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Nitrogen Biogeochemistry of Aquaculture Pond Sediments.

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NITROGEN BIOGEOCHEMISTRY OF AQUACULTURE POND SEDIMENTS

A Dissertation

**Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy**

in

The School of Forestry, Wildlife, and Fisheries

by

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B.A., Amherst College, 1980
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May 1995**

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ABSTRACT

Aquaculture pond water quality is dominated by planktonic processes, although the high surface-to-volume ratio suggests that sediment has the potential to effect important changes in water quality. The objectives of this research were to evaluate annual variation of sediment nitrogen biogeochemistry and develop a model of annual variation in ammonia concentration in a commercial channel catfish pond. In addition, the effects of physical and chemical sediment management techniques on water quality in aquatic mesocosms were evaluated.

Sediment nitrogen was dominated by organic (92-96%) and adsorbed ammonium (2-4%) fractions. Porewater ammonium comprised the smallest yet most dynamic component of sediment N, with maximum concentrations measured during summer. Porewater ammonium concentrations were 4-20x higher than in the water column. Sediments were sinks for oxygen and oxidized nitrogen and sources of carbon dioxide and ammonium. Molecular diffusion accounted for only 1.4-14.9% of ammonium flux suggesting that mineralization of organic matter at the sediment surface was rapid. Denitrification rates were limited by low ambient oxidized nitrogen concentrations, although denitrification potential was substantial.

A simulation model accurately described annual variation of catfish pond ammonium concentration. Ammonium production was partitioned between fish excretion (67-75%) and sediment diffusion (25-33%). Phytoplankton ammonium uptake exceeded nitrification during the growing season (April-October); nitrification was a more important removal mechanism than phytoplankton uptake during the

winter (November-March). Model output was most sensitive to changes in the partition of nitrogenous excretion, average feeding rate, and the phytoplankton specific uptake rate.

Sediment disturbance in organically-enriched aquatic mesocosms improved water quality for fish production. Dissolved oxygen concentration was higher and ammonia concentration was lower in mesocosms stocked with fish. Whole-tank respiration was inversely related to sediment disturbance. Nitrification associated with suspended particles was inversely related to C:N ratio of organic inputs.

Nitrate enrichment of aquatic mesocosms did not improve water quality for fish production. Elevated concentrations of ammonia and nitrite were associated with high dissimilatory nitrate reduction rates. Substantial quantities of organic matter mineralization mediated by nitrate were inferred from alkalinity accumulation. Dissolved oxygen concentrations were increased and soluble phosphorus concentrations were decreased only slightly as a function of nitrate enrichment.

INTRODUCTION

The future of aquaculture in the face of global environmental problems is not clear, but it is certain to play an important role. Aquaculture may be a component of techniques developed to restore degraded land and alleviate water scarcity. However, the most daunting challenge facing aquaculture is related to feeding a world population projected to reach 6.25 billion by 2000 and 8.5 billion by 2025.

Global fisheries harvest is expected to reach maximum sustainable yield of approximately 100 million metric tons (mmt) by the end of the decade. Most fish stocks are fully exploited or depleted. In addition, an anticipated 1-m rise in sea level by the end of the next century will inundate vast areas of critical coastal habitats that function as nursery areas in support of important fisheries and force large economic and personal dislocation.

Further increases in global fisheries supply will be derived from aquaculture. In order to maintain annual per capita consumption of fish at current levels (19.1 kg), aquaculture production must expand to 38 mmt by 2010 and 62 mmt by 2025 (New 1991). Currently, global aquaculture production accounts for 16.6 mmt (million metric tons) or 15% of the total fish harvest (FAO 1993). Finfish production accounts for 8.7 mmt or 52.7% of total aquaculture production and has an estimated value of \$16.14 billion. Brackishwater aquaculture production has doubled between 1984 and 1991 and stands at 1.32 mmt. The majority of brackishwater aquaculture production consists of penaeid shrimp (0.80 mmt), which is valued at \$5.25 billion.

Assuming an average annual yield of 500 kg ha⁻¹ from inland and brackishwater aquaculture, approximately 17 million ha of ponds are involved in global aquaculture production. Clearly, ponds are the predominant culture system, particularly in inland freshwater aquaculture. Inland freshwater aquaculture accounts for 7.69 mmt or 88% of total finfish production. The proportion of the total inland fish harvest from aquaculture is growing and now exceeds 50%. The bulk of inland aquaculture production consists of non-carnivorous fish, predominately cyprinids (>6 mmt).

Given current development pressures and the potential effects of global climate change on the coastal zone, further growth in global aquaculture production is likely to be derived from inland pond-based aquaculture. It is likely that further increases in aquaculture production will come from intensification of existing areas of production rather than expansion into new areas, although the development of marginal lands, unsuitable for other forms of agriculture, will continue.

Intensification will require improved management and understanding of nutrient utilization in aquaculture. It has been estimated that 20-25% of global fish meal supplies will be utilized in aquaculture feeds by 2000 (New 1991). Fish meal is one of the most important protein sources in fish feeds, which typically account for approximately 50% of the operating costs of an aquaculture operation. Therefore, improvements in nutrient efficiency (feed conversion) will improve the economic performance of the operation.

However, most farmed fish are cultured in ponds receiving organic fertilization. Such systems are designed to maximize the utilization of natural productivity or to convert low-quality organic inputs into high-quality fish flesh. Nutrient flow in integrated animal agriculture-aquaculture, rice-fish, and sewage-based aquaculture systems requires further elaboration in order to maximize system productivity. Increasing the efficiency of nutrient use in such pond-based systems can therefore have a dramatic effect on global aquaculture production. The development of management techniques that promote nutrient retention and recycling within the production system are required.

Pond management to assure maximum fish yield is still very much an art, particularly in pond-based systems in which fish production depends upon manipulation of detrital or plankton-based food chains. The pond has been largely managed as a "black box" in which inputs are applied and an output (a fish crop) is harvested. Scientifically-based management of water quality is only some 50 years old, compared to the thousands-year-old history of aquaculture production. Research into the principles of pond-based aquaculture has proceeded steadily, although opportunities for "intervention" are limited by only basic understanding of pond dynamics and the effects of management techniques.

This research was conducted to further develop our understanding of pond nutrient dynamics. Although water column processes are fairly well-understood, the role of the sediment is less appreciated. The abrupt discontinuity between the

aerobic water column and the (essentially) anaerobic sediment has important implications for water quality in aquaculture ponds.

The objectives of this research were to examine the magnitude of nitrogen pools and flows in aquaculture ponds, particularly with reference to interactions with other important nutrients, e.g., oxygen and carbon. The pond production system point-of-reference of this study was semi-intensive channel catfish culture, the most important aquaculture activity in the southeastern United States. Specifically, the annual variation of sediment nitrogen biogeochemistry in a commercial channel catfish pond was evaluated. Some of these data were used to develop a simulation model of annual variation in ammonia concentration. Finally, the effect of one physical and one chemical sediment management technique on water quality and fish production were evaluated.

LITERATURE CITED IN INTRODUCTION

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CHAPTER 1

NITROGEN BIOGEOCHEMISTRY OF AQUACULTURE PONDS

INTRODUCTION

The efficiency of fish nitrogen assimilation has important implications for water quality and profitability of pond aquaculture. Results from a variety of culture systems indicate that on average about 25% (range: 11-36%) of N added as feed or other nutrient input is recovered by the target organism (Table 1). Protein sources such as fish meal and soybean meal are the most expensive components of formulated feeds utilized in fish culture diets and improvement in the efficiency of N assimilation and utilization will thus improve the economics of fish production.

The inherent inefficiency of nutrient utilization implies that N loading of aquaculture ponds may be limited by the capacity to assimilate nitrogenous excretion, which may have a deleterious impact on water quality and fish growth. Following dissolved oxygen, the accumulation of dissolved inorganic nitrogen is the factor most likely to limit feeding rate in aquaculture ponds. Fish excrete ammonia¹ as the end product of protein catabolism, which may be toxic if allowed to accumulate. The toxicity of ammonia in aquaculture ponds is most likely expressed as the sublethal

¹Ammonia exists as a component of a pH-dependent equilibrium in natural waters. Across the range of pH most commonly encountered in natural waters (6.5-8.0), the equilibrium favors the aqueous, ionized form (NH_4^+), or "ammonium". Elevated pH (>9.3) favors the gaseous, unionized form (NH_3), or "ammonia". The convention adopted to describe this compound varies and is dependent upon the field of inquiry. Limnological literature tends to describe the compound as "ammonium". I have adopted a convention of the aquaculture literature, "total ammonia-nitrogen" (abbreviated "TAN"), which refers to the concentration of ammonia plus ammonium expressed in terms of the mass of nitrogen per unit volume. This convention will be used throughout the text unless otherwise explicitly indicated.

Table 1. Estimates of the range (%) of nitrogen recovered by fish and released to the environment in various aquaculture production systems.

Fish Species	Production System ¹	Recovered	Released			Reference
		Fish	Total	Dissolved	Solid	
polyculture	P	11-16	84-89			Schroeder <i>et al.</i> 1990
<i>Anguilla japonica</i>	P	14-25	75-86			Chiba 1986
<i>Oreochromis niloticus</i>	P	18-21	79-82			Green and Boyd (in press)
<i>Oreochromis</i> spp.	P	25-29	75-81			Avnimelech and Lacher 1979
<i>Morone saxatilis</i>	P	22	78			Daniels and Boyd 1989
<i>Ictalurus punctatus</i>	P	27	73			Boyd 1985
<i>Sparus aurata</i>	P	36	64			Krom <i>et al.</i> 1985
<i>Sparus aurata</i>	P	26	74			Neori and Krom 1991
<i>S. aurata</i>	T	27		66	7	Neori and Krom 1991
<i>S. aurata</i>	T	30		60	10	Porter <i>et al.</i> 1987
<i>Oncorhynchus mykiss</i>	C	21		49	30	Philips and Beveridge 1986

(table continued)

Fish Species	Production System ¹	Recovered	Released			Reference
		Fish	Total	Dissolved	Solid	
<i>O. mykiss</i>	C	25		60	15	Pillay 1992
<i>O. mykiss</i>	C	25-29	71-75			Penczak <i>et al.</i> 1982
<i>Salmo salar</i>	C	25		62	13	Folke and Kautsky 1989
<i>S. salar</i>	C	25		65	10	Gowen and Bradbury 1987
<i>Clarias macrocephalus</i>	C	24	76			Lin <i>et al.</i> 1993
<i>I. punctatus</i>	R	14	86			Worsham 1975
<i>O. mykiss</i>	R	19		74	7	Foy and Rosell 1991a, 1991b

¹ Production system codes: P=earthen pond, T=tank, C=cage, R=raceway.

reduction of fish growth, rather than as acute toxicity leading to mortality. Colt and Tchobanoglous (1978) demonstrated linear reduction of channel catfish growth over the range $0.05\text{--}1.0\text{ mg L}^{-1}\text{ NH}_3\text{-N}$ with a calculated 50% reduction of growth at approximately $0.5\text{ mg L}^{-1}\text{ NH}_3\text{-N}$. The mechanisms of ammonia toxicity have not been firmly established. However, a combination of plasma sodium depletion (Colt and Tchobanoglous 1978, Tomasso *et al.* 1980), biochemical effects of Krebs cycle suppression by depletion of α -ketoglutarate (Sousa and Meade 1977, Smart 1978) resulting in reduced ammonia excretion, and other factors (see Meade 1985) may be implicated. Ammonia is more toxic to fish at elevated pH and temperature, which shifts the ionization equilibrium toward the toxic, unionized gaseous form. The risk of elevated pH and unionized ammonia is more substantial in poorly buffered (low alkalinity) ponds in the late afternoon.

The contribution of ammonia excretion to nitrogen flow in aquaculture ponds is substantial. If 25% of input N is retained by fish, then 75% of input N is excreted. N excretion can be partitioned into dissolved (62%) and particulate (13%) fractions (Folke and Kautsky 1989). Assuming a feeding rate of $100\text{ kg ha}^{-1}\text{ d}^{-1}$ (32% protein feed), then $317\text{ mg N m}^{-2}\text{ d}^{-1}$ is excreted as ammonia. Alternately, using a direct measure of ammonia excretion of $30\text{ g N kg}^{-1}\text{ feed}$ (Colt and Orwicz 1991), then $300\text{ mg N m}^{-2}\text{ d}^{-1}$ are excreted.

Fish also excrete fecal solid wastes that settle to the sediment along with senescent phytoplankton and other particulate organic matter. By the mass balance approach (13% N as particulate solids), then $67\text{ mg N m}^{-2}\text{ d}^{-1}$ are excreted as fecal

solids at a feeding rate of $100 \text{ kg ha}^{-1} \text{ d}^{-1}$. Alternately, fecal solids can account for up to 50% by weight of dry weight feed applied to the pond (Colt and Orwicz 1991). Feces from catfish fed a 32% protein feed are 13.1% protein (Brown *et al.* 1989). By this analysis, fecal solids contribute $104 \text{ mg N m}^{-2} \text{ d}^{-1}$. This organic matter is rapidly hydrolyzed and mineralized, and becomes an additional source of ammonia.

Nitrite is produced as an intermediate product in nitrification and may be toxic to fish. Nitrite can competitively bind to hemoglobin to form methemoglobin, which does not have the capacity to carry oxygen.

Third, primary production may be limited by N in ponds in which the development of autotrophic food webs is important for improved fish yields. Thus, organic and inorganic fertilizer programs may be directed toward increasing the availability of N for phytoplankton (Noriega-Curtis, 1979, Green *et al.* 1989, Schroeder *et al.* 1990, Knud-Hansen *et al.* 1991a, Knud-Hansen *et al.* 1991b).

Fourth, nitrogen discharged in fish pond effluents may degrade the quality of receiving waters. Given the inherent nutrient inefficiency described above, it is clear that large quantities of nutrients may be released to the environment. Compared to concentrations in influent water, Ziemann *et al.* (1992) measured increases of total N and total ammonia N and decreases of nitrate N in the effluent from freshwater fish and prawn ponds and marine fish and shrimp ponds. Similarly, Tucker and Lloyd (1985) measured higher concentrations of total N and total ammonia N and lower concentrations of nitrate N in channel catfish ponds as compared to nearby receiving streams.

PROCESSES RELATED TO N FLUX IN AQUACULTURE PONDS

Feeding and Fertilization

The main (>90%) input of N in semi-intensive fish ponds is from fish feeds. For example, at a feeding rate of $100 \text{ kg ha}^{-1} \text{ d}^{-1}$ ($10 \text{ g m}^{-2} \text{ d}^{-1}$) of 32% protein feed, more than $500 \text{ mg N m}^{-2} \text{ d}^{-1}$ are added to ponds. Organic and inorganic fertilizers may also supply significant quantities of N to fish ponds. In systems managed for autotrophic productivity, a net fish yield of $30\text{-}40 \text{ kg ha}^{-1} \text{ d}^{-1}$ ($3\text{-}4 \text{ g m}^{-2} \text{ d}^{-1}$) is possible at a loading of $700\text{-}800 \text{ mg N m}^{-2} \text{ d}^{-1}$ from a combination of organic and inorganic sources (Schroeder *et al.* 1990, Knud-Hansen *et al.* 1991b). Smaller amounts of N may be added by water used to replace losses due to evaporation and seepage or by atmospheric deposition, particularly during the dry season in the tropics.

N Fixation

Nitrogen may be added to fish ponds by the reduction of atmospheric dinitrogen by cyanobacteria, many of which contain heterocysts, the sites of active nitrogen fixation. Nitrogen fixation ranged from $6\text{-}23 \text{ mg N m}^{-2} \text{ d}^{-1}$ during the dry season and $21\text{-}57 \text{ mg N m}^{-2} \text{ d}^{-1}$ during the rainy season in tropical fish ponds (Lin *et al.* 1988). Nitrogen fixation averaged $24 \text{ mg N m}^{-2} \text{ d}^{-1}$ in a tropical freshwater fish pond and accounted for 10% of estimated N input (Acosta-Nassar *et al.* 1994). El Samra and Oláh (1979) measured an average nitrogen fixation rate of $4 \text{ mg m}^{-2} \text{ d}^{-1}$ in a temperate aquaculture pond. The quantity of nitrogen added to aquaculture ponds by fixation depends largely upon species composition of the phytoplankton bloom

(significant proportion of heterocystic cyanobacteria) and ammonia concentration.

The extent of inhibition of nitrogen fixation is inversely related to ammonia concentration (Lin *et al.* 1988). Nitrogen fixation is a minor, but occasionally important contributor to the N budget of aquaculture ponds receiving formulated feeds.

Phytoplankton Uptake of Inorganic N

Phytoplankton uptake of dissolved inorganic nitrogen from the water column of aquaculture ponds is the primary pathway of nitrogenous removal. Semi-intensive aquaculture ponds often develop dense phytoplankton populations (chlorophyll *a* >250 $\mu\text{g L}^{-1}$, SDV <20 cm) in response to a high rate of nutrient input.

Phytoplankton blooms in most aquaculture ponds are likely light-limited (Laws and Malecha 1981, Smith and Piedrahita 1988), suggesting nutrients are available at concentrations exceeding those limiting uptake or are supplied in excess of cellular requirements.

Nitrogen uptake by phytoplankton can be estimated by several methods, all of which yield results of similar magnitude. Phytoplankton fix carbon over an annual cycle in temperate environments that varies from 1 to 3 $\text{g C m}^{-2} \text{d}^{-1}$ (Boyd 1990). In tropical ponds, carbon fixation by phytoplankton can approach 5 to 10 $\text{g C m}^{-2} \text{d}^{-1}$ (Krom *et al.* 1989, Schroeder *et al.* 1991). Assuming phytoplankton uptake of nutrients corresponds to the Redfield ratio (C:N:P=106:16:1), N uptake ranges from 151 to 453 $\text{mg N m}^{-2} \text{d}^{-1}$ in temperate ponds, and from 750-1,500 $\text{mg N m}^{-2} \text{d}^{-1}$ in tropical ponds. Sustained (1-3 month) phytoplankton production of 15-25 g dry

matter $\text{m}^{-2} \text{d}^{-1}$ in algal mass cultures has been demonstrated, with higher production ($30\text{-}40 \text{ g m}^{-2} \text{d}^{-1}$) possible over shorter periods (Goldman 1979). Assuming the N content of phytoplankton with no nutrient deficiency is 5-10% of dry matter (Jørgensen *et al.* 1991), maximum (light-limited) N assimilation is equivalent to approximately $750\text{-}2,000 \text{ mg N m}^{-2} \text{d}^{-1}$, with higher uptake rates possible.

A third approach toward assessment of the magnitude of phytoplanktonic nitrogen uptake can be derived from maximum uptake rates (V_{max}) determined for natural populations of *Microcystis aeruginosa* and *Oscillatoria agardhii*, common cyanobacteria in eutrophic aquaculture ponds. Assuming a chlorophyll *a* concentration of $250 \mu\text{g L}^{-1}$, a chlorophyll *a* composition of 1.5% of phytoplankton dry weight and V_{max} ranging from $1.5\text{-}4.0 \mu\text{g N mg dry weight}^{-1} \text{h}^{-1}$ (Kappers 1980), then nitrogen uptake rates range from $600\text{-}1600 \text{ mg N m}^{-2} \text{d}^{-1}$. Clearly, phytoplankton uptake is a powerful mechanism for conversion of potentially-toxic inorganic N to relatively-stable organic N.

Ammonium is the preferred N substrate for phytoplankton, and only after ammonium is depleted ($<0.03 \text{ mg N L}^{-1}$) will significant quantities of nitrate be assimilated (Syrett 1981, McCarthy 1981). Nitrate assimilation and incorporation is an energetically less-favorable pathway of N nutrition for phytoplankton, as enzymatic-reduction to ammonium within the phytoplankton cell is necessary prior to incorporation into cellular amino acids.

Dissolved inorganic nitrogen uptake generally follows Michaelis-Menten enzyme-substrate kinetics in which uptake rate is a hyperbolic function of

concentration. Half-saturation concentrations (K_s) for the assimilation of dissolved inorganic nitrogen by marine phytoplankton range from 0.01-0.10 mg N L⁻¹ (Eppley *et al.* 1969), although internal concentrations required to saturate internal enzyme systems may be 1-2 orders of magnitude greater (Syrett 1981) suggesting that nutrients are concentrated within the phytoplankton cell. Given the generally elevated dissolved inorganic nitrogen concentrations in aquaculture ponds (0.5-3 mg L⁻¹), it is not likely that substrate concentrations limit phytoplankton growth.

In aquaculture ponds, phytoplankton has an important effect on water column dissolved inorganic nitrogen concentrations (Tucker *et al.* 1984, Krom *et al.* 1989). In these studies, concentrations of total ammonia nitrogen (TAN) were inversely related to phytoplankton density. During phytoplankton die-offs, TAN concentrations increased dramatically. As phytoplankton density increased, ammonia concentrations declined. In addition, seasonal changes in phytoplankton density may affect dissolved inorganic nitrogen concentrations in aquaculture ponds (Tucker and van der Ploeg 1993). Dissolved inorganic nitrogen concentrations are greatest in winter when phytoplankton density is lowest.

Dissolved inorganic nitrogen concentrations may be affected by phytoplankton species composition. In Israeli brackishwater fish ponds, chlorophytes and chrysophytes dominated phytoplankton blooms in winter and spring, whereas cyanobacteria were dominant in summer (Van Rijn *et al.* 1986). The presence of cyanobacteria was coincident with low (<0.01 mg N L⁻¹) concentrations of dissolved inorganic nitrogen. A similar successional pattern was observed in channel catfish

ponds (Tucker and van der Ploeg 1993), although cyanobacteria were dominant throughout the year.

Ammonia Volatilization

pH has an important effect on the equilibrium between gaseous, unionized ammonia (NH_3) and aqueous, ionized ammonium (NH_4^+), which has a pK_a of 9.24 at 25 C. Alkaline pH favors the unionized, gaseous form. At pH 9.3, approximately 50% of total ammonia is unionized; at pH 8.3, approximately 10% is unionized; and, at pH 7.3, approximately 1% is unionized. Volatilization is thus enhanced at elevated pH due to equilibrium relationships and the resultant increase in the partial pressure of ammonia gas. Ammonia volatilization is not important at $\text{pH} < 7.5$.

Ammonia volatilization may be important as a mechanism of ammonia removal during the late afternoon in poorly-buffered (total alkalinity $< 20 \text{ mg L}^{-1}$ as CaCO_3) ponds, when pH may exceed 9 in response to the depletion of CO_2 from solution by phytoplankton (Hariyadi *et al.* 1994). Murphy and Brownlee (1981) calculated ammonia volatilization rates in a hypereutrophic lake dominated by *Aphanizomenon flos-aquae* during the late afternoon ($\text{pH} < 9$) of a windy day that were an order of magnitude greater than the rate of phytoplankton uptake of ammonia. Schroeder (1987) estimated ammonia volatilization of $50 \text{ mg N m}^{-2} \text{ d}^{-1}$ from manure-loaded polyculture ponds at pH 8 and 0.5 mg N L^{-1} as ammonia. In general, ammonia volatilization is enhanced by increased ammonia concentration, pH, temperature, evaporation rate and wind speed.

Sediment Organic Matter Accumulation

Sediments play an active role in the chemistry of the water column of aquaculture ponds. Organic inputs, senescent phytoplankton, fish fecal solids and uneaten feed settle out of the water column to the sediment. In ponds fertilized with manures or agricultural by-products, direct consumption is minimal and most of the input settles to the sediment. In addition, as much as 50% of the algal standing crop (about 10 g algal dry weight $\text{m}^{-2} \text{d}^{-1}$) settles to the sediment surface each day (Schroeder *et al.* 1991). Sediment traps in freshwater fish ponds collected 200-500 g dry matter $\text{m}^{-2} \text{d}^{-1}$, most of which was derived from previously-deposited material that had been resuspended by fish (Schroeder *et al.* 1991). The settling rate of organic matter in intensive shrimp ponds can exceed 800 g dry matter $\text{m}^{-2} \text{d}^{-1}$ (Wyban and Sweeney 1989).

The rate of organic matter accumulation in the sediment of aquaculture ponds is variable. Increases in sediment organic matter (0.23% yr^{-1}) and nitrogen (0.02% yr^{-1}) were measured in channel catfish ponds over 8 years (Tucker 1985). Organic carbon in the upper 5-cm sediment layer of tropical fish ponds enriched with chicken manure increased by approximately 0.1% mo^{-1} (Ayub *et al.* 1993). Using nutrient budget estimates of Schroeder (1987) and assuming accumulation in the 0-5 cm layer only, the magnitude of N accumulation in sediment was approximately 0.07% after a 4-month culture period. Organic N of a tropical fish pond sediment increased by approximately 0.06% after only 4 months, accounting for 65% of input N (Acosta-Nassar *et al.* 1994). Similarly, 70% of input N accumulated in the sediments of

tropical fish ponds enriched with chicken manure (Green and Boyd, in press). Chiba (1986) recovered 8-13% of input N as sediment organic N in an intensive eel pond with continuous water circulation. Hopkins *et al.* (1994) recovered 15-22% of N inputs as bottom organic deposits (sludge) of semi-intensive shrimp ponds in which sediments were periodically suspended by aeration or allowed to settle in place.

Particulate matter settling to the sediment will develop into a dynamic, flocculent, organic layer (Visscher and Duerr 1991, Hopkins *et al.* 1994). Schroeder (1987) demonstrated maximum heterotrophic activity in the flocculent sediment layer extending 2 cm above the firm sediment surface by measuring the rate of weight loss of cotton cloth. In shrimp ponds enriched with bagasse-based pellets, microbial biomass and density increased with the depth of the flocculent layer (Visscher and Duerr 1991). Microbial density of all functional groups was maximum in the turbid layer above the sediment of an experimental fish tank when compared to densities in the water column and sediment (Ram *et al.* 1981).

Benthic Nitrogen Regeneration (Mineralization)

Dissolved organic nitrogen (DON) is produced by the autolysis of settled phytoplankton cells or the hydrolysis of other, high-quality particulate organic nitrogen. DON is further mineralized by proteolytic bacteria to dissolved inorganic substances (e.g. ammonia produced by deamination of DON). Mineralization of organic matter at the sediment-water interface of aquaculture ponds is important as a source of N for primary production and a sink for dissolved oxygen.

Oxygen consumed by sediment is used for (1) heterotrophic microbial respiration, (2) chemical oxidation of reduced iron, manganese and sulfides, and (3) autotrophic oxidation of ammonium (Reddy and Patrick 1984, Oláh *et al.* 1987). Differences in reported estimates of sediment oxygen demand (SOD) can be related to differences in measurement techniques, salinity, organic fertilization history, climate and other edaphic factors. The SOD in aquaculture ponds is typically $<100 \text{ mg m}^{-2} \text{ h}^{-1}$ (Table 2). Such a rate can be expected to reduce dissolved oxygen concentration in a 1-m deep pond by 1.2 mg L^{-1} over a 12-h night.

Despite the relatively high efficiency of organic matter decomposition mediated by aerobic heterotrophic bacteria and the deposition and rapid mineralization of high-quality organic matter at the sediment surface, most decomposition in sediments takes place under reduced conditions. Organic matter in reduced sediments is derived from that deposited during previous cropping cycles or from that present during pond construction. Most organic matter in reduced sediment is relatively recalcitrant to decomposition (e.g. fulvic and humic acids). Anaerobic decomposition is characterized by (1) incomplete oxidation of organic matter, (2) reduced microbial cell yield per unit substrate, and (3) reduced assimilatory requirement for nitrogen by anaerobic microbes (Reddy and Patrick 1984). Thus, in general, relatively more N is released from organic matter under anaerobic conditions. Ammonia accumulates in the reduced sediment layer because the biochemical pathway of ammonia transformation requires oxygen.

Table 2. Estimates of sediment oxygen demand ($\text{mg O}_2 \text{ m}^{-2} \text{ h}^{-1}$) in aquaculture ponds.

