested to determine if they differed from 1.0 and 0.0, respectively.

Statistical significant was accepted at $P \leq 0.05$. All statistical analyses were conducted with SAS 9.3 (SAS Institute, Cary, NC, USA).

RESULTS

Spike recoveries by the different methods were compared among the different types of water and TAN concentrations (Tables 2 and 3). The salicylate method had excellent accuracy based on spike recovery tests – average recoveries for ten replicates ranged from 97.6 to 103.3% – grand mean of 100.4%. The CVs of spike recoveries among replicates of spiked sample by the salicylate method were less than 5% in all instances.The phenate I method performed about as well as the salicylate method in recovery of TAN spikes in freshwater (97.1 – 107.9%), and it tended to have spike recoveries closer to 100% than did the phenate II method across all freshwater comparisons. In saline water, both thephenate I nor II methods had a wide range in spike recoveries – values varied from 81.5 – 114.4% and 75.9 – 78.0%, respectively.

The Nessler II procedure had spike recoveries of 101.0 – 107.4% in freshwaters of both low and high hardness. This method had spike recoveries over 125% at low and medium TAN concentration in saline water, but it gave a spike recovery of 102.5% at the high TAN

concentration in saline water. The Nessler I method usually had spike recoveries between 126 and 189% – only at the high TAN concentration in hard freshwater and saline water was spike recovery by this method near 100%.

The NH_3 and NH_4^+ sensing electrodes had spike recoveries of 75.4 – 109.7% and 93.5 – 123.27%, respectively, in freshwater. The electrodes had spike recoveries of 122.0 – 147.5% and 92.9 – 101.0%, respectively, in saline water.

The Nessler kit had spike recoveries of 102.3 – 171.7% in freshwater and 106.3 – 139.1% in saline water. The best spike recoveries were for high TAN concentration. The salicylate kit, however, performed well with respect to spike recovery across all TAN concentrations and types of waters – values ranged from 99.1 to 117.4% with only one instance of a recovery greater than 110%.

The repeatability of spike recovery estimate among the replicates for each type of water and TAN concentration tended to be reasonably good. Of the CVs for the 90 combinations of methods, waters, and TAN concentrations, 56 were less than 5%, only 10 were above 10%, and the greatest was 23.0%.

When the means across the spike recoveries for the three TAN concentrations were averaged, there were significant differences in average spike recovery betweenNessler I method and salicylate method, phenate I or II methods in soft freshwater; and Nessler I technique and phenate II technique in hard freshwater and saline water. There were no significant differences in average spike recovery amongsalicylate method, NH_3 and NH_4^+ sensing electrodes and salicylate kit in freshwater except that the NH_3 sensing electrode method provided results different from the standard salicylate method, salicylate kit and NH_4^+ sensing electrode method in saline water.

Although there were differences in spike recovery among several of the TAN analysis techniques relative to the salicylate method, TAN concentrations measured in 27 water

samples by all methods were highly correlated (R^2 = 0.919 to 0.996) with the salicylate method. However, the slopes and intercepts for the regression lines were equal to 1.0 and 0.0, respectively, only for the regression between the salicylate method and the phenate I method (Table 4). For the phenate II method, the slope of the regression line with the salicylate method did not differ from 1.0, but the intercept differed from 0.0, while the opposite was true for the regression line for the salicylate method and the salicylate kit. Slopes and intercepts for the regression line between the salicylate method and other procedures differed from 1.0 and 0.0, respectively.

The replicate analyses for precision estimates allowed a statistical comparison of mean TAN concentration measured on the same samples by the salicylate method and the other methods (Tables 5 and 6). The phenate I method gave a different mean than the salicylate method for only one of the nine combination of water type and TAN concentration. The number of differences for the other methods were: phenate II, five; Nessler I, nine; Nessler II, six; NH_3 sensing electrode, eight; NH_4^+ sensing electrode, eight; Nessler kit, eight; salicylate kit, one. Thus, only the phenate I and salicylate kit could be considered as providing TAN concentration roughly comparable to the standard salicylate method.

The precision estimates for the salicylate method ranged from 0.85 to 5.68% – average of 2.02%; the other methods tended to have lower but reasonably good precision. The numbers of CVs greater than 5.0% for the other methods were as follows: phenateI, two; phenate II, three; Nessler I, eight; Nessler II, eight; NH_3 sensing electrode, nine; NH_4^+ sensing electrode, two; Nessler kit, seven; salicylate kit, five. In general, the precision increased (CVs decreased) at greater TAN concentration.

DISCUSSION

Results of accuracy and precision tests (Table 2, 3, 5 and 6) showed that the phenate I method performed much better than thephenate II method. The regression equation between the phenate I method and the salicylate method in freshwater was $Y_{salicylate} = 0.987X_{phenate\ I} - 0.011$ with slope and intercept not different from zero, and R^2 was 0.996. In spite of the good performance of the phenate I method of Soloranzo (1969), the experience in water quality laboratory in the School of Fisheries, Aquaculture and Aquatic Sciences, Auburn University (Alabama, USA) has been that in the phenate I technique, the blue color of indophenol sometimes is replaced by yellow or green color, resulting in unreliable results. This problem was sometimes encountered in the present study, and when it was, the aliquot was discarded and another aliquot was taken from the sample for analysis. Because of the occasional problem with inconsistency of color, we feel that the standard salicylate method is better suited for use in aquaculture.

The addition of Rochelle salt solution to samples for which the Nessler II method was used resulted in better accuracy and precise measurements by the Nessler method.The NesslerII method performed best in samples of the high TAN concentration category. Nevertheless, neither Nessler method was equal or superior to the standard salicylate method with respect to overall accuracy and precision.

Ammonia electrodes do not present concernsrelated to sample color or turbidity. The electrode methods also are less expensive; the basic ISE setup including a meter capable of reading millivolts, are 2 to 4 times less costly than a spectrophotometer. Average CVs in the three TAN concentration categories demonstrated that the NH_4^+electrode was more precise than the NH_3 probe (Table 6). However, neither electrode procedure was as accurate or precise asthe standard salicylate method. In saline water, the NH_4^+ electrode gave much

higher TAN results — 2.7 to 8 times as high than results reported by the standard salicylate technique. This was the result of the NH_4^+ electrode being strongly affected by sodium and potassium in shrimp pond water. The effect of sodium and potassium interference on the TAN measurement by the NH_4^+ electrode decreased as TAN concentration in water increased.

Aquaculture producers in Alabama and elsewhere often monitor TAN concentrations using ammonia test kits,because these devices are cheap, portable, rapid, and easy to use in field and require little knowledge of quantitative chemistry. The Nessler kit tested in this study was neither highly precise nor accurate except at high TAN concentration. The salicylate kit, however,gave excellent precision and accuracy in most instances.

The least expensive method for TAN analysis was the Nessler kit, and analyses by the gas and ion sensing probes also were of relatively low cost (Table 7). All laboratory methods were more expensive because of the need to use filter paper, but the standard salicylate method was the least expensive of the other five techniques. The salicylate kit method incurs about 25% less expensive for chemicals than the standard salicylate method incurs for chemicals and filter paper.

Another issue in TAN analysis relates to disposal of hazardous waste. In order to improve waste management, the United States Congressin 1976 passed the Resource Conservation and Recovery Act (Horinko, 2002). In 1980, chemicals hazardous because of ignitability, corrositivity, reactivity (explosion risk), and toxicity were included in this act (US Environmental Protection Agency, 1980a,b). Nessler reagent contains mercury, which is a hazardous waste because of its toxicity and danger to the environment. Phenol and the by-product o-chlorophenol from the phenateI and II methodsalso are human health hazards. Hazardous waste disposal must follow specific protocols that increase the complexity and cost of laboratory operation. The salicylate method does not result in a hazardous waste that needs to be disposed by a special procedure, making its use in the laboratory less problematic.

CONCLUSION

The results of this study confirm that the standard salicylate method for TAN measurement is appropriate for wide application in aquaculture water. Results for thephenate I and salicylate method were not different (slope=1.0, intercept=0.0, R^2=0.996) in freshwater, but the phenate I method occasionally presents improper color development and samples must be re-run.Nessler's method gives better results in water of high TAN concentration (0.8 mg L^{-1}),but Rochelle salt should be added to negate the interference from calcium, magnesium and other ions. Electrodes for sensing NH_3 and NH_4^+ were less precise or accurate in most comparison than the other methods.Nevertheless, in spite of the variation in precision and accuracy among the methods, there was an excellent correlation (R^2 ranged from 0.919 to 0.996) when the various methods being evaluated were used to predict the concentration obtained in the same samples by the standard salicylate.

The YSI salicylate kit is an alternative to the standard salicylate method for use at aquaculture facilities.

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Table 1. Detect range without dilution,references for analytical protocol for each of total ammonia nitrogen (TAN) determination method in this study

Method	Detect range without dilution (mg L^{-1})	Reference
Salicylate	0 - 1.0	Bower and Holm-Hansen, 1980
Phenate I[§]	0.02 - 1.25	Eaton et al., 2005
Phenate II[§§]	0 - 2.0	Boyd and Tucker, 1992
Nessler I[§]	0.02 - 5.0	Eaton et al., 2005
Nessler II[§§]	0.02 - 5.0	Eaton et al., 2005
NH_3 electrode (VWR Symphony TM)	0.05 – 14000	VWR User Manual
NH_4^+ electrode (Radiometer Analytical, ISE25NH4)	0.04 – 14000	Radio-meter Analytical User Manual
Nessler kit (Hach Company, Model NI-8)	0 - 3.0	Hach User Manual
Salicylate kit (Yellow Spring Institute Company)	0 - 1.0	YSI User Manual

[§] Without Rochelle salt solution
[§§] With Rochelle salt solution

Table 2. Means, SD and coefficient of variations (CV) for recovery of 0.3 mg L^{-1} ammonia nitrogen spikes to samples of waters from different TAN concentration ranges and locations by salicylate, phenate and Nessler methods. Means are tested for difference by Tukey'sStudentized Range (HSD) test; homogeneity of variances was tested by F-tests with salicylate method as the standard method. Means indicated by the same letter in a column do not differ ($P = 0.05$) according to HSD test.

Method	Low Concentration		Medium Concentration		High Concentration		
	Spike recovery (%)	CV (%) (F test)	Spike recovery (%)	CV (%) (F test)	Spike recovery (%)	CV (%) (F test)	Average SR (%)
Low Hardness							
Salicylate	98.8±0.57 a	0.6	100.5±0.84 ac	0.8	97.6±0.560 a	0.6	99.0 a
Phenate I	107.8±2.42 a	2.3(*)	97.1±1.43 ac	1.5	101.5±1.53 b	1.5(*)	102.1 a
Phenate II	94.2±8.19 a	8.7(*)	96.7±03.17 a	3.3(*)	94.2±1.607 a	1.7(*)	95.0 a
Nessler Kit	171.7±17.23 b	10.1(*)	136.1±5.66 b	4.2(*)	104.8±3.28 b	3.1(*)	137.6 ab
Nessler I	167.3±14.67 b	8.8(*)	145.7±9.57 d	6.6(*)	127.7±5.03 c	3.9(*)	146.9 b
Nessler II	102.4±5.31 a	5.2(*)	103.9±5.60 c	5.4(*)	103.7±2.15 b	2.1(*)	103.3 ab
Medium to high hardness							
Salicylate	99.9±0.97 a	1.0	98.7±1.41 a	1.4	98.7±0.79 a	0.8	99.1 ac
Phenate I	100.2±2.82 a	2.8(*)	103.4±4.03 a	3.7(*)	107.9±3.51 b	3.3(*)	103.8 ac
Phenate II	94.7±3.97 a	4.2(*)	84.4±4.28 b	5.1(*)	89.1±3.03 c	3.4(*)	89.4 ab
Nessler Kit	132.3±14.12 a	10.7(*)	133.6±7.95 c	6.0(*)	102.3±3.02 d	3.0(*)	122.7 ac
Nessler I	187.4±13.15 b	7.0(*)	138.2±6.98 c	5.0(*)	103.1±1.87 d	1.8(*)	142.9 c
Nessler II	107.4±5.63 a	5.3(*)	104.5±2.76 a	2.6(*)	101.0±2.84 ad	2.8(*)	104.3 ac
Saline							
Salicylate	102.0±0.56 a	0.6	99.0±1.73 a	1.8	103.3±0.63 a	0.6	101.5 ac
Phenate I	81.5±8.35 b	10.3(*)	83.5±1.95 b	2.4	114.4±7.77 ac	6.8(*)	93.1 ac
Phenate II	75.9±3.20 b	4.2(*)	76.8±1.78 b	2.3	78.0±2.64 b	3.4(*)	76.9 ab
Nessler Kit	126.6±7.41 c	5.9(*)	139.1±9.91 c	7.1(*)	106.3±4.24 a	4.0(*)	124.0 ac
Nessler I	189.6±22.43 d	11.8(*)	137.5±9.03 c	6.6(*)	109.9±2.90 c	2.6(*)	145.7 c
Nessler II	126.1±9.50 c	7.5(*)	126.8±11.37 d	9.0(*)	102.5±5.66 a	5.5(*)	118.5 ac

Table 3. Means, SD and coefficient of variations (CV) for recovery of 0.3 mg L^{-1} ammonia nitrogen spikes to samples of waters from different TAN concentration ranges and locations by salicylate, salicylate kit and ammonia probes. Means are tested for difference by Tukey'sStudentized Range (HSD) test; homogeneity of variances was tested by F-tests with salicylate method as the standard method. Means indicated by the same letter in a column do not differ ($P = 0.05$) according to HSD test.

Method	Low Concentration		Medium Concentration		High Concentration		Average SR (%)
	Spike recovery (%)	CV (%) (F test)	Spike recovery (%)	CV (%) (F test)	Spike recovery (%)	CV (%) (F test)	
Low hardness							
Salicylate	102.6±0.92 a	0.9	100.6±1.22 a	1.2	102.1±0.89 ab	0.9	101.8 a
Salicylate kit	104.9±2.37 a	2.3(*)	98.9±5.18 a	5.2(*)	105.1±2.58 a	2.5(*)	103.0 a
NH$_4^+$ probe	123.3±13.17 b	10.7(*)	96.6±1.70 a	1.8	99.1±2.63 bc	2.7(*)	106.3 a
NH$_3$ probe	106.8±15.70 a	14.7(*)	85.3±7.60 b	8.9(*)	94.6±8.57 c	9.1(*)	95.6 a
Medium to high hardness							
Salicylate	99.9±2.16 ab	2.2	102.5±1.47 a	1.4	100.5±2.13 a	2.1	101.0 a
Salicylate kit	109.0±2.91 a	2.7	105.2±5.93 a	5.6(*)	117.4±6.48 b	5.5(*)	110.5 a
NH$_4^+$ probe	93.5±5.03 a	5.4(*)	108.1±3.17 a	2.9(*)	97.6±2.48 a	2.5	99.7 a
NH$_3$ probe	109.7±17.68 b	16.1(*)	75.4±8.83 b	11.7(*)	106.5±13.56 a	12.7(*)	97.2 a
Saline							
Salicylate	99.0±1.71 a	1.7	103.5±0.93 a	0.9	100.5±0.83 a	0.8	101.0 a
Salicylate kit	99.1±4.18 a	4.2(*)	104.3±4.13 a	4.0(*)	103.1±4.43 a	4.3(*)	102.1 a
NH$_4^+$ probe	92.9±4.05 a	4.4(*)	101.0±3.46 a	3.4(*)	98.9±3.51 a	3.6(*)	97.6 a
NH$_3$ probe	147.5±22.91b	23(*)	133.8±22.88 b	17.1(*)	122.0±8.38 b	6.9(*)	134.4 b

*Significant at α=0.05.

Table 4. Results of regression analyses of TAN concentrations measured by the salicylate method (X) and the other method (Y) in freshwater. Slopes of X and Y intercepts of regression lines were tested to determine if they differed from 1.0 and 0.0, respectively

Method	Regression equation	R^2	Slope=1.0	Intercept=0.0
PhenateI	Y = 0.987X-0.011 (*P*<.0001)	0.996	Yes	Yes
PhenateII	Y = 1.023X+0.083 (*P*<.0001)	0.985	Yes	No
Nesslerkit	Y = 2.045X-0.201 (*P*<.0001)	0.967	No	No
NesslerI	Y = 1.355X-0.268 (*P*<.0001)	0.919	No	No
NesslerII	Y = 2.707X-0.275 (*P*<.0001)	0.959	No	No
Salicylate kit	Y = 1.071X + 0.013 (*P*<.0001)	0.993	No	Yes
NH_4^+ probe	Y = 1.112X + 0.167 (*P*<.0001)	0.971	No	No
NH_3 probe	Y = 1.105X + 0.056 (*P*<.0001)	0.978	No	No

Table 5. Means, SD and coefficient of variations (CV) for TAN determinations of ten replicates of each sample from different TAN concentration ranges and locations by salicylate, phenate and Nessler methods. Means are tested for difference by Tukey'sStudentized Range (HSD) test; homogeneity of variances was tested by F-tests with salicylate method as the standard method. Means indicated by the same letter in a column do not differ (P = 0.05) according to HSD test.

Method	Low Concentration		Medium Concentration		High Concentration		Average CV (%)
	Mean ±SD in mg/L	CV (%) (F test)	Mean ±SD in mg/L	CV (%) (F test)	Mean ±SD in mg/L	CV (%) (F test)	
Low hardness							
Salicylate	0.206±0.0077 a	3.75	0.532±0.0057 a	1.08	1.517±0.0279 a	1.85	2.23 a
Phenate I	0.194±0.0011 a	0.56(*)	0.521±0.0059 a	1.14	1.456±0.0424 a	2.91	1.54 a
Phenate II	0.207±0.0112 a	5.42	0.514±0.0133 a	2.6(*)	1.234±0.0419 b	3.39	3.80 a
Nessler Kit	0.372±0.0214 b	5.76(*)	1.153±0.1346 b	11.68(*)	2.683±0.1465 c	5.46(*)	7.63 a
Nessler I	0.836±0.2252 c	26.93(*)	1.420±0.1353 d	9.53(*)	3.033±0.2057 d	6.78(*)	14.41 a
Nessler II	0.365±0.1008 b	27.61(*)	0.675±0.0651 c	9.64(*)	1.543±0.1049 a	6.80(*)	14.68 a
Medium to high hardness							
Salicylate	0.162±0.0009 a	5.68	0.472±0.0051 a	1.08	1.744±0.0342 a	1.96	2.91 a
Phenate I	0.147±0.0187 a	12.68(*)	0.462±0.0201 a	4.35(*)	1.635±0.0698 a	4.27(*)	7.10 a
Phenate II	0.227±0.0143 b	6.32	0.505±0.0184 ab	3.66(*)	1.316±0.0387 b	2.95	4.31 a
Nessler Kit	0.186±0.0513 ab	27.59(*)	0.590±0.0575 b	9.75(*)	2.675±0.0945 c	3.53(*)	13.62 a
Nessler I	0.581±0.0894 c	15.40(*)	1.393±0.1937 c	13.9(*)	3.369±0.2324 d	6.90(*)	12.07 a
Nessler II	0.245±0.0213 b	8.69(*)	0.547±0.0285 ab	5.22(*)	1.750±0.0449 a	2.57	5.49 a
Saline							
Salicylate	0.234±0.0061 a	2.60	0.620±0.0053 a	0.85	1.607±0.0168 a	1.05	1.22 a
Phenate I	0.170±0.0193 a	11.34(*)	0.456±0.0091 b	2.01	1.475±0.0461 a	3.13(*)	4.83 a
Phenate II	0.206±0.0112 a	5.43(*)	0.450±0.0129 b	2.86(*)	1.102±0.0225 b	2.21	2.56 a
Nessler Kit	0.328±0.0581 b	17.73(*)	0.625±0.0855 a	13.67(*)	2.324±0.1077 c	4.63(*)	7.50 a
Nessler I	0.552±0.0636 c	11.52(*)	1.082±0.0500 d	4.62(*)	2.977±0.3884 d	13.0(*)	8.21 a
Nessler II	0.323±0.0808 b	25.05(*)	0.704±0.0546 c	7.75(*)	2.193±0.1526 c	6.96(*)	10.70 a

*Significant at α=0.05

Table 6. Means, SD and coefficient of variations (CV) for TAN determinations of ten replicates of each sample from different TAN concentration ranges and locations by salicylate, salicylate kit and ammonia probes. Means are tested for difference by Tukey'sStudentized Range (HSD) test; homogeneity of variances was tested by F-tests with salicylate method as the standard method. Means indicated by the same letter in a column do not differ (P = 0.05) according to HSD test.

Method	Low Concentration		Medium Concentration		High Concentration		Average CV (%)
	Mean ±SD in mg/L	CV (%) (F test)	Mean ±SD in mg/L	CV (%) (F test)	Mean ±SD in mg/L	CV (%) (F test)	
				Low hardness			
Salicylate	0.169±0.0082 a	4.84	0.582±0.0052 a	0.89	1.359±0.0085 a	0.63	2.12 a
Salicylate kit	0.213±0.0298 b	14.01(*)	0.669±0.0557 a	8.32(*)	1.426±0.0604 a	4.24(*)	8.86 ab
NH_4^+ probe	0.275±0.0144 c	5.23	0.800±0.0336 b	4.20(*)	1.358±0.0390 a	2.93(*)	4.12 a
NH_3 probe	0.168±0.0328 a	19.59(*)	1.040±0.1907 c	18.33(*)	1.043±0.1328 b	12.74(*)	16.89 b
				Medium to high hardness			
Salicylate	0.209±0.0046 a	2.20	0.502±0.0006 a	1.23	1.540±0.0297 a	1.93	1.79 a
Salicylate kit	0.211±0.0129 a	6.10(*)	0.544±0.0230 a	4.22(*)	1.656±0.1005 a	6.07(*)	5.46 a
NH_4^+ probe	0.909±0.0520 b	5.72(*)	1.227±0.0462 b	3.77(*)	2.199±0.0798 b	3.63(*)	4.37 a
NH_3 probe	0.568±0.1124 c	19.81(*)	1.052±0.1717 c	16.33(*)	2.538±0.2928 c	11.53(*)	15.89 b
				Saline			
Salicylate	0.194±0.0033 a	1.72	0.568±0.0077 a	1.35	1.440±0.0225 a	1.56	1.54 a
Salicylate kit	0.189±0.0145 a	7.67(*)	0.588±0.0217 a	3.69(*)	1.518±0.0577 ac	3.80(*)	5.05 ab
NH_4^+ probe	1.547±0.0538 b	3.48(*)	3.160±0.0784 b	2.48(*)	3.951±0.0813 b	2.06(*)	2.67 a
NH_3 probe	0.338±0.0629 c	18.6(*)	0.783±0.0611 c	7.81(*)	1.550±0.1373 c	8.85(*)	11.75 b

*Significant at α=0.05.

Table 7. Cost comparisons for chemicals used indifferent TAN determination methods in this study.

	Grade	Price		Cost per sample (US$)							
			Salicylate	Phenate I	Phenate II	Nessler Kit	Nessler I	Nessler II	Salicylate kit	NH4 probe	NH3 probe
Sodium salicylate	Reagent	$61.86/kg	0.0163								
Sodium nitroprusside	Reagent	$2164/kg	0.0004	0.0004							
Sodium hydroxide	ACS	$293.90/kg	0.0049	0.0071	0.0044		0.0353	0.0353			
Sodium citrate	ACS	$50.46/kg	0.0045	0.0030							
Phenol crystal	ACS	$480.40/kg		0.0605	0.0288						
Rochelle salt	ACS	$402.42/kg			0.0101						
Manganese sulfate	ACS	$57.68/kg			0.0000						
Sodium phosphate tribasic	ACS	$184.30/kg		0.0111							
EDTA	ACS	$412.86/kg		0.0048							
Lithium chloride	Reagent	$127.50/kg								0.0108	
Ammonia ionic strength adjustor	—	$0.273/ pillow									0.2730
Salicylate kit reagent	—	$0.335/ reagent							0.3347		
Nessler reagent	Reagent	$0.256/mL				0.0384	0.2560	0.2560			
Rochelle salt solution	Reagent	$0.436/mL						0.0436			
Zinc sulfate heptahydrate	Reagent	$55.42/kg					0.0055	0.0060			
Commercial bleach	Technical	$0.01/mL		0.0010	0.0010						
No 42 Whatman filter paper	—	0.423/sheet	0.4230	0.4230	0.4230		0.4230	0.4230			
Total			0.4492	0.5108	0.4673	0.0384	0.7198	0.7639	0.3347	0.0108	0.2730

IV. AN ASSESSMENT OF TOTAL AMMONIA NITROGEN CONCENTRATION INALABAMA NITROGEN CONCENTRATION IN ALABAMA (USA) ICTALURIDCATFISH PONDS AND THE POSSIBLE RISK OF AMMONIA TOXICITY

ABSTRACT

An assessment of total ammonia nitrogen (TAN) concentration was conducted for 31, ictalurid catfishponds on six farms, in the Blackland Prairie region of Alabama (USA). Five farms that provided production data had average annual feed inputs and harvest weights of 15,579 – 21,739 kg ha^{-1} and 8,104 – 12,344 kg ha^{-1}, respectively. Concentrations of TAN were measured 26 times (weekly June through September and less frequently other months) between May 2013 and May 2014. The farm average, annual TAN concentrations were 1.05-1.78 mg L^{-1} at five farms and 4.17 mg L^{-1} at the other. Correlations were not found (P > 0.05) when pond average TAN concentration was regressed individually against feed input, weight fish harvested, and aeration rate. Nearly half of the TAN concentrations were < 1 mg L^{-1}, the majority was<5 mg L^{-1}, but some ranged from 5 to 15 mg L^{-1}.

Analysis of the literature on ammonia toxicity to channel catfish suggested that the no-observed-effect level (NOEL) is around 1.0 mg L^{-1} NH$_3$-N in ponds with pH of 7.5 and above where NH$_3$-N concentration fluctuates greatly because of daily change in temperature and especially pH. Based on the daily pH fluctuation of 7.5 to 9.5 observed in ponds, and typical monthly average water temperatures, the NOEL for NH$_3$-N was oftenexceeded. At pH 8.5 – 8.9, depending upon the month, up to 14.5% of ponds exceeded the NOEL for NH$_3$-N. The NOEL was exceeded by up to 31.5% of pond at pH ≥ 9.0. The findings reveal that TAN concentrations often are at chronically toxic levels for ictalurid catfish in Alabama ponds.

There usually is no practical emergency treatment for reducing NH_3-N (or TAN) concentration in ponds exceeding the NOEL. Thus, good management practices for avoiding excessively high TAN concentrations in ponds, e.g., efficient feed management, adequate aeration to promote nitrification, and treatments maintaining buffering capacity in pond water should be applied.

INTRODUCTION

In feed-based aquaculture, 20 to 40% of nitrogen contained in protein of feed applied to ponds is recovered in harvested biomass. The rest of the nitrogen enters the pond in uneaten feed and feces or is excreted as ammonia nitrogen by the culture species. Nitrogen in uneaten feed and feces is released into the water as ammonia nitrogen by bacteria and other decomposer organisms (Boyd and Tucker, 2014).

Ammonia nitrogen occurs in water as un-ionized ammonia (NH_3) and ammonium ion (NH_4^+):
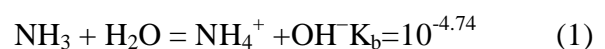
$$NH_3 + H_2O = NH_4^+ + OH^- \quad K_b = 10^{-4.74} \qquad (1)$$

Biological membranes are more permeable to NH_3 than to NH_4^+, and ammonia toxicity is attributed primarily to NH_3. Nevertheless, high NH_4^+ concentration in the water interferes with the outward movement of ammonia through the gills (Liew et al., 2013). Thus, NH_4^+ has some degree of toxicity, but much less than that of NH_3.

The ratio NH_3:NH_4^+ increases with greater pH as obvious from Eq. 1. Moreover, examination of K_b of Eq. 1 for different temperatures (Bates and Pinching, 1949) shows that the NH_3:NH_4^+ ratio also increases with rising temperature.

The usual analytical procedures do not distinguish between ammonia and ammonium, and results are reported as total ammonia nitrogen (TAN) consisting of NH_3-N and NH_4^+-N. The concentrations of each of the two forms can be calculated with Eq.1 using the measured pH and the appropriate K_b for the observed water temperature. However, convenient tables for estimating the percentage of TAN present as NH_3-N at different pHs and water temperatures are available (Trussell, 1972; Emerson et al., 1975), and even more convenient NH_3-N calculators are available on-line – an excellent one can be found at http://www.hbuehrer.ch/Rechner/Ammonia.html.

Concern over possible toxic effects of ammonia in aquaculture system has increased in recent years, because of the intensification of production by greater use of feeds. For example, in ictalurid catfish farming in the southern United States, average production in ponds has increased from less 2,000 kg ha^{-1} in the 1960s to over 5,000kg ha^{-1}in recent years(Hanson and Sites, 2012).Annual production at some farms in Alabama has exceeded 10,000 kg ha^{-1} in recent years. Such high production results in greater nitrogen input that favors higher TAN concentration. Nitrogen and phosphorus that enters water as a result of feeding stimulate phytoplankton productivity, and greater photosynthesis often increases pH during the day, increasing the proportion of NH_3-N (Boyd and Tucker, 2014).

There seems to be reason for concern over possible negative effects of ammonia in ictalurid catfish ponds and other types of intensive pond aquaculture. Nevertheless, a careful analysis of the situation has not been made despite there being considerable information on the toxicity of ammonia to aquaculture species including channel catfish *Ictaluruspunctatus*. The 96-hr LC50 to channel catfish ranged from 1.50 to 3.30 mg L^{-1} with an average of 2.28 mg L^{-1} (Hargreaves and Kucuk, 2001).

Water temperature and pH fluctuate daily in ponds with highest values typically occurring in early to mid-afternoon (Boyd and Tucker, 2014). In order to investigate the

effect of daily fluctuations in NH_3-N, Hargreaves and Kucuk (2001) exposed channel catfish in laboratory systems for 22 to 43 days to pH regimes that mimicked those in ponds. Exposure of fish to a daily maximum NH_3-N concentration of 0.91 mg L^{-1} NH_3-N did not influence growth compared to the control, while a 42% reduction in growth occurred at a maximum daily NH_3-N concentration of 1.81 mg L^{-1} NH_3-N. They concluded that in ponds, a high daily maximum NH_3-N concentration would persist for no more than 5 to 10 days, while the high daily concentration persisted throughout their trial. They suggested that in ponds, an effect on growth might not be elicited at as low a NH_3-N concentration as observed in their study.

The intensity of ictalurid catfish culture in ponds in the United States has increased, and farmers sometimes measure TAN concentrations of 10 mg L^{-1} with ammonia analysis kits. A few such high TAN concentrations have been verified by laboratory analyses at the Alabama Fish Farming Center in Greensboro.The major concern about the high TAN concentration is that disease outbreaks have been noted during or after these episodes (William Hemstreet, Alabama Fish Farming Center, personal communication). The present study was conducted to determine the range in TAN concentration in Alabama catfish ponds, and to ascertain if the concern over high TAN concentration is justifiable.

MATERIALS AND METHODS

Six commercial catfish farms in the Blackland Prairie region of west-central Alabama that have high production were selected, and a total of 31 ponds – five each on five farms and six at the other – were chosen because they had the highest stocking densities on the farms.

The ponds had total alkalinity and total hardness concentrations ranging from 85-128 mg L^{-1} and 91-142 mg L^{-1}, respectively.

The ponds were watershed-type ponds maintained by runoff. Pond water surfaces varied (Table 1), and catfish ponds in Alabama are typically about 1.5 m in average depth (Boyd et al., 2000). The ponds were stocked with channel catfish (*Ictaluruspunctatus*), hybrid catfish (*I. punctatus*♀×*I. furcatus* ♂), or a combination of both. Management was similar among farms. Fish were produced by the multiple-batch system (Boyd et al., 2000) in which marketable-size fish are harvested by a tractor-drawn grading seine at intervals determined by the manager, and advanced fingerlings are stocked as replacements. Ponds usually are drained about twice over a 15-yr period.

Fish were provided a 32% crude protein, floating, pelleted ration daily by truck-mounted feeders that propelled the feed over the water surface around the sides of the pond. Feed usually was applied to apparent satiation, often resulting in more feed being offered than consumed. Floating, electric, paddlewheel aerations in each pond (Table 1) were operated – mainly at night – between May and October.

Water samples were collected from the ponds by dipping surface water with a dipper attached at the end of a 3-m plastic rod. Samples were placed in 1-L plastic bottles and held on ice in insulated chests during transport to the laboratory at Auburn University. Samples were collected weekly from May to September 2013 (late spring and summer), twice weekly in October, and once per month until May 2014. Water samples were filtered by gravity through No. 42 paper, and TAN concentrations in filtrates were measured by the salicylate method (Bower and Holm-Hansen, 1980; Le and Boyd, 2012).

Fluctuations in water temperature and pH were measured over a 24-hr period in two ponds each with light [Secchidisk (SD) visibility > 40 cm], medium [SD visibility 15 – 30 cm], and dense [SD visibility <15 cm)] phytoplankton abundance at Farm K. This farm was

chosen because there were six ponds that met the desired phytoplankton abundance categories. Some of these ponds, however, were not included in monitoring of TAN concentration. Surface and bottom water samples were collected every 3 hr and measurement of temperature and pH were attained with a handheld WaterproofpHTestr® 30 (Oaklon Instruments, Vernon Hills, IL, USA).

Data on water surface areas, total feed input, and total weights of harvested fish were attained from farm owner on managers.

The nitrogen input to ponds was calculated by the equation:

$$N_i = (F_i)(N_f/100) \tag{2}$$

where N_i = nitrogen input in feed (kg ha^{-1}), F_i = feed input (kg ha^{-1}), and N_f = nitrogen concentration in feed (%),

The nitrogen waste load to ponds (feed nitrogen- fish nitrogen) was estimated for each pond as follows:

$$N_w = N_i - (B)(N_b/100) \tag{3}$$

where N_w = nitrogen waste load (kg ha^{-1}), B=harvested biomass (kg ha^{-1}); and N_b = nitrogen concentration in harvested biomass (kg ha^{-1}). Live channel catfish contain 2.38% nitrogen (Boyd et al., 2007).

The nitrogen waste load as equivalent TAN concentration was estimated with the equation:

$$TAN_{eq} = \frac{N_w \times 10^{-3}}{D \times 10^4} \tag{4}$$

where TAN_{eq} = TAN equivalent (g m^{-3} = mg L^{-1}), D = average pond depth (1.5 m was used), 10^{-3} = kg g^{-1}, 10^4 = m^{2}ha^{-1}.

Statistical analysis including estimating of means and standard deviation, analysis of variance (ANOVA) followed by Tukey multiple range test, box plots, and correlation

analyses were conducted using Prism software, Version 6 (GraphPad Software, Inc., La Jolla, CA, USA).