SOD	Reference	Comments
12-27	Teichert-Coddington <i>et al.</i> 1991	newly-renovated ponds
21-24	Teichert-Coddington and Green 1993	chemically/organically-fertilized ponds
37-64	"	organically-fertilized and fed ponds
42 (8-114)	Mezainis 1977	channel catfish ponds
42	Shapiro and Zur 1981	tilapia/carp polyculture (7,000 fish ha^{-1})
92	"	" (20,000 fish ha^{-1})
30 (max.=49)	Boyd and Teichert-Coddington 1994	tropical, manured ponds
16-141	Olah <i>et al.</i> 1987	Indian undrainable polyculture ponds
42-125	Schroeder 1975	manured polyculture ponds
43	Roos and Eriksen 1995	semi-intensive polyculture ponds
46-51	Green and Boyd, in press	manured tilapia ponds
60	Costa-Pierce <i>et al.</i> 1984a	prawn ponds
62.5	Schroeder <i>et al.</i> 1991	20 °C
125	"	30 °C
67-80	Blackburn <i>et al.</i> 1988	marine finfish ponds

(table continued)

SOD	Reference	Comments
45-55	Holmer 1991	seasonal variation, 1 cage culture site
115-595	Holmer 1991	6 farms, annual mean
80-307	Holmer 1991	seasonal variation, 1 cage culture site
95 (max.=132)	Hargrave <i>et al.</i> 1993	Atlantic salmon cage culture site
146	Boyd <i>et al.</i> 1978	channel catfish ponds
221	Fast <i>et al.</i> 1983	prawn ponds
246	Costa-Pierce <i>et al.</i> 1984a	prawn/carp polyculture ponds
298	Madenjian 1990	marine shrimp ponds
324 (136-748)	Madenjian <i>et al.</i> 1987	prawn ponds

The rate of anaerobic mineralization depends on temperature, pH, the C:N ratio of organic material, nutrient availability in soil, and soil texture and structure (Reddy and Patrick 1984). Mineralization processes can be described by the Arrhenius equation, with a Q_{10} of 2 between 15 and 40 C, and proceed optimally over a pH range between 6.5 and 8.5. First-order (specific) rate constants for organic matter decomposition in fish ponds range from 0.001-0.002 d⁻¹ (Avnimelech 1984). Jana and Roy (1985) measured seasonal variation in the abundance of mineralizing bacteria in fish pond sediment over three years. Abundance of protein mineralizing bacteria (10^4 - 10^5 cells g⁻¹) and ammonifying bacteria (10^5 - 10^6 cells g⁻¹) was maximum during winter (November-January) and minimum during March and September. Bacterial density was directly related to management intensity, although site-specific differences were also apparent.

Concentrations of porewater or interstitial ammonium produced during mineralization may be an order of magnitude greater than that of the water column. Schroeder (1987) measured porewater ammonium concentrations of 10 mg N L⁻¹ at 1-4 cm sediment depth one month after filling a fish pond and 100 mg N L⁻¹ in a manure pile in the same pond. Porewater TAN concentration increased with pond age and water temperature (Masuda and Boyd 1994). Porewater TAN concentration of a 40-year old fish pond was over 20 mg N L⁻¹. Elevated porewater ammonium concentrations are mostly of concern with respect to the growth and survival of cultivated species with benthic feeding or burrowing habits, particularly crustaceans.

The distribution of porewater ammonium in sediment is typified by a low concentration at the sediment-water interface that increases rapidly with depth. Mineralized ammonium diffuses from the reduced sediment layer to the oxidized surface where it is subject to oxidation to nitrate or further diffusion to overlying water. The depth of maximum porewater ammonium concentration is a function of organic matter concentration and the rate of diffusion to the sediment-water interface. Beyond the depth of maximum porewater ammonium concentration, organic matter concentration decreases and porewater ammonium concentration declines.

Ammonium flux from the sediment can be enhanced by the burrowing activities of macrofauna (bioturbation). Sediment macrofauna can increase the effective surface area by 125% (Hyllenberg and Henriksen 1980). Benthic invertebrates increased the flux of ammonium from marine sediment by 50%, primarily by the irrigation of burrows, which may extend up to 8-12 cm into the sediment (Henriksen *et al.* 1980, Blackburn and Henriksen 1983). Similarly, 30% of the solute flux from the sediment of a marine fish pond was attributed to disturbance by fish (Blackburn *et al.* 1988).

To summarize, sediments are a source of ammonium to the water column of aquaculture ponds (Table 3). Most of this ammonium is derived from the regeneration of nitrogen from the mineralization of organic matter at the sediment-water interface. A smaller and variable source of ammonium is derived from mineralization of organic matter in the reduced sediment layer. Ammonium diffusion into the water column is driven by molecular flux along a concentration gradient

Table 3. Estimates of total ammonia-nitrogen (TAN) flux ($\text{mg N m}^{-2} \text{ d}^{-1}$) from marine and freshwater sediments.

TAN flux	Reference	Comments
0.03 - 0.45 0.20 (mean)	Brannon <i>et al.</i> 1985	reservoirs (porewater profiles)
0.13 - 0.81	Gunnison <i>et al.</i> 1985	reservoirs (model)
0.17 - 0.58	Blackburn and Henriksen 1983	Danish marine sediments
0.3 0.3 2.7 3.1	Acosta-Nassar <i>et al.</i> 1994	tropical fish pond (July) (August) (September) (November)
0.5 2.3 5.1	Nixon <i>et al.</i> 1976	coastal sediment (10 C) (20 C) (25 C)
1.9	Reddy and Rao 1983	Lake Okeechobee
2	Reddy <i>et al.</i> 1988	Lake Okeechobee
2.5 - 3.6	Reddy <i>et al.</i> 1990	Lake Okeechobee
-1.5 - 22 4.1 (mean)	Boynton <i>et al.</i> 1980	Patuxent River estuary
6.5	Jenkins and Kemp 1984	Patuxent River estuary
3.9	Vanderborght <i>et al.</i> 1977	Belgian Coast
4.2	Schroeder 1987	manured polyculture pond -diffusive flux + benthic regeneration

(table continued)

TAN flux	Reference	Comments
4.4	Seitzinger <i>et al.</i> 1984	Narragansett Bay
5 - 15	Fillos and Swanson 1975	eutrophic lake and river sediments
8.8 - 11.7 10.5 (mean)	Blackburn <i>et al.</i> 1988	marine fish pond
11.4	Avnimelech 1984	intensive fish pond -mineralization kinetics model
11 (mean) 22 (maximum)	Hargrave <i>et al.</i> 1993	Atlantic salmon cage culture site

extending from the reduced sediment layer to the sediment-water interface, a process that may be enhanced by macrofauna.

Ammonium Adsorption

A substantial quantity of mineralized ammonium in the porewater of the reduced sediment layer may weakly adsorb to negatively-charged cation exchange sites on the surface of clay minerals or organic matter. Adsorbed (exchangeable) ammonium is important as a regulator of ammonium supply to the overlying water and as a sink for mineralized ammonium. Acosta-Nassar *et al.* (1994) estimated that approximately 2% of the N added to a freshwater fish pond was stored in the adsorbed pool, although undoubtedly greater amounts of adsorbed ammonium were derived from the mineralization of autochthonous sediment organic matter.

Adsorbed and porewater ammonium are in equilibrium, so profiles of adsorbed and porewater ammonium are similar. The ratio of adsorbed to porewater ammonium (partition coefficient) is variable, but generally much greater than 1. Differences in the partition coefficient are related to the cation exchange capacity of soil, adsorbed and porewater ammonium concentrations, season (temperature) and sediment depth.

The concentration of adsorbed ammonium is affected by sediment drying and re-wetting. A rapid decline in the exchangeable ammonium pool to very low levels after 6 weeks of drying a fish pond sediment was measured by Diab and Shilo (1986). Following refilling, the adsorbed ammonium pool increased within 10 days to levels equivalent to approximately 50% of that prior to draining and increased

during the cropping cycle (Shilo and Rimón 1982, Diab and Shilo 1986).

Presumably, nitrification was responsible for reduction in exchangeable ammonium concentration, although evidence that adsorbed ammonium can be utilized by nitrifying bacteria is equivocal. The loose adsorption of exchangeable ammonium to sediment and the association of nitrifying bacteria with particles are cited as evidence in support of the importance of this process (Seitzinger 1990).

The dynamic nature of the adsorbed ammonium pool is further illustrated by measurement of the complete and rapid desorption of ammonium from a sandy sediment after two hours following suspension by wind-driven water turbulence (Simon 1989). Ammonium supplied to the water column by desorption of ammonium from sediment solids was estimated to exceed that supplied by diffusive flux. Suspension of aquaculture pond sediments by aeration or wind-driven water turbulence may increase, at least temporarily, the concentration of ammonium in the water column.

Nitrification

Nitrification is the sequential, two-step oxidation of ammonium to nitrate. The process is mediated by predominately two bacterial genera. The oxidation of ammonium is mediated by *Nitrosomonas* and the oxidation of nitrite is mediated by *Nitrobacter*. The organisms are chemautotrophic, gram-negative, motile rods with long generation times (20-40 h). The reaction proceeds as follows:



and



Thus, two moles of oxygen are required for each mole of ammonium oxidized. These organisms derive energy from the oxidation of ammonium and nitrite. The free energy yield (ΔG) from the oxidation of ammonia is approximately $-65 \text{ kcal mole}^{-1}$, and that from the oxidation of nitrite is approximately $-18 \text{ kcal mole}^{-1}$. Thus, over three times as much nitrite must be oxidized to support an equivalent microbial growth to that derived from the oxidation of ammonia.

Nitrification rates for estuarine sediments were similar and ranged from $15\text{-}25 \text{ mg N m}^{-2} \text{ d}^{-1}$ (Henriksen and Kemp 1988) and are probably representative of those of aquaculture pond sediments (Table 4). Assuming 5-10% of SOD is utilized for nitrification (Henriksen and Kemp 1988) then approximately $25\text{-}50 \text{ mg N m}^{-2} \text{ d}^{-1}$ is oxidized, equivalent to 5-10% of daily N input. Thus, nitrification is a relatively unimportant mechanism of ammonia transformation during the production cycle. Nitrification rates are elevated only during periods between cropping cycles when ponds may be dried.

Nitrification is affected by dissolved oxygen concentration, temperature, substrate concentration, pH, numbers of nitrifying bacteria, and availability of surfaces. Many of these factors are interrelated and their effect on nitrification is complex.

Nitrifying bacteria require oxygen to derive energy from reduced nitrogen. The K_m (half-saturation concentration) for oxygen ranges from $0.3\text{-}0.9 \text{ mg L}^{-1}$ and is directly related to temperature (Painter 1970). The K_m for oxygen is higher for

Table 4. Nitrification rate ($\text{mg N m}^{-2} \text{ d}^{-1}$) estimates in the sediments of marine and freshwater systems.

Nitrification Rate	Reference	Location/Comments
0	Blackburn <i>et al.</i> 1988	tropical marine fish pond
0.4-0.9 (mean=0.5)	Acosta-Nassar <i>et al.</i> 1994	tropical freshwater fish pond
0-42	Henriksen 1980	Danish coast
4-18	Henriksen <i>et al.</i> 1981	Danish coast
3-48	Billen 1978	Belgian coast (North Sea)
11	Blackburn and Henriksen 1983	Danish coast
11	Lindau <i>et al.</i> 1988b	rice soil
13 15	DeLaune and Lindau 1989	Lac des Allemands, LA Little Lake, LA
16 28-35	Henriksen <i>et al.</i> 1980	Danish coast -without fauna -with fauna
7-37 (mean=20)	Hansen <i>et al.</i> 1981	Danish coast
7-45 (mean=20)	MacFarlane and Herbert 1984	Scottish estuary
8-34 (mean=22)	Nishio <i>et al.</i> 1983	Japanese coast

(table continued)

Nitrification Rate	Reference	Location/Comments
24	Boynton <i>et al.</i> 1980	Patuxent River estuary
26-30	Jenkins and Kemp 1984	Patuxent River estuary
30	DeLaune and Smith 1987	Lake Verret, LA
7-45 (mean=39)	Seitzinger <i>et al.</i> 1984	Narragansett Bay
27-67 (mean=45)	Koike and Hattori 1978	Japanese coast
29 69	Chaterpaul <i>et al.</i> 1980	freshwater stream sediment -without fauna -with fauna
63	Vanderborght <i>et al.</i> 1977	Belgian coast (North Sea)
60-152	DeLaune <i>et al.</i> 1991	Calcasieu River, LA

Nitrobacter than for *Nitrosomonas* at 30 C suggesting that nitrite oxidation is more sensitive to low dissolved oxygen concentrations at warm temperature. Nitrification rate is constant at dissolved oxygen concentrations above 2 mg L⁻¹. The K_m for oxygen by nitrifiers is several orders of magnitude greater than that of heterotrophic aerobes, suggesting that heterotrophic bacteria may be competitively more successful than nitrifying bacteria.

Oxygen penetration into sediments is a key factor regulating nitrification (Reddy and Patrick 1984). The depth of oxygen penetration into aquatic sediments is typically on the order of 1-5 mm and is inversely related to temperature (Revsbech *et al.* 1980). Although nitrification increases with temperature, the volume of sediment involved in nitrification is restricted by oxygen penetration, which exerts control on overall nitrification rate. Nitrification potential was demonstrated in reduced sediment (6-8 cm depth), although actual nitrification was restricted to the sediment surface (Hansen *et al.* 1981, Henriksen *et al.* 1981). Nitrification potential was minimum during the summer, coincident with minimum oxygen penetration.

The optimum temperature range for growth of pure cultures of nitrifying bacteria (25-35 C) is fairly narrow, although the scope for growth (3-45 C) is much wider (Focht and Verstraete 1977). The Q₁₀ of nitrification ranges from 1.7 to 3.3 between 20 and 30 C (Fenchel and Blackburn 1979). Evidence of differential sensitivity of the two principal nitrifying genera to temperature is equivocal, but tends to implicate the greater sensitivity of nitrite oxidizers to low temperature, particularly at pH values outside the optimum range (Focht and Verstraete 1977).

However, climatic and other environmental variables exert strong selection pressures on populations of nitrifying bacteria suggesting adaptation is likely. Thus, information derived from studies of pure cultures should be viewed with qualification.

Nitrification rates are also affected by substrate concentration. In aquaculture pond sediments, ammonium is supplied (1) by the mineralization of organic N at the sediment-water interface, (2) diffusion of ammonium from the reduced sediment layer to the sediment-water interface, and (3) the bulk water. Half-saturation concentrations (K_m) for substrate increase with temperature (20-32 C) and range from 1-10 mg N L⁻¹ for ammonium oxidation, and from 5-9 mg N L⁻¹ for nitrite oxidation (Painter 1970). TAN concentrations in channel catfish ponds are usually <3 mg N L⁻¹ (Tucker and van der Ploeg 1993), with the highest concentrations occurring during the winter when phytoplankton biomass is minimal. Nitrite concentrations in channel catfish are usually <0.2 mg N L⁻¹ with seasonal peaks in the spring and fall. Such low concentrations in the bulk water impose substrate limitation on nitrification in aquaculture ponds, suggesting that the kinetics of nitrification are first-order with respect to substrate concentration. However, nutrient regeneration at the sediment-water interface and ammonium diffusion from reduced sediment may be sufficient to surmount substrate limitation.

Nitrifiers require slightly alkaline pH (7-8.5) for optimal growth. Under slightly alkaline conditions the pattern of nitrification is characterized by the rapid oxidation of ammonium, an accumulation of nitrite coincident with a decline in

ammonium, and, after a lag period, a decline in nitrite. At pH >8.5, *Nitrobacter* may be inhibited more than *Nitrosomonas*, resulting in an accumulation of nitrite (Fenchel and Blackburn 1979). Increased nitrification at alkaline pH suggests that NH_3 may be the substrate for nitrification. Additionally, unionized ammonia can inhibit nitrite oxidation at 0.1-1.0 mg $\text{NH}_3\text{-N L}^{-1}$ (Belser 1979). However, these concentrations are rarely observed in fish ponds as they are toxic to fish as well. Finally, pH is important because 2 hydrogen ions are released for each mole of ammonium oxidized. Natural waters usually contain sufficient alkalinity to buffer an increase in hydrogen ion concentration from nitrification.

Nitrifiers are lithotrophic, requiring organic or mineral surfaces for attachment. Nitrifier density on the sediment surface ($10^6\text{-}10^9 \text{ cm}^{-3}$) is approximately 3 orders of magnitude greater than that in the water column ($10^3\text{-}10^4 \text{ ml}^{-1}$). The abundance of ammonium oxidizers ($10^4\text{-}10^5 \text{ cells g}^{-1}$) in sediment is greater than that of nitrite oxidizers ($10^3 \text{ cells g}^{-1}$) (Ram *et al.* 1981, Ram *et al.* 1982). The sediment surface is the locus for mineralization of particulate organic matter settling from the water column. In addition, ammonium may be concentrated on sediment mineral particles (clays) as part of the cation exchange complex. Competition for surfaces between heterotrophic and nitrifying bacteria may contribute to limitation of population density of the latter group.

Nitrification at the sediment-water interface is more important than nitrification in the water column in stratified or periodically-mixed fish ponds. Nitrification in the water column is restricted by the availability of surfaces and

possibly by light-inhibition. Nitrification may increase temporarily following phytoplankton die-offs in response to elevated ammonia concentration. Water-column nitrification is an important mechanism of ammonia transformation in high-intensity pond systems in which particles suspended by mechanical aeration are sites of active mineralization and nitrification.

In flooded soils, nitrification and denitrification are closely coupled. A two-layer model has been developed to describe the interdependence of these two processes (Reddy and Patrick 1984). In the two-layer model, ammonium diffuses from the reduced sediment layer along a concentration gradient to the surface, where it is oxidized by nitrifying bacteria. Nitrate diffuses in response to a concentration gradient back to the reduced sediment layer where it is denitrified to dinitrogen gas, which evolves to the atmosphere through gas ebullition. Thus, although oxygen inhibits denitrification, the reaction indirectly requires oxygen for the production of nitrate.

A complementary theory has been developed to explain the seemingly contradictory coexistence of oxic and anoxic processes within an oxic environment. Jørgensen (1977) found that detrital particles of 100 μm to several mm may have anoxic centers. Paerl (1984) estimated that reduced microzones can occur in cyanobacterial mats ranging in thickness from 10 to several 100 μm . Jenkins and Kemp (1984) argued that calculation of the effective nitrate diffusion distance ($\approx 80 \mu\text{m}$) suggests that denitrification must be tightly coupled with nitrification and occur in reduced microzones within the oxidized layer of the sediment surface. Reduced

microsites depend on (1) oxygen consumption rate, (2) oxygen diffusion rate, and (3) particle geometry (Focht and Verstraete, 1977).

Two annual patterns of nitrification have been described in estuaries. Most investigators have observed a depression of nitrification in mid-summer due primarily to limited penetration of oxygen into sediments (Hansen *et al.* 1981, Jenkins and Kemp 1984). The other pattern is directly related to annual temperature fluctuation, indicating that oxygen was not limiting nitrification in those estuaries (Seitzinger *et al.* 1984, MacFarlane and Herbert 1984).

Spring and fall seasonal peaks of nitrite concentration have been observed in temperate fish ponds (Tucker and van der Ploeg 1993). There has been some speculation about whether the source of this nitrite is derived from nitrification or denitrification. Hollerman and Boyd (1980) suggested that nitrite produced during denitrification was released to the water column after suspension of sediments by aeration. In channel catfish ponds, nitrate concentration was inversely related ($R = -0.220$, $P = 0.021$) to soluble reactive phosphorus concentrations (data of Tucker and van der Ploeg 1993). Principal component (factor) analysis of these data (not shown) extracted a factor associated with positive loadings by nitrite and nitrate and negative loading by soluble reactive phosphorus. These results suggest that sediment oxygenation is a key process regulating concentrations of nitrite, nitrate and soluble reactive phosphorus in channel catfish ponds. When sediments are relatively oxygenated, nitrification can proceed and nitrite and nitrate may accumulate, at least temporarily, and phosphorus adsorption to sediment is enhanced. Under relatively

anoxic conditions, nitrification ceases and phosphorus may be desorbed and diffuse to the water.

Nitrification in aquaculture pond sediments is most likely limited by the depth of oxygen penetration (typically 1-5 mm) (Figure 1). Although operation of aerators prevents dissolved oxygen in the water column from declining to concentrations $<4 \text{ mg L}^{-1}$, it is likely that a laminar benthic boundary layer ($\approx 100 \text{ }\mu\text{m}$) depleted of dissolved oxygen develops at the sediment-water interface in the summer. Stirring of sediments, such as occurs during pond aeration, may increase the depth of oxygen penetration (Revsbech *et al.* 1980).

Reduced sediment redox potential may extend a short distance (millimeters) into the water column during summer when temperatures are elevated and sediment oxygen demand is high, allowing the passage of reduced substances (Fe^{2+} , Mn^{2+} and S^{2-}) into the water column. As a result, phosphorus loosely bound to amorphous oxides and oxyhydroxides of iron is desorbed and diffuses to the water column. Although phytoplankton density is maximum during summer, soluble reactive phosphorus concentrations are paradoxically maximum.

As temperature declines during the fall, dissolved oxygen concentration of the water column increases due to reduced feeding rate and pond respiration and increased oxygen solubility. Sediment oxygen demand declines as metabolic activity is depressed and the depth of oxygen penetration into the sediment surface increases. Nitrite and nitrate in the water column increases in response to nitrification at the sediment-water interface and soluble phosphorus concentrations decline due to

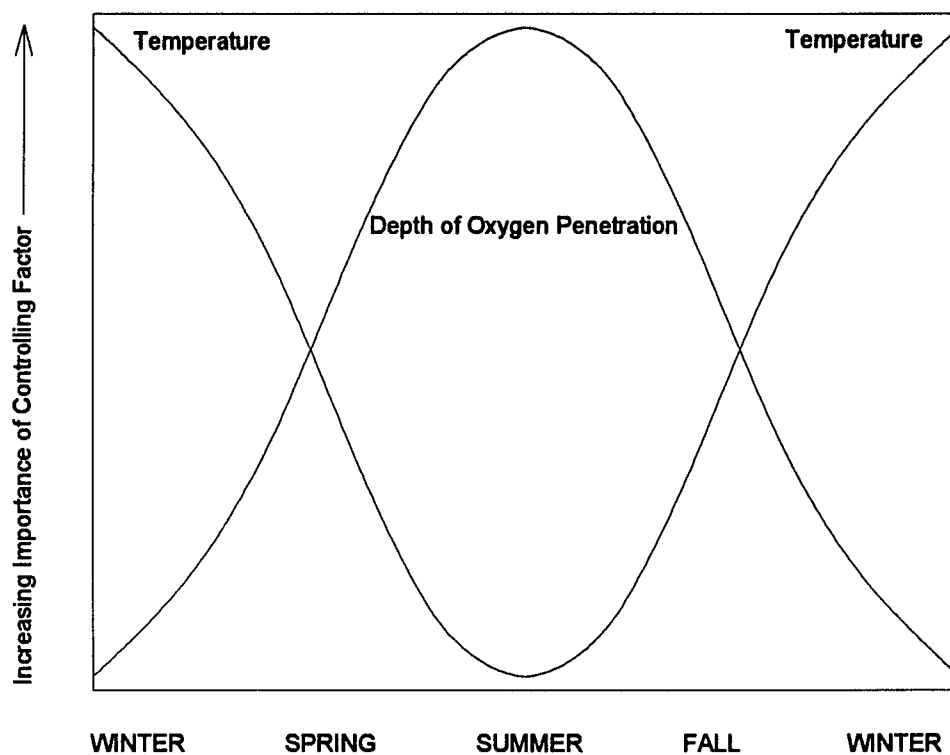


Figure 1. Conceptual model of the relative importance of temperature and sediment oxygen penetration as factors controlling coupled nitrification-denitrification in temperate fish pond sediments.

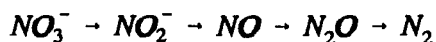
physical and chemical processes that bind phosphorus to sediment (adsorption, co-precipitation with calcium, aluminum, and iron).

Nitrification rate is reduced during the winter due to seasonally minimal temperatures. In the spring, as temperature increases, organic N accumulated over the winter is rapidly mineralized to ammonium and the rate of nitrification is once again stimulated, producing another sharp increase in water column nitrite concentration. As temperature increases further, water column dissolved oxygen declines, sediment oxygen penetration and nitrification are reduced.

In summary, nitrification rate during summer is controlled (limited) by oxygen penetration into the sediment, despite seasonally maximum temperatures. During the fall, control of nitrification gradually shifts from oxygen penetration to temperature. During the winter, temperature controls nitrification. During the spring, control of nitrification shifts from temperature to sediment oxygen penetration.

Nitrate Reduction

Nitrate may follow several biochemical pathways following production by nitrification. Plants and microbes may reduce nitrate to ammonia for incorporation into cellular amino acids (assimilatory nitrate reduction). Nitrate may function as a terminal electron acceptor during the oxidation of organic matter and thereby supply energy for microbial growth. Dissimilatory nitrate reduction, or nitrate respiration, results in the reduction of nitrate to dinitrogen or ammonium. Denitrification is a special case of nitrate respiration in which nitrate is reduced to gaseous dinitrogen by the following pathway:



Oxygen is the energetically-preferred terminal electron acceptor for the oxidation of organic matter. However, when oxygen concentration becomes limiting ($\sim 0.1\text{-}0.2 \text{ mg L}^{-1}$ or $E_h < 220\text{mV}$), heterotrophic facultative anaerobes shift to nitrate as the terminal electron acceptor. The energetic yield from the oxidation of organic carbon (e.g. glucose) by nitrate ($\Delta G = -649 \text{ kcal mole}^{-1}$) is only slightly less than that by oxygen ($\Delta G = -686 \text{ kcal mole}^{-1}$).

Unlike the limited species diversity mediating nitrification, at least 14 genera of bacteria are involved in denitrification, with *Pseudomonas*, *Bacillus* and *Alcaligenes* the most prominent numerically (Focht and Verstraete 1977). In addition, the growth, activity and population density of denitrifying bacteria exceed that of nitrifying bacteria. Most denitrifying bacteria are considered facultative anaerobes. Although denitrification is inhibited by oxygen, the reaction occurs primarily near the sediment surface, quite possibly in reduced (suboxic) microzones in the oxidized sediment surface layer.

The rate of denitrification depends on concentrations of nitrate, organic carbon, and oxygen, temperature, and the population density of denitrifying bacteria (Table 5). Denitrification rates increase with substrate concentration. Denitrification rates in estuaries correspond with seasonal (spring) peaks in nitrate loading (Andersen *et al.* 1984). However, denitrification rates in most natural aquatic systems are first-order with respect to nitrate concentration, and can be considered substrate-limited. In aquaculture ponds, nitrate is typically $< 0.5 \text{ mg N L}^{-1}$ (Ziemann

Table 5. Denitrification rate ($\text{mg N m}^{-2} \text{ d}^{-1}$) estimates in the sediments of marine and freshwater systems.

Denitrification Rate	Reference	Location/Comments
1.4-3.6 3.6-7.1	Messer and Brezonik 1983	Lake Okeechobee -acetylene blockage -mass balance
0.1-7.4	Acosta-Nassar <i>et al.</i> 1994	tropical freshwater fish pond
2.3	Oren and Blackburn 1979	Kysing Fjord, Denmark ($\sim 0.15 \text{ mg L}^{-1} \text{ NO}_3^- \text{-N}$)
2.7-10.9	Kaspar 1982	intertidal mud flat
3.6-7.2	Chan and Knowles 1979	eutrophic ponds
5	Tirén 1977	oligotrophic Swedish lake
3.4-13	Nishio <i>et al.</i> 1983	Japanese coast
3.6-18	Sweerts and de Beer 1989	eutrophic lake (Vechten)
3.8	Smith and DeLaune 1983	freshwater/estuarine eutrophic lake sediments
0-29	Billen 1978	Belgian coast
4-55	Cerco 1989	Potomac River (10-30 C, $8 \text{ mg L}^{-1} \text{ DO}$, $0.21\text{-}0.63 \text{ mg L}^{-1} \text{ NO}_3^- \text{-N}$)
4-71	Andersen <i>et al.</i> 1984	Danish estuary; seasonal variation

(table continued)

Denitrification Rate	Reference	Location/Comments
10-40	Henriksen <i>et al.</i> 1980	Danish coast
14	Sørensen 1978	Danish coast
14-20	Chan and Campbell 1980	eutrophic Canadian lake
18-35	Nielsen 1992	eutrophic stream bed
17-34	Seitzinger <i>et al.</i> 1984	Narragansett Bay
25-40	Tirén 1977	3 eutrophic Swedish lakes
26-30	Jenkins and Kemp 1984	Patuxent River estuary (spring)
29	Vanderborght <i>et al.</i> 1977	Belgian coast
1.5-57	Lindau <i>et al.</i> 1990	urea-treated rice plot
47-81	Roos and Eriksen 1995	semi-intensive polyculture pond
14-25 71-119	Blackburn <i>et al.</i> 1988	marine fish ponds -acetylene blockage -nitrite+nitrate reduction
56-69	Krom, unpublished (cited in Blackburn <i>et al.</i> 1988)	marine fish ponds
52	D'Angelo and Reddy 1993	Lake Okeechobee
58 110 34 85	Andersen 1977	Byrup Langsø (lab cores) " (mass balance) Kvind Sø (lab cores) " (mass balance)

(table continued)

Denitrification Rate	Reference	Location/Comments
45	DeLaune and Smith 1987	Lake Verret, LA -nitrate reduction
34-52	DeLaune <i>et al.</i> 1991	Calcasieu River, LA
33-342	Lindau <i>et al.</i> 1990	KNO ₃ -treated rice plots
95-160	Van Kessel 1977	enriched ditch sediment
100-200	Nishio <i>et al.</i> 1982	polluted estuary, Japan
101-296	Seitzinger and Nixon 1985	enriched marine mesocosm
367	Lindau <i>et al.</i> 1988a	enriched bottomland hard- wood forest swamp plot
100-500	Andersen 1977	enriched lake sediment
420-490	Binnerup <i>et al.</i> 1992	enriched, bioturbated marine sediment

et al. 1992, Tucker and van der Ploeg 1993), a concentration likely below reported half-saturation constants (K_m) for denitrification. Nitrate concentration in temperate aquaculture ponds is maximum during winter, when phytoplankton blooms are minimal and aerobic water column and sediment surface conditions promote nitrification, despite seasonally minimal temperatures.

Kinetic constants vary with available carbon (reductant). Reported K_m values range from 0.1 to 170 mg N L⁻¹, and increase in direct relation to carbon (Focht and Verstraete 1977). In a multiple (stepwise) regression model, dissolved organic carbon concentration was the most important predictor of denitrifying bacteria abundance in tropical fish ponds (Jana and Patel 1985). The large quantity and low C:N ratio of settled organic matter in aquaculture ponds suggests that carbon limitation of denitrification is not likely.

A wide range of Q_{10} values have been reported, quite possibly reflecting the broad generic diversity of denitrifying bacteria. Most Q_{10} values range from 1.4 to 3.4 between 15-35 C (Focht and Verstraete 1977). Denitrifier activity is sharply curtailed below 15 C. Q_{10} values are affected by the concentration of oxidant (nitrate) and reductant (organic carbon) as well as oxygen concentration. At 34 C denitrification rate was much less affected by oxygen concentration than at 19 C (Focht and Verstraete 1977). The abundance of denitrifying bacteria in tropical fish pond sediment was maximum during summer (10⁴ cells g⁻¹) and minimum during winter (10³ cells g⁻¹) (Jana and Patel 1985). Comparable abundance and seasonal patterns were observed in the water column. In Israeli fish pond sediment, the

abundance of denitrifying bacteria increased with intensity of management and ranged from 10^4 - 10^6 cells g^{-1} (Ram *et al.* 1982).

MANAGEMENT PRACTICES AFFECTING NITROGEN BIOGEOCHEMISTRY

Feeds and Feeding Practices

Feeds and feeding practices have a dramatic impact on the concentration of ammonia in fish pond water. TAN was strongly correlated with daily feeding rate in channel catfish ponds over the range 0-224 kg $ha^{-1} d^{-1}$ (Tucker *et al.* 1979, Cole and Boyd 1986). TAN increased in response to dietary protein concentration and total protein fed over the range 24%-40% (Li and Lovell 1992). However, un-ionized ammonia nitrogen was not affected by dietary protein concentration. Temporary withdrawal of feed (9 days) did not reduce TAN in channel catfish ponds (Tidwell *et al.* 1994).

Water Exchange

Various water management practices have been evaluated to reduce TAN. Water exchange rates of 0, 1, 2 or 4 pond volumes over three months (July to September) were insufficient to affect water quality in channel catfish ponds (McGee and Boyd 1983). A water exchange rate of 2.5% d^{-1} was sufficient to reduce ($P < 0.05$) TAN in intensive shrimp (44 m^2) ponds compared to ponds with no water exchange (Hopkins *et al.* 1993). TAN in ponds with a water exchange rate of 25% d^{-1} was not different from that of inlet water.

In high-biomass ($>10 \text{ kg m}^{-2}$), intensive fish ponds (500 m^3), a hydraulic retention time $>24 \text{ h}$ was necessary for nitrification to proceed (Diab *et al.* 1992).

Greater water exchange rates led to washout of nitrifying bacteria.

Water Circulation and Aeration

Semi-intensive pond systems for the culture of channel catfish and penaeid shrimp employ paddlewheel aeration to maintain dissolved oxygen concentration. The effects of aeration and circulation on dissolved inorganic nitrogen concentrations are a complex function of feeding rate, sediment suspension, duration of aeration and phytoplankton density. Ammonia concentration was only slightly lower ($P>0.05$) and nitrite concentration slightly higher ($P>0.05$) in channel catfish ponds ($50 \text{ kg feed ha}^{-1} \text{ d}^{-1}$) aerated for 6 hours during the night as compared to ponds aerated on an emergency basis only (Lai-Fa and Boyd 1988). TAN concentration increased slightly and nitrite concentration increased substantially in channel catfish ponds (up to $90 \text{ kg feed ha}^{-1} \text{ d}^{-1}$) aerated nightly compared to those of unaerated control ponds (Hollerman and Boyd 1980). Similarly, concentrations of TAN, nitrite and nitrate were directly related to duration of aeration (emergency, nightly, continuous) in channel catfish ponds (Thomforde and Boyd 1991). The suspension of sediment particles by aeration may have promoted the rapid desorption of exchangeable ammonium and nitrification in the water column. Continuous paddlewheel aeration reduced ammonia concentrations slightly in brackishwater shrimp ponds, but concentrations were not different from unaerated control ponds (Sanares *et al.* 1986). Un-ionized ammonia concentrations were not affected by continuous paddlewheel

aeration in freshwater ponds stocked with tilapia at 3000 kg ha⁻¹ (Ver and Chiu 1986).

In Taiwan, Israel and Hawaii, intensive pond systems have been established in which water is circulated continuously by paddlewheel aeration, and water and settled organic matter are periodically or continuously removed from a center drain (Avnimelech *et al.* 1986, Wyban and Sweeney 1989, Fast and Boyd 1992).

Phytoplankton uptake is insufficient to assimilate the large quantity of ammonium generated as a consequence of high feeding rate and stocking density. In such high-intensity ponds, nitrification in the water column is the most important mechanism of ammonium removal. Although nitrate accumulated in the water of such circulated systems, substantial denitrification was assumed to occur in the sediment. Total nitrogen may exceed 30 mg N L⁻¹ (most as nitrate) (Avnimelech *et al.* 1986).

Although the quantity of effluent released from such ponds is low, the concentration of N is high.

Such high-intensity pond systems have been integrated with larger, extensive reservoirs. Water retention in the intensive component is sufficient to allow water-column nitrification to proceed to completion. High-nitrate effluent is directed to the reservoir, where solids are deposited and denitrification takes place prior to recirculation to the high-intensity ponds.

Pond Depth

In shrimp ponds of variable depth, TAN was not significantly affected by pond depth, although nitrite and nitrate were significantly decreased as pond depth

increased (Carpenter *et al.* 1986). Presumably, reducing water depth in a pond with a high phytoplankton density will reduce light-limitation of phytoplankton growth and nutrient uptake.

Organic Carbon Addition

Avnimelech *et al.* (1989) demonstrated a practical technique to recycle excess nitrogen into fish flesh. Dissolved inorganic nitrogen limitation can be established in intensive, circulated fish ponds by adding a carbon-rich substrate (e.g. cellulose, sorghum meal) that promotes the formation of microbial biomass. The resulting heterotrophic production (single-cell protein) may be utilized as a food source by carp and tilapia (Schroeder 1978).

Sediment Management

Most sediment management techniques are undertaken while the pond is drained between cropping cycles. Drying is probably the most important sediment management technique. Drying promotes oxidation of accumulated organic matter (Ayub *et al.* 1993) and nitrification of mineralized nitrogen (Diab and Shilo 1986), although the optimum soil moisture content for organic matter decomposition is 20% (Boyd 1992). Shrimp pond soils in Ecuador are dried for an average of 10 days following harvest (Peterson and Daniels 1992). Pond soils may be tilled to hasten the decomposition process, although 5 weeks of drying are required before heavy equipment can enter the pond. Drying also kills undesirable competitors and potential pathogens. Organic matter may be physically removed from intensive

shrimp ponds by suction dredges, by hand, or by earthmoving equipment or flushed out by hydraulic jets (Clifford 1992).

Ponds may be limed following draining and drying to raise soil pH to levels promoting decomposition, particularly in brackishwater ponds constructed with acid-sulfate soil and in areas with low alkalinity water. Certain liming agents (e.g. hydrated lime) may reduce pathogens in the soil. Ponds are limed at a rate of 1,000-3,000 kg ha⁻¹ depending on soil pH (Boyd 1992). Fertilization of brackishwater shrimp pond soils with 50-200 kg urea ha⁻¹ reduced sediment organic matter (Peterson and Daniels 1992) although urea application did not reduce organic carbon in manured freshwater fish ponds (Ayub *et al.* 1993).

Sediment management techniques during the cropping cycle while the pond is full have not been fully evaluated. Brackishwater shrimp ponds may be dredged during the cropping cycle to remove accumulated organic matter or a chain may be dragged across the pond bottom periodically (Fast and Boyd 1992).

Sediment Management Implications

Management approaches toward sediments can be divided into two separate types depending upon the desired goal. In ponds receiving nutrients supplied in excess of requirements, or where autotrophic food webs are not important for increased fish yields, but are only important for improved water quality, then it is desirable to promote conditions that maximize the potential of the pond to remove excess nitrogen. The process with the greatest potential to remove nitrogen is denitrification, which requires the transformation of reduced (ammonium) to oxidized

(nitrate) nitrogen. Management techniques that improve oxic conditions at the sediment-water interface will promote rapid mineralization and coupled nitrification-denitrification. Likewise, techniques that promote water-column nitrification will increase substrate in the bulk water to concentrations that will stimulate sediment denitrification.

In ponds managed for primary productivity, promotion of nutrient recycling within the pond will maximize fish yield. Anaerobic sediment conditions will allow the diffusion of ammonium mineralized under reduced conditions or regenerated at the sediment-water interface to diffuse into the water column where it will be available for phytoplankton.

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CHAPTER 2

ANNUAL VARIATION OF SEDIMENT NITROGEN CHEMISTRY IN A COMMERCIAL CHANNEL CATFISH POND

INTRODUCTION

Large quantities of nitrogen are added to channel catfish ponds ($>500 \text{ mg N m}^{-2} \text{ d}^{-1}$ during mid-summer), yet a relatively small proportion of input N is retained by fish (Chapter 1). Consequently, large quantities of N are released to the culture environment, with potentially deleterious consequences for water quality, fish growth and pond production.

Although phytoplankton play the major role in assimilating excess dissolved nutrients in aquaculture ponds, the high surface-volume ratio of aquaculture ponds suggests that sediments may have an important impact on water quality in this environment. Few studies have evaluated the importance of sediments to overall nutrient budgets in aquaculture ponds.

Denitrification is potentially an important mechanism of N loss in shallow aquatic systems such as aquaculture ponds. However, few direct measurements of N loss by this mechanism have been made. Nutrient budgets prepared for freshwater channel catfish and brackishwater striped bass ponds using a mass balance approach estimated that 55-57% of the nitrogen applied was lost by denitrification and ammonia volatilization (Boyd 1985, Daniels and Boyd 1989). The difference remaining following measured gains and losses was assumed to be equivalent to the magnitude of N loss by these processes.

Seasonal variation of water quality in channel catfish ponds has been described (Tucker and van der Ploeg 1993). However, few studies have examined seasonal effects on the rates of sediment processes and how these processes affect water quality.

The objectives of this study were to evaluate seasonal effects on sediment-water nutrient exchange rates and to evaluate the magnitude and potential of N removal by denitrification in a commercial channel catfish pond.

MATERIALS AND METHODS

A 2.1-ha earthen pond located on a commercial channel catfish farm located in the Atchafalaya River Basin near St. Martinville, LA (30° 10' N, 91° 42 ' W) was selected for sediment sampling. Ponds were managed for semi-intensive culture by a single-batch cropping system, but water was not drained at harvest. Fish were stocked at approximately 20,000 fish m⁻². A 7.5-kW (10-hp) paddlewheel aerator located at one end of the pond was operated during periods of high feeding rates and low dissolved oxygen. The selected pond had been in continuous fish production for 5 years. Alkaline ground water was pumped to the surface to fill ponds and to replace losses derived from seepage and evaporation as required. The pond was constructed on a silty, clay-loam soil (62% silt, 38% clay). Clays were montmorillonitic, and the soil was slightly alkaline (pH=7.68) with very high shrink-swell potential (Table 6).

Table 6. Soil classification of Sharkey clay (after Murphy *et al.* 1977) used to construct channel catfish ponds and evaluated in a study of sediment-water nutrient interactions.

Order	Inceptisols
Subgroup	vertic haplaquepts
Family	very-fine, montmorillonitic, nonacid, thermic
Series	Sharkey

Eight sampling stations were established along an S-shaped meander extending the length of the pond (Figure 2).

Sediment was collected at each station in a 5-cm ID clear PVC coring tube attached to a coring device consisting of a length of 5-cm PVC pipe, a brass check valve, and a rubber coupling attached with hose clamps. The core tube-coring device assembly was gently lowered vertically through the water column and forced into the sediment. Upon assembly withdrawal, closure of the check valve retained sediment in the core tube. The bottom of each core tube was capped with a rubber cap and secured with a hose clamp. The core tube was removed from the coring device, capped with a rubber stopper, and placed vertically in a matrix of crushed ice. Four cores were collected at each station. Sediment temperature was determined by inserting a mercury thermometer approximately 5 cm into a sediment core. Water was collected from the near surface of the pond. Water transparency was measured with a Secchi disk.

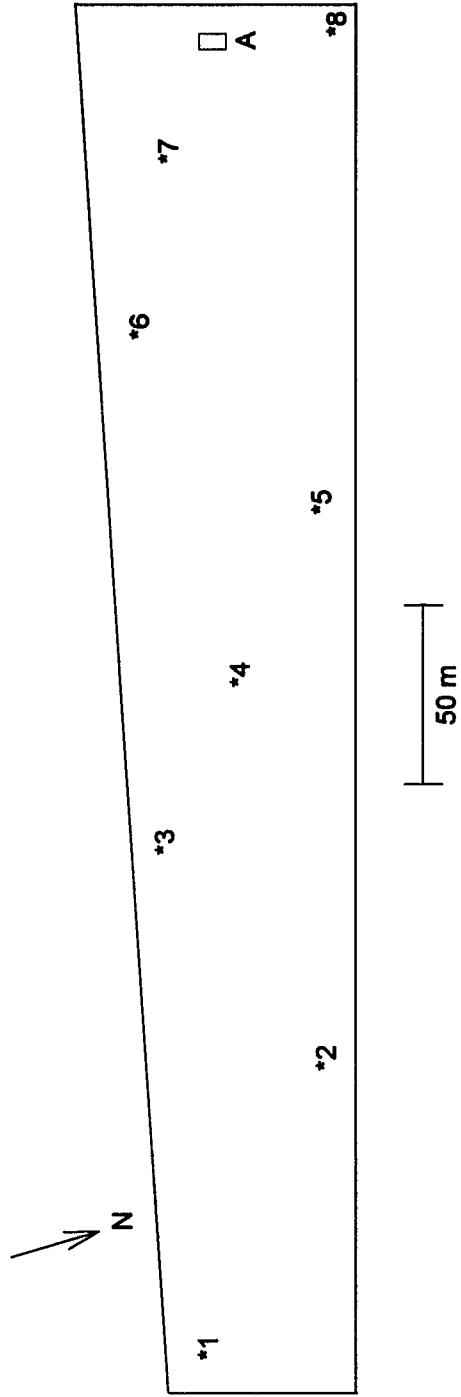


Figure 2. Location of sampling stations and a paddlewheel aerator (A) in a 2.1-ha channel catfish pond located near St. Martinville, Louisiana.

Sediment cores and pond water were transported to the laboratory (approximately 100 km) within 3 hours of collection. In the laboratory, cores were incubated in the dark at the temperature (± 0.5 C) of sediment collection. Pond water was filtered through 0.45- μ m membranes and placed in an incubator. Alkalinity (titration with dilute HCl to the methyl orange end point), total ammonia-N (phenol-hypochlorite method), nitrite-N (diazotization), and nitrate-N (Cd reduction followed by diazotization) of a sample of filtered (Whatman No. 42) pond water was measured (Boyd and Tucker 1992, APHA *et al.* 1992).

Sediment cores were collected at 3-month intervals beginning in the Fall 1993. Samples were collected in Fall (11/3) 1993, Winter (2/2) 1993/1994, Spring (4/27) 1994 and Summer (8/1) 1994. Between the Spring and Summer 1994 sampling period the pond was drained, dried for approximately 6 weeks, treated with lime, and refilled.

Sediment Nitrogen Pools

One core collected from catfish pond sediment at each station in winter (February) and summer (August) 1994 was sectioned at 5-cm intervals by removing the overlying water and extruding sediment into a section of core tubing placed on top of the core tube used in sediment collection. Core sections were sliced with a wide spatula and transferred to a tared weighing vessel. Wet sediment was weighed to the nearest 0.01 g and dried at ambient laboratory temperature (25-28 C) for 72 h. Dried sediment was weighed and ground with a mortar and pestle. Sediment bulk density was calculated from these measurements. Sediment porosity was calculated

by the following equation using a particle density of 2.65 g cm^{-3} (Blake and Hartge 1986):

$$\text{Porosity} = 1 - [\text{bulk density}/\text{particle density}].$$

KCl-extractable total ammonia-N was determined by extracting approximately 10 g of dry soil from each section with 100 mL of 2.0 N KCL. Soil-KCl mixtures were placed in screw-top plastic bottles that were capped and placed on a rotary shaker at 150 rpm for 1 hour. Soil-KCl suspensions were allowed to settle for 0.5 h. Total ammonia-N of filtered (Whatman No. 42) extracts was measured by the phenol-hypochlorite method after appropriate dilution with ammonia-free deionized water.

Organic (Kjeldahl) nitrogen was determined by digesting an aliquot of dry soil from each section with concentrated H_2SO_4 in the presence of a salt (K_2SO_4) and a catalyst ($\text{CuSO}_4 \cdot \text{H}_2\text{O}$ and Se) for 5 hours at 350 C. Digests were neutralized with approximately 20 mL of 10 N NaOH prior to determination of total ammonia-N by steam distillation. Distillates were collected in boric acid indicator and titrated with dilute HCl.

Porewater TAN was determined as described below. Porewater TAN concentration was averaged over each 5-cm section and expressed on a sediment volumetric basis using porosity values for each section, which were determined separately.

Porewater TAN Profiles

The concentration gradient of TAN in the upper 15 cm of sediment was determined using diffusion-controlled, pore water equilibrators consisting of an acrylic block measuring 40 cm x 10 cm x 4 cm, beveled at one end, into which sampling cells were cut at 1-cm intervals. Equilibrators were prepared by filling the cells with distilled, deionized water and covering with a 0.20- μ m mesh polycarbonate membrane followed by a 250- μ m mesh polyethylene membrane for protection. Preparation was completed by screwing a matching cover to the base of the equilibrator. Prepared equilibrators were placed in a bucket filled with distilled, deionized water.

At the pond, equilibrators were carefully driven into the sediment at each station bevelled end first. A float attached to the upper end of the equilibrator facilitated location and retrieval. Equilibrators were placed into the sediment at the time of core collection and recovered after a 10-day equilibration period. Upon recovery, the location of the sediment-water interface was noted and the equilibrators placed in a plastic bag under crushed ice for transport to the laboratory.

In the laboratory, membranes were removed and discarded. Water from each well (approximately 7 mL) ranging from 5 cm above the sediment-water interface to 15-cm below was removed with a syringe and placed in a test tube, covered with plastic (Parafilm®) and frozen for later analysis. TAN of thawed and diluted porewater was determined by the phenol-hypochlorite method. Analysis was completed within one week of equilibrator recovery.

TAN diffusion rate due to the concentration gradient at the sediment-water interface was estimated using the equation used to describe Fickian (molecular) diffusion:

$$J = \theta D_s \left(\frac{\delta C}{\delta z} \right)_{z=0}$$

where J =flux rate ($\text{mg cm}^{-2} \text{ d}^{-1}$)
 θ =sediment porosity at sediment-water interface ($\text{cm}^3 \text{ cm}^{-3}$)
 D_s =molecular diffusion coefficient ($\text{cm}^2 \text{ d}^{-1}$)
 $(\delta C/\delta z)$ =porewater concentration gradient at the sediment water interface ($\text{mg cm}^{-3} \text{ cm}^{-1}$).

Solute Flux

Filtered (0.45- μm) pond water was aerated with an aquarium air pump and diffusers for 12 hours. Dissolved oxygen (polarographic probe) and pH (combination electrode) were measured in a sample of aerated water.

Water overlying intact cores was carefully removed to avoid disturbance of surficial sediment and replaced with 150-200 mL of filtered, aerated pond water. Cores were incubated in the dark at *in situ* sediment temperature for 4 hours. A control core without sediment was incubated simultaneously.

After 4 hours, the dissolved oxygen concentration in the overlying water of each core was measured. Overlying water was removed, volume measured with a graduated cylinder, and pH measured. Total ammonia-N, nitrite-N, and nitrate-N were determined from filtered (Whatman No. 42) samples of overlying water as described above. Free CO_2 was estimated from equilibria among components of the

carbonate system as determined by total (methyl orange) alkalinity and pH (APHA *et al.* 1992).

The rate of solute (O_2 , CO_2 , TAN, NO_2^- -N, NO_3^- -N) flux between the sediment and water was determined from differences between initial and final concentrations adjusted for changes in the control core over the 4-h interval.

Nitrous Oxide Production Rate

Initial evaluation of denitrification rate by acetylene blockage of nitrous oxide reduction indicated that the rate was very low. Nitrous oxide is produced as an intermediate product of denitrification, dissimilatory nitrate reduction to ammonia, and nitrification, but most is produced by denitrification in flooded soil systems (Granli and Bøckman 1994). Therefore, nitrous oxide emission was measured as an indicator of the magnitude of N_2 flux by denitrification. The ratio of $N_2:N_2O$ produced during sediment denitrification is variable and depends on temperature, nitrate concentration, oxygen concentration, pH and carbon content (Focht and Verstraete 1977, Setzinger and Nixon 1985, Granli and Bøckman 1994). The $N_2:N_2O$ ratio is inversely related to nitrate concentration, which is the most important parameter affecting the ratio. Therefore, a liberal estimate of the ratio (100) was used to estimate denitrification rate at *in situ* nitrate concentration and a lower ratio (50) was used to estimate denitrification rate at elevated concentrations (Reddy and Patrick 1984, Lindau *et al.* 1988).

Overlying water was carefully replaced with 203 mL (10 cm H) of 0.45- μ m filtered pond water. One core from each station was incubated with pond water at

the *in situ* concentration of NO_3^- -N ($n=8$). Overlying water in eight additional core tubes was amended with laboratory-grade KNO_3 to an initial concentration of 10 mg L^{-1} (stations 1-4) or 20 mg L^{-1} (stations 5-8) NO_3^- -N. After 12 h, core tubes were capped with rubber stoppers. Approximately 4 cm^3 of headspace gas was removed with a plastic syringe and needle inserted through a serum stopper in the rubber cap. Gas was injected into evacuated 10 mm diameter \times 60 mm glass vials (Vacutainer®) that were sealed with silicone adhesive. Cores were incubated in the dark at sediment temperature at the time of collection. After 12 hours, headspace gas was sampled as described. The rubber stopper capping the core tube was then removed for 12 hours to allow equilibration of overlying water with the atmosphere. This cycle (capping-incubation-sampling-cap removal-equilibration) was repeated four times. At the end of the fourth incubation, the water in the 8 cores amended with KNO_3 was removed and the residual nitrate measured. The rate of nitrate depletion was calculated as an additional measure of denitrification and compared to rates estimated from nitrous oxide production.

Nitrous oxide was measured with a gas chromatograph (Shimadzu GC-14A) equipped with a 1 m Porapak Q (80/100 mesh) pre-column, a 3 m Porapak Q analytical column and a ^{63}Ni electron capture detector. Ultra-high purity nitrogen was the carrier gas (25 ml min^{-1}). Temperatures were 40 C in the column, 100 C in the injection port and 270 C in the detector. Changes in headspace N_2O concentrations were used to calculate the rate of N_2O production ($\text{mg N m}^{-2} \text{ h}^{-1}$), which in turn was used to estimate denitrification rate.

Denitrification Potential

Overlying water of one core from each station was carefully removed to avoid disturbance of surficial sediment and replaced with 203 mL of 0.45- μm filtered pond water enriched with KNO_3 to a concentration of 100 mg N L^{-1} (10 g N m^{-2}). Cores were incubated in the dark at sediment collection temperature. Nitrate-N concentration in overlying water was measured at 1-3 day intervals for 2 weeks and compared with that of 10% formalin-killed control cores. Nitrate concentration values were fitted to a first-order decay function to estimate the specific removal rate.

Statistical Analysis

A one-way analysis of variance (ANOVA) using PROC GLM was conducted to evaluate the effect of season on mean solute flux rates (SAS Institute, Inc. 1989b). If the ANOVA was significant ($P < 0.05$) then pairwise t-tests (LSD) were performed to further separate means. Solute flux rates were also fitted to a multiple regression model as a function of incubation temperature and initial concentration. A correlation matrix among solute flux measurements was used to produce Pearson product-moment correlation coefficients (R) with associated P-values (PROC CORR).

To evaluate heterogeneity of the pond bottom selected for this study, cluster analysis (PROC CLUSTER) was performed on stations independent of season (SAS Institute, Inc. 1989a). Variables included in the evaluation were solute flux rates, maximum porewater TAN concentration, and denitrification potential. Stations were separated by the normalized root-mean-square procedure (METHOD=AVERAGE).

Additionally, coefficients of variation were calculated for solute flux rates, porewater TAN profiles, N_2O production rates, and denitrification potential.

RESULTS

Sediment Nitrogen Pools

Organic nitrogen constituted the largest pool (92-96%) of nitrogen in the sediment (Table 7). Concentrations of organic N were greatest near the surface of the sediment and decreased with increasing sediment depth. The magnitude of organic N reduction following draining and drying decreased with increasing sediment depth. Organic N was reduced from 0.23% to 0.18% in the surface layer (0-5 cm), from 0.16% to 0.11% in the 5-10 cm layer, and was unchanged at 0.09% in the 10-15 cm layer.