RESULTS

Production data were provided by only five of the farms (Table 1), but TAN concentration was measured at all six farms. Ponds were large, ranging in average watersurface area from 2.53 ha at Farm W to 4.17 ha at Farm K. Total feed input for the 12-month period averaged from 15,580 kg ha^{-1} at Farm A to 21,740 kg ha^{-1} at Farm D; the grand mean and standard deviation were 10,960$\pm$ 2,230. Feed was applied almost entirely between late March and late October – a period of roughly 210 days. Thus, daily feed input averaged around 81 – 103 kg ha^{-1} day^{-1} (grand mean = 92 kg ha^{-1} day^{-1}).

Harvest biomass ranged from 8,010 kg ha^{-1} at Farm A to 13,650 kg ha^{-1} at Farm K (grand mean = 10,960$\pm$ 1,990 kg ha^{-1}). The variation in harvest biomass among ponds was great with coefficients of variation from 31.0 to 48.4% at Farm R and W, respectively.

Because of the great variability in production variables, there were no differences (P > 0.05) among farms for average feed input and harvest biomass. Moreover, the linear relationship between feed input and harvest biomass for all ponds, although significant, was not strong (R^2=0.178, P < 0.05).

Average TAN concentrations did not differ (P > 0.05) among Farms A, D, K, U, and W, but Farm R had a higher average concentration than other farms (Fig. 1). Variation in TAN concentration among ponds and dates was considerable at all farms. The lower end of the range was similar at all farms and concentrations above 5 mg L^{-1} TAN were measured at all farms. However, at Farm A and D, TAN concentration above 10 mg L^{-1} were noted, and at Farm R, a concentration above 15 mg L^{-1} occurred. Roughly 50% of samples had less than 1

mg L^{-1} TAN, and the frequency declined as concentration increased – 10.79% samples were > 5 mg L^{-1} TAN, 2.23% > 10 mg L^{-1}, and 0.12% were > 15 mg L^{-1} (Fig. 2).

Concentrations of TAN were plotted across sampling dates (Fig. 3)for each of three ponds selected to represent the low, medium, and high TAN concentration categories. The TAN concentration tended to fluctuate greatly over time in the low and medium categories, but a concentration of 5 mg L^{-1} was reached in only one of the six ponds of these categories. In ponds of the high TAN concentration category, the concentration steadily increased from May until late August and September. Afterwards, there was considerable variation among dates. On many dates, TAN concentration in all three ponds exceeded 5 mg L^{-1}, and on 11 September all concentrations exceeded 10 mg L^{-1}.

Concentration analysis also revealed that total feed input (R^2= 0.119; P>0.05) and weight of harvested fish (R^2= 0.004; P>0.05) were not related to TAN concentration when all data were pooled. Moreover, concentrations of TAN were not correlated with aeration rate when data from all ponds were considered (R^2= 0.002; P>0.05).

In ponds with light, medium, and dense phytoplankton blooms at Farm K, the pH range was from 7.31 at 0830 hr to 9.50 at 1730 hr (Fig. 4), while the water temperature range was from 26.2 °C at 0530 hr to 31.8 °C at 1730 hr (Fig. 5). Daily trends of change and averages in pH and water temperature were similar among the ponds with different phytoplankton abundance levels.

DISCUSSION

In the multiple-batch production system, fish are partially harvested at intervals followed by restocking of replacement fingerlings. The intervals between partial harvest vary because of delays caused by presence of off-flavor in fish (Boyd and Tucker, 2014), fish

price, and a need for cash or other reasons specific to the producers. The weight of fish harvested and amounts of feed applied varies in individual ponds, farms, and years. It is not possible to make an accurate estimate of actual fish production, feed conversion ratio (FCR), or survival on an annual basis. These estimates require several years of data to which we did not have access. An indication of production performance in multiple batch culture was provided by a yield trial conducted over a 3-yr period in two channel catfish and three hybrid catfish ponds in Alabama (Southern Regional Aquaculture Center, 2007). Net production averaged 6,849 kg ha^{-1} yr^{-1}; mean survival was 67.4%; net FCR averaged 2.10.

Despite the inability to obtain highly reliable production statistics, it was possible to obtain accurate estimates of nitrogen input to ponds during the study period from feed input and feed nitrogen concentration (Eq. 2). These data allowed estimates of nitrogen waste load to ponds (Eq. 3), but nitrogen waste loads (Table 1) must be considered approximate, because actual fish production cannot be estimated over a 12-month period in multiple-batch culture systems.

The daily feed input to multiple-batch culture systems changes during the season because of periodic, partial harvests. There is not a steady increase in feed input from beginning to end of the growing season as in single-batch culture. Nevertheless, there is nearly always a relatively high feed and nitrogen input to ponds in multiple-batch channel catfish culture. This results in a rather continuous and large input of ammonia nitrogen to the water from metabolic excretion by fish utilizing the feed and the microbial community decomposing uneaten feed, feces, and dead plankton (Boyd and Tucker, 2014).

The average waste nitrogen load for the ponds (Table 1) was equal to a combined TAN concentration of 48.44 mg L^{-1} or an average daily TAN concentration input of 0.23 mg L^{-1}per day. The measured TAN concentrations usually did not consistently increase during the grow-out period, and they were far below the average maximum concentration possible

from feed input. This attests to the effectiveness of the pond ecosystem in lessening ammonia nitrogen through nitrification, ammonia diffusion to the air, uptake of ammonium by phytoplankton, and sequestration of ammonia nitrogen in protein of dead organic matter (Gross et al., 2000; Boyd and Tucker, 2014).

Despite the finding that TAN concentration in ponds of this study was not correlated with aeration rate, adequate aeration is important in ammonia management. Low dissolved oxygen concentration inhibits ammonia oxidation by nitrification (Avnimelech et al., 1992) and increases the toxicity of NH_3 to fish (Lloyd, 1961; Thurston et al., 1981).

There obviously was spatial variation in TAN concentration within a pond. However, time constraints allowed us to take only onewater sample per pond because ponds were large, distances between farms were 5 to 20 km, and a large number of ponds were sampled on each date. A study of variation in water quality measurements conducted in 14, un-aerated fish culture ponds ranging from 0.04 to 10.32 ha in water surface area (Boyd et al., 1994) revealed that variation in water quality measurements was in the order: analytical technique < within a pond on a single date < among dates for a single pond < among replicate ponds on a single date. Although that study did not include TAN concentration, it included soluble reactive phosphorus (SRP) concentration and pH that like TAN are influenced greatly by biological activity. Variability decreased from low to high concentration of the measured variables. Results of the study showed that in a pond with an average pH of 8.5 and an SRP concentration of 0.10 mg L^{-1}, one sample per pond usually would provide a concentration within 5% of the standard error of the mean. This suggests that the single samples gave a reasonable estimate of pond average TAN concentration. Of course, fish could seek out areas of lower TAN concentration avoiding exposure to concentrations as great or greater than the average (Boyd and Tucker, 1998)

The abrupt increase in TAN concentration noted in some ponds and illustrated in Fig. 3 apparently was the effect of temporary disruption of one or more of the processes that removed ammonia nitrogen from the water. The most likely explanation for greater TAN concentration in ponds at Farm R was that a cattle pasture surrounded the ponds. Boyd (1976) reported that runoff from cattle pastures increased TAN concentration in ponds. At this farm, rainfall probably caused sudden inputs of ammonia nitrogen from the watershed. The findings clearly show that catfish ponds have elevated TAN concentrations, but concentrations usually are below 5 mg L^{-1}, and greater concentrations are much less frequent.

Schwartz and Boyd (1994) measured TAN concentrations in surface and bottom waters of 25 channel catfish ponds in the Alabama Blackland Prairie region during the four seasons for 2 yr (October 1980 – November 1992). The TAN concentration did not vary greatly between surface and bottom water, and the 2-yr means (and range) for the 400 samples follow: winter 0.49 (0.03-2.48) mg L^{-1}; spring 0.99 (0.01-4.08) mg L^{-1}; summer 1.37 (0.05-4.71) mg L^{-1}; fall 1.89 (0.09-7.71) mg L^{-1}. Concentrations tended to increase from spring to summer, peak in the fall, and diminish in winter. The distribution of TAN concentration measured in 1990-92 was compared to the distribution of concentration measured in the present study in Fig. 6. The histogram shows that there was a greater percentage of samples with < 1 mg L^{-1} TAN in 1990-92 than in 2013, while the percentage of samples with > 4 mg L^{-1} TAN was greater in 2013 than 1990-92. Moreover, the average TAN concentration was somewhat greater in 2013 than 1990-92 – 1.98 mg L^{-1} versus 1.18 mg L^{-1}, respectively. Annual production of channel catfish in ponds of the survey by Schwartz and Boyd (1994) did not exceed 6,000 kg ha^{-1} and feed input did not exceed 12,000 kg ha^{-1} – values considerably less than reported for the 2013 study (Table 1). Thus, it is not surprising that TAN concentration tended to be greater in 2013 than in 1990-92.

The pH of waters in the 1990-92 study differed little between surface and bottom water as also found in the present study (Fig. 4). The pH of 94% of samples of the 1990-92 study of Schwartz and Boyd (1994) was between 7 and 9 when measured between 10:00 and 14:00 hr – only 1% of the 400 samples had pH above 9. The pH range did not differ much among seasons, and a few pHs> 9 were observed in winter. A study by Silapajarn et al. (2004) reported pH values for 223 channel catfish ponds in the Alabama Blackland Prairie averaging 8.1 $\pm$ 0.6 with 209 of the ponds having pH of 7 – 9.5. However, 9 ponds had pH above 9.5 with the highest being 10.4.

The average daily temperature in Alabama catfish ponds will vary from a low of 6 to 8 ℃ in winter to a high of 30 to 32 ℃ in summer. The daily variation in water temperature – especially during warm months – may be as much as 3 to 4 ℃. The pH in the ponds will vary on a daily bases from a low of 7.0 to 7.5 to a high of 8.5 to 9.5. Thus, there is much variation in the proportion of TAN concentration in NH_3-N form at different times of the day and seasons of the year. For example, on a summer day when TAN concentration is 1 mg L^{-1}, a pH change from 8.0 to 9.0 with water temperature at a constant 28 ℃ will raise NH_3-N concentration from 0.066 to 0.412 mg L^{-1}. If pH remains constant at 8.0, a 1 ℃ increase in water temperature will increase NH_3-N concentration from 0.066 to 0.070 mg L^{-1}. However, both temperature and pH usually increase on a summer afternoon. In a pond where temperature and pH increase from 27 ℃ and 7.5 in the morning to 31 ℃ and 9.0 in the afternoon, NH_3-N concentration will raise from 0.020 to 0.463 mg L^{-1}.

Nearly all data on the effects of NH_3-N concentration on fish are for exposure to a fixed TAN concentration at more or less constant pH and temperature. The US Environmental Protection Agency (1999, 2009) developed criteria maximum concentration (CMC) for "safe" TAN concentration for continuous long-term exposure of coldwater and warmwater fish (all species combined) based on pH and temperature. The CMC

concentrations for warmwater species suggest a somewhat lower NOEL for NH_3-N concentration than reported in studies conducted specifically with channel catfish. This difference probably is related to channel catfish being less sensitive to elevated NH_3-N concentration than many other warmwater species (Colt and Tchobanoglous, 1978). This conclusion was confirmed by Hargreaves and Kucuk (2001) who showed that channel catfish excluded ammonia from their blood more effectively than did hybrid striped bass hybrid striped bass *Moronechrysops* ♀ × *M. saxatilis* ♂ ,and blue tilapia *Oreochromisaureus*.

The "safe" or no-observed-effect level (NOEL) of common toxins such as ammonia to aquatic animals often is considered to be 5% of the 96-hr LC50 (Boyd and Tucker, 1998). Based on the average 96-hr LC50 of 2.28 mg L^{-1}, the NOEL of NH_3-N for channel catfish is 0.114 mg L^{-1}. Colt and Tchobanoglous (1978) exposed channel catfish to a range of constant NH_3-N concentrations for 31 days in laboratory systems. Growth averaged 131.9 g in the control (0.048 mg L^{-1} NH_3-N), 109.1 g at 0.217 mg L^{-1} NH_3-N, and progressively declined to 15.3 g at 0.989 mg L^{-1} NH_3-N. Fish mortality occurred at NH_3-N concentrations above 0.989 mg L^{-1}. The NOEL estimated from the 96-hr LC50 of NH_3-N of 0.114 mg L^{-1} fell between the control concentrations of 0.048 mg L^{-1} and next lowest concentration of 0.217 mg L^{-1} included in the study by Colt and Tchobanoglous (1978).

Hargreaves and Kucuk (2001) demonstrated that long-term afternoon exposure to 0.91 mg $L^{-1}NH_3$-N did not affect channel catfish survival and growth, while 1.81 mg $L^{-1}NH_3$-N lessened growth by 42%. Based on results of the aforementioned laboratory studies, we selected 1.0 mg $L^{-1}NH_3$-N as a reasonable NOEL for continuous exposure of ictalurid catfish under conditions of fluctuating pH and temperature.

The NOEL for TAN concentration was calculated for typical average monthly water temperature in west-central Alabama and pHs of 8.0, 8.5, 9.0 and 9.5. The NOEL estimates were plotted by month for each pH (Fig. 7). The NOEL estimates were rather constant during

spring and summer, but increased during fall to a maximum in January. The average TAN concentration was below the estimated NOEL concentration at pH≥ 8.5, about equal to it in summer at pH 9.0, and equal to or above it at pH 9.5 year around. In the "average" pond, TAN concentration will be below the NOEL throughout the year if pH does not rise to pH 9.0 in summer.

The percentage of ponds that had TAN concentration above the NOEL for each month was calculated and presented as a histogram (Fig. 8). No ponds had TAN concentration greater than the NOEL when pH was < 8.5, but from June to October, 3.2% to 14.5% of ponds exceeded the NOEL each month when pH was 8.5. At a pH of 9.0, TAN concentrations exceeded the NOEL each month of the year with the percentage of ponds ranging between 19.4% and 31.5% from June to December. At a pH of 9.5, the NOEL was exceeded monthly throughout the year − 19.4% of ponds in February to 45.2% of ponds in March.

Estimates of the NOEL of NH_3-N to channel catfish by Hargreaves and Kucuk (2001)relied on a daily fluctuating NH_3-N concentration in which the daily maximum was always the same. Thus, the researchers cautioned that their estimated NOEL was probably too lower, because in ponds, conditions for a NH_3-N concentration equal to the NOEL − specifically high temperature, pH, and TAN concentration − would rarely persist for more than 5 to 10 days.Data in Fig. 3, however, do not support the supposition that high peak NH_3-N concentrations would always be brief. In ponds of the high TAN category, TAN concentration often were high for several weeks − even longer in duration than the fluctuating NH_3-N exposures of 22 – 43 days conducted by Hargreaves and Kucuk (2001). Moreover, sustained periods of exceptionally high TAN concentration occurred in the summer when water temperature and afternoon pH were high. Of course, in some ponds, periods conductive to sustained high NH_3-N concentration were brief. But, the NOEL selected for use in this

study of 1.0 mg L^{-1} NH_3-N seems to be a conservative safe level for catfish ponds – it would be better to overestimate NOEL than to underestimate it for obvious reasons.

The ammonia effect data from Colt and Tchobanoglous (1978) and Hargreaves and Kucuk (2001) that were the basis for the NOEL of 1.0 mg L^{-1} NH_3-N were obtained in waters of pH 7.7 and above – similar to pHs in Alabama catfish ponds. However, Sheehan and Lewis (1986) and Miron et al. (2008) found the toxicity of NH_3 to fish increased as pH declined. At pH 6.0, NH_3 was two to fourfold more toxic than at pHs above 8.0. The NOELs for TAN in Fig. 7 should not be considered reliable for pH < 8.0.

The observation that TAN concentrations in the study ponds were not correlated with the harvest weight of fish during the 12-month study does not imply that fish growth was not affected when TAN concentrations exceeded the NOEL. The quantity of fish harvested from a pond during the study resulted from the number of partial harvests and the amount of biomass removed by each – both factors are greatly influenced by managerial decision. Much of the biomass harvested during the study had been produced earlier, and considerable biomass produced during the study remained for later harvest. Negative impacts of elevated TAN concentration on fish health, survival, and growth could have gone undetected during the study.

Once the TAN concentration exceeds the NOEL in a pond, measures such as adding an acid to lower pH, applying an algicide to lessen phytoplankton photosynthesis and reduce pH, or exchange water in ponds to flush out ammonia nitrogen could be used in emergency treatments. However, such treatments are expensive and have possible negative effects in ponds (acidification and algicide treatment), and may have negative environmental impacts, or be impossible to implement at particular sites (water exchange). The practice of applying living bacterial amendments to ponds to lessen TAN concentration is popular in Asia and is

now being used by some ictalurid catfish producers in the United States. However, there is no evidence that this practice is effective (Boyd and Tucker, 2014).

The high TAN concentrations are the result of intensification of production leading to high feed inputs and more nitrogenous waste. As producers are unlikely to lessen stocking and feeding rates, the best approach for avoiding high TAN concentration is to apply practices that lessen TAN concentration and episodes of high pH. These practices include better feed management to improve FCR and reduce nitrogen input in waste, adequate aeration to avoid low dissolved oxygen concentration that inhibits nitrification, and to prevent low total alkalinity and hardness that favor high afternoon pH (Hargreaves and Tucker, 2004; Boyd and Tucker, 2014).