KCl-extractable TAN is a measure of the quantity of ammonium adsorbed onto the surface of clays as part of the cation exchange complex. The ratio of adsorbed TAN to porewater TAN increased from the surface downward, indicating a relative depletion of TAN from exchange sites on near-surface sediments. Adsorbed ammonium was greatest in the 5-10 cm layer in winter 1994 (120.4 $\mu\text{g N/g dry soil}$) and lowest in the 10-15 cm layer in summer 1994 (67.6 $\mu\text{g N/g dry soil}$).

Reduction of adsorbed ammonium was greatest near the surface and declined with depth. Drying significantly reduced adsorbed ammonium in the 0-5 cm layer ($t=-3.37$, $p<0.005$) and the 5-10 cm layer ($t=-3.90$, $p<0.005$), but did not reduce adsorbed ammonium in the 10-15 cm layer.

Table 7. Sediment nitrogen pools (\pm SD) ($\mu\text{g N cm}^{-3}$) for three sediment layers (N=8) in a commercial channel catfish pond before¹ and after² drying.

Layer	Organic N ³		Adsorbed TAN		Porewater TAN		Ratio ⁴	
	before	after	before	after	before	after	before	after
0-5 cm	904 (118)	871 (83)	41 (15)	22 (6)	2.0 (0.6)	3.2 (2.2)	22.7 (10.9)	9.8 (6.3)
5-10 cm	878 (116)	816 (33)	70 (17)	41 (11)	2.3 (0.7)	5.0 (3.1)	32.3 (12.8)	11.4 (6.5)
10-15 cm	729 (144)	787 (110)	62 (18)	61 (14)	2.0 (0.1)	6.9 (5.9)	30.4 (9.3)	15.2 (10.9)

¹ February 1994

² July 1994

³ Total Kjeldahl N - (KCl-extractable TAN+porewater TAN)

⁴ KCl-extractable TAN \div porewater TAN

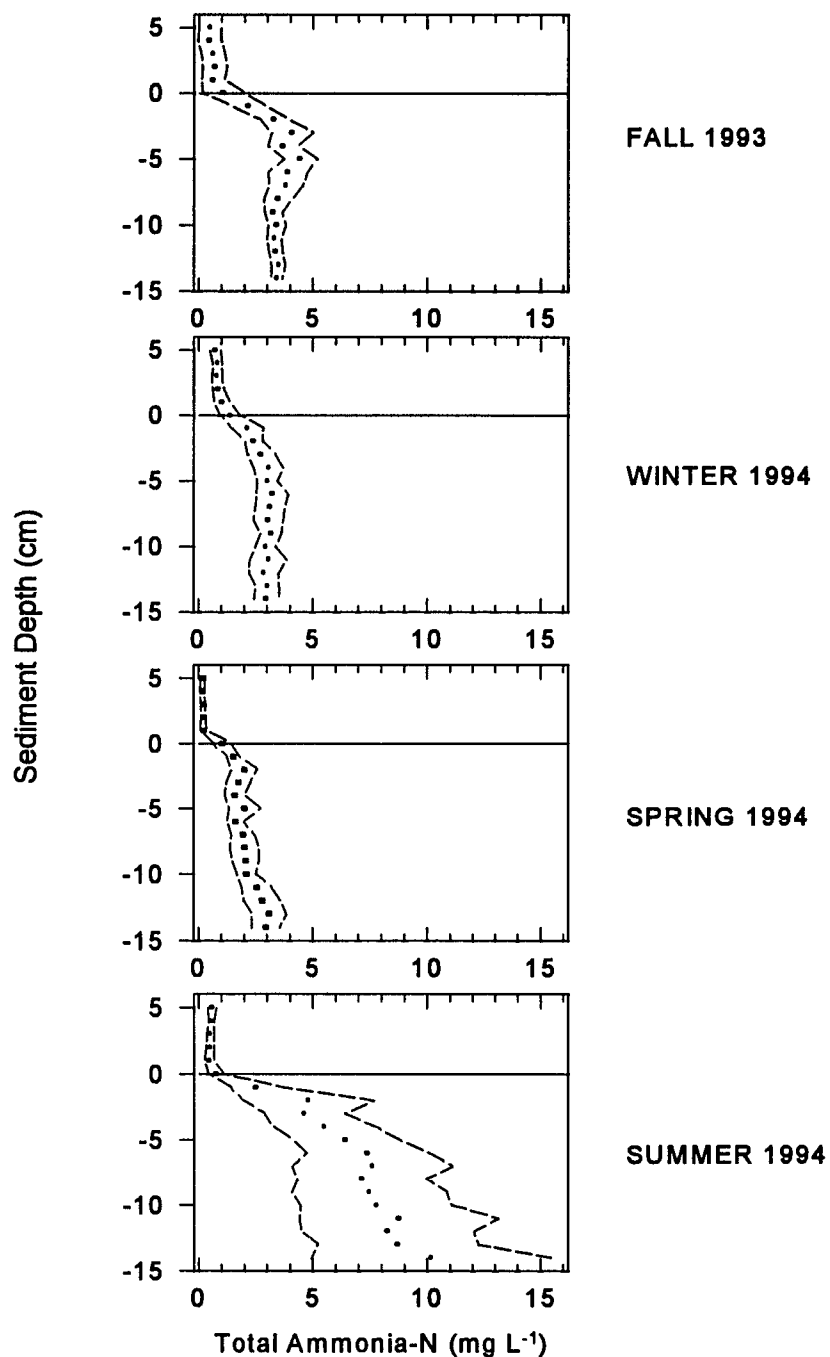


Figure 3. Mean ($\pm 95\%$ confidence interval) TAN concentration (mg L^{-1}) in the sediment porewater of a commercial channel catfish pond through four seasons.

Porewater TAN Profiles

TAN concentration in sediment porewater was 4-20x higher than in the water column with the greatest differential in summer and lowest in winter (Figure 2).

TAN concentration in sediment porewater increased rapidly with depth from the sediment-water interface. The source of ammonium in the sediment at a depth greater than that of oxygen penetration (typically a few mm) was likely the anaerobic mineralization of organic matter. The porewater pool of TAN is dynamic over the time scale of months with the greatest concentrations during periods of elevated temperature and therefore metabolic activity.

Solute Flux

Sediments were sinks for oxygen in the channel catfish pond. Sediment oxygen demand (SOD) ranged from $31 \text{ mg m}^{-2} \text{ h}^{-1}$ in Summer 1994 to $87 \text{ mg m}^{-2} \text{ h}^{-1}$ in Summer 1993 (Table 8). SOD was significantly lower in winter and in the summer following pond drying than in spring or summer prior to drying. However, initial dissolved oxygen concentration and incubation temperature were not significant predictors in a multiple regression model of SOD (Table 9). The rate of increase in SOD decreased as temperature increased (Table 9) and the Q_{10} in all cases was <2 suggesting factors other than temperature affected the rate of SOD. SOD of the pond bottom was most heterogeneous in spring (C.V.=51.3%).

Sediments were sources of CO_2 in the channel catfish pond (Table 8). The pattern of CO_2 flux was similar to that of SOD. The molar ratio of O_2 : CO_2 is expected to be near unity if the oxygen consumed during organic matter

mineralization was equivalent to the carbon dioxide released. This was the case only for the Winter 1994 sampling. In general, the molar ratio was >2.5 , suggesting a portion of SOD was due to chemical oxidation of reduced substances diffusing from the reduced layer of sediment and the chemautotrophic oxidation of ammonium at the sediment surface. Additionally, a portion of the CO_2 released during organic matter mineralization may have been consumed by the sediment.

Sediments were sources of ammonium in the channel catfish pond (Table 8). Incubation temperature was a strong predictor of TAN flux to the water column indicating that mineralization activity increased with temperature. The Q_{10} of TAN flux between 16 and 26 C was 3.2 suggesting a threshold temperature beyond which mineralization increases more rapidly (Table 9). TAN flux was also strongly correlated with SOD ($R=0.870$, $P=0.0001$) and CO_2 flux ($R=0.530$, $P=0.002$) indicating that most of the N produced during mineralization was released to the water column. TAN flux was exceptionally high during Spring 1994 ($T=26$ C). Although not enumerated, a substantial number of macrofaunal burrows were observed in sediment cores collected at this time.

Sediments were sinks for nitrite and nitrate in the channel catfish pond (Table 8), although reduction rates appear to be limited by substrate concentration, particularly in the case of nitrite (Table 9). The flux of nitrite ($R=0.717$, $P=0.0001$) and nitrate ($R=0.672$, $P=0.0001$) was positively correlated with initial concentration of these compounds. Nitrite flux into sediment was significantly greater in Spring 1994 than at any other time of the year. The Q_{10} of nitrite reduction was greatest

Table 8. Mean (\pm SD) (N=8) solute (O_2 , CO_2 , TAN, NO_2^- -N, NO_3^- -N) flux ($mg\ m^{-2}\ hr^{-1}$) between water and sediment determined in intact sediment cores incubated for 4 hours at the temperature of sediment collection. Negative sign indicates flux from water to sediment; positive sign indicates flux from sediment to water.

Season	Temperature ($^{\circ}C$)	Solute				
		O_2	CO_2	TAN	NO_2^- -N	NO_3^- -N
Summer 1993	32	-87 (20) a ¹	+79 (38) a	+5 (4) b	-	-
Fall 1993	16	-59 (8) b	+31 (10) cd	+6 (3) b	-0.225 (0.041) b	-0.414 (0.076) a
Winter 1994	9.5	-43 (16) bc	+66 (37) ab	+6 (2) b	-0.159 (0.022) b	-0.420 (0.162) a
Spring 1994	26	-86 (44) a	+52 (21) bc	+19 (10) a	-0.685 (0.302) a	-0.094 (0.260) b
Summer 1994 ²	32	-31 (8) c	+17 (8) d	+4 (1) b	-0.069 (0.015) b	-0.115 (0.082) b
Overall Mean		-61	+48	+8	-0.284	-0.261

¹ Means with the same letter in a column are not different ($P>0.05$); comparisons valid within a column only.

² Pond was dried for 6 weeks prior to pond refilling and sampling.

Table 9. Qualitative effects of initial concentration and incubation temperature on solute (O_2 , CO_2 , TAN, NO_2^- -N and NO_3^- -N) flux rates in sediments collected from a commercial channel catfish pond (1993-1994).

Solute	Qualitative Effect ¹		Adjusted R^2	Temperature Range (°C)	Q_{10}
	Initial Concentration	Incubation Temperature			
O_2	0	0	0.330	9.5 - 16	1.6
				16 - 26	1.5
				26 - 32	1.0
CO_2	0	0	NS	16 - 26	1.6
				26 - 32	2.0
TAN	+	++	0.533	9.5 - 16	1.0
				16 - 26	3.2
NO_2^- -N	++	+	0.619	9.5 - 16	1.7
				16 - 26	3.0
NO_3^- -N	0	0	0.430	9.5 - 16	1.0
				16 - 26	0.2

¹ 0=no effect;+=moderate effect;+=strong effect.

between 16-26 C and was similar to the pattern for TAN flux. NO_2^- -N flux into sediment was positively correlated with TAN flux out of sediment ($R=0.924$, $P=0.0001$) although it was not correlated with NO_3^- -N flux. Overall mean nitrite and nitrate flux rates to the sediment were low, similar to each other, and were more than an order of magnitude lower than TAN flux from the sediment.

The molecular flux of TAN from sediment to water calculated on the basis of the porewater TAN gradient was <15% of that calculated by incubation (Table 10). This suggests that regeneration of nutrients at the sediment-water interface is a much more important process than molecular diffusion of TAN affecting the magnitude of TAN flux.

Table 10. Comparison of diffusion of TAN from sediment to water ($\text{mg m}^{-2} \text{ h}^{-1}$) in a channel catfish pond estimated from porewater profiles to TAN flux measured in 4-hour incubations.

Method	Season			
	Fall	Winter	Spring	Summer
molecular diffusion (J) ¹	0.29	0.25	0.27	0.66
incubation	6.1	6.0	19.4	4.5
proportion (%) ²	4.8	4.9	1.4	14.9

¹ $J=\theta D_s(\delta C/\delta z)_{z=0}$, $\theta=0.66$, $D_s=1.2 \text{ cm}^2 \text{ d}^{-1}$

² (molecular diffusion flux \div incubation flux) $\times 100$

Nitrous Oxide Production Rate

Nitrous oxide production in sediments incubated at the concentration of nitrate in the pond at the time of core collection was extremely low (Figure 4). Maximum nitrous oxide emission from channel catfish pond sediment cores incubated over 4 days ranged from $0.8 \mu\text{g N}_2\text{O-N m}^{-2} \text{ h}^{-1}$ in fall to $15.3 \mu\text{g N}_2\text{O-N m}^{-2} \text{ h}^{-1}$ in spring. Nitrous oxide production increased when cores were enriched with nitrate. Nitrous oxide production in cores incubated at reduced temperature ($<16^\circ\text{C}$) was lower than those of elevated temperatures ($>26^\circ\text{C}$) at all concentrations. In cores incubated at elevated concentrations and reduced temperatures, nitrous oxide production increased with time, suggesting a period of acclimation was required before nitrous oxide emission increased. At elevated concentrations and temperature, nitrous oxide production decreased with time, suggesting rapid depletion of substrate.

Denitrification Potential

The potential of aquaculture pond sediments to remove nitrate was extremely high (Table 11). Nitrate was usually depleted to within 10% of initial concentration within 10-14 days following initiation of the incubation. The high potential denitrification rate suggests that denitrification is likely limited by substrate diffusion from the overlying bulk water.

Cluster Analysis

Cluster analysis on stations was used to characterize the heterogeneity of pond sediments with respect to measured process rates. In general, sediment process rates clustered in relation to distance from the paddlewheel aerator and/or pond depth

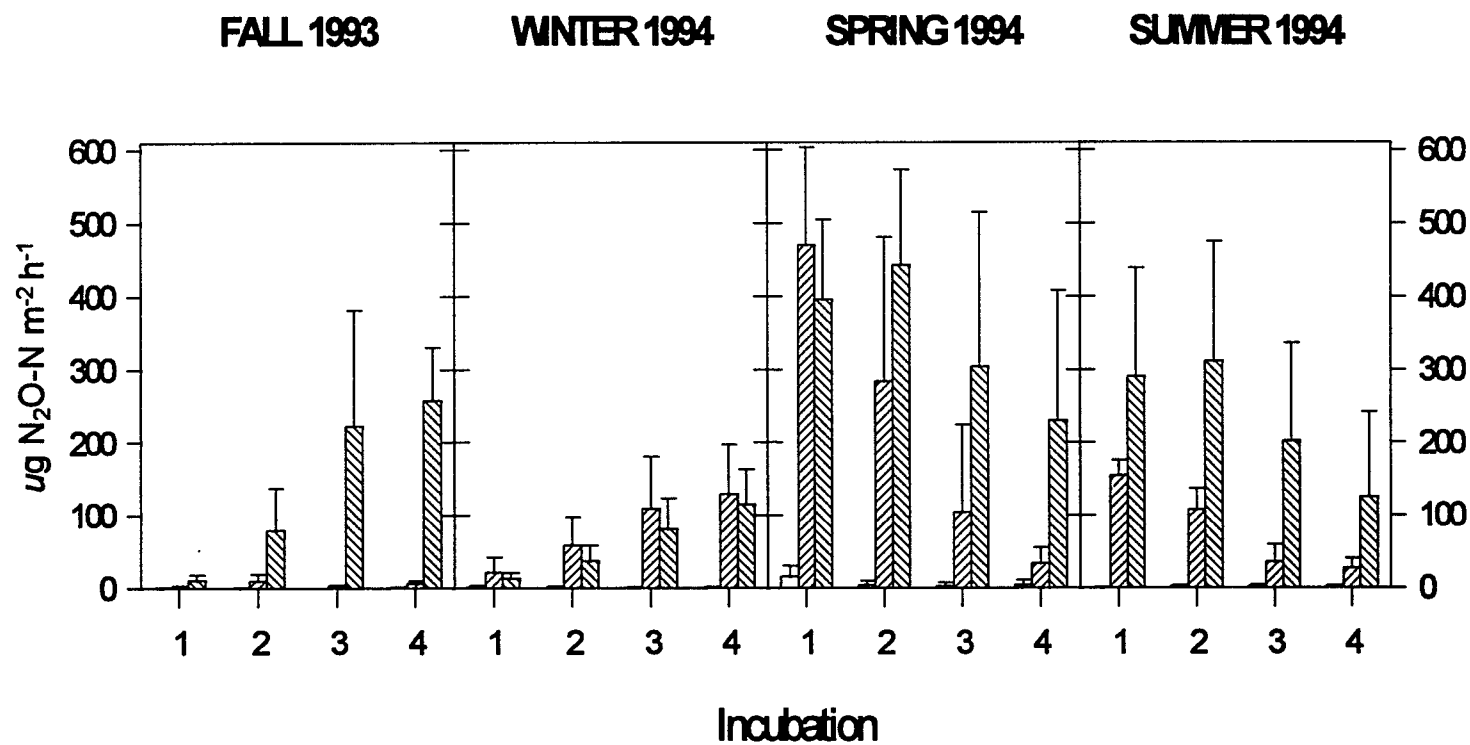


Figure 4. Nitrous oxide emission rate from channel catfish pond sediment cores collected during fall 1993, winter, spring and summer 1994 and incubated over 108 h at *in situ* NO_3^- -N concentration ($N=8$), $10 \text{ mg NO}_3^- \text{N L}^{-1}$ ($N=4$) and $20 \text{ mg NO}_3^- \text{N L}^{-1}$ ($N=4$).

Table 11. Denitrification rate estimates (\pm SD) ($\text{mg N m}^{-2} \text{d}^{-1}$) as a function of nitrate concentration and season in a commercial channel catfish pond.

NO ₃ ⁻ -N Concentration (mg L ⁻¹)	Method	Season			
		Fall	Winter	Spring	Summer
<i>in situ</i> (≈ 0)	solute flux (NO ₂ ⁻ +NO ₃ ⁻)	15.3 (1.4)	13.9 (2.2)	18.7 (6.7)	4.5 (1.2)
	N ₂ O flux ¹	0.5 (1.0)	2.7 (1.4)	14.8 (14.8)	4.1 (2.6)
10	NO ₃ ⁻ reduction during N ₂ O flux incubation	128 (5)	150 (24)	223 (13)	212 (38)
	N ₂ O flux ²	11(11)	156 (81)	565 (160)	186 (25)
20	NO ₃ ⁻ reduction during N ₂ O flux incubation	181 (55)	240 (70)	446 (80)	287 (36)
	N ₂ O flux ²	309 (87)	138 (57)	531 (157)	374 (196)
100	denitrification potential	511 (231)	431 (90)	3421 (2629)	1017 (541)

¹ assumes N₂:N₂O ratio = 100

² assumes N₂:N₂O ratio = 50

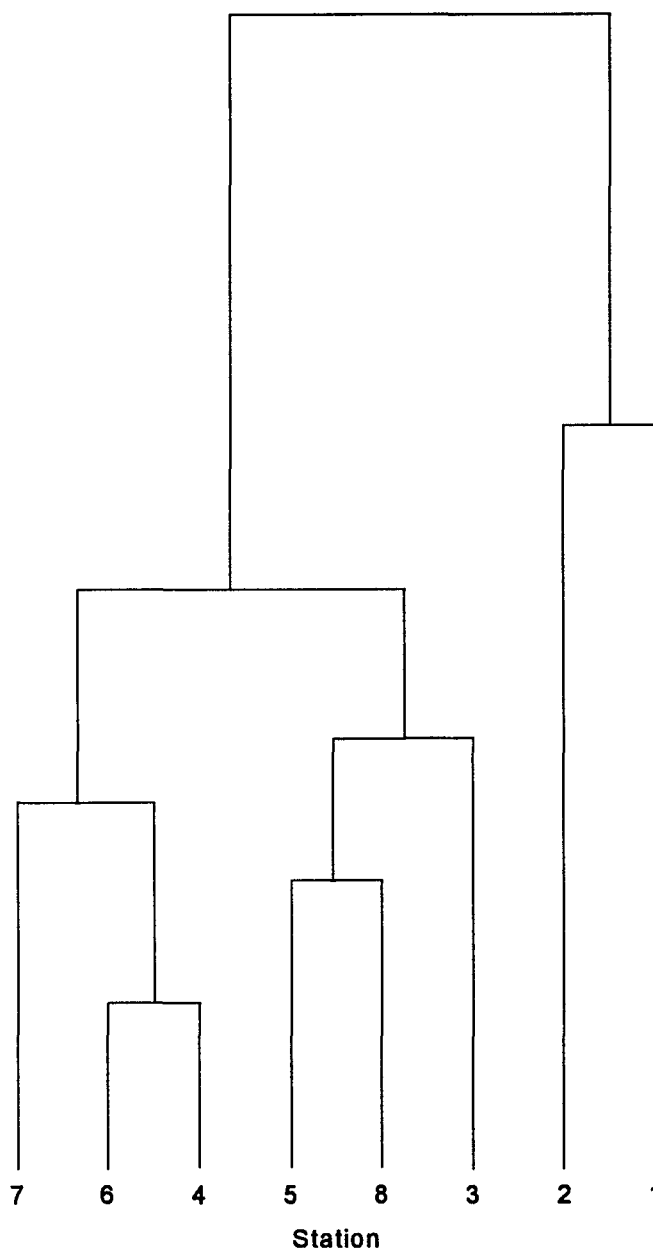


Figure 5. Cluster analysis on station independent of season. Variables include solute flux rates, denitrification potential and maximum porewater TAN concentration.

(Figure 5). Stations 1 and 2 were most distant from the aerator and located near the deep (drain) end of the pond. Sediments at this end of the pond were the least consolidated (high porosity) and had the highest concentrations of porewater TAN and highest solute flux rates.

DISCUSSION

Organic N was the largest pool measured in the sediment of a channel catfish pond. However, the concentration of organic N measured is in the "low" range (0.2-0.3% N) when compared to that of sediments from 358 freshwater aquaculture ponds (Boyd *et al.* 1994), but similar to sediment samples from 32 channel catfish ponds in Mississippi (Tucker 1985), and similar to research ponds in Alabama and Mississippi (Hariyadi *et al.* 1994). The pool of organic N is the least dynamic of those measured. Organic matter and nitrogen accumulate slowly in aquaculture ponds (Tucker 1985, Green and Boyd, in press). However, Acosta-Nassar *et al.* (1994) measured a rapid increase in organic N in sediments from about 0.08% to 0.14% over a 5-month culture period and estimated that sediments accumulated 65% of input N.

Draining and drying a pond will reduce organic matter and N concentrations (Diab and Shilo 1986, Ayub *et al.* 1993). In this study drying of pond sediment reduced surface (0-5 cm) sediment organic N from 0.23% to 0.18%. Ayub *et al.* (1993) demonstrated that approximately equal amounts of organic matter were lost from the surface layer (0-5 cm) of aquaculture ponds sediments during discharge of pond water and during subsequent drying for 5 weeks.

Organic nitrogen has a strong effect on other pool sizes of sediment N. In sediments containing approximately 2x the organic N concentration, porewater ammonium concentrations were 2-7x greater than those of the sediments evaluated in this study (Reddy *et al.* 1988).

Adsorbed ammonium was the second largest pool of N in the catfish pond sediment. Ammonium adsorbed to negatively-charged exchange sites on clay minerals is an important regulator of ammonium supply to the overlying water and an important sink for mineralized ammonium. Acosta-Nassar *et al.* (1994) estimated that approximately 2% of the N added to a freshwater fish pond was stored in the adsorbed pool. Blackburn and Henriksen (1983) suggested that negatively-charged carboxyl groups of mineralized organic matter may also function as exchange sites. However, organic matter concentration usually decreases with depth and adsorbed ammonium concentration increases with depth (Diab and Shilo 1986, Acosta-Nassar *et al.* 1994) indicating the greater importance of mineral exchange sites.

Adsorbed ammonium is in equilibrium with porewater ammonium, although the ratio of adsorbed to porewater ammonium (partition coefficient) is variable, but generally much greater than 1. Blackburn and Henriksen (1983) calculated a partition coefficient that was <6 in November and increased to about 10 in July. Partition coefficients of 6.4 and 7.55 were used in a model of eutrophic lake sediment (Reddy *et al.* 1988, Reddy *et al.* 1990). Differences in the partition coefficient are related to the cation exchange capacity of soil, adsorbed and porewater ammonium concentrations, season (temperature) and sediment depth.

The capacity of Israeli pond sediments to adsorb ammonium was limited and ranged from 300 to 900 $\mu\text{g N g}^{-1}$ dry soil (Diab and Shilo 1986). Differences in adsorption capacity can be related to the cation exchange capacity of pond soils. The maximum KCl-extractable ammonium measured in this study was substantially less (120 $\mu\text{g N g}^{-1}$ dry soil) than that measured in the Israeli fish ponds. Although the cation exchange capacity of the channel catfish pond sediment was not measured and that of the Israeli pond sediment was not given, the capacity of the channel catfish pond sediment to adsorb ammonium was not likely saturated given the soil composition of 38% (primarily montmorillonitic) clay.

The effect of draining and drying the pond bottom on reduction of the adsorbed ammonium pool was limited to the upper 10 cm in this study. Diab and Shilo (1986) measured a rapid decline in the KCl-extractable ammonium pool to very low levels after 6 weeks of drying. Evidence that exchangeable ammonium can be utilized by nitrifying bacteria is equivocal. Seitzinger (1990) pointed out that exchangeable ammonium is only loosely sorbed to sediment and that nitrifying bacteria are associated with particles. Following refilling, the KCl-extractable ammonium pool increased within 10 days to levels equivalent to approximately 50% of that prior to draining. Adsorbed ammonium increases during the cropping cycle and constitutes an important sink for ammonium in intensive fish ponds (Shilo and Rimon 1982, Diab and Shilo 1986).

The dynamic nature of the adsorbed ammonium pool is further illustrated by measurement of the complete and rapid desorption of ammonium from a sandy

sediment after 2 hours following suspension by wind-driven water turbulence (Simon 1989). Ammonium supplied to the water column by desorption of ammonium from sediment solids was estimated to exceed that supplied by diffusive flux. Suspension of aquaculture pond sediments by aeration or wind-driven water turbulence may increase, at least temporarily, the concentration of ammonium in the water column (Hollerman and Boyd 1980). Further research is required to evaluate the magnitude and importance of this process in nutrient flow in aquaculture ponds and the potentially deleterious effects on water quality and fish growth.

The porewater ammonium pool was the smallest yet most dynamic of all measured in this study. Porewater ammonium is produced by the anaerobic mineralization of organic matter in the reduced sediment layer and is present in equilibrium with adsorbed ammonium. The profile of porewater ammonium concentration measured in this study is consistent with that observed in other flooded soil systems, in which ammonium concentration increases rapidly with depth near the sediment surface and reaches an asymptotic concentration as depth increases. The steepness of the gradient near the surface determines the ammonium flux rate based on molecular diffusion. Porewater ammonium concentration is low at the sediment-water interface due to oxidation (nitrification) and diffusion into the bulk water, where a low concentration is maintained by phytoplankton uptake.

Porewater TAN concentration changed in response to seasonal temperature fluctuation with a large increase in summer. A similar seasonal increase in porewater TAN concentration was observed in channel catfish ponds in Alabama (Masuda and

Boyd 1994). In addition, porewater (10 cm sediment depth) mid-summer TAN concentration increased with pond age and was 2.2 mg L^{-1} (new pond), 6.2 mg L^{-1} (20 year-old pond), and 21.6 mg L^{-1} (40 year-old pond). These concentrations were comparable to those of the channel catfish pond sediment (5 year-old) evaluated in this study ($5\text{-}11 \text{ mg L}^{-1}$).