CONCLUSION

The survey of ponds on six ictalurid catfish farms in Alabama revealed that TAN concentrations usually are < 5 mg L^{-1}; but, concentration between 5 and 15 mg L^{-1} were observed. The NOEL for NH_3-N of 1.0 mg L^{-1} for channel catfish suggested in this study would be reached at the typical summer condition of 30 ℃ and pH 8.5 at a TAN concentration of 4.93 mg L^{-1}– the critical concentration. Of 806 samples taken during this study in a total of 31 ponds, there were 89 samples in which the critical (NOEL) TAN concentration was exceeded.

This study confirms that TAN concentrations high enough to be chronically toxic to ictalurid catfish occur rather commonly in ictalurid catfish ponds in Alabama. There are no practical emergency treatments for quickly reducing NH_3-N concentrations in these ponds when the critical TAN concentration has been exceeded. Therefore, the use of good management practices to avoid excessively high TAN concentrations seems appropriate.

Additional research on effects of elevated TAN concentration in ponds for intensive culture of ictalurid catfish and other species is needed. The studies should focus on the relationship of TAN concentrations to disease outbreaks, physiological well-being of fish, feed intake, survival rate, and growth. In the case of ictalurid catfish, toxicity tests should be conducted to ascertain if channel catfish and hybrid catfish have similar response to elevated NH_3-N concentration.

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Table 1. Average pond size, feed input, production, aerator use, and nitrogen waste load over a 1-yr period for catfish farms of this study.(Means were tested by Tukey'sStudentized Range (HSD) test; entries indicated by the same letter in a column do not differ at P = 0.05)

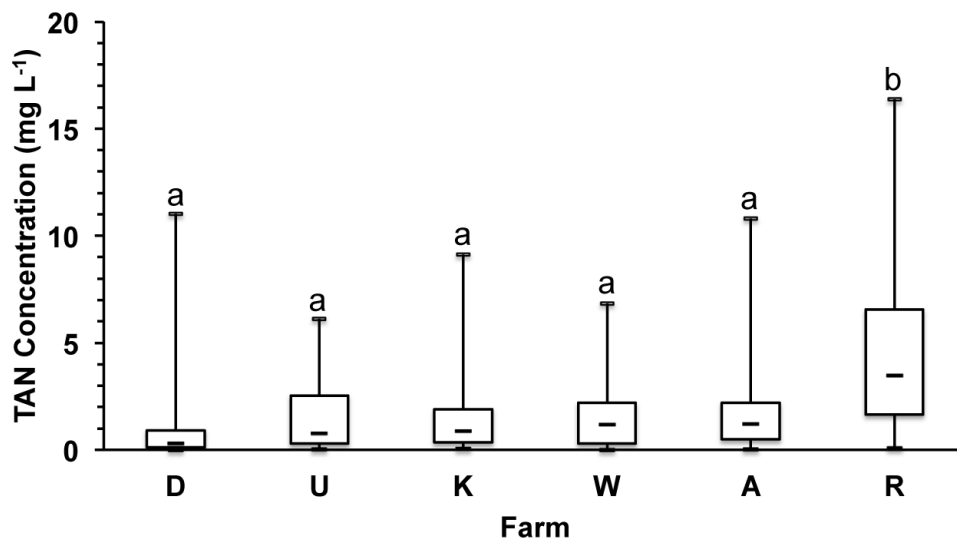| Farm	Average pond area (ha)	Average production (kg/ha)	Average feed input (kg/ha)	Average aerator use (kw/ha)	N waste load (mg/L)
W	2.53	9560 ±4630 a	19680 ±1890 a	7	52 ±10.8 a
A	2.83	8010 ±2641.0 a	15580 ±4540 a	6	40 ±12.2 a
D	3.89	12340 ±5124. a	2174 ±1980 a	17	55 ±12.7 a
R	2.53	11250±3490 a	21390 ±3960 a	10	55 ±11.6 a
K	4.17	13650 ±5900.6 a	18070 ±5690 a	5	40 ±15.7 a
U					

Figure 1. Boxplots of total ammonia nitrogen (TAN) concentrations in ponds for six catfish farms in west Alabama (Entries indicated by the same letter shows the means do not differ at P = 0.05).
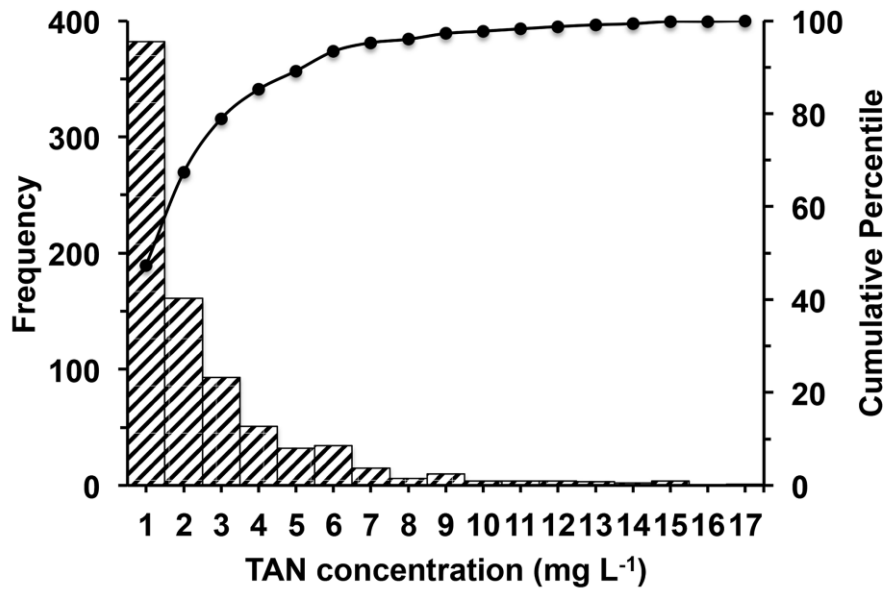
Figure 2. Summary of frequency distributions and cumulative percentiles of total ammonia nitrogen (TAN) concentrations for the period May 2013 to May 2014 in 31 catfish ponds located in west Alabama.
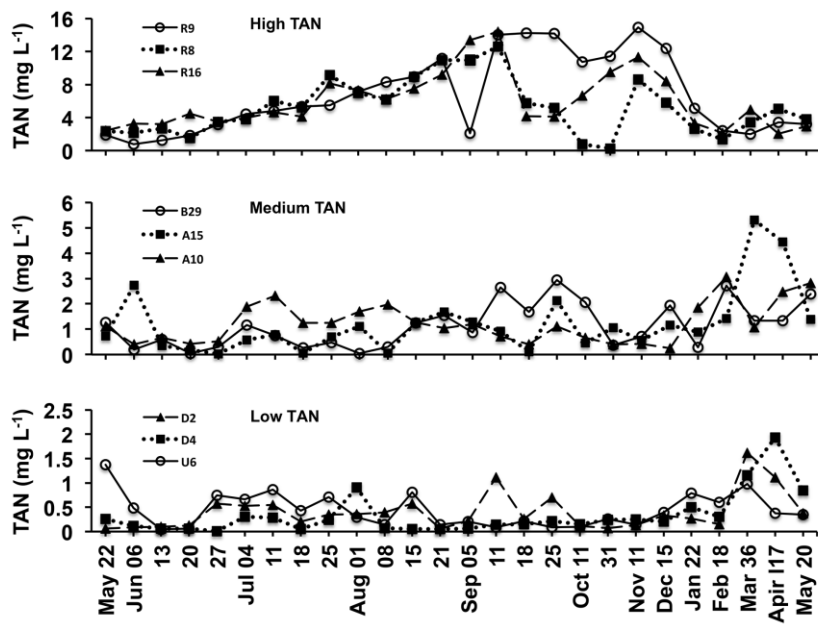
Figure 3. Fluctuations in total ammonia nitrogen (TAN) concentrations betweenMay 2013 and May 2014 in three ponds each with high, medium, and low TAN concentrations.

Figure 4. Fluctuation in pH over a 24-hr period in surface water (T) and bottom water (B) of ponds with dense, medium and light plankton blooms.

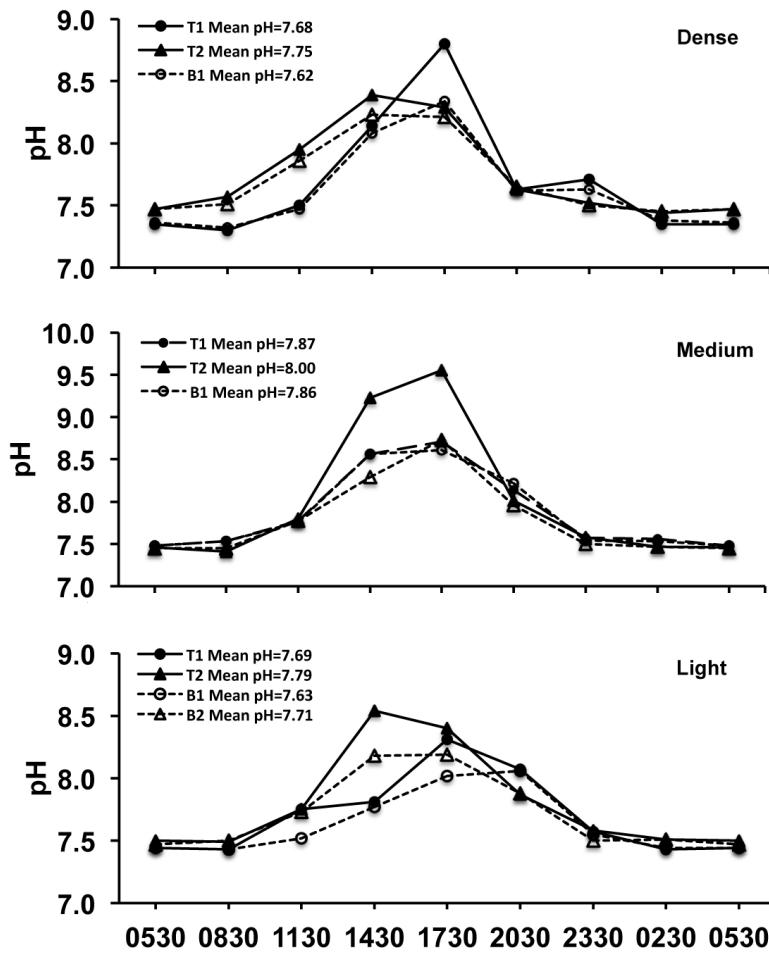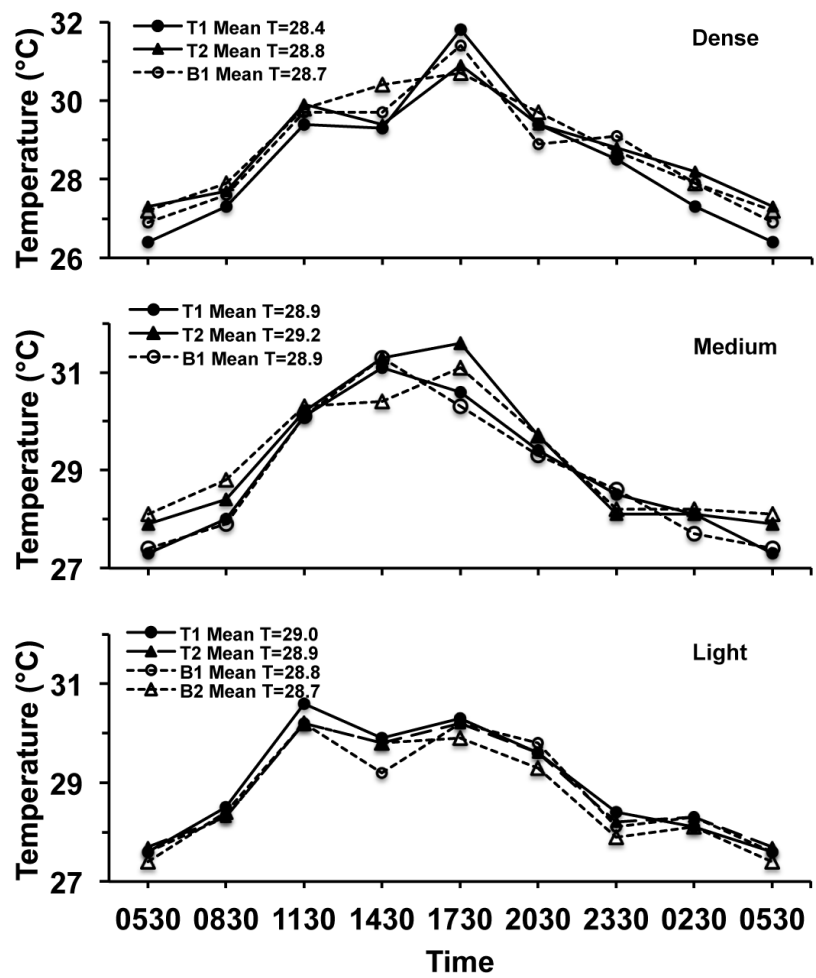Figure 5. Temperature fluctuation patterns over a 24-hr period in surface water (T) and bottom water (B) of ponds with dense, medium and light plankton blooms.
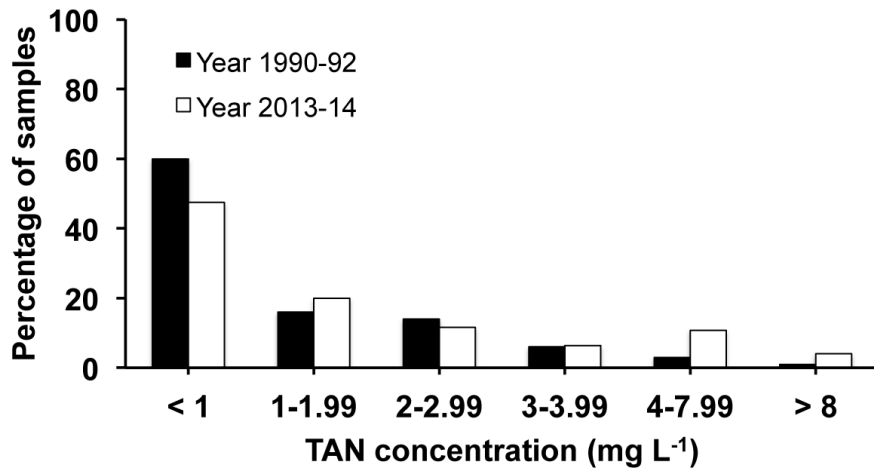
Figure 6. Distribution of total ammonia nitrogen (TAN) concentrations measured in spring, summer, fall, and winter in 25 Alabama catfish ponds during the period 1990-92 (Schwartz and Boyd) compared with the period 2013-14 (present study).

Figure 7. The estimated no observed effect level (NOEL) for total ammonia nitrogen (TAN) concentration to ictalurid catfish. The NOELs were estimated for average monthly water temperatures at different pHs.

Figure 8. Cumulative percentage of ponds having total ammonia nitrogen (TAN) concentration above the no observed effect level (NOEL) for each month at three pH levels.

V. TOTAL AMMONIA NITROGEN REMOVAL FROM AQUEOUS SOLUTIONS BY THENATURAL ZEOLITE, MORDENITE: A LABORATORY TEST AND EXPERIMENT STUDY

ABSTRACT

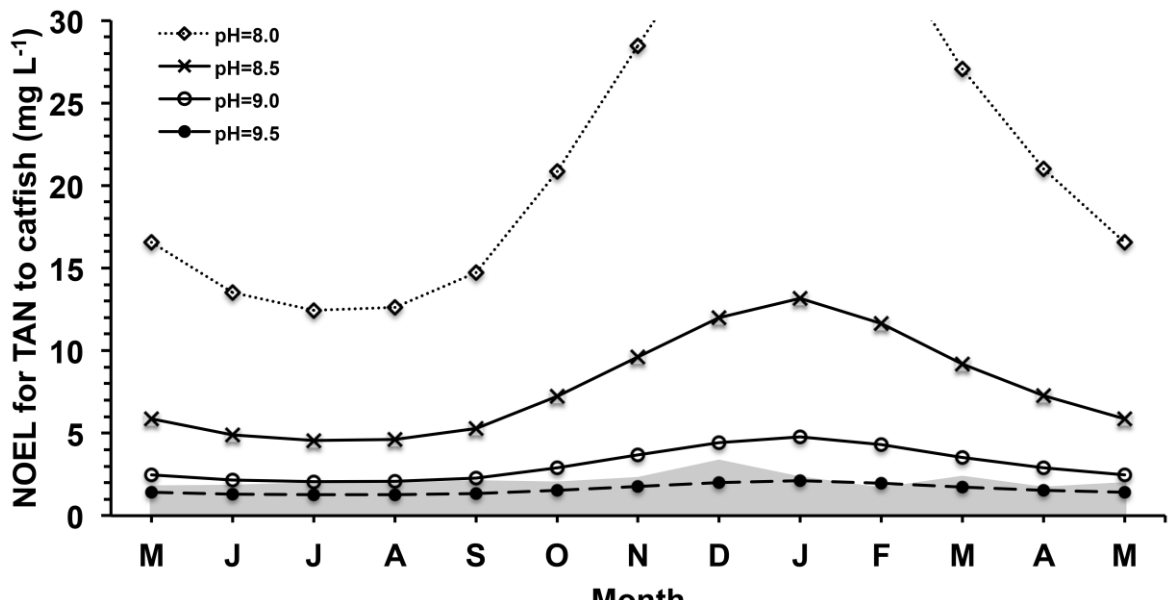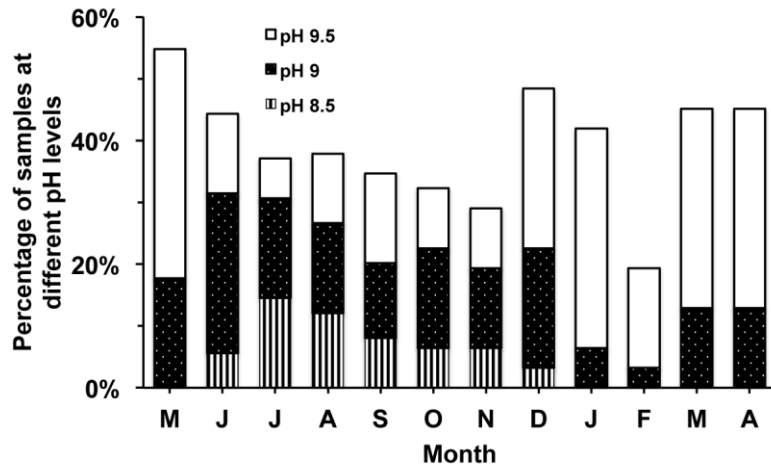The effectiveness of two processed samples of New Zealand mordenite for possible use in removing total ammonia nitrogen (TAN) in aquaculture application was studied. The percentage reduction in TAN concentration in 100-mL solutions held on a rotating shaker increased linearly with greater mordenite application rate, while the amount of TAN removed per gram of mordenite (adsorptive efficiency) declined. At a TAN concentration of 200 mg L^{-1}, both mordenite samples had an adsorption efficiency of about 8.7 mg TAN g^{-1}. Ammonia removal and adsorptive efficiency decreased with increasing salinity up to 30 g L^{-1}. In aquarium tests with less vigorous mixing, mordenite at concentrations equivalent to 10 to 500 kg ha^{-1} in ponds removed only 1 to 8% of TAN from water. Mordenite and other zeolites are not useful for removing TAN from ponds. The New Zealand mordenite was of good quality and like other zeolites could be used for other applications in aquaculture such as small, intensively-stocked transport and holding units for aquatic animals, water recirculating culture systems, and possibly as a feed additive.