Aquaculture pond sediments are a source of reduced inorganic nitrogen (ammonium) and a sink for oxidized inorganic nitrogen (nitrite and nitrate). The magnitude of TAN flux from sediment to the overlying water is over an order of magnitude greater than that of $\text{NO}_2^- \text{-N}$ and $\text{NO}_3^- \text{-N}$ flux into the sediment. The flux of ammonium from the sediment is much higher than that reported for estuarine and eutrophic lake sediments (Table 3, Chapter 1), although similar to that reported for aquaculture ponds (Blackburn *et al.* 1988) and an Atlantic cage culture site (Hargrave *et al.* 1993). In this study, TAN flux due to molecular diffusion generally accounted for <15% of total flux. This suggests that regeneration of nutrients at the sediment-water interface is more important than diffusion-driven flux in systems supplied with large quantities of allochthonous inputs. Organic matter inputs to channel catfish pond sediments are extremely labile and subject to rapid degradation.

The magnitude of TAN flux from aquaculture pond sediment can be estimated by considering the fate of net primary production. Typically 50% of primary production is deposited in the sediment of shallow aquatic systems (Nixon *et al.* 1976, Billen 1978, Blackburn and Henriksen 1983, Schroeder *et al.* 1991). Assuming a dissolved inorganic carbon fixation rate of $3 \text{ g C m}^{-2} \text{ d}^{-1}$ and a phytoplankton

composition equivalent to the Redfield ratio (C:N:P=106:16:1), then approximately 226 mg N m⁻² d⁻¹ is supplied to sediments. Blackburn *et al.* (1988) suggested that nitrification and denitrification were insignificant mechanisms of nitrogen transformation in earthen marine fishponds. Therefore, assuming complete mineralization of organic matter at the sediment-water interface and negligible coupled nitrification-denitrification, all sedimented nitrogen is released to the water column. This rate (9.4 mg N m⁻² h⁻¹) is comparable to the range of TAN flux rates observed in this study (4-19 mg N m⁻² h⁻¹) and that measured in earthen marine fish ponds (10.5 mg N m⁻² h⁻¹) by Blackburn *et al.* (1988).

Klump and Martens (1981) measured a seasonal pattern of ammonium flux from the sediment that primarily reflected seasonal changes in porewater ammonium concentration. Ammonium flux from the sediment was maximum in the summer, coincident with maximum porewater ammonium concentration. Seasonal (temperature) effects on solute flux rates were also very important rate determinants in sediments underlying an Atlantic salmon cage culture site (Hargrave *et al.* 1993). Variability of sediment ammonium flux was partitioned between temperature (42%) and dissolved oxygen (12%) in an empirical regression model of eutrophic river sediment (Cерco 1989). Ammonium flux was enhanced by high temperature and low dissolved oxygen.

Ammonium flux from sediment can be increased by sediment macrofauna. Presence of the polychaete *Nereis virens* can increase ammonium flux by 1.8x (Hylleberg and Henriksen 1980) to 3.9x (Henriksen *et al.* 1980) as compared to that

of defaunated control sediments. Macrofaunal burrows increase the effectively oxidized area of sediment by 50-200% (Hylleberg and Henriksen 1980). Macrofauna increase ammonium flux by irrigation of surface sediment and direct excretion. Nitrification and denitrification rates may also be increased by benthic macrofauna. The high solute flux rates measured in this study during the spring may be partially explained by the presence of macrofauna.

Although nitrification was not measured in this study, the magnitude of the process can be estimated by sediment oxygen demand. Two moles of oxygen are consumed for each mole of ammonium oxidized during nitrification. The proportion of sediment oxygen demand due to nitrification ranges from 5 to 20% (Henriksen and Kemp 1988). Average annual nitrification therefore ranges from 16-46 mg N m⁻² d⁻¹. Mean nitrification rates in a number of estuaries were similar and ranged from approximately 15-25 mg N m⁻² d⁻¹ (Henriksen and Kemp 1988). However, nitrification rates are likely much lower in stratified aquaculture ponds, especially during summer when near-bottom water is chronically undersaturated with dissolved oxygen (Losordo 1991). For example, Acosta-Nassar *et al.* (1994) measured an average nitrification rate of 0.5 mg N m⁻² d⁻¹ in a tropical freshwater fish pond.

Coupled nitrification-denitrification reactions are important only if oxygen penetration into the sediment is sufficient to allow the development of a significant population of nitrifying bacteria able to compete with heterotrophic bacteria for dissolved oxygen. Such conditions do not likely occur during the summer when feeding rate and temperature are maximum and average dissolved oxygen is

minimum. Coupled nitrification-denitrification reactions are probably only important in the fall and spring when average dissolved oxygen is sufficiently high to allow oxygen penetration into the sediment.

Similar to other wetland soil systems, aquaculture pond sediments have a considerable capacity for nitrate reduction, which appears to be limited by substrate concentration. The flux of oxidized inorganic nitrogen into sediment depends on the production at the sediment-water interface and subsequent diffusion to reduced microsites or sediment layers (coupled nitrification-denitrification). A strong positive correlation between denitrification and sediment oxygen demand (Seitzinger 1990) is further evidence that coupled nitrification-denitrification is the primary process responsible for nitrogen loss in most coastal marine and lacustrine sediments. Denitrification rates also depend on nitrate diffusion from the overlying bulk water. In channel catfish ponds, nitrate concentrations in the overlying water are usually very low ($<0.5 \text{ mg N L}^{-1}$) and in the summer, are extremely low ($<0.05 \text{ mg N L}^{-1}$) (Ziemann *et al.* 1992, Tucker and van der Ploeg 1993). Estimated nitrate reduction/denitrification rates at *in situ* nitrate concentrations were $<20 \text{ mg N m}^{-2} \text{ d}^{-1}$ and were more likely $<5 \text{ mg N m}^{-2} \text{ d}^{-1}$. Denitrification rates in eutrophic lakes range from $6.7\text{-}98 \text{ mg N m}^{-2} \text{ d}^{-1}$, although rates $<20 \text{ mg N m}^{-2} \text{ d}^{-1}$ are more common (Seitzinger 1990).

Nitrous oxide fluxes from aquatic systems are generally less than $28 \mu\text{g N}_2\text{O-N m}^{-2} \text{ h}^{-1}$ and account for $<2\%$ of dinitrogen production (Seitzinger 1990). Maximum N_2O emission at *in situ* nitrate concentrations measured in this study was

16 $\mu\text{g N}_2\text{O-N m}^{-2} \text{ h}^{-1}$ and was often $<1 \mu\text{g N}_2\text{O-N m}^{-2} \text{ h}^{-1}$. Maximum N_2O emission from channel catfish pond sediments was 642 $\mu\text{g N m}^{-2} \text{ h}^{-1}$ in the spring in a core enriched to 10 mg N L^{-1} . Nitrous oxide fluxes ranged from 7.8 to 714 $\mu\text{g N m}^{-2} \text{ h}^{-1}$ in marine mesocosms enriched along a geometrically-increasing nutrient gradient (Seitzinger and Nixon 1985).

Nitrate reduction/denitrification increased in direct relation to enrichment concentration. Engler and Patrick (1974) measured an average nitrate removal rate by a freshwater swamp (mineral) soil of 333 $\text{mg N m}^{-2} \text{ d}^{-1}$ when the overlying water was enriched to 25 mg N L^{-1} . This rate is similar to estimated nitrate reduction/denitrification rates of catfish pond sediment cores in which the overlying water was enriched to 20 mg N L^{-1} . Seitzinger and Nixon (1985) measured denitrification fluxes ranging from 54 to 336 $\text{mg N m}^{-2} \text{ d}^{-1}$ in the marine mesocosm nutrient enrichment study.

In highly enriched catfish pond sediment cores, estimated nitrate reduction/denitrification potential was extremely high, particularly in the spring. As mentioned previously, large numbers of macrofaunal burrows were observed in sediment cores collected in spring. Denitrification was likely increased by the bioturbation and burrow irrigation activities of macrofauna and the increase in the effective sediment surface area available for coupled nitrification-denitrification. Increased nitrification-denitrification rates as a result of the presence of macrofauna in sediments were demonstrated by comparison of defaunated and macrofauna-stocked coastal marine sediments (Henriksen *et al.* 1980).

Nitrate removal by four different swamp and marsh soils ranged from 586 to 976 mg N m⁻² d⁻¹ when the overlying water was enriched with nitrate to 100 mg N L⁻¹ (Patrick *et al.* 1976). When bottomland hardwood forest soils were enriched by 10 g m⁻² KNO₃ (equivalent to 100 mg N L⁻¹ in water overlying cores from the channel catfish pond) the combined flux of dinitrogen and nitrous oxide was equivalent to 367 mg m⁻² d⁻¹ (Lindau *et al.* 1988), all within the range of results reported here for channel catfish pond sediment.

These results suggest that denitrification in aquaculture pond sediments has the potential to remove all of the nitrogen added to fish ponds. Limitations on nitrogen loading to aquaculture ponds are imposed by characteristics of the N input and by processes within the pond. The form of nitrogen imposes the first restriction on nitrogen loading. The low density of nitrogen (typically <5% of dry matter) in organic inputs, such as feeds, manures and agricultural by-products, suggests that biochemical oxygen demand (BOD) is a more important determinant of maximum loading rate. The limitation of inorganic nitrogen application is also related to form. The application rate of certain inorganic nitrogen sources (e.g. ammonium salts, urea) is limited by the capacity of pond processes, primarily phytoplankton assimilation, to reduce potentially toxic concentrations of ammonia related to inorganic loading. The third restriction on nitrogen loading is also related to the assimilative capacity of the pond. Ammonium produced within the pond by fish excretion and nutrient mineralization/regeneration in the water column and at the sediment-water interface is removed by phytoplankton uptake and coupled nitrification-denitrification at the

sediment-water interface. Thus, concentrations of dissolved inorganic nitrogen in aquaculture ponds represent the balance between processes that generate and remove inorganic nitrogen.

Process rates involved in sediment-water nutrient flux as well as the chemical characteristics of pond soil from a single catfish pond were very heterogeneous. Boyd (1976) measured an increase in the proportion of clays, soil organic matter and nitrogen as pond depth increased. Ayub *et al.* (1993) calculated coefficients of variation of 20 - 50% in soil organic matter concentration from samples collected from small research ponds. Only 35% of the variation in sediment organic matter of Mississippi channel catfish ponds could be explained by pond age (Tucker 1985). Spatial variability in solute flux rates of sediments underlying an Atlantic salmon cage culture facility was high (Hargrave *et al.* 1993). Although stations were established randomly as part of a meandering pattern in the catfish pond evaluated in this study, spatial variability was substantial and reflected distance from the paddlewheel aerator, pond depth and the annual pattern of wind direction. All of these factors promote erosion of finer particles from shallow or mid-pond areas and favor deposition in the deeper end of the pond.

It is possible that a stratified sampling program reflecting such spatial variability would reduce variability in measured rates. A statistical model to estimate the number of samples and ponds required to demonstrate a detectable change in soil organic matter concentration indicated that the likelihood of detecting changes was increased by increasing the number of samples per pond rather than increasing

the number of ponds (Ayub *et al.* 1993). Such a sampling program would collect greater than 30 samples per ha. In this study, approximately 4 cores per ha were collected.

Evidence from this study indicates that regeneration of nutrients at the sediment-water interface is rapid and the most important sediment-water column interaction with respect to nitrogen. In addition, losses of nitrogen by coupled nitrification-denitrification are of minor significance in the overall N budget of aquaculture ponds, due largely to the overwhelming effect of input N, high phytoplankton biomass, and minimal sediment oxygen penetration. The inherent inefficiency of nutrient utilization coupled with high rates of nutrient input in semi-intensive aquaculture production systems suggests that N may be accumulating in sediments over time. Further research is required to evaluate rates of N accumulation, organic matter mineralization at the sediment-water interface, and the relative importance of regenerated ammonium to nitrogen budgets in aquaculture ponds. In addition, sediment management methods to promote N removal by nitrification-denitrification during the production cycle require evaluation.

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CHAPTER 3
A SIMULATION MODEL OF AMMONIUM DYNAMICS
IN A CHANNEL CATFISH POND
IN THE SOUTHEASTERN UNITED STATES

INTRODUCTION

Most mechanistic models describing water quality in aquaculture ponds have focused on prediction of diurnal variation in dissolved oxygen concentration. Models describing annual variation in pond water quality are rare. Although dissolved oxygen is undoubtedly the most important water quality parameter affecting fish growth and production in ponds, other water quality variables, particularly sublethal concentrations of nitrogen, may affect fish production. Empirical (Boyd 1985) and theoretical (reviewed by Piedrahita 1991) evidence indicates that fish have a minimal effect on pond dissolved oxygen, and the primary effect of fish on water quality is related to nitrogenous excretion.

A conceptual model of the nitrogen biogeochemistry of aquaculture ponds has been developed (Shilo and Rimon 1982), although mathematical description of the processes described in this model has not been accomplished. Few models have quantified the magnitude of nitrogen flux between the various nitrogen pools in a fish pond. The pond ecosystem model of Piedrahita *et al.* (1984) simulated dissolved inorganic nitrogen component concentrations, but over short (24-48 h) simulation intervals. The effect of water exchange rate on dissolved inorganic nitrogen concentration in intensively aerated fish ponds was evaluated in a model developed by Kochba *et al.* (1994).

The purpose of this modeling exercise was to quantify the primary nitrogen flows in an aquaculture pond using a simple mass balance approach. The specific objective of the model was to develop an accurate simulation of the annual cycle of total ammonia-nitrogen (TAN) concentration in channel catfish ponds in the southeastern United States. Another goal of the model was to evaluate the effects of various management methods related to feeds and feeding practices on nitrogen pool sizes and flux rates by conducting a sensitivity analysis on model parameters. The magnitude of such changes may suggest options and opportunities for management related to the effects of feeds and feeding on water quality. The development of a mechanistic model for ammonium dynamics in an aquaculture pond can enhance our understanding of pond nitrogen dynamics and can be incorporated into existing pond ecosystem models as an integral component.

MATERIALS AND METHODS

Pond Description

A theoretical model was developed to simulate nitrogen transformations in commercial (levee-type) channel catfish ponds in the southeastern United States. Simulated ponds were stocked with two fish m⁻² and operated by a multiple-batch cropping system in which several cohorts of fish were present simultaneously (Tucker *et al.* 1992). Fish were fed a pelleted diet (32% protein) at rates ranging from approximately 20 kg ha⁻¹ d⁻¹ (winter) to 100 kg ha⁻¹ d⁻¹ (summer). Simulated ponds were assumed to be 1 m deep so that relationships of mass to area and volume were equivalent.

Model Structure

A simple mass balance approach was used to model water column TAN concentration (NH_4_w), which represents the balance (residual) between nitrogen sources and sinks. The processes supplying ammonium to the fish pond included fish excretion (N_{ammexcr}) and sediment ammonium diffusion (N_{diff}). Smaller, but significant sources of nitrogen may be added by cyanobacterial nitrogen fixation and atmospheric deposition, although these sources were considered to be unimportant for the purposes of this model due to the overwhelming importance of nitrogen input derived from feed addition. Sink processes included phytoplankton uptake (N_{up}) and planktonic nitrification (N_{nitrif}).

$$\text{NH}_4_w = (N_{\text{ammexcr}} + N_{\text{diff}}) - (N_{\text{up}} + N_{\text{nitrif}})$$

The only allochthonous source of nitrogen to the simulated fish pond was feed. Feeding rate (FDRT) was modeled as a sinusoidal function of time (Julian Date=JD):

$$\text{FDRT} = \text{FDRT}_{\text{avg}} + [(0.5 \times \text{FDRT}_{\text{range}}) \times \sin(2 \times \pi \times (\text{JD} - 90) / 365)]$$

Feeding rate was transformed into nitrogen input rate by considering the dietary protein concentration (PROT) and assuming an average protein N content of 16%. Thus, nitrogen input rate ($\text{g N m}^{-2} \text{ d}^{-1}$) can be described by the equation:

$$N_{\text{feed}} = (\text{FDRT} / 10) \times \text{PROT} \times 0.16$$

Total consumption of feed by fish was assumed ($N_{\text{cons}} = N_{\text{feed}}$) although the model provides for opportunities to evaluate changes in feed consumption as affected by appetite, which may be related to temperature, water quality or other factors.

Table 12. Parameters, symbols, units of variables used in a pond nitrogen model.

Symbol	Description	Value/Units
<u>State Variables</u>		
N_{feed}	feed nitrogen	g m^{-2}
N_{cons}	consumed nitrogen	g m^{-2}
N_{fecal}	fish fecal nitrogen	g m^{-2}
N_{pton}	phytoplankton nitrogen	g m^{-2}
NH_4_{w}	freewater ammonium nitrogen (=TAN)	g m^{-2}
NH_4_{sed}	sediment ammonium nitrogen	g m^{-2}
<u>Forcing Functions</u>		
T	water temperature	C
FDRT	feeding rate	$\text{kg ha}^{-2} \text{ d}^{-1}$
<u>Auxiliary Variables</u>		
T_{avg}	average annual water temperature	20 C
T_{range}	annual water temperature range	26 C
FDRT_{avg}	average annual feeding rate	$60 \text{ kg ha}^{-2} \text{ d}^{-1}$
$\text{FDRT}_{\text{range}}$	annual feeding rate range	$80 \text{ kg ha}^{-2} \text{ d}^{-1}$
$N_{\text{diff},20}$	sediment ammonium-N flux rate @ 20 C	$65 \text{ mg m}^{-2} \text{ d}^{-1}$
$\mu_{\text{max,nitrif}}$	maximum sediment nitrification rate	$70 \text{ mg m}^{-2} \text{ d}^{-1}$
<u>Coefficients</u>		
PROT	feed protein fraction	0.32
N_{RETN}	nitrogen retention fraction	0.25
DISS_FRAC	dissolved nitrogen excretion fraction	0.80
Q_{10}	van't Hoff constant	2
β	Arrhenius temperature constant	1.07
k_{up}	phytoplankton uptake constant	0.053 d^{-1}
$k_{\text{nit},T}$	nitrification rate temperature multiplier	0.0025
k_{nitrif}	water column nitrification constant	$0.002 + (k_{\text{nit},T} \times T) \text{ d}^{-1}$

The fate of consumed nitrogen was modeled by assuming a relatively small proportion (base case=25%, Table 1, Chapter 1) was retained (N_{RETN}) by fish for growth (Table 12). Of the excreted nitrogen, a large fraction (base case=80%, Table 1, Chapter 1) was excreted directly as dissolved nitrogen (DISS_FRAC) and the balance was excreted as solids. These transformations were modeled by the following equations:

$$N_{\text{fish}} = N_{\text{cons}} \times N_{\text{RETN}}$$

$$N_{\text{ammexcr}} = N_{\text{cons}} \times (1 - N_{\text{RETN}}) \times \text{DISS_FRAC}$$

$$N_{\text{fecexcr}} = N_{\text{cons}} \times (1 - N_{\text{RETN}}) \times (1 - \text{DISS_FRAC})$$

Sediment ammonium diffusion (N_{diff}) was considered the second most important source of TAN in fish ponds. Sediment ammonium was derived from the mineralization of organic matter at the sediment-water interface and, to a lesser extent, diffusion from the anaerobic sediment layer. Sediment ammonium diffusion was modeled assuming the process was a function of temperature (Cerco 1989):

$$N_{\text{diff}} = N_{\text{diff},20} \times \beta^{(T-20)}$$

Phytoplankton uptake is an important sink of TAN in aquaculture ponds. Phytoplankton biomass in fish ponds increases in response to the large quantity of nutrients added as feed. Phytoplankton growth in aquaculture pond ecosystem models is usually described as a function of maximum growth rate (μ_{max}) limited by temperature, solar irradiance and nutrient concentration. In this model, simplifying assumptions were made to model uptake as a function of phytoplankton biomass (specific uptake) and temperature. In this model, growth and loss processes were

ignored in favor of an approach that related phytoplankton biomass to feeding rate. This relationship was determined by linear regression of chlorophyll *a* concentration with respect to nitrogen added as feed (data of Tucker and van der Ploeg 1993) and described by the following equation:

$$\text{CHLA} = 127.0 + (734.7 \times N_{\text{feed}}) \quad r^2 = 0.599$$

Chlorophyll *a* concentration was converted to phytoplankton nitrogen by assuming a ratio of 5 mg N (mg chlorophyll *a*)⁻¹ (Laws and Bannister 1980). Ammonium uptake by phytoplankton was assumed to follow the Vant Hoff relationship with temperature:

$$N_{\text{up}} = N_{\text{pton}} \times k_{\text{up}} \times Q_{10}^{((T-20)/10)}$$

Planktonic (water column) nitrification was assumed to be temperature-dependent and first-order with respect to ammonium concentration (Fritz *et al.* 1979). Nitrification was:

$$N_{\text{nitrif}} = \text{NH}_4_{\text{w}} \times k_{\text{nitrif}}$$

Dissolved oxygen was related to feeding rate according to the relationship depicted in Figure 6 in which average dissolved oxygen declined as a linear function of feeding rate (Andrews *et al.* 1973, Cole and Boyd 1986). At feeding rates exceeding 100 kg ha⁻¹ d⁻¹, dissolved oxygen concentration was maintained at a concentration no less than the critical threshold of 2 mg L⁻¹ by paddlewheel aeration. Although a greater threshold (e.g., 4 mg L⁻¹) than that modeled here is more likely the case in practice, the dissolved oxygen concentration of bottom waters is chronically undersaturated in the middle of the summer when water temperature and

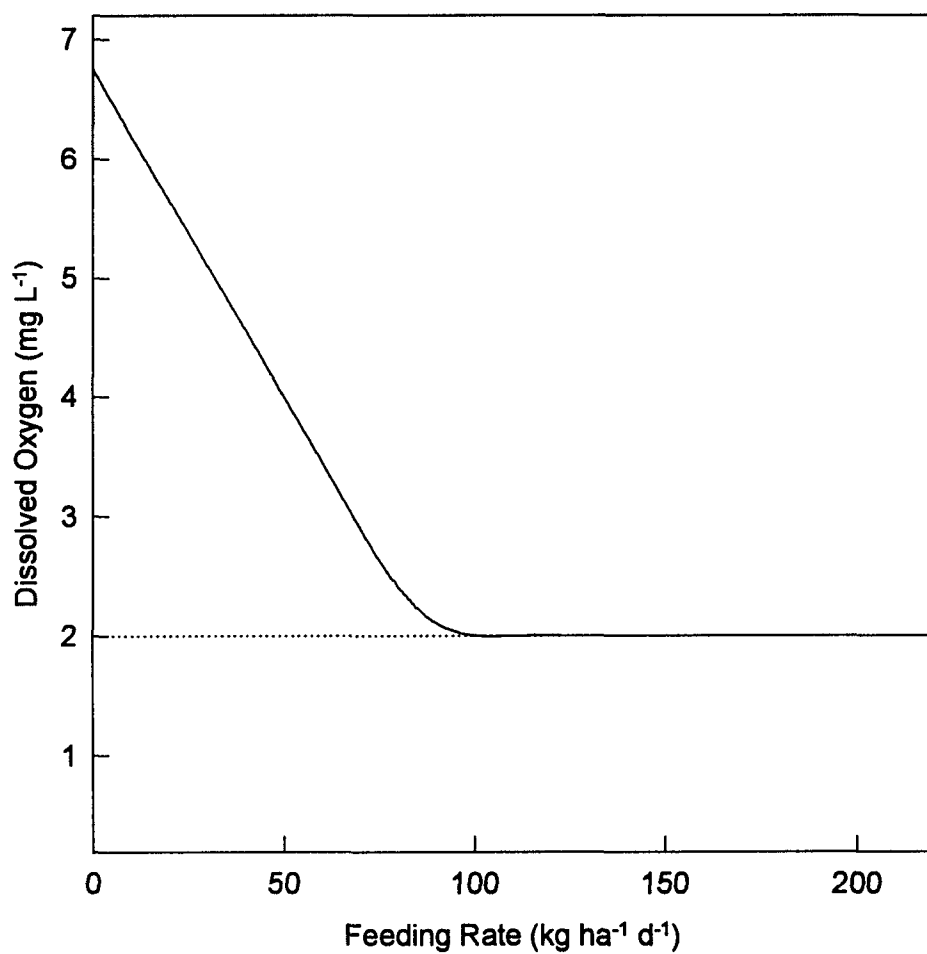


Figure 6. The effect of feeding rate on average dissolved oxygen concentration.

feeding rate are elevated, especially in the stagnant diffusive boundary layer at the sediment-water interface.

Control of sediment nitrification was assumed to be a function of temperature and the depth of oxygen penetration (Figures 7 and 8). The depth of sediment oxygen penetration was estimated by the dissolved oxygen concentration of the overlying water. The relationship between nitrification rate and the dissolved oxygen of the overlying water was derived from data of Jensen *et al.* (1993, 1994) and Blackburn *et al.* (1994) for nearshore marine sediments (Figure 2). Diurnal variation in dissolved oxygen was not considered in this model although there is some evidence (Diab *et al.* 1993) that fluctuations in dissolved oxygen may depress nitrification.

Sediment nitrification was modeled as a process independent of water column total ammonia-nitrogen. The sources of ammonium for sediment nitrification were assumed to be derived from organic matter mineralization at the sediment-water interface and diffusion of ammonium from anaerobic sediment to the sediment-water interface.

Model Implementation

The aquaculture pond nitrogen model was constructed using the STELLA[®] (Systems Thinking Experiential Learning Laboratory with Animation) II version 3.0.5 software (High Performance Systems, Inc., Manchester, NH) and executed on a 486-SX IBM-compatible personal computer. The model consisted of state variables (stocks) that were interconnected by regulated flows (Figure 9). Flows were

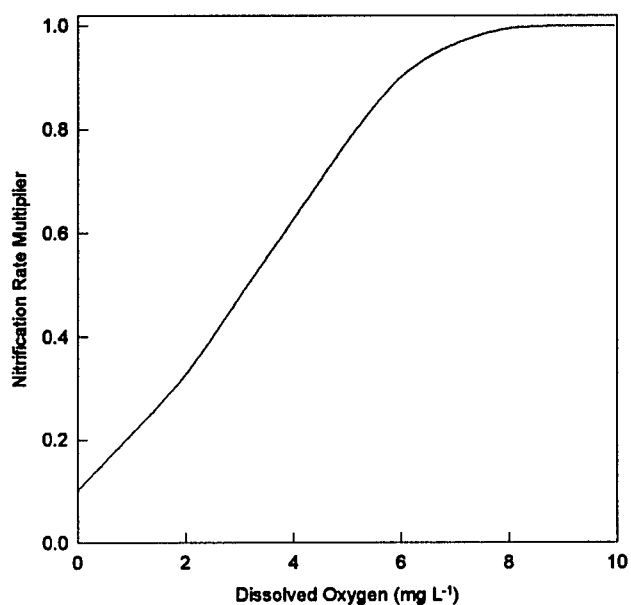


Figure 7. Sediment nitrification rate multiplier as a function of dissolved oxygen concentration overlying sediment.

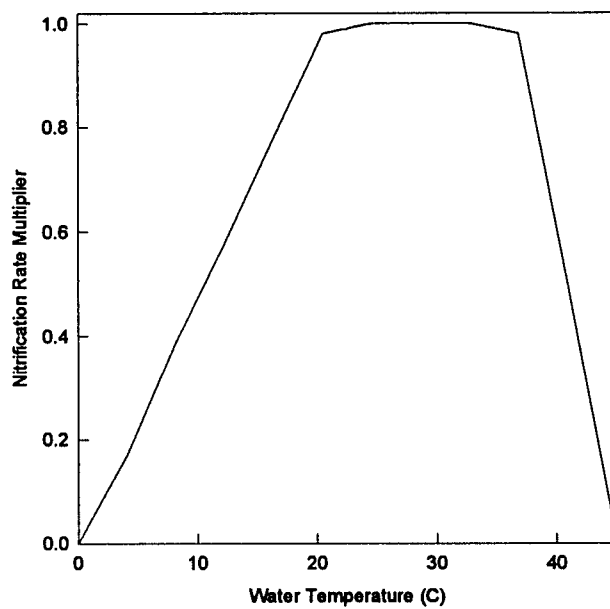


Figure 8. Sediment nitrification rate multiplier as a function of temperature.