INTRODUCTION

Zeolites are naturally occurring, synthetic minerals with a three-dimensional framework formed by silica-oxygen tetrahedrals in which some of the Si^{4+} has been replaced by Al^{3+} in the porous latticework resulting in a negative charge that imparts cation exchange properties to zeolite (Mumpton and Fishman, 1977).Zeolite filters are used for softening water for household use; sodium on zeolite is exchanged for calcium and magnesium in water. Zeolites are used in industry for cation exchange, molecular sieving, catalysis, and sorption processes (Jorgensen et al., 1976; Yusof et al. 2010). Zeolite also has been used to remove ammonia nitrogen from wastewater (Reeves, 1972; Yusof et al., 2010).

Mordenite, the zeolite studied here, is a common alteration product of pyroclastic sediment, sedimentary rock, and lava flows of worldwide distribution. It is an orthorhombic zeolite of high silica content (Deer et al., 2004). The ideal composition of mordenite is $[(Na_2K_2Ca)_4Al_8Si_{40}O_{96}]\cdot28H_2O$. Mordenite contains 0.45% potassium, 2.29% calcium, and 2.89% sodium, while clinoptilolite, the zeolite commonly used in research on zeolite applications, is 3.21% potassium, 1.25% calcium, and 1.79% sodium (Passaglia, 1975; Simoncic and Armbruster, 2004).

Mordenite often is modified during processing to tailor its pore size and shape to meet the criteria for specific applications (Corma et al., 1994; Lin et al., 2013). It has been widely used as a catalyst in industrial processes such as hydrocracking, hydroisomerization, alkylation and reforming (Bajpai, 1986; Shaikh et al., 1993), and for adsorptive separation for gas or liquid mixtures (Shao, 2002). Mordenite also is used as a host matrix in semiconductors, chemical sensors and selective membrane (Gilbert and Mosset, 1998).

The main interest in zeolites in aquaculture relates to control of total ammonia nitrogen (TAN) concentration. Ammonia is toxic to fish, shrimp, and other aquatic animals,

and zeolite is used to lessen TAN concentrations in aquaria, fish holding tanks, water recirculating aquaculture systems, and containers of water for transporting aquatic animals (Bower and Turner, 1982; Boyd and Tucker, 1998; Johnson and Sieburth, 1974). Ammonia removal by zeolite is possible, because ammoniacal nitrogen exists as ammonium (NH_4^+) and ammonia (NH_3) in a pH and temperature equilibrium as follows:

$$NH_3 + H_2O = NH_4^+ + OH^- \tag{1}$$

The proportion of ammonium decreases with increasing pH, but even at pH 9.0, ammonium comprises about 70% of the ammoniacal nitrogen in water (Trussell, 1972). Removal of ammonium by zeolite will lower the TAN concentration and thereby lessen the concentration of ammonia at equilibrium.

Shrimp farmers in Thailand and other Asian countries often apply zeolite to ponds at 180 to 350 kg ha^{-1} in attempts to lower the concentration of ammonia to which culture animals are exposed, but there are no research findings to support this practice (Tonguthai, 2000). Moreover, zeolite products sold in Thailand (and presumably in other Southeast Asian countries) usually have a cation exchange capacity (CEC) less than 50 meq 100 g^{-1} and do not absorb appreciable ammonium (Silapajarn et al., 2006). Chiayvareesajja and Boyd (1993) suggested that zeolite was not effective in removing TAN from pond waters – especially those filled with brackishwater and ocean water – because of competition for cation exchange sites on zeolite by other cations in water.

According to Yusof et al. (2010), there have been many studies of natural zeolites – particularly clinoptilolite – for removing ammonium from wastewater. The results of these studies do not provide much useful information related to use of zeolites in aquaculture, because TAN concentrations in wastewater are much greater than in waters of aquaculture

systems. A laboratory study was undertaken to ascertain if New Zealand mordenite has potential to lower TAN concentrations in waters of aquaculture systems.

MATERIALS AND METHODS

Two samples of mordenite were supplied by Blue Pacific Minerals, Tokora, New Zealand. Bulk density of the samples was determined by weighing 100-cm^3 of each. Cation exchange capacity was obtained by the magnesium chloride technique (Rhoades, 1982) and by the neutral ammonium acetate method (Jackson, 1958; Kitsopoulos, 1999). Particle size was determined by passing known quantities of the two products through nested sieves of the following aperture sizes: 850, 425, 250, 150, 106, 75, 53, and 43 μm and weighing the amount of particles retained on each sieve. The pH was determined by glass electrode in 1:2 mixtures of mordenite:distilled water (Thunjai et al., 2001). Color was assessed with a Munsell color chart (Kollnorgen Instruments Corporation, New Windsor, New York).

Ammonium adsorption by the two mordenite samples was assessed under different conditions by adding weighted quantities to triplicate, 100-mL solutions containing known TAN concentrations. The following trials were conducted: (1) 1.00-g mordenite and TAN concentrations ranging from 0 to 200 mg L^{-1} and prepared in distilled water; (2) mordenite amounts ranging from 0 to 100.0 g L^{-1} in TAN concentration of 1 mg L^{-1} prepared in distilled water; (3) 1.00-g mordenite and TAN concentration of 1.00 mg L^{-1} in selected salinities between 0 and 30 g L^{-1}; (4) 1.00-g mordenite in TAN concentration of 5.00 mg L^{-1} in solutions also containing either sodium, potassium, calcium, or magnesium at selected concentrations between 0 and 50 meq L^{-1}.

In the trials, TAN concentrations were obtained from ammonium chloride (NH_4Cl). The different salinities were prepared by adding appropriate amounts of Instant Ocean® to

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distilled water. The different concentrations of major cations were made in distilled water using sodium chloride (NaCl), potassium chloride (KCl), magnesium sulfate ($MgSO_4 \cdot 5H_2O$), and calcium nitrate [$Ca(NO_3)_2$]. The trials were conducted in a room where temperature varied from 22 to 26 ^{o}C. The flasks were agitated for 24 h on a mechanical shaker at 150 oscillations min^{-1}.The pH of solutions was measured with a glass electrode before shaking. After shaking, solutions were filtered (Whatman Number 42 paper) to remove mordenite particles and TAN concentrations in filtrates were measured by the salicylate technique (Bower and Holm-Hansen, 1980; Le and Boyd, 2012).

The capacity of mordenite to lower TAN concentration also was tested in 20-L aquaria filled with freshwater (0.1 g L^{-1} salinity) and containing 3.0 mg L^{-1} TAN from ammonium chloride. Mordenite was applied to each of four aquaria at 0, 1, 5, 20, and 50 mg L^{-1}; equivalent to approximately 10, 50, 200, and 500 kg ha^{-1} in a 1-ha pond of 1 m average depth. Aeration was applied continuously via an air stone (5 cm long × 1.5 cm diameter) to gently mix the water in two aquaria at each mordenite concentration, but the other two aquaria were not aerated. Water samples were removed from each aquarium after 24 h for measurement of pH and TAN concentration.

RESULTS

The two mordenite samples were yellowish in color, acidic, and of low bulk density (Table 1). Sample 1 was of much finer particle size than sample 2 (Fig. 1). Nearly 60% of particles in sample 1 passed a sieve with 250 μm apertures while only about 0.5% of particles in sample 2 were that small. The CEC values ranged from 93.3 to 119.8 meq 100 g^{-1}. The CEC was around 6% greater for sample 1 than for sample 2 by both CEC methods. However, the CEC measured by the ammonium acetate method was approximately 20 meq 100 g^{-1}

greater than the CEC determined by the magnesium chloride procedure for both samples. The ammonium acetate technique has been reported to give high results for acidic soil samples (Ross and Ketterings, 1995). Nevertheless, Kitsopoulos (1999) recommended using the ammonium acetate method for determining the CEC of mordenite. The manufacturer's website states that the New Zealand zeolites have a CEC typically greater than 100 meq 100 g^{-1} – this agrees fairly well with the CECs measured here.

Some studies have reported higher CECs for mordenite samples. For example, 80 mordenite samples from Santorini and Polyegos Islands of Greece (Kitsopoulos, 1999) had an average CEC of 130 meq 100 g^{-1} (range = 1.70 to 200.4 meq 100 g^{-1}). Wang (2009) reported that mordenite had a CEC of 187 meq 100 g^{-1}, and a value of 164 meq 100 g^{-1} was given by Sprynskyy et al. (2005). However, samples mentioned above had been pulverized to pass a sieve with 125 μm apertures. Particles of sample 1 that passed a screen with 250 μm apertures and particles that passed a screen with 53 μm apertures were analyzed for CEC; the results were 136 meq 100 g^{-1} and 208 meq 100 g^{-1}. Thus, the lower CEC in the commercial products from New Zealand than in mordenite samples studied in previous research probably was related to particle size rather than to a natural property of the New Zealand mordenite.

The main reason for this investigation relates to zeolite use in aquaculture; therefore, the properties of the two mordenite samples were compared with properties of 25 different zeolite products purchased in shrimp farming supply stores in Thailand (Silapajarn et al., 2006). Only four of those samples were yellowish in color – the others were various shades of white and gray. The density of the samples from Thailand averaged 1.01 g cm^{-3} (range – 0.57 to 1.40 g cm^{-3}), and the average pH was 6.98 (range = 4.11 to 12.03). Most of the zeolite samples from Thailand were finely pulverized – more than 50% of all but two samples passed a screen with 250 μm apertures. The CEC of the samples from Thailand tended to be

quite low; the average was 34.4 meq 100 g^{-1} (range = 2.7 to 147.8 meq 100 g^{-1}). Only four of 25 samples had CECs above 80 meq 100 g^{-1}. The two mordenite samples of the present investigation had a greater CEC than all but two of the Thailand samples.

The percentage reduction in TAN concentration from solution and the quantity of TAN removed per gram of mordenite are presented in Fig. 2. In the 100-mL solutions with 1.00 g of mordenite, as the concentration of TAN in the water increased from 1 mg L^{-1} to 200 mg L^{-1}, the removal percentage bymordenite decreased quickly, while the amount of TAN removed per gram increased drastically.

When TAN concentration was 200 mg L^{-1}, the adsorptive efficiency for these two mordenite samples was roughly 8.70 mg TAN g^{-1} zeolite, while TAN removal percentage was about 43% for both mordenite samples. This result agrees well with those obtained by Marking and Bills (1982) and Chiayvareesajja and Boyd (1993) for clinoptilolite − maximum uptake in both studies was about 9 mg TAN g^{-1}clinoptilolite.

At TAN concentrations less than 10mg L^{-1}, the two mordenite samples removedabout 90% of TAN,but adsorptive efficiency was quite low (less than 1 mg TAN g^{-1}mordenite).Pairwise t-test showed that there was no difference between the two samples in ammonium adsorptive capacity at different TAN concentrations (t = 0.974; P > 0.05).

Experimental data werefitted to the Freundlich model and Langmuir model to describe the equilibrium uptake behavior of ammonium ion on to mordenite(Barrow, 1966). Efficiency of TAN uptakeby mordenite(absorbent)increased with greater TAN (absorbate) concentration in the liquid phase (Fig. 3). The Langmuir model provided a better fit for the experimental data from sample 1 than did the Freundich model. While for sample 2, the Freundlich model agreed more closely with the experimental data than did the Langmuir model at lower solution concentration (<40mg/L), while at higher concentration, neither model provided a good fit.

Ammonium removal capacity was a function of the amount of mordenite placed in solutions (Fig. 4).As mordenite concentration in water of 1 mg L^{-1} TANincreased, the removal percentage of TANincreased linearly, while the efficiency of mordenite decreased. When the mordeniteconcentration was 100.0mg L^{-1}, percentage removal of TAN reached 57% for both samples, but the adsorptive efficiency for these two samples was roughly 2.8 mg TAN $g^{-1}$of mordenite. The highest adsorption efficiency was attained at a mordenite concentration of 2mg L^{-1}; however, theTAN removal percentage was only 15%. Greater absorptive efficiency could be obtained by either applying a small amount of mordenite or by increasing TAN concentration in solution. Bower and Turner (1982) reported that clinoptilolite had an equilibrium exchange capacity for ammonium of 1.2 to 2.25 meq g^{-1}. For Modenite used in this study, the ammonium equilibrium capacity ranged from 0.3 to 3.7 meq g^{-1}. Thus, under the best conditions for ammonium exchange, mordenite appears to be superior to clinoptilolite.

The removal percentage of TAN decreased exponentially (sample 1, $R^2=$ 0.952;sample 2, $R^2=0.957$; $P < 0.05$) as salinity of the solution increased(Fig. 5).Considering both samples, when salinity was less than 0.6 g L^{-1}, 90% of TAN could be removed by mordenite; at salinity of 8.4 g L^{-1}, roughly 50% of the TAN in water was removed, but at 30 g L^{-1} salinity the removal rate was about 23%. The decrease in TAN removal as salinity increasedresulted fromgreater concentrations of cations at higher salinities that competed for the adsorption sites on the mordenite. The rate of decrease in the percentage TAN removal declined slightly with increasing salinity. Pairwise t-test showed that there was no difference (t = 0.134; $P > 0.05$) between ammonia adsorptive capacity of the two samples at different salinities. Johnson and Sieburth (1974) also reported that clinoptilolite was much more effective for removing TAN in fresh water than in salt water, and the efficiency rate decreased slowly as water salinity increased.

The influence of competing cations (potassium, sodium, calcium, and magnesium) on the capacity of mordenite to remove TAN is presented in Fig. 6.At low cationic concentration (0.5 meq L^{-1} to 2 meqL^{-1}), the presence of potassium, sodium, calcium and magnesium ions caused little inhibition of TAN adsorption. Moreover, as the ionic concentration increased,magnesium and calcium had little effect on TAN adsorption with roughly 90% of TAN being removed at 50 meq L^{-1}. The influence of sodium and potassium – especially potassium – on TAN uptake by mordenite was greater, and the presence of 50 meq L^{-1} potassium with 1 mg L^{-1} TAN caused a significant decrease in TAN adsorption (less than 10% ammonia removal). The order of cation preference by mordenite was $K^+>$ $Na^+> Ca^{2+}> Mg^{2+}$; this is the same order of preference noted for clinoptilolite(Ames, 1960).

The average concentrations of major cations in seawater are as follows: potassium, 380 mg L^{-1} (9.71 meq L^{-1}); calcium, 400 mg L^{-1} (19.96 meq L^{-1}); magnesium, 1,350 mg L^{-1} (111.02 meq L^{-1}); sodium, 10,500 mg L^{-1} (456.52 meq L^{-1}). Neither calcium nor magnesium at concentrations up to 50 meq L^{-1} caused a great decrease in TAN removal by mordenite. Moreover, at a potassium concentration equal to that of normal seawater, there was only a 50% reduction in TAN adsorption. The large reduction in TAN adsorption with increasing salinity (Fig. 5) apparently was caused mainly by sodium that is present in seawater at much higher concentration than the other three cations. Even at 5 ppt salinity, seawater should contain about 65.2 meq L^{-1} of sodium.

In the 20-L aquaria, applications of 1 mg L^{-1} to 50 mg L^{-1}mordenite wereequivalent to application rates of 10 kg ha^{-1} to 500 kg ha^{-1}in a 1-ha pond of 1 m in depth. The TAN concentrations initially of 3 mg L^{-1} decreased by only 1 to 8% (Fig. 7) regardless of whether or not aeration was used to mix the water ($t = 0.600$; $P > 0.05$). Reduction would have been even less in saline water. Although zeolite is capable of reducing TAN concentration in

water of laboratory tests, it probably would not be effective for this purpose in aquaculture ponds.

DISCUSSION

Previous research on zeolite applications in aquaculture revealed that a zeolite filter can be an effective backup system to biological filters for TAN removal during culture of marine or brackishwater species in water recirculating systems. In addition to use in event of failure of the biological filter, the zeolite filter could be used when diseased fish are treated with antibiotics to avoid harm to the biological filter (Johnson and Sieburth, 1974). It has been suggested that zeolite filters could function as the primary method of TAN removal in freshwater recirculating aquaculture systems (Johnson and Sieburth, 1974; Dryden and Weatherley, 1987), but their use in recirculating aquaculture systems is limited mainly to backups or supplements to biological filters for TAN removal (Yanong, 2012). However, Gendel and Lahav (2013) developed a technique for removing TAN from water of recirculating aquaculture systems using a zeolite filter that is regenerated by chemical desorption and indirect electrochemical ammonia oxidation. This method of TAN removal possibly could be used instead of a biological filter.

Zeolite can remove TAN from water and thereby prevent fish toxicity. But, the treatment rates necessary to do so were reported as 10 kg m^{-3} (Emadi et al., 2001), 12 kg m^{-3} (Farhangi and Rostami-Charate, 2012), and 15 kg m^{-3} (Asgharimoghadam et al., 2012). Zeolite treatment also caused large reductions in TAN concentrations in sediment pore water, but treatment rates of 10% of sediment volume were necessary (Besser et al., 1998). Zeolite application to polluted water at a treatment rate of 4 kg m^{-3} prevented cadmium toxicity to tilapia presumably by removing cadmium by cation exchange (James and Sampath, 1999).

Zeolite filters have been promoted for removal of TAN from aquaculture wastewater production units (Bergero et al., 1994). This application was considered feasible where TAN concentration exceeded 0.35 mg L^{-1}, and water discharge rate was less than 0.60 m^3 m^{-1} per tonne of fish production (Bergero et al., 2001). There is considerable interest in zeolite for removal of TAN from wastewater, e.g., Huang et al. (2010) and Guo et al. (2013), and such reports often mention aquaculture as a possible application.

Zeolite has been added to fish feeds in amounts of 5 to 10%, but there was no reduction in effluent TAN concentration from the culture units in which this feed was used (Edsall and Smith, 1989). There is evidence, however, that inclusion of zeolite in aquaculture feeds can improve nutrient utilization and growth in fish (Khodanazary et al, 2013; Obradović et al., 2006).

At present, the major use of zeolite in aquaculture is for the purpose of reducing TAN concentrations in shrimp ponds in Asia (Arthur et al., 2000). Zeolite is broadcast over pond surfaces at rates of 180 to 350 kg ha^{-1} (Tonguthai, 2000). These rates equal about 18 to 35 mg L^{-1} in 1.0-m average depth ponds – much less than application rates found necessary to lessen TAN concentrations in sediment pore water (Besser et al., 1998) and to avoid ammonia toxicity to fish (Emadi et al., 2001; Farhangi and Rostami-Charate, 2012); Asgharimoghadam et al., 2012). Moreover, Chiayvareesajja and Boyd (1993) and Briggs and Funge-Smith (1996) reported that zeolite applications to aquaculture ponds did not result in lower TAN concentrations. Based on previous findings and aquarium tests of the present study, it may be concluded that zeolite is not effective in lessening TAN concentrations in pond aquaculture at application rates typically used. Application rates necessary to be effective possibly would be as great as 100,000 kg ha^{-1} based on data for TAN removal in laboratory trials by Emadi et al. (2001).

Previous research confirms that zeolite filters can effectively remove ammonia nitrogen from water – especially when the TAN concentration is elevated and the flow rate is moderate. More research is needed on the use of zeolite for removing TAN from water in recirculating aquaculture systems and in reducing TAN concentrations in aquaculture effluents. There also should be further investigation of the benefits of zeolite on growth and nutrient utilization when it is included in aquaculture feed.

Most of the earlier work on zeolite in aquaculture focused on clinoptilolite, but mordenite is equal to clinoptilolite in CEC and ability to remove TAN from water. The mordenite of the present study was of high quality – much better in quality than all but two of 25 zeolite samples from Thailand that were analyzed by Silapajarn et al. (2006). Although we do not recommend mordenite, or any other zeolite, for application to shrimp or other aquaculture ponds for TAN control, mordenite is a good product for TAN removal from water in aquaria, intensively-stocked holding and transport containers, water recirculating aquaculture systems, TAN removal from small volumes of aquaculture effluent, and in research on use of zeolite as a feed additive.

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Table 1. Physical and chemical properties of two, New Zealandmordenite samples.

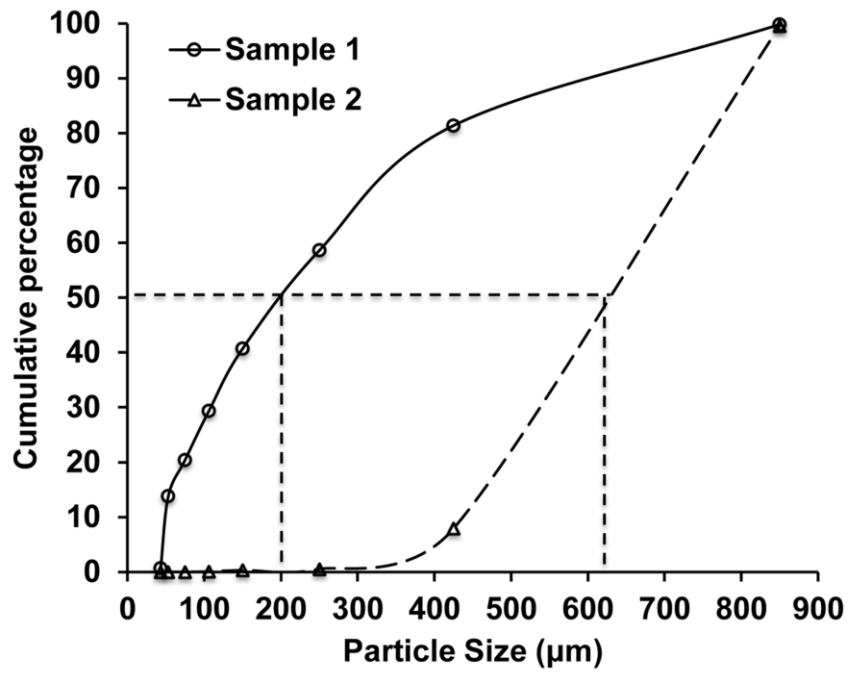| Variable	Sample 1	Sample 2
Color (Munsell color units)	8/6 (yellow)	8/4 (pale yellow)
Bulk density (g cm^{-3})	0.606	0.727
Median particle diameter (μm)	200	660
pH	5.48	6.06
Cation exchange capacity (meq 100 g^{-1})		
Magnesium chloride method	98.8	93.3
Neutral ammonium acetate method	119.8	112.0

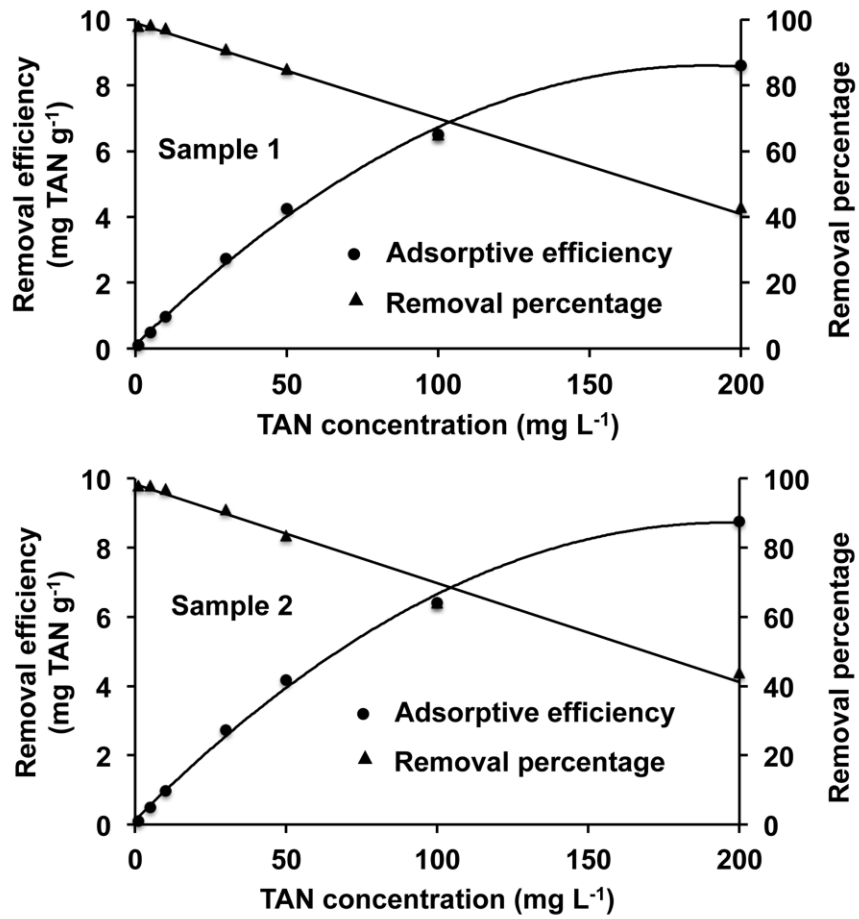Figure 1. Particle size of two, New Zealand mordenite samples.

Figure 2. Effect of total ammonia nitrogen(TAN) concentration on adsorptive and percentage removal of TAN of two mordenite samples applied at 1 g 100 mL^{-1}.
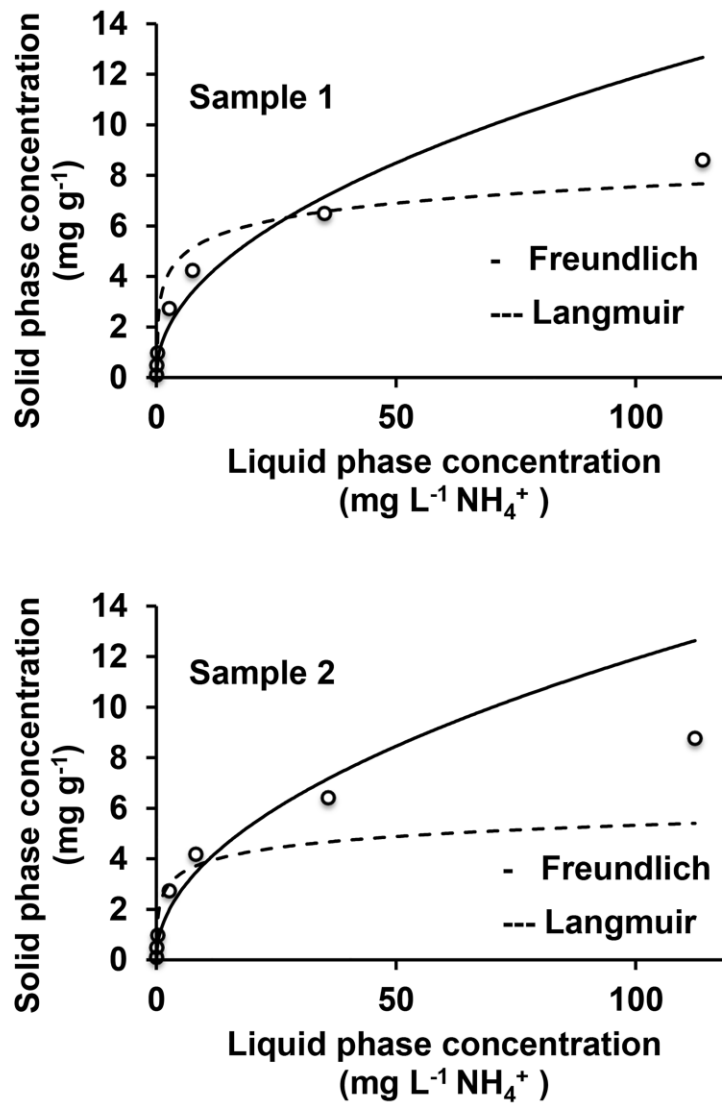
Fig. 3. Equilibrium isotherm data for ammonium uptake ontomordenite fitted to the Langmuir and the Freundlich uptake models with two mordenite samples applied at 1 g 100 mL^{-1}.
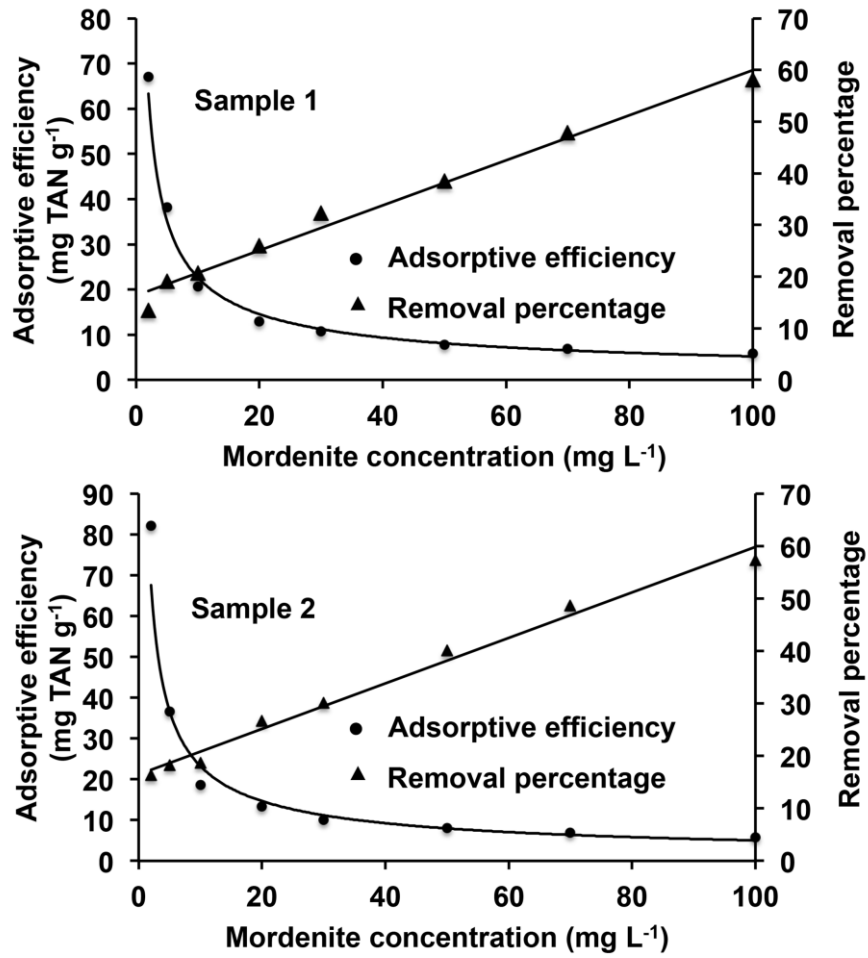
Figure 4. Effect of application rate of two mordenite samples on adsorptive efficiency and percentage removal of total ammonia nitrogen (TAN) from solution containing 1 mg L^{-1} TAN.
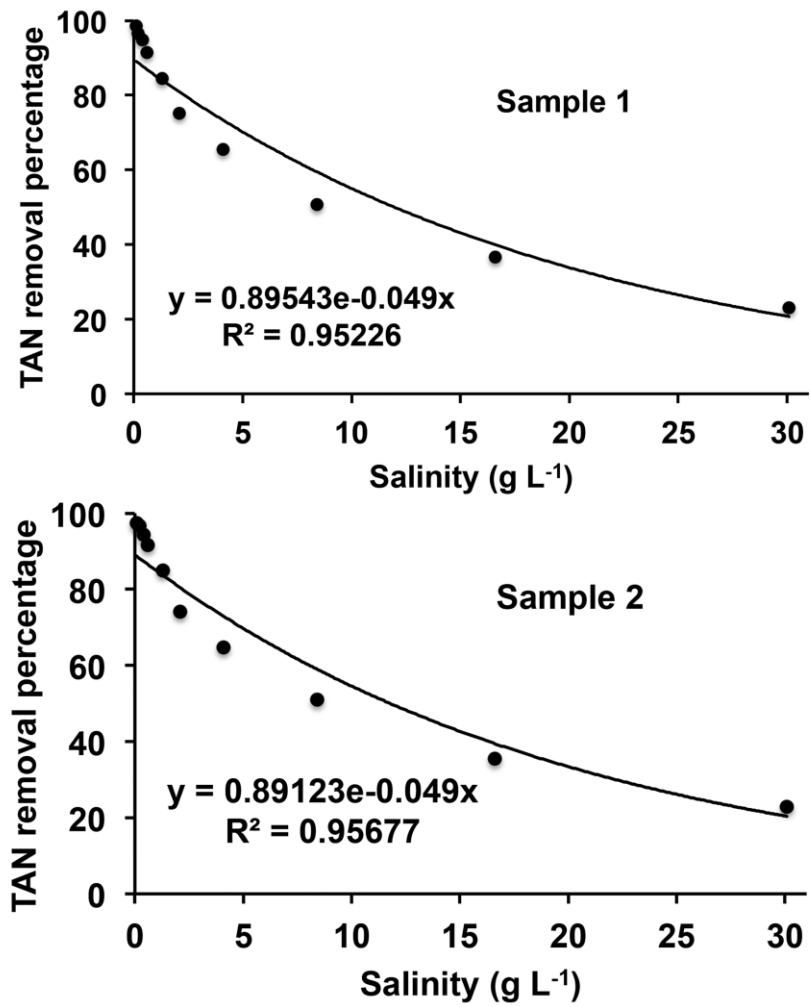
Figure 5. Exponential relationship between percentage removal of total ammonia nitrogen (TAN) from sample containing 5 mg L^{-1} TAN by two mordenite samples (1 g 100 mL^{-1}) at different salinities.