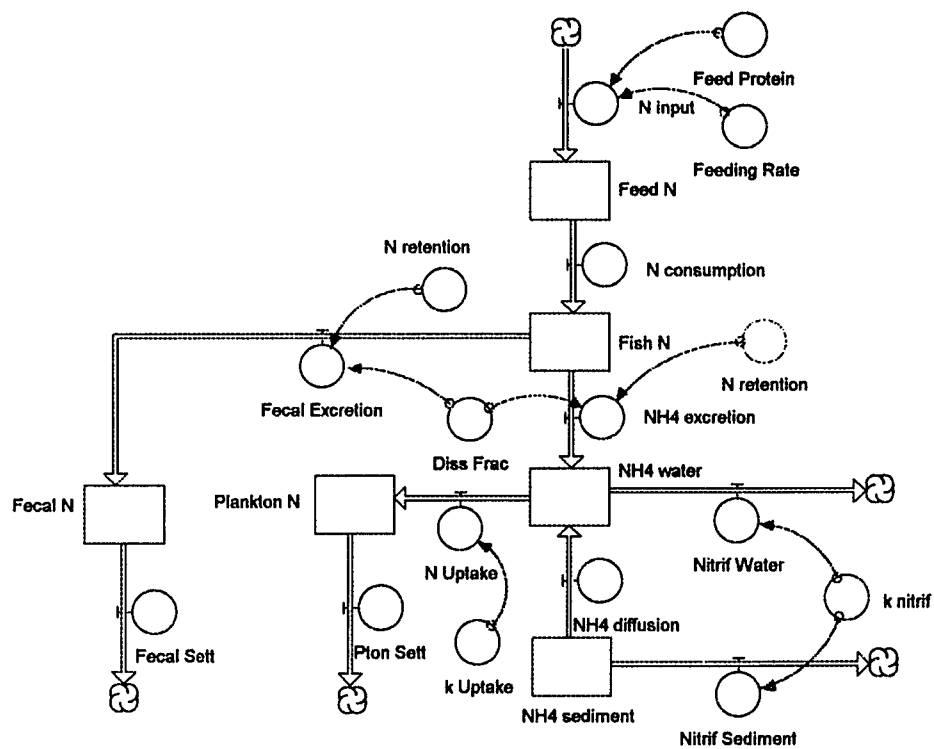


Figure 9. Graphic representation of a simulation model of ammonium dynamics in a channel catfish pond in the Southeastern United States showing main pools, flows, and auxiliary variables.

regulated by auxiliary variables, which may be a function of other parameters (e.g. temperature). Input data requirements included average annual temperature, annual temperature range, average annual feeding rate and annual feeding rate range, nitrogen retention by fish, and the fraction of excreted nitrogen that is dissolved. Model differential equations were integrated by Euler's method with a time step (DT) equivalent to one day. Simulations were executed for two annual cycles beginning January 1 (JD 1) and ending December 31 (JD 730). Output from the first annual cycle was discarded due to model instability associated with the selection of initial values for N state variables.

Model Calibration

The model was calibrated from a small empirical data base describing annual variation in water quality in ten commercial channel catfish ponds in Mississippi (Tucker and van der Ploeg 1993). Water quality was measured in samples collected from ten commercial channel catfish ponds at monthly intervals. The model was calibrated by adjusting the parameter related to the specific ammonium uptake rate of phytoplankton.

Model Verification

The effect of dietary protein on TAN concentration was evaluated by comparing model output average TAN concentration during the growing season (210 days) with the results of a study by Li and Lovell (1992) in which the effect of feeds of variable dietary protein, over the range 24-40%, on TAN concentration in channel catfish ponds was evaluated.

The effect of feeding rate was evaluated in two respects. First, average TAN concentration for the growing season determined by the simulation model were compared to those measured in channel catfish ponds by Cole and Boyd (1986), in which maximum feeding rates ranged from 0-224 kg ha⁻¹ d⁻¹, and Tucker *et al.* (1979), in which maximum feeding rates ranged from 33.6-78.4 kg ha⁻¹ d⁻¹. Second, the effect of average feeding rate on sediment nitrification rates was evaluated. Average feeding rate was increased in 10 kg ha⁻¹ d⁻¹ increments over the range of 40-80 kg ha⁻¹ d⁻¹, while fixing the annual range of feeding rate, and the sediment nitrification rate was determined for an annual cycle at each feeding rate. The studies of Cole and Boyd (1986) and Li and Lovell (1992) permitted further evaluation and verification of the model constructed on the basis of the data of Tucker and van der Ploeg (1993).

Sensitivity Analysis

Sensitivity analysis was performed by defining a base case and changing the value of the parameter of interest in 10% increments across a range (70-130%) of base case parameter values, while keeping all other parameter values constant. Average TAN values were calculated from output values at 5-day intervals over the second year of each simulation run. Elasticity with respect to changes in parameter value was calculated by the following formula:

$$\text{Elasticity} = (\Delta \text{TAN} / \text{TAN}_{\text{avg}}) \div (\Delta \text{Parameter Value} / \text{Parameter Value}_{\text{avg}})$$

in which average TAN concentration and parameter values represented the average of the base case value and the concentration or parameter value of the sensitivity run.

Mean elasticity was calculated as the average of six sensitivity runs in which parameter values were set at values 10-30% above (3) or below (3) the base case value.

RESULTS

The model was able to accurately predict annual variation in water column TAN concentration in commercial channel catfish ponds (Figure 10). Simulated TAN concentrations were within one standard error of the mean values measured by Tucker and van der Ploeg (1993) in all months except March, October and November, in which measured concentrations were substantially less than simulated. Rates of ammonium excretion and sediment ammonium diffusion corresponded closely to seasonal variation in temperature and feeding rate (Figure 11). Sediment ammonium diffusion accounted for approximately 25% (winter) to 33% (summer) of total ammonium production in the simulated channel catfish pond.

Phytoplankton uptake and water column nitrification were the two mechanisms simulated to remove ammonium from the water column. Phytoplankton uptake was a more important removal mechanism than planktonic nitrification during the growing season (April-October) when phytoplankton density was correspondingly maximum, whereas planktonic nitrification was a more important removal mechanism during fall, winter and spring, albeit at a lower rate (Figure 12). Assuming the Redfield ratio of nutrients (C:N:P=106:16:1), simulated phytoplankton uptake of ammonium corresponded to C fixation rates of $0.2\text{--}2.6 \text{ g C m}^{-2} \text{ d}^{-1}$, which closely

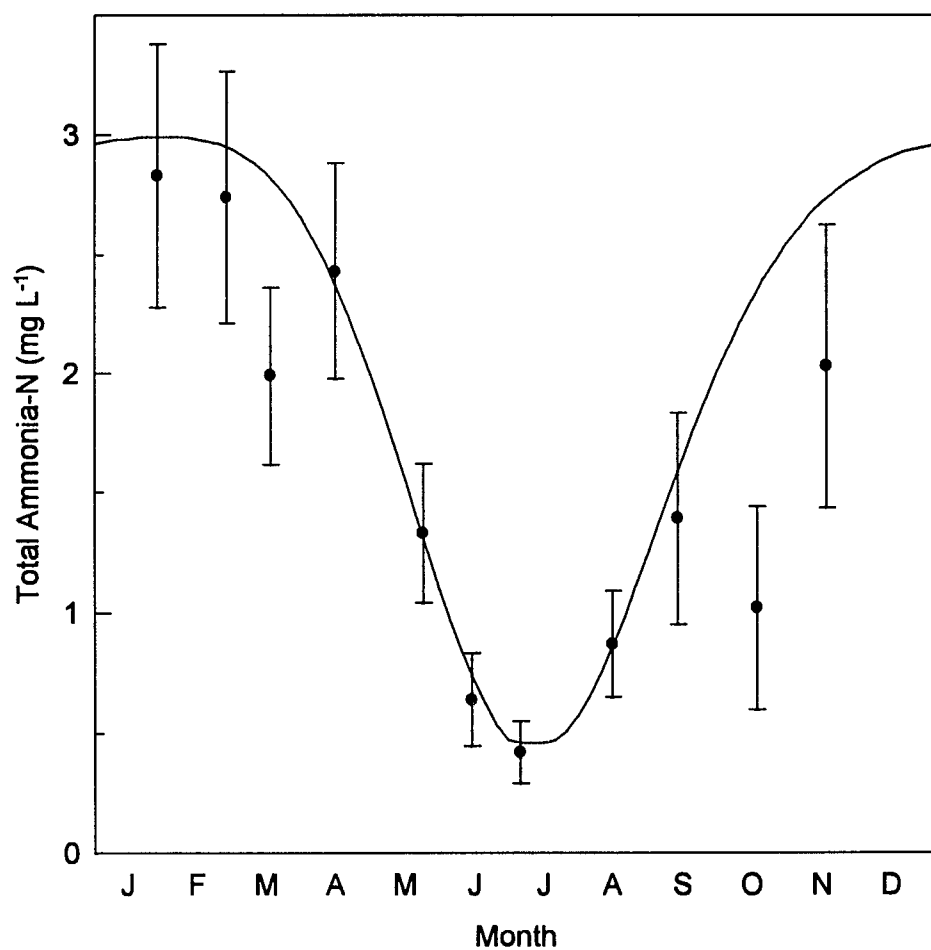


Figure 10. Simulated annual variation in total ammonia-nitrogen concentration (solid line) in a channel catfish pond as compared to measured (\pm SE) concentrations (●) from Tucker and van der Ploeg (1993).

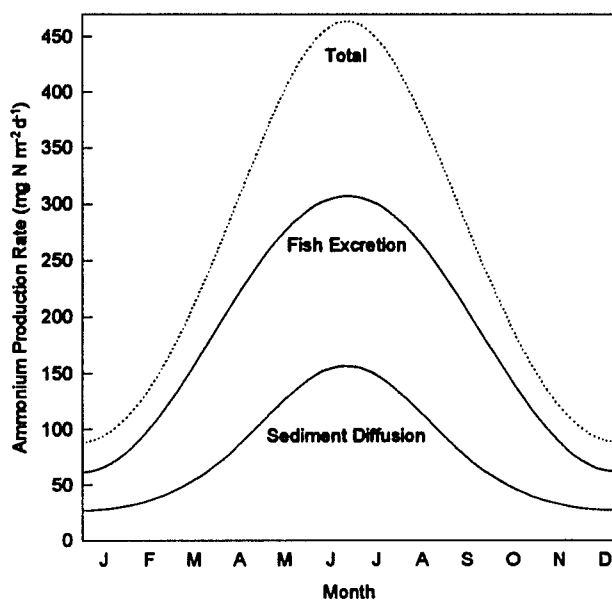


Figure 11. Simulated ammonium production rates from fish excretion and sediment diffusion in a channel catfish pond.

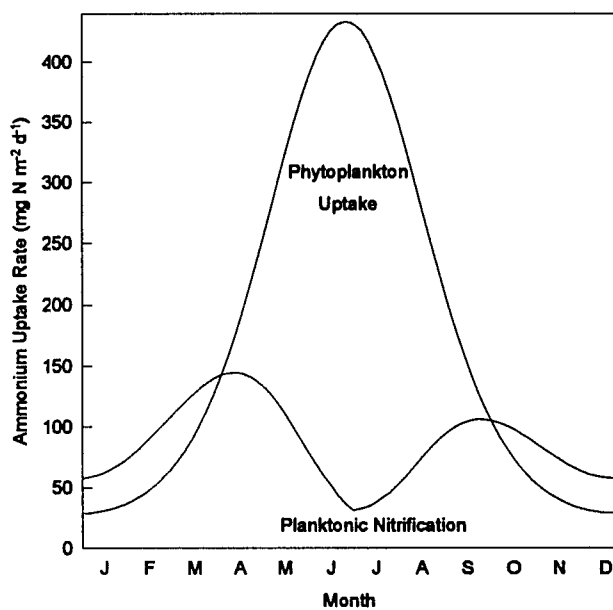


Figure 12. Simulated ammonium uptake rates by phytoplankton and planktonic nitrification in a channel catfish pond.

approximate the range of fixation rates ($0\text{--}3 \text{ g C m}^{-2} \text{ d}^{-1}$) reported for channel catfish ponds (Boyd 1990).

The annual pattern of simulated planktonic nitrification revealed two peaks of activity during late-March to mid-April and late-September to early-October. The spring peak of planktonic nitrification was slightly greater than that occurring during fall. Seasonal peaks of nitrite concentration corresponding to simulated seasonal variation in planktonic nitrification have been measured in channel catfish ponds (Tucker and van der Ploeg 1993). The lowest planktonic nitrification rates occurred during mid-summer when water column ammonium concentration was depressed by elevated rates of phytoplankton uptake. Although temperature was less favorable for planktonic nitrification in the winter, TAN concentration was sufficiently higher than in summer to overcome depression of activity by low temperature.

The seasonal variation of sediment and planktonic nitrification was similar (Figure 13) although the magnitude of sediment nitrification was approximately 50% of that occurring in the water column. Sediment nitrification was depressed to minimum levels during the winter, corresponding to control of nitrification by temperature. Nitrification increased during spring in relation to increased microbial activity as a result of increasing temperatures to a peak of activity in mid-spring (April to early May). Nitrification then decreased in response to elevated feeding rate, reduced dissolved oxygen and therefore reduced oxygen penetration into sediments. Sediment nitrification declined to low rates during summer, although not as low as rate depression by low temperature during the winter. In the fall, sediment

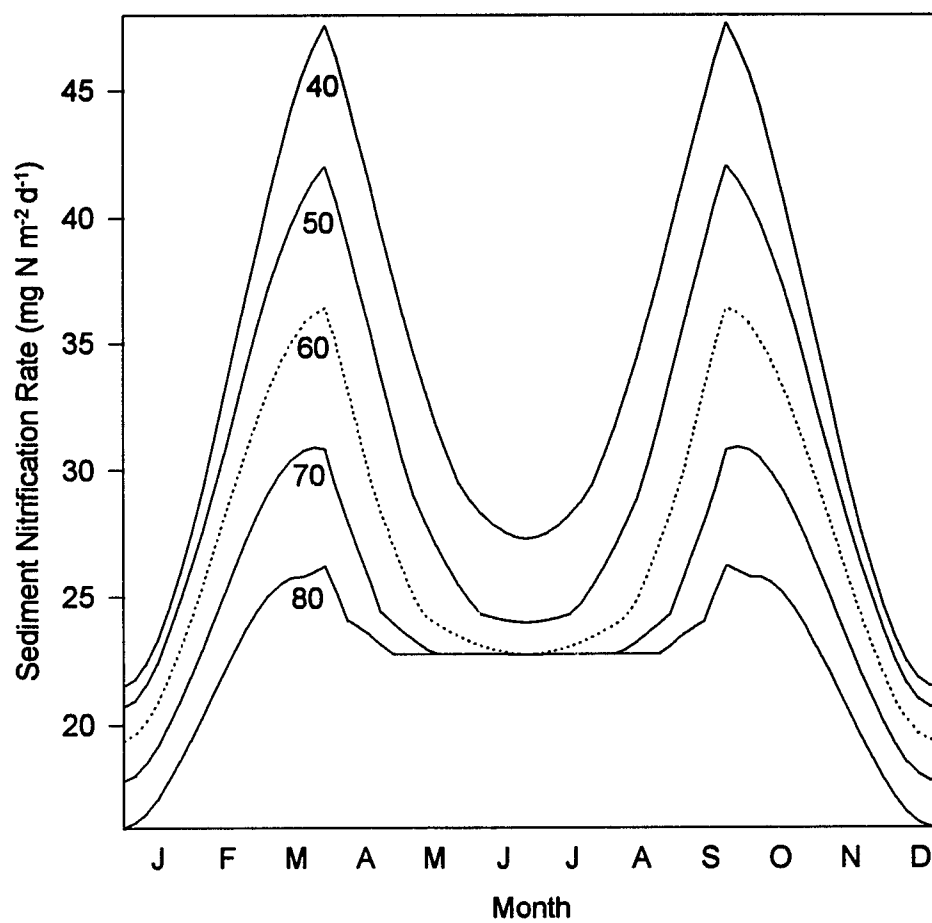


Figure 13. The effect of feeding rate on annual variation of sediment nitrification rate (mg N m⁻² d⁻¹). Dotted line denotes base case scenario.

nitrification increased once again as feeding rates declined, dissolved oxygen concentration increased and the depth of sediment oxygen penetration increased, but temperatures remained within the range of optimum nitrification. During late fall sediment nitrification declined corresponding to declining temperature.

Model Verification

A linear relationship between average TAN and protein concentration of channel catfish diets ranging from 24-40% protein has been demonstrated (Li and Lovell 1992). The pond ammonium model adequately simulated the average ammonia concentrations measured by Li and Lovell (Figure 14). The model most accurately simulated total ammonia-nitrogen concentration at 24 and 28% dietary protein, although measured average total ammonia-nitrogen concentrations were within one standard deviation of simulated results for catfish fed 32 and 40% protein diets.

Cole and Boyd (1986) measured a linear increase in TAN and maximum feeding rate over the range 0-224 kg ha⁻¹ d⁻¹. Simulated TAN concentration over the range of maximum feeding rates evaluated by Cole and Boyd systematically underestimated measured average TAN concentration at maximum feeding rates greater than 50 kg ha⁻¹ d⁻¹, although measured concentrations were within one standard deviation of simulated values for the growing season (Figure 15). The slope of the regression line of average TAN concentration with respect to feed calculated by Cole and Boyd (1986) was approximately 2.5x that estimated by the simulation model. However, over the range of maximum feeding rates most common in commercial

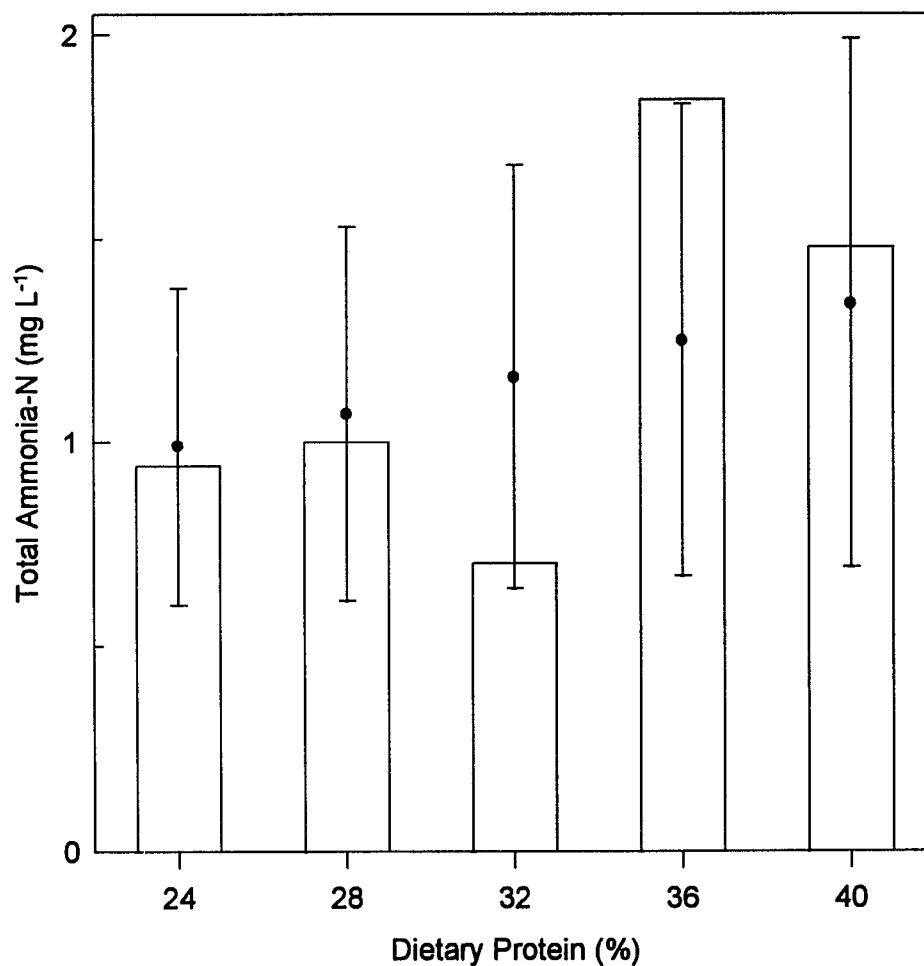


Figure 14. Effect of dietary protein concentration on average TAN concentration. Closed circles and error bars indicate simulated values; open bars indicate values measured by Li and Lovell (1992).

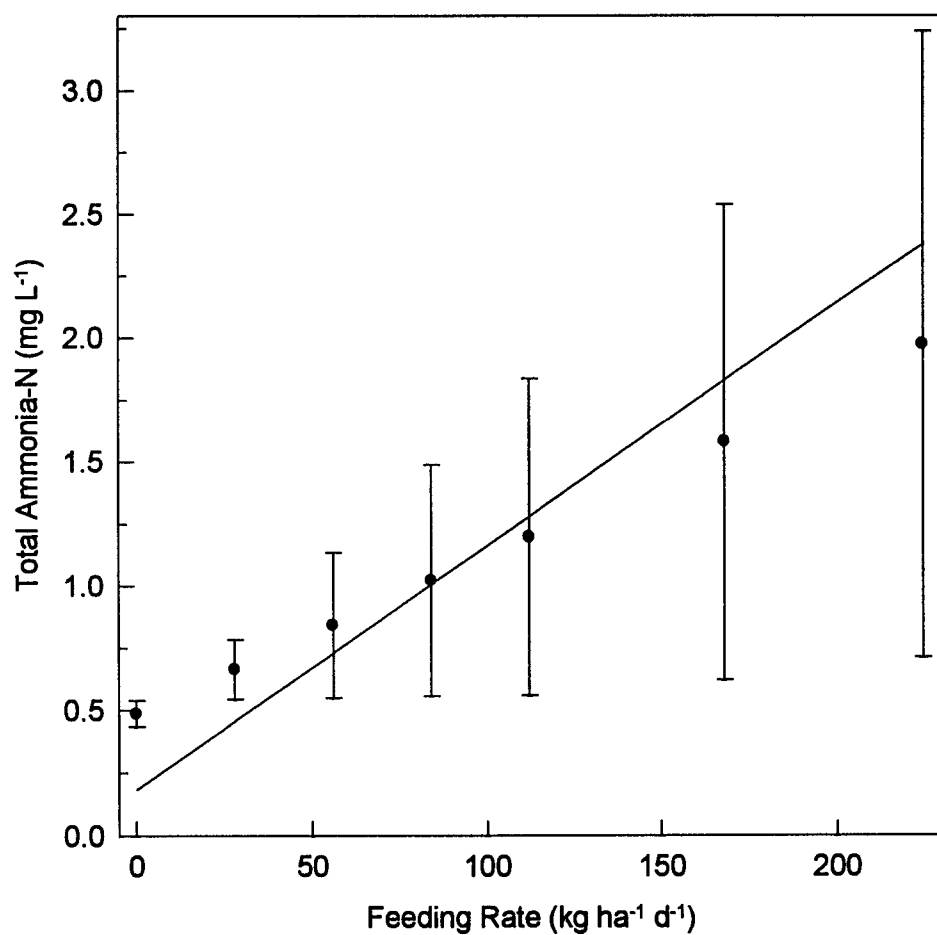


Figure 15. Effect of maximum feeding rate on average TAN concentration. Closed circles and error bars represent simulated values; regression line derived from data of Cole and Boyd (1986).

channel catfish culture ($<150 \text{ kg ha}^{-1} \text{ d}^{-1}$), the model adequately simulated TAN concentrations. Simulated maximum TAN concentrations were similarly underestimated, although of similar magnitude (Table 13).

Similar feeding rate effects on TAN concentration were measured by Tucker *et al.* (1979), although over a more restrictive range of feeding rates than evaluated by Cole and Boyd (1986). The simulation model overestimated TAN concentration early in the growing season and overestimated TAN concentration at all times at a maximum feeding rate of $33.6 \text{ kg ha}^{-1} \text{ d}^{-1}$ (Figure 16). However, the trend of increasing TAN concentration as the growing season progressed matched that of measured concentrations. Simulated concentrations exceeded measured values at maximum feeding rates of 56.0 and $78.4 \text{ kg ha}^{-1} \text{ d}^{-1}$ later in the growing season, although, in general, agreement between simulated and measured values was good.

Sensitivity Analysis

The most important model parameters as indicated by sensitivity analysis are those related to feeding practices and how they interact with protein digestibility, assimilation and excretion by fish. The model was most sensitive (elastic) to changes in the partition of fish nitrogenous excretion between dissolved and solid components (Table 14). As the proportion of nitrogen excreted as ammonia increased, average annual total ammonia-nitrogen increased. Average annual total ammonia-nitrogen concentration increased in response to increases in average feeding rate and dietary protein. Unexpectedly, simulated average annual TAN concentration was relatively inelastic with respect to fish nitrogen retention, which is an indicator

Table 13. Simulated and measured maximum total ammonia-nitrogen concentrations with respect to maximum feeding rate.

Maximum Feeding Rate (kg ha ⁻¹ d ⁻¹)	Maximum Total Ammonia-Nitrogen (mg L ⁻¹)		
	Simulated	Measured ¹	Δ
0	0.55	0.9	-0.35
28	0.94	1.0	-0.06
56	1.50	2.6	-1.10
84	2.05	4.2	-2.15
112	2.60	4.1	-1.50
168	3.71	4.5	-0.79
224	4.81	4.7	+0.11

¹ Cole and Boyd (1986)

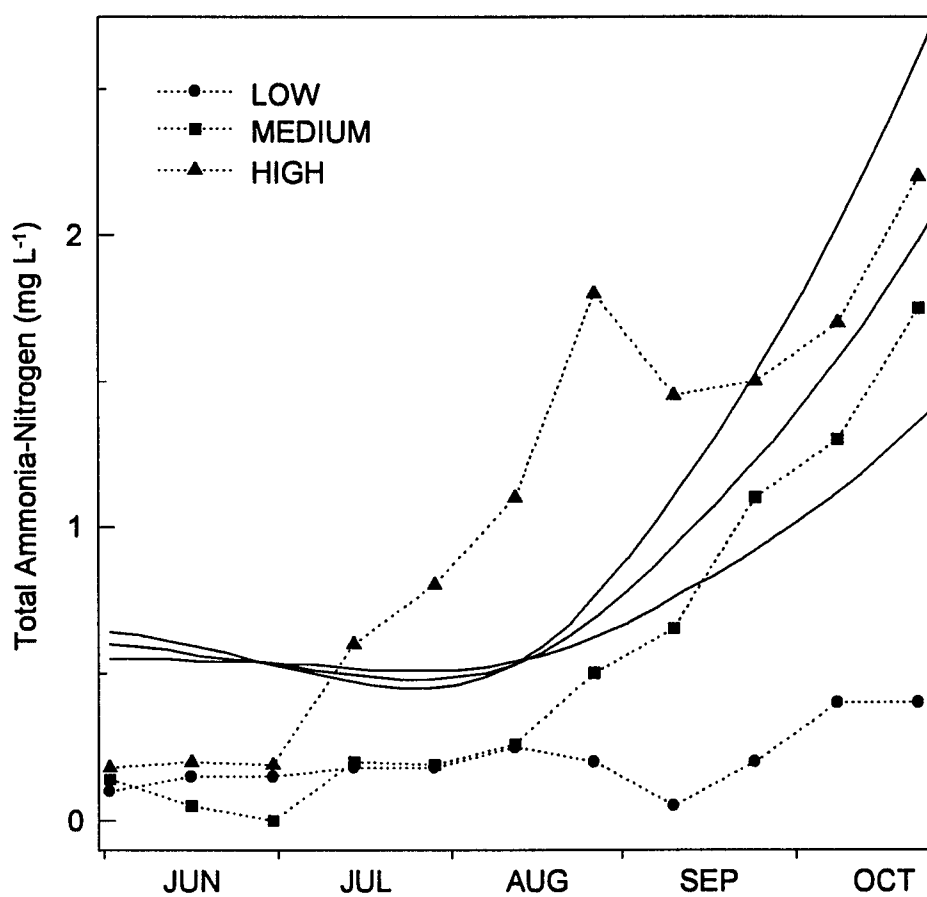


Figure 16. Simulated (solid lines) and measured (symbols) TAN concentrations in ponds fed a maximum of 33.6 (low), 56.0 (medium), or 78.4 (high) kg ha⁻¹ d⁻¹ (after Tucker *et al.* 1979).

Table 14. Effects of change in model parameters on average annual total ammonia-nitrogen concentrations.

Parameter ¹	Base Case	Range Evaluated	Elasticity ²	Directional Effect of Parameter Increase ³
DISS_FRAC	0.80	0.65-0.95	1.59	+
FDRT _{avg}	60	42-78	1.20	+
k _{up}	0.07	0.05-0.09	1.19	-
k _{nit,T}	0.0025	0.00175-0.00325	0.94	-
PROT	0.32	0.24-0.42	0.72	+
N _{diff,20}	0.065	0.0455-0.0845	0.71	+
N_RETN	0.25	0.175-0.325	0.52	-

¹ Parameters are defined in Table 1.

² Elasticity defined as % change in average annual total ammonia-nitrogen concentration divided by % change in parameter value from base case.

³ Directional effect indicated as an increase (+) or decrease (-) in average annual total ammonia-nitrogen concentration as parameter value increases.

of net protein utilization and feed conversion, although an increase in N retention reduced average TAN concentration.

Model output was also sensitive to parameters related to pond processes involved in ammonium production and transformation. Average annual TAN concentration was sensitive to the specific uptake rate (k_{up}) of phytoplankton. As the specific nitrogen uptake rate of phytoplankton increased, average annual TAN concentration decreased. Modeled TAN concentrations were relatively inelastic with respect to the temperature multiplier for the specific planktonic nitrification rate ($k_{nit,T}$). As the specific rates of planktonic nitrification increased, average annual TAN decreased.

DISCUSSION

The simulation model accurately predicted annual variation of TAN concentration in commercial channel catfish ponds. Perhaps the greatest descriptive utility of the model relates to partition of the magnitude of the flow rates between sources and sinks of ammonium in a fish pond. The importance of fish ammonia excretion to water quality in any culture system is well appreciated. However, the contribution of sediment diffusion to total ammonia-nitrogen in fish ponds is less well understood. The model indicates that sediment ammonium diffusion is an important source of total ammonia-nitrogen in fish ponds. The rate of sediment diffusion is greater in the summer in response to rapid, high-temperature enhanced mineralization of organic matter at the sediment-water interface. In addition, the

proportional contribution of sediment diffusion to overall ammonium flux is most important in summer.

Similarly, the overwhelming importance of phytoplankton as a regulator of TAN concentration in fish ponds is well appreciated. The relative importance of planktonic and sediment nitrification is less well understood. Although seasonal maxima in nitrification products have been observed in fish ponds (Boyd 1990, Tucker and van der Ploeg 1993), the relative magnitude of uptake processes has not been clarified. Planktonic nitrification is a more important sink than phytoplankton uptake from late-fall until mid-spring. The interaction between temperature and TAN concentration determines the planktonic nitrification rate. Maximum planktonic nitrification rates correspond to average water temperature and TAN concentration. While the model adequately predicted TAN concentrations for most months of the year, during March and October measured concentrations were considerably lower than expected by the model. It is possible that planktonic nitrification rates, which are maximum during mid-spring and mid-fall, are greater than indicated by the model. Alternatively, sediment nitrification may be utilizing TAN from the overlying water during those short periods during the spring and fall when conditions are optimum.

The potential of denitrification in fish pond sediments to remove substantial quantities of nitrogen is high. However, the magnitude of this loss is poorly understood as few direct measurements of denitrification in fish ponds have been made. Denitrification requires oxidized nitrogen as a substrate. Thus denitrification

derives substrate from oxidized nitrogen produced by nitrification in the water column (planktonic nitrification) or at the sediment-water interface. Nitrification-denitrification reactions at the sediment-water interface are tightly coupled, and interaction with the overlying water is important only with respect to oxygen concentration, which restricts nitrification rate in the summer. However, sediment denitrification that derives substrate from the overlying water is likely more important than coupled nitrification-denitrification at the sediment-water interface.

Oxidized nitrogen rarely accumulates in channel catfish ponds and is usually present at concentrations that limit denitrification rate. Assuming all oxidized nitrogen is subsequently removed by denitrification, rates of planktonic ($86 \text{ mg N m}^{-2} \text{ d}^{-1}$) and sediment ($28 \text{ mg m}^{-2} \text{ d}^{-1}$) nitrification integrated over an annual cycle indicate the potential importance of denitrification as a mechanism of nitrogen removal in fish ponds. The potential loss of nitrogen from denitrification of substrate derived from planktonic and sediment nitrification accounts for 28% and 9%, respectively, of nitrogen added as feed. As fish remove approximately 25% of nitrogen added as feed, the majority of the balance (38%) likely accumulates in the sediment.

Sensitivity analysis indicated that average annual TAN concentration was most elastic with respect to the proportion of nitrogen excreted as ammonium, average feeding rate and the specific uptake rate of phytoplankton. The scope for managing the first factor is limited as fish physiology largely determines the partition of nitrogenous excretion between dissolved and solid components. Although the model was relatively inelastic with respect to nitrogen retention (an index of feed

conversion), improvement in the digestibility of feeds may reduce TAN concentration.

The positive relationship between feeding rate and TAN concentration has been clearly demonstrated (Tucker *et al.* 1979, Cole and Boyd 1986). Coincident with the goal of maximizing fish production, water quality is managed to allow maximum feeding rate. Higher average TAN concentration related to feeding rate is not a problem when feeding rates are maximum, and phytoplankton uptake, planktonic nitrification and other metabolic processes are similarly maximum. However, increased feeding rate during periods of seasonally depressed temperatures may result in elevated TAN concentration as uptake and removal mechanisms are similarly depressed. Thus, feeding rate practices are less important during than after the growing season.

The specific uptake rate of phytoplankton was the third most sensitive variable in the model. Although the specific phytoplankton uptake rate constant was assumed not to vary during an annual cycle, it is likely that this assumption is false. Specific uptake rate is likely maximum for phytoplankton populations that are growing rapidly. Thus, in the spring, when phytoplankton density is increasing and dissolved inorganic nitrogen concentrations begin to decline from maximum values, the specific uptake rate constant may be maximum. During the mid-summer, when phytoplankton density is maximum, phytoplankton growth rate becomes limited by light despite seasonally maximum temperature. Direct measurements of uptake by natural assemblages of phytoplankton in fish ponds are needed.

Increased phytoplankton density is an inevitable consequence of nutrient addition. Although it is not likely that feeding rates will be reduced for reasons cited above, there may be opportunities to regulate TAN concentrations in fish ponds by management of phytoplankton populations. Smith and Piedrahita (1988) determined that intermediate phytoplankton densities ($150\text{--}350\ \mu\text{g chl } a\ \text{L}^{-1}$) will maximize oxygen production. Oxygen production will be maximized when phytoplankton growth rates are maximized. Thus, maximum phytoplankton growth rates will improve pond water quality by increasing dissolved oxygen production and ammonium uptake.

Agreement between simulated results and studies conducted by Li and Lovell (1992), Cole and Boyd (1986) and Tucker *et al.* (1979) was good, indicating the general applicability of the model to pond aquaculture in the southeastern United States. One possible explanation for the systematic bias in simulated results relative to those of the feeding rate study by Cole and Boyd (1986) may be related to differences in temporal variation in maximum feeding rate between the simulation model and the feeding rate study. In the model, feeding rate was modeled as a sinusoidal function of time and maximum feeding rate was therefore sustained for a short period (days), whereas maximum feeding rates were sustained for 6 weeks in the feeding rate study of Cole and Boyd. Another possibility for systematic bias may be related to the fairly low frequency (monthly) of data collection by Cole and Boyd, whereas average concentrations determined by the simulation model were derived from model output values at 5-day intervals over the duration of the growing season.

Finally, the model may overestimate algal uptake at high maximum feeding rates as phytoplankton concentrations were modeled as a linear function of nitrogen input rate.

A more complete integration of the model with respect to other nitrogen pools is required. Processes not modeled, but which require further elaboration, include the relative contribution of phytoplankton sedimentation and fecal excretion to sediment particulate organic nitrogen concentration. The rates of hydrolysis of particulate organic nitrogen to dissolved organic nitrogen and the subsequent mineralization to ammonium are required to fully integrate the model.

The fate of oxidized ammonium (nitrite and nitrate) must be considered. In the model phytoplankton uptake of ammonium only is assumed. Although phytoplankton prefer ammonium, and nitrate uptake is negligible if ammonium is present, it is possible that phytoplankton uptake of nitrate occurs, particularly in the mid-summer when ammonium concentrations are minimal, despite the fact that phytoplankton have a higher affinity for nitrogen than nitrifying bacteria. The relative importance of coupled nitrification-denitrification as well as denitrification derived from water column nitrate is poorly understood.

The relative effects of planktonic and sediment nitrification on the production of nitrite and nitrate in the water column are required. Direct measurements of sediment denitrification are required to validate nitrogen loss rates estimated by the model.

Additional studies are required to increase confidence in model parameters. In particular, temperature effects on phytoplankton uptake, planktonic nitrification, sediment ammonium diffusion and fish excretion are required.

In the future, the introduction of some stochastic or random elements into the model may provide a more realistic picture of pond processes. In particular, random fluctuation in temperature and chlorophyll a concentrations around mean values generated by sinusoidal curves would offer insights regarding short-term changes in water quality. Simulation of the water quality effects of a phytoplankton die-off may suggest management options to mitigate the effects of such a potentially-devastating event. Integration with other water quality models, particularly those related to dissolved oxygen dynamics and fish bioenergetics would provide a more complete picture of pond dynamics with respect to nutrient flows.

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CHAPTER 4
THE EFFECT OF SEDIMENT DISTURBANCE ON WATER QUALITY
IN ORGANICALLY-ENRICHED AQUATIC MESOCOSMS

INTRODUCTION

Water quality in shallow, eutrophic aquaculture ponds is dominated by water column processes, particularly related to phytoplankton dynamics. Efforts toward improvement of water quality in aquaculture ponds typically are directed to the water column. Ponds are aerated when dissolved oxygen declines to critical levels. Water may be flushed through the pond to remove organic matter and metabolites and supply water enriched in oxygen, nutrients and/or food items. Salt (NaCl) may be added to mitigate potential nitrite toxicity.

The high surface-to-volume ratio of aquaculture ponds suggests that pond sediments may strongly impact water quality. Pond soils may be limed to increase soil pH and the alkalinity of overlying water. However, management techniques are rarely directed to pond sediments. In some shrimp ponds, the surface layer of sediment may be removed following harvest and pond draining, although the efficacy of this treatment has been called into question (Boyd 1992). Ponds may be constructed and managed for periodic or continuous removal of settled organic matter by concentration at a center drain through the development of circular water currents in round ponds (Wyban and Sweeney 1989).

Mass balance studies conducted in a wide variety of culture systems indicate that a relatively small proportion (approx. 25%) of nutrients applied in the form of feeds are retained in fish (see Table 1, Chapter 1). The remainder is released to the

water column in dissolved and solid fractions. Phytoplankton and other water column microorganisms assimilate the majority of the dissolved nutrients. Fecal solids, uneaten feed, and senescent phytoplankton fall to the sediment. The sediment thus stores a large quantity of the nutrients supplied in feed. In intensive ponds, accumulation of organic matter in ponds will increase sediment oxygen demand and lead to areas of anaerobiosis, which may negatively impact fish growth (Avnimelech and Zohar 1986).

In intensive ponds it is desirable to promote conditions that enhance nutrient removal. In extensive or low-intensity, small-scale aquaculture ponds, maximizing the utility of applied inputs by recycling nutrients trapped in the sediment into the water column is desirable. Stirring of pond bottoms has been proposed as a method of directing detrital foods and nutrients into the water column (Costa-Pierce and Pullin 1989, Costa-Pierce *et al.* 1993).

This experiment evaluated the effects of sediment disturbance by mechanical and biological means on water quality in aquatic mesocosms. In addition, water quality in mesocosms enriched with inputs of variable quality applied at high rates was evaluated. Water quality was evaluated in the context of suitability for fish production. Exploratory multivariate statistical techniques (multiple regression and principal component/factor analysis) were used to identify the relative importance of environmental and treatment variables on water quality and evaluate the most important underlying ecological processes acting upon the system.

MATERIALS AND METHODS

Experimental Design

A 3x4 factorial experiment was designed to evaluate the effect of sediment disturbance and input type on water quality in fiberglass tank mesocosms. Two methods of sediment disturbance were compared to an undisturbed control (CL). By one method (CH), sediments were disturbed by dragging a 3.7 m section of 1.6 cm link chain around the tank bottom by fixing one end of the chain at the bottom to one side and pulling along the circumference of the tank bottom for two revolutions. Tanks were stirred with a chain weekly on Monday, one day prior to water quality sampling and before adding the daily allowance of organic inputs. In another method, sediments were disturbed by fish. Tanks were stocked with fish (FI) at a density of 2 m⁻² (20 per tank). Channel catfish (*Ictalurus punctatus*) and 'koi' carp (*Cyprinus carpio*) fingerlings were stocked at 1 m⁻² each at an initial weight (least squares means) of 68 g and 41 g, respectively.

In addition, the effect of four organic input types on water quality was evaluated. These included channel catfish feed (FD), distiller's grains with solubles (DG), aged chicken manure (CM), and rice bran (RB). Channel catfish feed consisted of floating, 4-mm pellets formulated primarily from grain meals (soybean, corn) and fish meal and fortified with vitamins and minerals. Distiller's grains with solubles consisted of the byproducts of cereal grain (primarily barley and corn) fermentation. Aged chicken manure was collected as needed (usually monthly) from laying hen houses located at the LSU Poultry Research Unit. Manure consisted of

fecal matter, spilled feed, and feathers. Manure was collected from beneath pens and had been in the house for approximately 2-3 months. Manure was ground in a mixer to homogenize the material and reduce particle size. Rice bran consisted of germ coat, husks and chipped rice produced in the milling process. Inputs were dried at 105 C to determine moisture and establish an equivalent dry matter loading rate. Dry weight carbon and nitrogen of inputs were determined with a CHN (Heraeus CHN-o-Rapid, UIC Inc., Joliet, IL) analyzer (Table 15). Input particle size distribution was evaluated by passing a quantity of the input through a Tyler screen series (Figure 17).

Table 15. Proportion (%) of dry matter, carbon, nitrogen and carbon to nitrogen ratio of organic inputs evaluated in a study of sediment disturbance.

Input	Dry Matter	Carbon	Nitrogen	C:N
Rice Bran	89.9	43.6	2.4	17.8
Distiller's Grains	90.5	45.4	4.3	10.5
Catfish Feed	90.0	41.4	6.0	6.9
Chicken Manure	82.1	30.3	6.1	5.0

Each treatment combination (total=12) was randomly assigned to three fiberglass tanks (experimental units). Fiberglass tanks (3.66 m D x 1.22 m H) were located at a facility consisting of four double rows of 26 tanks each. Each double row of tanks was serviced by an independent water and air supply system. Tanks

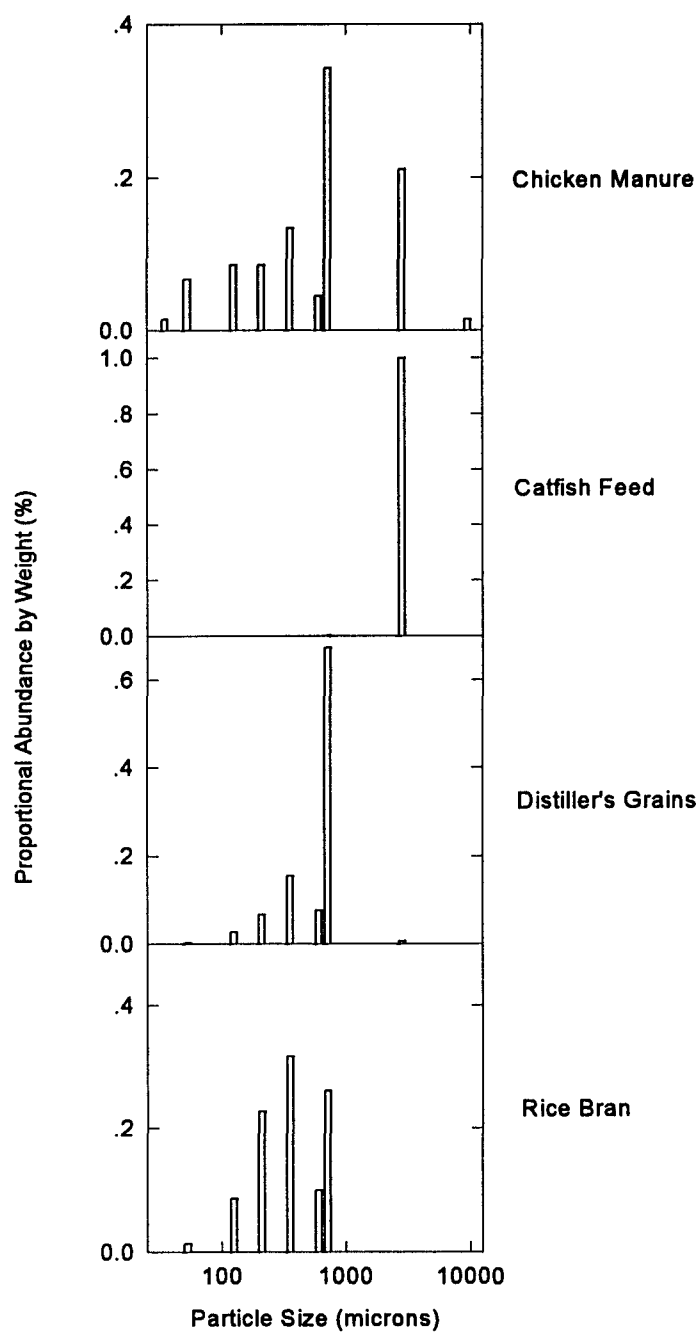


Figure 17. Particle size distribution of organic inputs used to enrich aquatic mesocosms.

utilized for this study were located in one double row and the first 10 tanks of the adjacent double row. A sandy clay-loam soil (58% sand, 26% clay, 16% silt) was added to fiberglass tanks to a depth of 15-30 cm. Each tank was filled with surface water from a reservoir (total alkalinity=150 mg L⁻¹ as CaCO₃) to a depth of 98 cm to provide a total water volume of 10.3 m³. Water was fertilized with 4-6 applications of liquid fertilizer (10-46-0) from late April through May to establish dense phytoplankton blooms. Water was added only as needed to replace evaporative loss and was maintained at a level 4-5 cm below the top of the standpipe to minimize overflow during rainfall events. Air was supplied to each tank through glass-bonded silica diffusers placed on the sediment surface. Completely mixed conditions were verified by no difference between water temperature near the surface and bottom.

Organic inputs were added to each tank daily, usually between 0800 and 1000. Initial dry matter (DM) enrichment rate was equivalent to 100 kg DM ha⁻¹ d⁻¹. The dry matter enrichment rate was increased by 25 kg DM ha⁻¹ d⁻¹ each month for the first 4 months, to a maximum of 175 kg DM ha⁻¹ d⁻¹. The enrichment rate was reduced during the fifth month to 150 kg DM ha⁻¹ d⁻¹ (Figure 18). The experiment was conducted during a period of elevated, seasonal and relatively stable temperature. Tanks were enriched for 20 weeks between 31 May and 19 October 1993.

Water Quality Analysis

Water samples (125 mL) were collected from the near surface of each tank in clean polyethylene bottles every 2 weeks at dawn and carried immediately to the

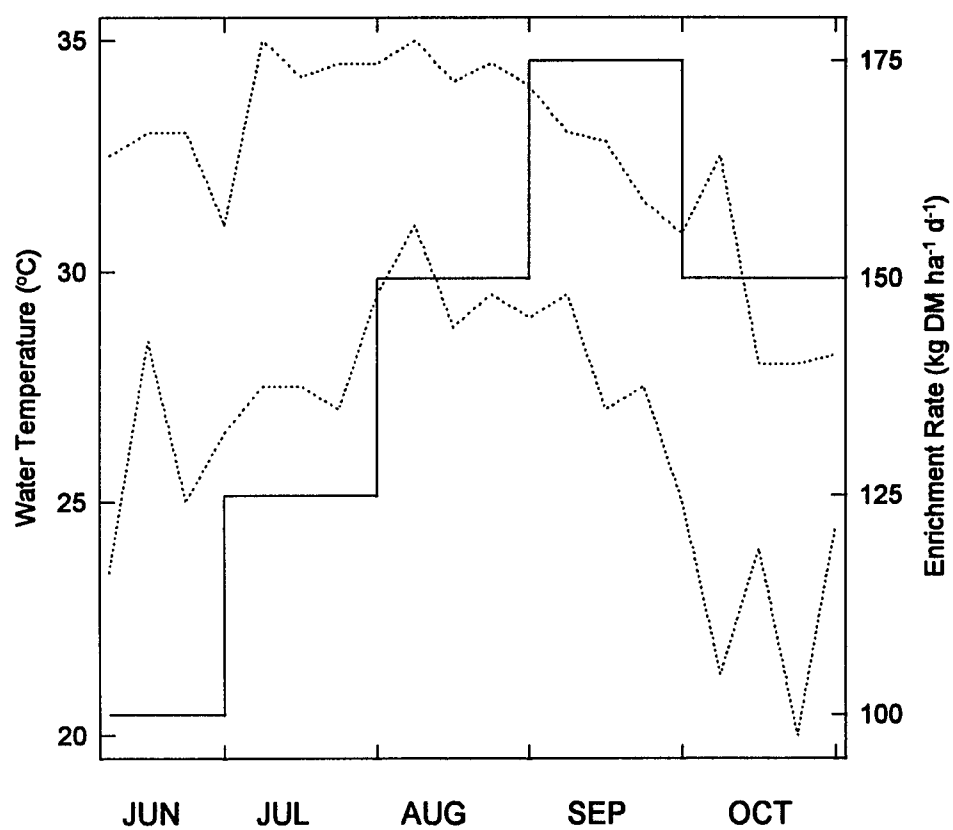


Figure 18. Minimum and maximum weekly water temperature and dry matter enrichment rate of aquatic mesocosms.

laboratory. Water samples from the same treatment combination were pooled (total=12 samples) and filtered (Whatman No. 42) prior to analysis (Table 16).

The method employed in this study to extract pigments from phytoplankton allowed for the quantitative distinction of chlorophyll *a* and phaeophytin, a degradation product of chlorophyll that interferes with chlorophyll *a* estimation. Resolution of chlorophyll *a* and phaeophytin concentrations from replicate (n=6) measurements of a single water sample resulted in a coefficient of variation of 24.4% when pigments were considered separately and only 4.8% when considered together. Therefore, pigments were not resolved for purposes of data analysis.

In addition, the unit of data analysis was treatment combination, whereas chlorophyll *a* and phaeophytin (C+P) were measured from samples collected from individual tanks, one from each treatment combination. However, Secchi disk visibility was measured in each tank. Regression (exponential decay) equations were fitted from plots of C+P concentration with respect to Secchi disk visibility for each treatment combination (Almazan and Boyd 1978). These empirical equations were used to predict average C+P concentrations for each treatment combination, which were then used in data analysis.

Sediment Physical Characteristics

The accumulation of organic matter at the sediment surface was evaluated every 5 weeks by measuring the depth of the flocculent organic layer in duplicate sediment cores (5 cm diameter) collected from each tank.

Table 16. Sampling protocol, frequency and methods used for water quality measurement in a study evaluating the effect of sediment disturbance and organic input type on water quality in tank mesocosms.

Parameter	Sampling Unit ¹	Frequency	Method ²
temperature	T	weekly	minimum-maximum thermometer
dissolved oxygen (DO)	T	weekly (dawn and dusk)	polarographic probe
pH	TC	every 2 weeks	combination probe
total ammonia-N (TAN)	TC	every 2 weeks	phenol-hypochlorite
nitrite-N	TC	every 2 weeks	diazotization
nitrate-N	TC	every 2 weeks	Cd reduction followed by diazotization
total alkalinity	TC	every 2 weeks	titration with dilute HCl to the methyl orange endpoint
total suspended solids (TSS)	TC	every 2 weeks	filtration through pre-ashed GF/C glass fiber filters; dry at 105 °C for 2 hours
volatile/fixed solids (VSS/FSS)	TC	every 2 weeks	loss-on-ignition of dried GF/C filters at 550 °C
chlorophyll <i>a</i> and phaeophytin	T	every 2 weeks	acetone-methanol extraction; acidification of extract
water transparency	T	every 2 weeks	Secchi disk visibility

¹ T=tank; TC=treatment combination (pooled sample: n=3).

² all methods after Boyd and Tucker (1992) and APHA *et al.* (1992) except chlorophyll *a* and phaeophytin after Pechar (1987).

At the conclusion of the study, duplicate cores (5 cm in diameter) from each tank were collected and frozen. Frozen cores were extruded and sectioned at 1-cm intervals. Core sections were weighed, dried at 105 °C for 24 hours and weighed again for determination of moisture content and bulk density. A subsample of each dried section was then weighed in pre-ashed crucibles, combusted at 400 °C for 24 h, and weighed again for estimation of organic matter content. Sediment bulk density was calculated from these measurements. Water, organic matter and mineral matter were transformed and expressed in terms of proportional volume using particle densities of 1.00, 1.14 and 2.65 g cm⁻³ for water, organic matter and mineral matter, respectively (Blake and Hartge 1986).

Sediment Solute Flux

The flux of solutes (O₂, CO₂, and TAN) was measured in short-term (4-h) incubations of cores collected one week following termination of organic enrichment. On each of three successive days, a single sediment core was collected from one tank from each treatment combination. Cores were transported to the laboratory and the overlying water was removed and carefully replaced with approximately 150 mL of 0.45 µm-filtered reservoir water that had been aerated overnight. Cores were incubated in the dark at 30 °C for 4 hours. Dissolved oxygen in overlying water was measured with a polarographic probe. Water was removed by gentle siphon, pH measured, and volume determined. TAN was measured on a filtered (Whatman No. 42) water sample by the phenol-hypochlorite method. Flux rates were calculated

from changes in concentration of DO, CO₂ (determined from alkalinity, pH and temperature), and TAN compared to control cores with no sediment.

Fish Growth and Production

At the termination of the experiment, tanks with fish were drained of water and the fish removed. The total length (cm) and weight (g) of each fish was measured. Growth performance indices were calculated with the following equations:

Daily Weight Gain (DWG)

$$DWG = \frac{W_f - W_i}{t_f - t_i}$$

Specific Growth Rate (SGR)

$$SGR = \frac{\log_e W_f - \log_e W_i}{t_f - t_i} \times 100$$

where W is fish weight (g) and t is time (days) and the subscripts "i" and "f" are initial and final references.

Condition factor (K)

$$K = \frac{W \times 100}{FL^3}$$

where W=fish wet weight (g) and FL=fork length (mm). Survival data were arc-sine transformed prior to analysis of variance.

Statistical Analysis

Water quality parameter treatment means for each treatment combination were compared by analysis of variance (ANOVA) using PROC GLM (SAS Institute, Inc. 1989b). Means were further separated by least significant difference (LSD) tests if the ANOVA was significant. Differences were declared significant at $\alpha < 0.05$.

Water quality data were further interpreted by multivariate statistical techniques (factor analysis and multiple regression). Factor analysis (PROC FACTOR) was used to reduce the dimensionality of the data set (SAS Institute, Inc. 1989a). Distributional characteristics of each water quality parameter were evaluated by examination of parameter skewness, kurtosis, and the W statistic of the Shapiro-Wilk test (PROC UNIVARIATE). Values were \log_{10} transformed to stabilize the variance between parameters and to normalize the distribution of some parameters.