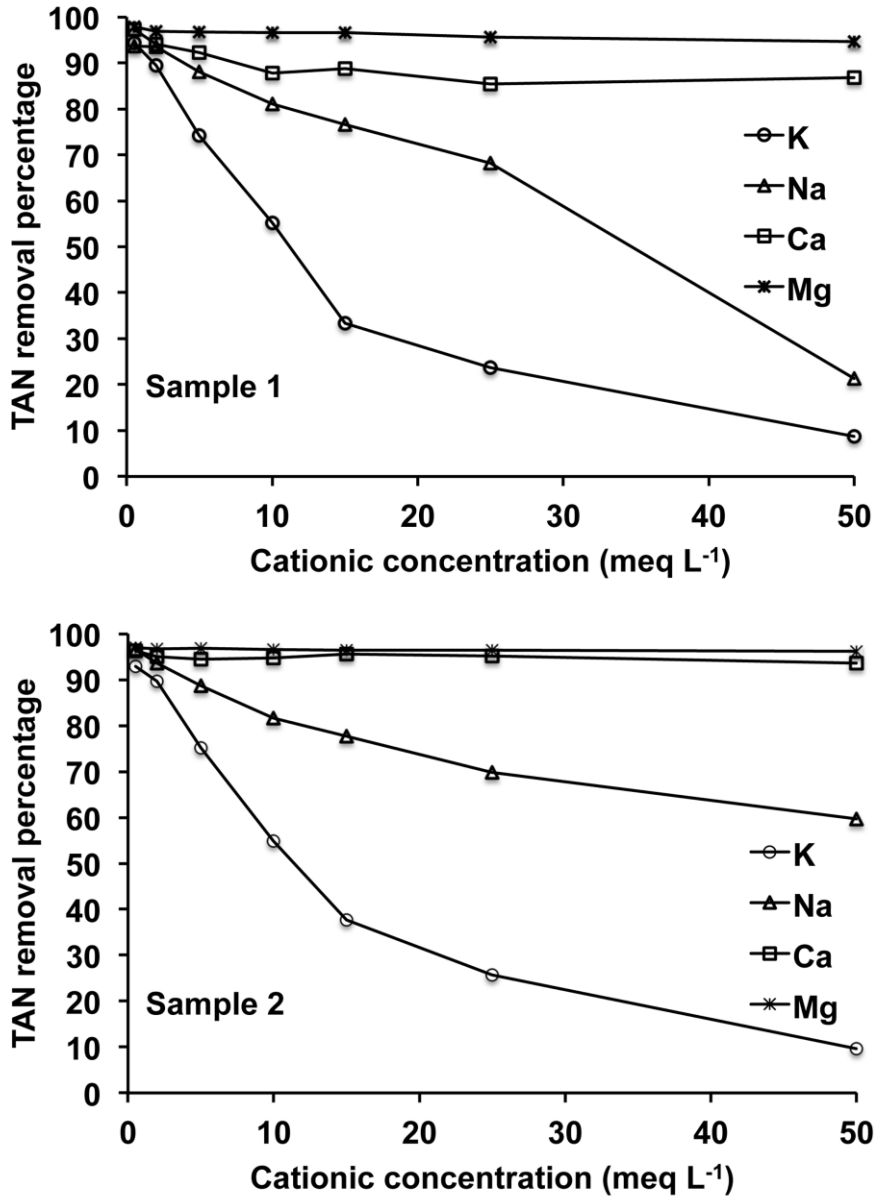Figure 6. Cation preference oftwo mordenite samples applied at 1 g 100 mL^{-1} in water of different cation concentration.
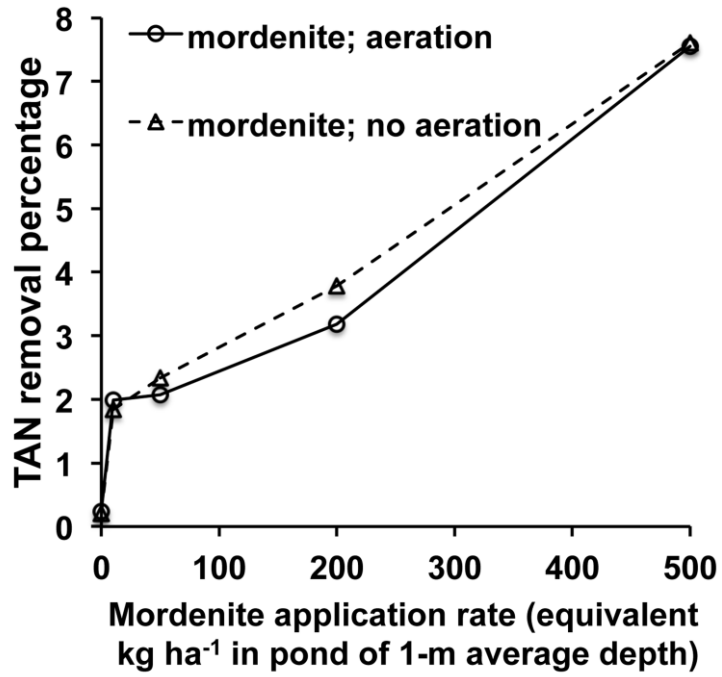
Figure 7. Removal of total ammonia nitrogen (TAN) from water containing 3 mg L^{-1} TAN by different application rate of mordenite sample 1 in aerated and non-aerated aquaria.

VI. BLUEGILL YIELD IN RESPONSE TO NITROGEN AND PHOSPHORUS VERSUS PHOSPHRUS-ONLY FERTILIZATION IN PONDS AT DIFFERENT TIMES SINCE SEDIMENT REMOVAL

ABSTRACT

Nitrogen often is omitted in fertilization of sportfish ponds in the southeastern United States that have been fertilized for several years with both nitrogen and phosphorus. This practice was tested in 40-yr-old research ponds at the Auburn University E. W. Shell Fisheries Center from which sediment had been removed 2 to 9yr earlier to restore bottoms nearly to their original soil composition. In ponds from which sediment had been removed 8 yr before, there was no difference ($P> 0.05$) in harvest weight of bluegill (*Lepomismacrochirus*) between ponds receiving nitrogen plus phosphorus fertilization (6 kg N and 3 kg P_2O_5 ha^{-1}application^{-1}) and those fertilized only with phosphorus (3 kg P_2O_5 ha^{-1} application^{-1}). Grass carp controlled aquatic macrophytes effectively in most ponds and did not interfere with bluegill yield. No correlation ($R^2 = 0.040$, $P> 0.05$) was found between time since sediment removal and bluegill yield in 13 ponds treated only with phosphate fertilizer at 3 kg P_2O_5 ha^{-1} application^{-1}.Concentrations of soluble reactive phosphorus and total ammonia nitrogen in the water column were correlated ($R^2 = 0.312$ and $R^2 = 0.514$, respectively, $P < 0.05$) with time since sediment removal. An earlier study showed that nitrogen fertilization was necessary in sportfishponds the year following sediment removal. In the present study, there was much variation in production among ponds treated with

nitrogen plus phosphorus as well as those treated only with phosphorus as typically encountered in pond fertilization trials. Nevertheless, results suggest that nitrogen fertilizationof sportfish ponds possibly can be ceased after only 2 yr of fertilization with nitrogen and phosphorus.

INTRODUCTION

Many sportfish ponds in the southeastern United States are treated with inorganic fertilizers to increase primary productivity and enhance the base of the food web culminating in sunfish (*Lepomis* spp.) and largemouth black bass (*Micropterussalmoides*) that are popular with anglers in the region. The application of fertilizer is adjusted to provide an acceptable phytoplankton bloom. The usual recommendation is to use enough fertilizer to maintain adequate phytoplankton for a Secchi disk visibility of 30 to 45 cm (Boyd and Tucker, 2014).

Many pond fertilization studies have been conducted in small (0.04-0.1 ha), earthen ponds at the Auburn University Fisheries Research Unit [now known as the E. W. Shell Fisheries Center (SFC)]. The typical procedure has been to stock only sunfish – usually bluegill (*L.macrochirus*) – and use the intensity of the phytoplankton bloom (usually estimated by particulate organic matter or chlorophyll *a* analysis), nitrogen and phosphorus concentrations, and the harvest weight of sunfish during a single growing season as indicators of the suitability of fertilization regimes (Swingle, 1947; Boyd, 1990).

The initial research on sunfish pond fertilization relied on broadcast applications of granular fertilizers, and resulted in a recommendation of 9 kg N, 9 kg P_2O_5, and 2.25 kg K_2O ha^{-1} $application^{-1}$ for 10 to 14 periodic applications at 2- to 4-wk intervals (depending upon the phytoplankton density) during the growing season (Swingle, 1947). Later, research with carp (*Cyprinuscarpio*), gold fish (*Carassiusauratus*) and channel catfish (*Ictaluruspunctatus*)

revealed that nitrogen and potassium fertilization was unnecessary to promote the production of these species in ponds with a 15-yr history of complete (N, P_2O_5, and K_2O) fertilization (Swingle et al., 1963). This finding led to the recommendation that nitrogen and potassium could be eliminated and phosphorus-only fertilization used in sportfish ponds with a history of complete fertilization (Byrd and Swingle, 1964). The source of nitrogen in older ponds was attributed to nitrogen fixation by blue-green algae common in waters with high phosphorus concentration and microbial mineralization of nitrogen from organic matter that accumulated in sediment (Swingle et al., 1963). However, at least to our knowledge, no bluegill pond fertilization studies to confirm the validity of this recommendation were made.

In an effort to verify whether nitrogen fertilization in older ponds was needed, studies were conducted in ponds on the SFC with 10- to 20-yr histories of fertilization or feed application. Bluegill production was not increased ($P > 0.05$) by nitrogen plus phosphorus fertilization as compared to phosphorus-only fertilization (Lichtkoppler and Boyd, 1977; Boyd and Sowles, 1978; Murad and Boyd, 1987). A study by Tepe and Boyd (2002) also revealed no benefit of nitrogen plus phosphorus fertilization over phosphorus-only treatment on golden shiner (*Notemigonuscrysoleucas*) production in ponds that had been fertilized with a complete fertilizer for 10 yr previously. Nevertheless, in these studies, fish production was numerically (but not statistically at $P = 0.05$) greater for ponds receiving both nitrogen and phosphorus than in the phosphorus-only treatments, and in one study (Tepe and Boyd, 2003), nitrogen plus phosphorous fertilization caused a slight increase ($P < 0.05$) in bluegill production over phosphorus-only fertilization. Thus, it does not seem prudent to rule out the possibility that nitrogen fertilizer addition may be beneficial in sportfish ponds with a history of complete fertilization.

Beginning in 2002, a project was initiated at the SFC to remove sediment and repair pond embankments over a period of several years. The physical and chemical condition of

the renovated pond bottoms was similar to that of the original pond bottoms (Yuvanatemiya and Boyd, 2006). Fertilization studies were conducted in newly renovated ponds that had not received nutrient inputs since renovation. The optimum fertilization rate using liquid instead of granular fertilizer (Metzger and Boyd, 1980) was 6 kg N and 3 kg P_2O_5 ha^{-1} application^{-1}, and potassium fertilization was unnecessary (Wudtisin and Boyd, 2005; Boyd et al., 2008; Viriyatum and Boyd, 2011). Because ponds were renovated over a period of several years, it was possible in the present study to select ponds that had been fertilized for different numbers of years since renovation and evaluate the response to nitrogen plus phosphorus fertilization and phosphorus-only fertilization.

MATERIALS AND METHODS

Seventeen, 0.04-ha earthen, research ponds of about 1 m average depth and located on Piedmont soils (TypicKandiudults, clayey, kaolinitic, and thermic) at the SFC near Auburn, Alabama were used in this study. Sediment had been removed from these ponds 2 to 9yr earlier (Table 1). Sediment samples were collected on 9 March 2012 before ponds were filled by taking 5-cm diameter by 5-cm deep samples from 10 random places in each pond bottom with a plastic tube and combining the cores to provide a composite sample from each pond. Samples were dried at 60 °C in a mechanical convection oven and pulverized by mortar and pestle to pass a 40-mesh sieve. Soil nitrogen was determined by the Auburn University Soil Testing Laboratory with a LECO CHN Analyzer (LECO Corporation, St. Joseph, Michigan, USA). Organic carbon (sulfuric acid-potassium dichromate oxidation method), pH (1:1 mixture of dry, pulverized soil and distilled water), and acid-extractable phosphorus (double acid extraction method) were measured by the Auburn University Soil Testing Laboratory using protocols described by Hue and Evans (1986).

Water to supply the ponds came from a reservoir filled with runoff from a wooded watershed. The ponds were filled with water on 9 and 10 March 2012, and water levels were maintained about 5 cm below the overflow structures by occasional additions from the supply line. Five ponds, from which sediment had been removed 8 yr earlier, were treated only with phosphorus fertilizer (3 kg P_2O_5 ha^{-1} $application^{-1}$ from triple superphosphate), and four other ponds, from which sediment also had been removed 8 yr earlier, were fertilized with nitrogen and phosphorus (3 kg P_2O_5 and 6 kg N ha^{-1} $application^{-1}$ from triple superphosphate and ammonium nitrate, respectively). Treatments were randomly assigned to the ponds. The remaining eight ponds, from which sediment had been removed 2 to 9 yr earlier, received 3 kg P_2O_5 ha^{-1} $application^{-1}$. Fertilizers were applied to ponds on 17 and 30 March, 6 and 26 April, 11 and 26 May, 17 June, 8 July, 12 August, and 17 September, 2012.

In early February 2012, 90 kg of agricultural limestone were applied to ponds with total alkalinity and total hardness concentrations below 25 mg L^{-1}, and 45 kg of agricultural limestone were applied to ponds with alkalinity and hardness below 30 mg L^{-1} (Boyd, 1990). On 16 March 2012, 220 juvenile bluegill, with an average weight of 1.38 g, were stocked into each pond. Five grass carp (*Ctenopharyngodonidellus*), with an average weight of 39.08 g, were stocked into each pond on 19 March 2012 for aquatic macrophyte control.

Water samples were collected weekly between 20 March 2012 and 15 October 2012, with a 90-cm water column sampler (Boyd and Tucker, 1992). Samples were transported to the laboratory and analyzed immediately for pH and concentrations of chlorophyll *a*, nitrate nitrogen (NO_3-N), total ammonia nitrogen (TAN), turbidity, and soluble reactive phosphorus (SRP). In addition, total hardness, total alkalinity, total suspended solids (TSS), particulate organic matter (POM), total nitrogen (TN), and total phosphorus (TP) were determined for the samples every other week. The TAN concentration was measured by the salicylate method (Le and Boyd, 2012); NO_3-N nitrogen was determined by the NAS reagent method

(van Rijn, 1993); total nitrogen and total phosphorus analyses were made according to Gross and Boyd (1998). Other analysis followed protocols recommended by Boyd and Tucker (1992).

Percentages of pond areas covered by aquatic macrophytes were determined monthly. Ponds were visually divided into 12 sections and the amount of aquatic macrophyte coverage in each section was sketched on a scaled map showing the 12 sections of ponds. Areas covered by aquatic macrophyte in each pond were estimated from maps using a polar planimeter.

Ponds were drained and fish were harvested between 22 and 25 October 2012. The weights of bluegill and crass carp in each pond were recorded.

Means and standard deviations, comparison of means of two treatments by t-test, and linear and nonlinear regression analyses were conducted using SAS 9.3 (SAS Institute, Cary NC, USA).

RESULTS AND DISCUSSION

Concentrations of organic carbon (1.15-2.71%), total nitrogen (0.08-0.37%), and acid-extractable phosphorus (48-132 mg kg^{-1}) in bottom soil samples had means and standard deviations of 1.78 $\pm$ 0.50%, 0.14 $\pm$ 0.07%, and 83 $\pm$ 28 mg kg^{-1}, respectively. Mean concentrations of these variables immediately after renovation had been 1.02% organic carbon, 0.10% total nitrogen, and 37 mg kg^{-1} acid-extractable phosphorus (Yuvanatemiya and Boyd, 2006). At the beginning of the study on 9 March 2012, the concentrations of organic carbon, total nitrogen, and acid-extractable phosphorus in the bottom soils tended to be greater than immediately after renovation, but there was no correlation ($P > 0.05$) between

time since pond renovation and concentrations of soil variables; $R^2 = 0.101$ for organic carbon, $R^2 = 0.001$ for total nitrogen, and $R^2 = 0.034$ for acid-extractable phosphorus.

The concentrations of TAN, NO_3-N, TN, SRP, TP, and chlorophyll a in water samples fluctuated greatly among ponds treated alike and across sampling dates as is typical in aquaculture pond water quality investigations (Boyd and Tucker, 2014). To simplify the data presentation, mean concentrations of water chemistry variables in each pond (with phosphorus-only fertilizer, n = 13) were regressed versus time since pond renovation (Fig. 1). There was no change ($P > 0.05$) with respect to TN concentration ($R^2 = 0.089$), NO_3-N concentration ($R^2 = 0.156$), chlorophyll a ($R^2 = 0.001$), or total phosphorus ($R^2 = 0.021$). Total ammonia nitrogen concentration ($R^2 = 0.514$) decreased slightly with time since pond renovation ($P < 0.05$). The concentration of SRP increased in older ponds ($R^2 = 0.312$, $P < 0.05$).

Concentrations of total hardness (31.0 to 58.6 mg L^{-1}) and total alkalinity (33.1 to 66.0 mg L^{-1}) averaged 43.4 mg L^{-1} and 47.6 mg L^{-1} for all ponds, respectively. The minimum concentration of these variables, considered acceptable for fertilized sportfish ponds, is 20 mg L^{-1}, but a higher concentration of 40 mg L^{-1} is recommended as a desirable goal for sportfish ponds (Boyd and Tucker, 2014). The ponds had adequate concentrations of total alkalinity and total hardness.

In the nine ponds from which sediment had been removed 8 yr previously and fertilized with either nitrogen plus phosphorus or phosphorus only (Table 2), there were no difference in mean concentrations of water chemistry variables between treatments ($P > 0.05$). The addition of nitrogen fertilizer did not result in greater concentrations of TN, TAN, or NO_3-N. This corroborates previous work suggesting that nitrogen fixation and microbial recycling of nitrogen from sediment organic matter supplies adequate nitrogen in sportfish ponds with a history of nitrogen and phosphorus fertilization. The findings that sediment

organic carbon, total nitrogen, and acid-extractable phosphorus were not correlated with time since sediment removal, but ponds – all of which had been fertilized for at least 2 yr – tended to have greater concentrations of these variables than found in the pond sediments immediately after renovation, suggest that the sediment quickly increases in concentrations of these variables. The concentrations of these variables in the sediment of the renovated ponds remained fairly constant over time. But, Munsiri et al. (1995) showed that the concentration of organic carbon and other variables in sediment of ponds on the SFC tended to reach an equilibrium concentration within 2 yr, but sediment continued to accumulate at a rate of about 1 cm yr^{-1}. Thus, the total amount of organic matter, nitrogen, and phosphorous in pond sediment increased over time. The depth to which sediment releases nutrients into the water column is only around 5 cm (Munsiri et al., 1995), and organic matter and nutrients in sediment below this depth do not normally enter the water column.

Grass carp were generally effective in controlling aquatic macrophytes. In April 2012, 12 ponds had macrophyte coverage of 5-100%, but by July, only three ponds contained macrophytes (64-96% cover). It was found that the grass carp had not survived in two of the three ponds containing aquatic macrophytes at harvest. The harvest weights of bluegill in those three ponds ranged from 213 to 375 kg ha^{-1}.

Considering all 13 ponds with phosphorus-only fertilization (Fig. 2), harvest weights of bluegill and grass carp were not correlated with time since sediment removal ($R^2 = 0.001$, $P > 0.05$ and $R^2 = 0.090$, $P > 0.05$, respectively). Bluegill yield was not negatively impacted by the grass carp in ponds; there was no correlation ($R^2 = 0.053$, $P > 0.05$) between the harvest weights of the two species. Variation in bluegill yield among the 13 ponds treated only with phosphorus was high (213-535 kg ha^{-1}), with a mean and standard deviation of 345 $\pm$ 98.7 kg ha^{-1}. The grass carp yield ranged from 68 to 296 kg ha^{-1} with a mean and standard deviation of 163 $\pm$ 86.6kg ha^{-1}, and total fish harvest (weight of bluegill plus grass carp) that

averaged 508 ± 150.6 kg ha^{-1}, with a range from 311 to 826 kg ha^{-1}. The range of bluegill yield is within the range reported in other bluegill pond fertilization studies at the SFC (Boyd, 2014). Doyle and Boyd (1984) reported a mean coefficient of variation of 27% in three to four replications of the same fertilization treatment repeated annually for 9 yr in ponds at the SFC.

In the ponds from which sediment removal occurred 8 yr previously, nitrogen plus phosphorus fertilization did not increase ($P > 0.05$) mean bluegill yield (362 kg ha^{-1}) above that of ponds fertilized with phosphorus only (353 kg ha^{-1}) (Table 2). Moreover, the mean yield for the five ponds with phosphorus-only fertilization (353 kg ha^{-1}) was not different ($P > 0.05$) than that of 347 kg ha^{-1} in 7 ponds from which sediment had been removed for 2 to 7 yr (Table 1) and also received phosphorus-only fertilization. The pond from which sediment had been removed for 9 yr had low sunfish yield (213 kg ha^{-1}), but this was thought to be the result of natural variation because four other ponds from which sediment had been removed for 3-8 yr had similarly low yield (227-241 kg ha^{-1}).

A previous study conducted in ponds at the SFC the year following sediment removal revealed a definite benefit of nitrogen fertilization on bluegill production (Boyd et al., 2008). In the present study, differences in bluegill production could not be attributed to nitrogen fertilization in ponds which had been fertilized with both nitrogen and phosphorus for 2-9 yr since sediment removal. Nevertheless, there is much variation in bluegill production in fertilized ponds constructed in the same type of soil according to an identical design, and sharing the same, regulated water source (Boyd and Tucker, 2014). If a particular fertilization schedule will not assure uniform results in ponds at a single location; it seems futile to attempt to develop a fertilization schedule for use across a wide range of pond design, soil type, water source, and hydrologic condition.

The results of the present study do suggest that after 2 yr of nitrogen plus phosphorus fertilization, pond manager might cease nitrogen fertilization and assess the response of the phytoplankton bloom. If the phytoplankton bloom wanes, then nitrogen fertilization could be resumed. However, the findings of this study likely are not applicable to food fish ponds where the standing crop of fish and the amount of nitrogen in fish biomass are much greater. Boyd (1976) and Lin et al. (1997) demonstrated that nitrogen fertilization increased tilapia production in older ponds with a history of nitrogen and phosphorus fertilization.

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Table 1. Pond renovation history and fertilization treatments in 2012.

Year renovated	Years since renovation	No. ponds	Fertilizer treatment (no. of ponds)	
			$P_2O_5{}^a$	$N^b + P_2O_5{}^a$
2003	9	1	1	0
2004	8	9	5	4
2005	7	1	1	0
2007	5	1	1	0
2008	4	2	2	0
2009	3	2	2	0
2010	2	1	1	0

[a]3 kg P_2O_5 ha^{-1} per application with triple superphosphate.

[b]6 kg N ha^{-1} per application with ammonium nitrate.

Table 2. Mean (± standard deviation) concentrations of 12 water chemistry variables and bluegill yield for ponds that were renovated 8 yr earlier. Five of the nine ponds received only triple superphosphate (TSP), while four ponds received TSP and ammonium nitrate (AN) fertilizer. Means within a row with a letter in common are not different at P-value = 0.05.

Variable	Treatment	
	TSP	TSP + AN
Total nitrogen (mg L^{-1})	1.11 ± 0.372 a	1.12 ± 0.108 a
Nitrate nitrogen (mg L^{-1})	0.05 ± 0.001 a	0.05 ± 0.001 a
Total ammonium nitrogen (mg L^{-1})	0.19 ± 0.009 a	0.20 ± 0.029 a
Total phosphorus (mg L^{-1})	0.27 ± 0.054 a	0.22 ± 0.037 a
Soluble reactive phosphorus (mg L^{-1})	0.08 ± 0.006 a	0.07 ± 0.010 a
Total alkalinity (mg L^{-1})	50.3 ± 9.83 a	46.8 ± 6.50 a
Total hardness (mg L^{-1})	45.6 ± 7.48 a	43.2 ± 6.20 a
Total suspended solids (mg L^{-1})	9.5 ± 2.06 a	12.8 ± 6.28 a
Particulate organic matter (mg L^{-1})	7.7 ± 1.49 a	7.2 ± 1.82 a
Chlorophyll a (µg L^{-1})	17 ± 3.6 a	15 ± 4.2 a
pH	8.3 ± 0.37 a	8.3 ± 0.06 a
Turbidity (NTU)	12 ± 5.4 a	16 ± 8.7 a
Bluegill yield (kg ha^{-1})	353 ± 105.9 a	362 ± 131.7 a
Total fish yield (kg ha^{-1})[*]	500 ± 222.7 a	443 ± 112.3 a

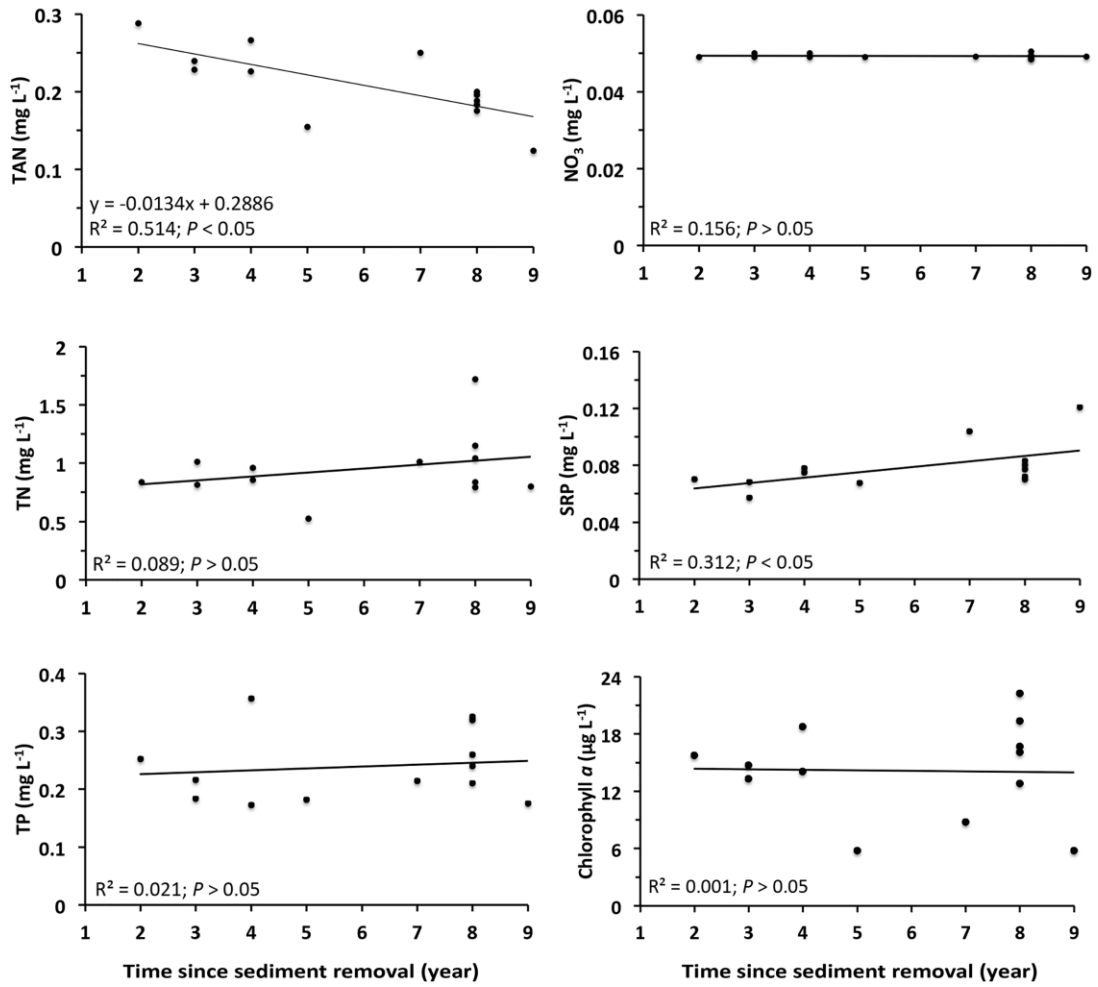[*] Total fish yield is the harvest weight of bluegill plus grass carp

Figure 1.Relationships between the mean concentrations of water chemistry variables and time (in years) since sediment removal in 13 bluegill ponds that received phosphorus-only fertilization
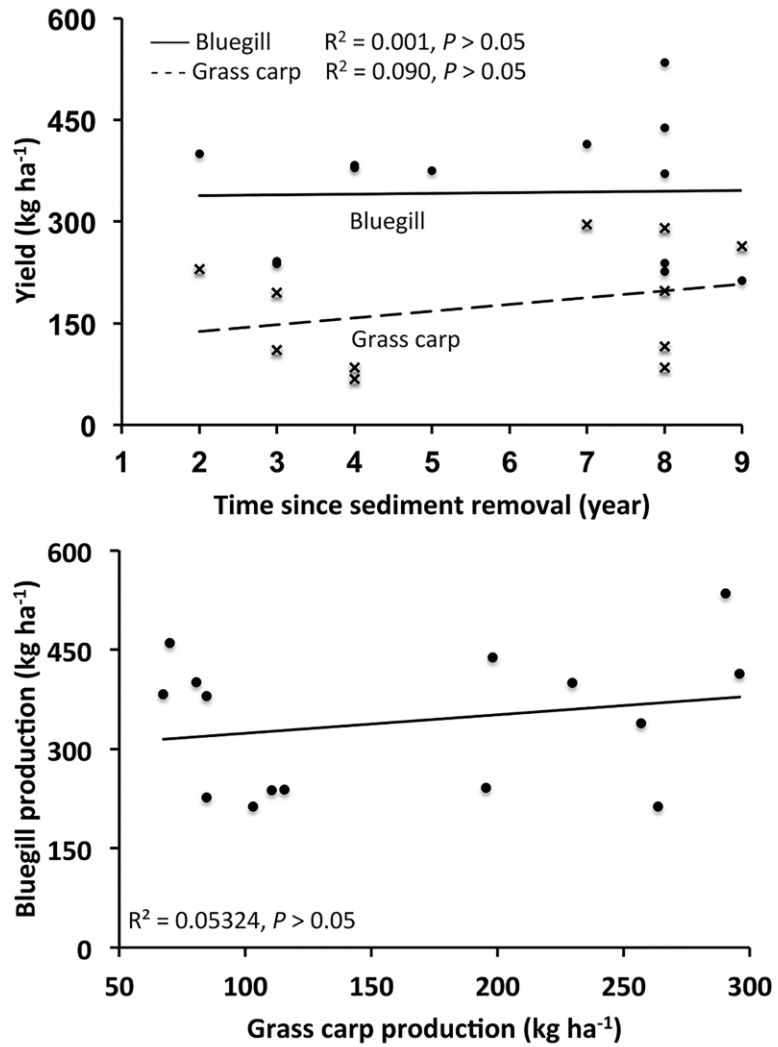
Figure2. Upper: The harvest weights of bluegill and grass carp versus time (in years) since sediment removal from 13 bluegill ponds that received phosphorus-only fertilization. Lower: Harvest weight of bluegill in 15 ponds versus harvest weight of grass carp

VII.AMMONIA MANAGEMENT

There are several ways to reduce ammonia nitrogen concentration. Although some are not long-term solutions or practical to use at production facilities, they are still worth mentioning. Measures such as adding an acid to lower pH, applying an algicide to lessen phytoplankton photosynthesis and reduce pH, or exchange water in ponds to flush out ammonia nitrogen can be used as emergency treatmentswhere the TAN concentration is too high. However, such treatments are expense, they also have possible negative effects on water quality in ponds (acid and algicide treatment), and in water receiving aquaculture effluents (release nutrient-enriched or pathogen-contaminated water) or be impossible to implement at particular sites (water exchange).

Fertilizing ponds with phosphorus promotes algae growth, thereby decreasing ammonia nitrogen through algae uptake. However, adding additive phosphorus to ponds already receiving phosphorus input from feed may causeunacceptably dense phytoplankton blooms(Hargreaves and Tucker, 2004).

Adding a source of organic matter such as manure, chopped hay can reduce ammonia nitrogen. This result because of organic matter with an elevated C/N ratio promotes immobilization of the ammonia from the water by microorganisms of decay. This practice, however, requires large amount of organic carbon and increases the oxygen demand(Hargreaves and Tucker, 2004).

Moreover, shrimp farmers in some Asian countries often apply zeolite to ponds in attempts to lower the ammonia concentration to which culture animals are exposed. This

method is practical to use in transport containers for ornamental freshwater fishes, and waters in aquaria, water recirculating aquaculture system, but not practical for large-volume fish ponds (Hargreaves and Tucker, 2004).

The practice of applying living bacterial amendments to ponds to lessen TAN concentration is popular in Asia and is now being used by some ictalurid catfish producers in the United States. However, there is no evidence that this practice is effective (Boyd and Tucker, 2014).

The best approach for avoiding high TAN concentration is to apply practices that minimize ammonia nitrogen input, increase nitrification, and lessen pH increase (Boyd and Tucker, 2014) as follows:

1. Use a good quality feed with optimal crude protein concentration.

2. Use moderate stocking and feeding rates.

3. Feed slightly less than fish will eat to avoid overfeeding and uneaten feed.

4. Use adequate mechanical aeration to avoid dissolved oxygen concentration from falling below 4 mg L^{-1} at night – around 1 hp for each 10 kg ha^{-1} day^{-1} usually is adequate.Aeration favors bacterial nitrification and enhances the diffusion of NH_3 from water to the air.

5. Avoid sources of ammonia nitrogen from watersheds. Livestock production on watershed will substantially increase TAN concentration in ponds.

6. Ponds with low alkalinity (< 40 mg L^{-1}) should be treated with agriculture limestone to increase alkalinity and buffer water against pH fluctuations.

7. Ponds with low total hardness but normal alkalinity should be treated with agricultural gypsum ($CaSO_4 \cdot 2H_2O$) to increase hardness and prevent high pH in response to high photosynthesis rates.

Adoption of moderate stocking and feeding rates will not appeal to many producers. But, even in ponds with high fish production, the other practices listed above can be beneficial in limiting the concentration of TAN.

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