A correlation matrix was used to calculate the loadings from each water quality parameter into the two common factors explaining the most variance. Factor loadings of $\geq 75\%$ of the highest value were retained for factor interpretation. The factor pattern was used to infer the most important ecological processes affecting water quality in this experiment. Factor scores were extracted from each observation, consisting of treatment and time identifiers and water quality concentrations (PROC SCORES), and compared by ANOVA (PROC GLM).

Water quality data were further examined by multiple regression analysis. Models were hypothesized to explain the extent to which environmental and treatment variables (predictors) influenced measured water quality (response)

variables. Multiple regression models were prepared for prediction of concentrations of dissolved oxygen at dawn (DO_AM), carbon dioxide at dawn (CO₂), total ammonia-nitrogen (TAN), nitrite plus nitrate nitrogen (NO_x), volatile suspended solids (VSS), fixed suspended solids (FSS), and chlorophyll plus phaeophytin (C+P). Additional multiple regression models were prepared for prediction of the two factors extracted by factor analysis that explained the most variance.

Treatment and environmental effects were decomposed into a number of quantitative (continuous) variables.

- (1) **FD_RT**: Daily dry matter input rate (kg DM ha⁻¹ d⁻¹).
- (2) **TEMP**: Average weekly water temperature calculated as 0.5x the sum of the weekly maximum and minimum water temperature (C).
- (3) **PAR**: Photosynthetically-active radiation (ly d⁻¹) on the sampling day.
- (4) **AV_PAR**: Average photosynthetically-active radiation (ly d⁻¹) for the week preceding water quality sample collection.
- (5) **DISTURB**: Variable quantifying the number of minutes that sediment was disturbed per week. Sediment in tanks stocked with fish was assumed subject to continuous disturbance.
- (6) **N**: Variable indicating nitrogen content (%) of input.
- (7) **C**: Variable indicating the carbon content (%) of input.
- (8) **C:N**: Variable indicating the carbon-to-nitrogen ratio of input.

Plots of residual values with respect to predicted concentrations were examined for evidence of systematic bias. Residual plots of four variables (TAN,

NO_x, CO₂, CP) indicated a systematic bias consisting of a positive relationship between the absolute value of residual concentration and predicted water quality variable concentration, i.e. increasing error variance. Data were transformed $[(\log_{10} \text{concentration})+1]$ to remove bias.

Parameter estimates (partial regression coefficients) were standardized (β -weights) by the following formula:

$$\beta = b_i \frac{SD_x}{SD_y}$$

where b_i is the partial regression coefficient and SD_y and SD_x are the standard deviation of the dependent and independent variables, respectively. β -weights of each predictor variable were utilized to interpret the magnitude and direction of effect (increase, decrease) on the concentration of water quality parameters and factors (Table 29).

An additional multiple regression procedure (stepwise selection) was conducted for each water quality parameter and the two primary factors to determine the rank of predictor variables and the proportional contribution of each to the adjusted model R^2 (Table 30).

RESULTS

Water Quality

Dissolved Oxygen. Sediment disturbance was the most important predictor of DO at dawn, explaining 33% of the variance of this parameter. In tanks disturbed by fish, average DO at dawn was more than 2 mg L⁻¹ greater than that of other

Table 17. Mean (\pm SD) early morning and late afternoon dissolved oxygen (mg L^{-1} and % saturation) in organically-enriched aquatic mesocosms arranged by disturbance treatment, input type, and dry matter enrichment rate ($N=720$).

Treatment	Dissolved Oxygen			
	Early Morning		Late Afternoon	
	(mg L^{-1})	(% saturation)	(mg L^{-1})	(% saturation)
Fish	4.65 (1.25) a ¹	60 (14)	8.17 (2.17)	111 (28)
Chain	2.49 (1.57) b	31 (18)	8.71 (2.38)	118 (33)
Control	2.32 (1.68) b	29 (20)	8.39 (2.71)	114 (38)
Catfish Feed	2.79 (1.68)	35 (20)	8.73 (2.41) b	119 (34)
Distiller's Grains	2.76 (1.89)	35 (23)	8.08 (2.32) c	110 (32)
Chicken Manure	3.80 (1.82)	48 (22)	9.42 (2.78) a	128 (38)
Rice Bran	3.28 (1.79)	42 (22)	7.47 (1.67) d	101 (21)
100 (JUN)	3.56 (1.54) b	46 (19)	8.77 (2.76) b	120 (39)
125 (JUL)	2.87 (1.65) c	38 (22)	9.32 (2.59) a	132 (37)
150 (AUG)	2.10 (1.52) e	28 (21)	7.26 (1.71) c	104 (25)
175 (SEPT)	2.37 (1.49) d	31 (19)	7.25 (2.07) c	98 (28)
150 (OCT)	4.87 (1.59) a	57 (18)	9.52 (1.85) a	118 (24)

¹ Means within a column followed by the same letter not significantly different ($P>0.05$); comparisons valid within treatment type only.

disturbance treatments (Table 17). Average DO at dawn was not different in tanks in which sediment was disturbed by dragging with a chain or left undisturbed. At no time did average DO at dawn in tanks with fish fall below the critical concentration of 2 mg L^{-1} , whereas average DO at dawn was below this threshold for 9 and 10 weeks during the middle of the summer in CH and CL tanks, respectively (Figure 19).

Water temperature was the second most important predictor of DO at dawn, explaining 26% of the variance. Temperature and the solubility of DO are inversely related, and temperature regulates metabolic processes. Temporal trends in dissolved oxygen for all treatments were generally inverse of temperature and enrichment rate trends. Dissolved oxygen was lowest in the middle of the summer when temperature was near maximum and highest near the end of the study with the onset of cool weather (Figure 19).

Other predictors (C, FD_RT, N and AV_PAR) had smaller yet significant effects on DO at dawn. The sign of the β -weights for these predictors indicated that DO at dawn was inversely related to both input carbon and nitrogen content and dry matter enrichment rate. Average DO at dawn was highest in tanks enriched by chicken manure and lowest in tanks enriched by catfish feed or distiller's grains. Contrary to expectations, dry matter enrichment rate was a relatively poor predictor of DO at dawn.

The multiple regression model explained only 28% of the variance of DO at dusk, with N and PAR the most important predictors. DO at dusk was directly

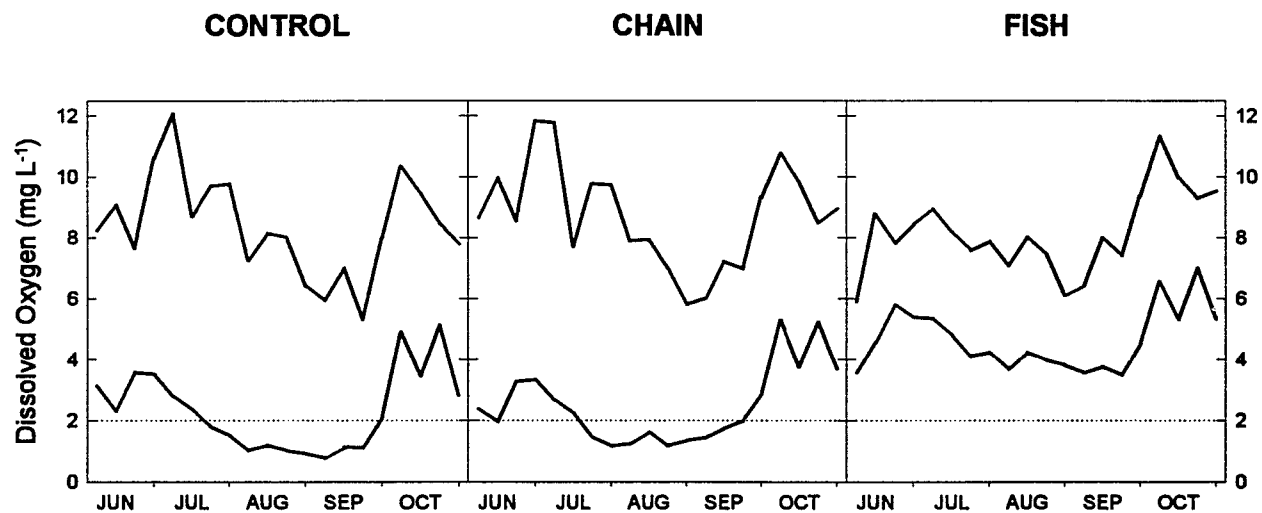


Figure 19. Dissolved oxygen at dawn (bottom line) and at dusk (top line) by time enriched aquatic mesocosms in which sediment was not disturbed (CONTROL), disturbed weekly with a chain (CHAIN), or disturbed by fish (FISH).

related to N content of inputs (greatest in tanks enriched with chicken manure and lowest in tanks enriched with rice bran) and solar radiation (PAR). Dissolved oxygen at dusk was highest in tanks in which sediment was disturbed weekly by a chain and lowest in tanks with fish.

Variance in the difference between DO at dawn and dusk (DELTA_DO) was explained primarily by sediment disturbance. DELTA_DO was lowest in tanks with fish and highest in undisturbed control tanks. DELTA_DO was also directly related to solar radiation (PAR) on the sampling day, and inversely related to C:N content of inputs and dry matter enrichment rate.

Carbon Dioxide. Carbon dioxide at dawn was calculated from equilibrium relationships between alkalinity components and pH, compensated by temperature (APHA *et al.* 1993). Dry matter enrichment rate was the most important predictor of CO₂ at dawn, explaining 23% of the variance of this parameter. CO₂ at dawn increased directly in relation to dry matter enrichment rate. The input C content was the second most important predictor of CO₂ concentration. CO₂ was highest in tanks enriched by catfish feed and distiller's grains, and lowest in tanks enriched by chicken manure (Table 18).

Although sediment disturbance was the most important predictor of DO at dawn, it was only the third most important predictor of CO₂ at dawn. Tanks with sediment disturbed by fish had significantly lower early morning CO₂ concentration than that of other disturbance treatments. Additional predictor variables explained smaller, yet significant proportions of variance. CO₂ at dawn was directly related to

Table 18. Mean (\pm SD) total alkalinity (mg L^{-1} as CaCO_3), early morning pH and calculated CO_2 concentration (mg L^{-1}) in enriched aquatic mesocosms arranged by disturbance treatment, input type and dry matter enrichment rate (N=120).

Treatment	Total Alkalinity	pH	CO_2
Fish	193 (36)	8.00 (0.20)	8.2 (5.5)
Chain	202 (36)	7.81 (0.27)	13.4 (8.4)
Control	189 (37)	7.78 (0.23)	13.7 (8.9)
Catfish Feed	191 (34)	7.69 (0.30)	16.7 (11.1)
Distiller's Grains	194 (30)	7.83 (0.21)	12.3 (6.7)
Chicken Manure	207 (46)	8.10 (0.25)	7.3 (5.4)
Rice Bran	187 (33)	7.88 (0.19)	10.6 (4.9)
100 (JUN)	156 (12)	8.18 (0.11)	4.3 (1.9)
125 (JUL)	168 (17)	7.77 (0.27)	11.7 (7.3)
150 (AUG)	193 (20)	7.74 (0.24)	14.3 (5.4)
175 (SEP)	216 (21)	7.83 (0.28)	13.6 (7.7)
150 (OCT)	242 (22)	7.86 (0.29)	14.8 (10.7)

solar radiation (PAR, AV_PAR) and input quality (N, C:N), and inversely related to temperature.

Dissolved Inorganic Nitrogen (DIN). Dissolved inorganic nitrogen was partitioned into fractions comprising total ammonia-nitrogen (TAN) and nitrite plus nitrate-nitrogen ($\text{NO}_x\text{-N}$). Sediment disturbance was the most important predictor of TAN, which decreased with increasing disturbance and was significantly different between disturbance treatments (Table 19). Dry matter enrichment rate was the next most important predictor of TAN, which increased directly with enrichment rate. Maximum TAN concentrations occurred in mid-September, coincident with maximum dry matter enrichment rate (Figure 20). TAN was generally inversely related to C:N ratio of inputs and was greatest in tanks enriched by distiller's grains and lowest in tanks enriched by rice bran.

The C:N ratio of inputs was the most important predictor of oxidized nitrogen ($\text{NO}_x\text{-N}$) concentration, explaining 48% of the variance of this parameter. $\text{NO}_x\text{-N}$ concentration was inversely related to C:N ratio of inputs and was greatest in tanks enriched by chicken manure and lowest in tanks enriched by rice bran. Sediment disturbance was a less important predictor of $\text{NO}_x\text{-N}$, explaining only 8% of variance. $\text{NO}_x\text{-N}$ in tanks with sediment disturbed by fish was three times greater than that in tanks of other disturbance treatments. $\text{NO}_x\text{-N}$ comprised 70% of DIN in tanks with fish, but only 25-30% of DIN in tanks of other disturbance treatments. The temporary accumulation of $\text{NO}_x\text{-N}$ (probably $\text{NO}_2^-\text{-N}$) in tanks with fish during the

Table 19. Mean (\pm SD) (n=10) total ammonia-nitrogen (TAN), nitrite+nitrate-nitrogen ($\text{NO}_x\text{-N}$), and total dissolved inorganic-nitrogen in enriched tank mesocosms arranged by disturbance treatment, input type, and dry matter enrichment rate (N=120).

Treatment	Dissolved Inorganic Nitrogen (mg L ⁻¹)		
	TAN	$\text{NO}_x\text{-N}$	Total
Fish	0.64 (0.29) c ¹	1.50 (1.63) a	2.14 (1.65) a
Chain	1.11 (0.72) b	0.49 (0.59) b	1.60 (0.87) b
Control	1.46 (1.05) a	0.50 (0.69) b	1.96 (1.28) ab
Catfish Feed	1.15 (0.81) ab	0.93 (0.77) b	2.08 (0.89) b
Distiller's Grains	1.47 (1.15) a	0.59 (1.17) b	2.06 (1.45) b
Chicken Manure	1.00 (0.56) bc	1.69 (1.50) a	2.69 (1.43) a
Rice Bran	0.66 (0.36) c	0.11 (0.19) c	0.78 (0.40) c
100 (JUN)	0.67 (0.50) b	0.47 (0.58) b	1.14 (0.69) b
125 (JUL)	0.98 (0.43) ab	0.97 (1.40) ab	1.95 (1.33) a
150 (AUG)	1.17 (0.78) a	0.73 (0.79) ab	1.90 (1.04) a
175 (SEP)	1.25 (1.07) a	1.18 (1.70) a	2.43 (1.72) a
150 (OCT)	1.28 (1.02) a	0.80 (0.98) ab	2.09 (1.31) a

¹ Means within a column followed by the same letter not significantly different ($P>0.05$); comparisons valid within treatment type only.

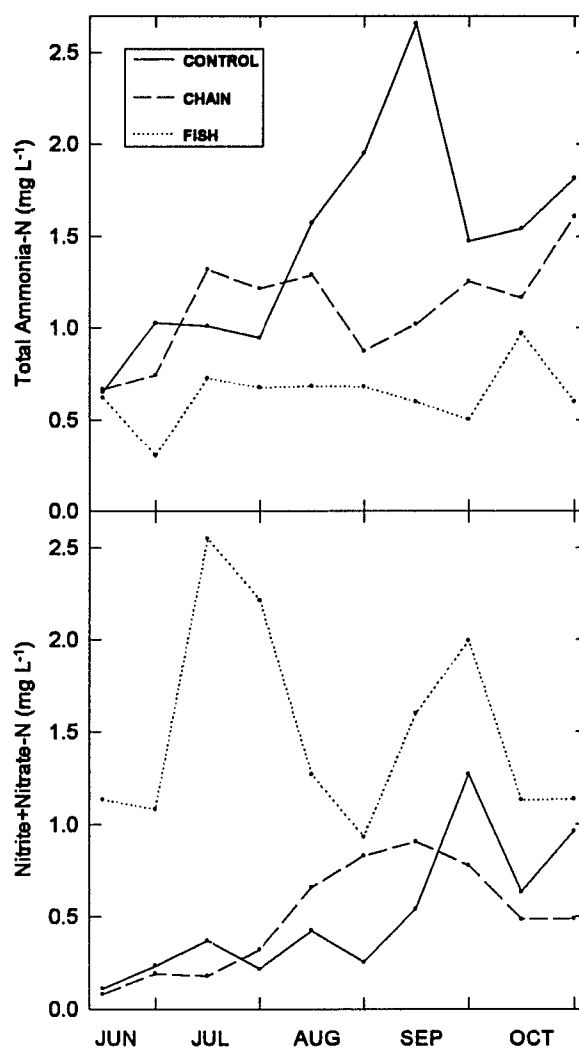


Figure 20. Total ammonia-nitrogen and nitrite+nitrate nitrogen with respect to time by disturbance treatment in enriched aquatic mesocosms.

first part of the study reflected the typical process lag associated with the initiation of nitrification in which the induction of ammonia oxidation precedes nitrite oxidation.

Solids. Total suspended solids (TSS) were partitioned into an organic or volatile fraction (VSS) and an inorganic or fixed fraction (FSS). Average TSS was greatest in tanks with fish and was more than 50% greater than average TSS in tanks disturbed with a chain or undisturbed controls (Table 20). Average TSS in tanks disturbed with a chain and in undisturbed controls were not significantly different.

Sediment disturbance was the most important predictor of FSS, explaining 56% of variance. Average FSS in tanks with fish was more than 3.5x greater than in tanks of other disturbance treatments. FSS comprised 62% of TSS in tanks with fish compared with 19% and 27% of TSS in tanks of the control and chain treatments, respectively. FSS in tanks with sediment disturbed by a chain was only slightly greater than undisturbed controls. Water temperature was a smaller yet significant predictor of FSS. FSS reached a maximum around the sixth week of the study (July) and declined linearly thereafter until termination of the experiment (Figure 21). FSS was similar among input types, although the FSS in tanks enriched with chicken manure was significantly greater than that in tanks enriched with rice bran.

N content of inputs was the most important predictor of VSS, explaining 27% of variance (Table 16). VSS was directly related to N content of inputs and was highest in tanks enriched by chicken manure and lowest in tanks enriched by rice bran. Dry matter enrichment rate explained a similar (24%) proportion of variance in VSS. VSS was directly related to dry matter enrichment rate. VSS was inversely

Table 20. Mean (\pm SD) (n=10) volatile, fixed, and total suspended solids in enriched tank mesocosms arranged by disturbance treatment, input type and dry matter enrichment rate (N=120).

Treatment	Suspended Solids (mg L ⁻¹)		
	Volatile	Fixed	Total
Fish	56 (26) b ¹	91 (39) a	146 (48) a
Chain	69 (35) a	25 (25) b	94 (43) b
Control	77 (43) a	19 (19) b	96 (50) b
Catfish Feed	86 (37) a	44 (39) ab	130 (41) a
Distiller's Grains	51 (32) b	52 (48) ab	103 (46) b
Chicken Manure	83 (36) a	55 (43) a	139 (56) a
Rice Bran	40 (17) c	37 (45) b	77 (45) c
100 (JUN)	36 (15) a	50 (55) a	83 (56) c
125 (JUL)	60 (24) b	56 (56) a	115 (60) ab
150 (AUG)	77 (44) c	54 (44) a	131 (55) a
175 (SEP)	89 (37) c	37 (30) ab	126 (47) ab
150 (OCT)	74 (32) d	31 (20) b	105 (27) b

¹ Means within a column followed by the same letter not significantly different ($P>0.05$); comparisons valid within treatment type only.

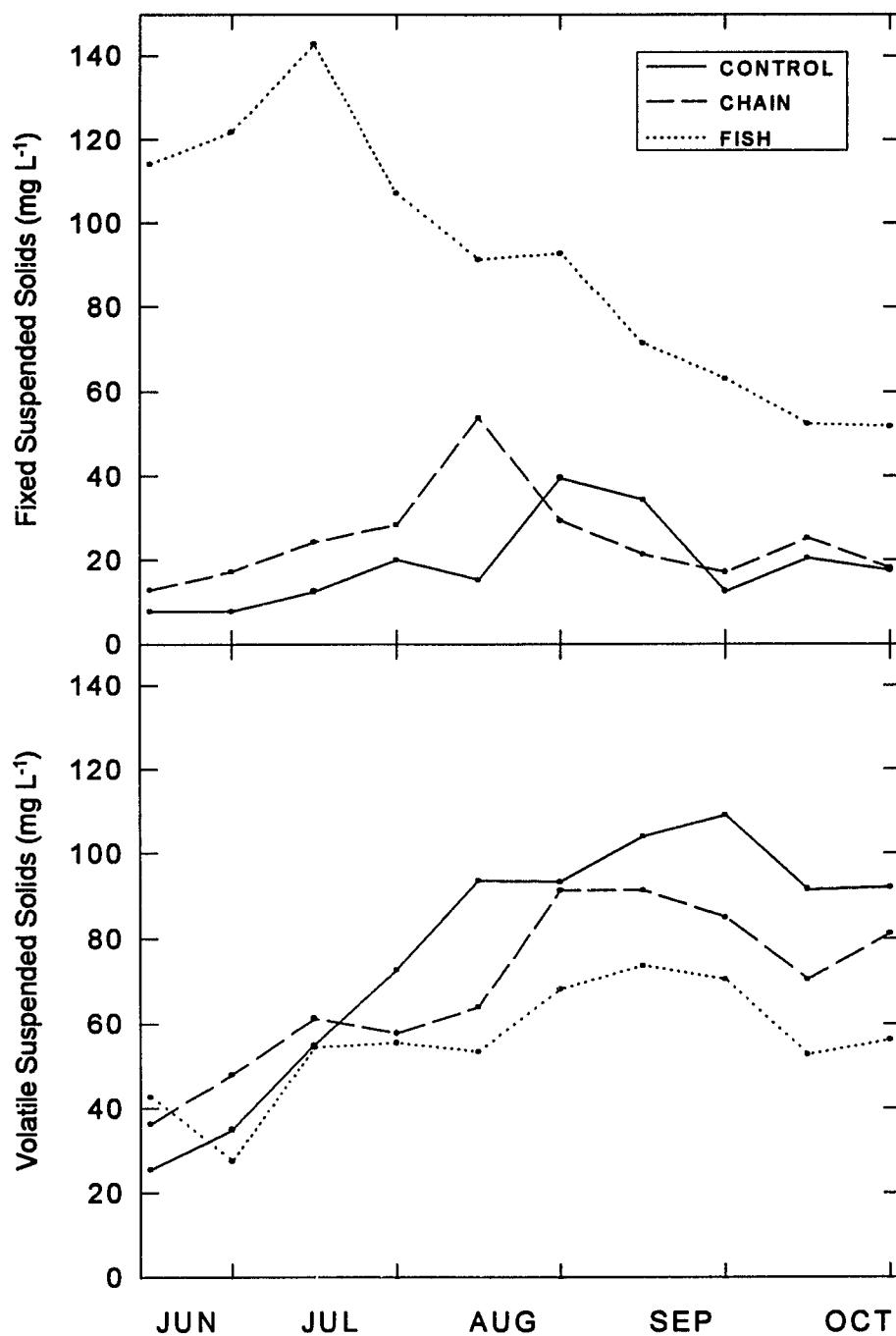


Figure 21. Fixed and volatile suspended solids with respect to time by disturbance treatment in enriched aquatic mesocosms.

related to sediment disturbance, although differences between disturbance treatments were relatively small.

Chlorophyll+Phaeophytin. Chlorophyll and phaeophytin concentration was inversely related to C:N ratio of inputs, which explained 30% of the variance of this parameter. C+P was also inversely related to sediment disturbance, which explained 16% of the variance, and was significantly lower in tanks with fish than that of other disturbance treatments (Table 21). C+P was directly related to dry matter enrichment rate, which contributed an additional 16% of variance explained.

Total Alkalinity. Total alkalinity accumulated in the water of all tanks. The rate of alkalinity accumulation was derived from linear regression models for each treatment combination that fitted total alkalinity concentration with respect to time as the independent variable. All models were significant ($P < 0.001$) with a mean adjusted coefficient of determination of 0.92 (range=0.82-0.98, $n=12$). The slope of the regression equation was equivalent to the rate of accumulation of total alkalinity and ranged from 3.4 to 7.2 mg L⁻¹ d⁻¹ as CaCO₃ (mean=5.5 mg L⁻¹ d⁻¹). Total alkalinity accumulation rate was significantly greater ($P=0.0168$) in tanks receiving chicken manure (7.5 mg L⁻¹ d⁻¹) than in tanks receiving other organic inputs. The accumulation rate was not significantly different among disturbance treatments.

Factor Analysis

Two common factors explained 66% of the variance in water quality data (Table 22). Concentrations of chlorophyll *a* + phaeophytin (C+P), total ammonia-nitrogen (TAN), early morning dissolved oxygen (DO_AM), carbon dioxide (CO₂),

Table 21. Mean chlorophyll plus phaeophytin (\pm SD) concentration ($\mu\text{g L}^{-1}$) in organically enriched mesocosms arranged by disturbance treatment, input type and dry matter enrichment rate.

Treatment	Chlorophyll + Phaeophytin
Fish	372 (134)
Chain	692 (355)
Control	846 (715)
Catfish Feed	792 (424)
Distiller's Grains	784 (749)
Chicken Manure	685 (323)
Rice Bran	285 (139)
100 (JUN)	357 (147)
125 (JUL)	472 (224)
150 (AUG)	726 (420)
175 (SEP)	818 (466)
150 (OCT)	813 (815)

and volatile suspended solids (VSS) loaded most heavily into Factor 1, which explained 39% of the variance in water quality data. Factor 1 was interpreted with respect to common factor loadings by water quality variables (Table 22) as follows: high (+) algal biomass (C+P) resulted in low (-) concentrations of early-morning dissolved oxygen (DO_AM) and high (+) concentrations of early-morning carbon dioxide (CO₂). Thus, Factor 1 represents whole-tank respiration, primarily due to phytoplankton. More specifically, Factor 1 represents the amplitude of diel respiratory fluctuation. TAN and DO_AM were inversely correlated (Table 15) suggesting that net ammonium production in the tank was enhanced under conditions of low DO_AM.

Sediment disturbance was the best predictor of whole-tank respiration (Factor 1) in a multiple regression model (Table 23). The sign of the β -weight indicates that whole-tank respiration was inversely related to sediment disturbance. Factor 1 was significantly different among all sediment disturbance treatments (Table 24). Mean factor score was lowest in tanks stocked with fish, intermediate in tanks in which sediment was disturbed by a chain, and highest in tanks in which sediment was undisturbed (control).

Dry matter enrichment rate (FD_RT) was also an important predictor of whole-tank respiration (Table 23). Whole-tank respiration increased in direct relation to dry matter enrichment rate (Table 24). Factor 1 was maximum in September, coincident with maximum dry matter enrichment rate and was significantly greater than that of all other enrichment rates.

Table 22. Loadings of water quality variables on, and variance explained by, common factors.

Variable	Factor 1	Factor 2
C+P	0.81¹	0.44
VSS	0.63	0.52
FSS	-0.38	0.71
TAN	0.76	-0.07
NO _x	-0.00	0.86
CO ₂	0.65	-0.12
DO_AM	-0.72	0.38
Variance Explained (%)	39	27

¹ Bold face indicates most important ($\geq 75\%$ of maximum) factor loadings.

Table 23. Partial coefficients of determination ($R^2 \times 100$) and sign of the standardized estimate (β -weight) of predictor variables used in a multiple regression model for the two factors explaining the most variance in water quality data.

Predictor Variable	Factor 1	Factor 2
DISTURB	38.5 (-)	18.7 (+)
FD_RT	19.6 (+)	2.2 (+)
C:N	0.7 (-)	45.2 (-)
C	8.7 (+)	0.7 (-)
N		
T		
PAR	0.7 (+)	
AV_PAR	4.0 (+)	
Total Explained	80.7	67.7

Table 24. Mean factor scores by disturbance treatment, input type and feeding rate (month) with results of multicomparison (LSD) test.

Treatment	Factor 1	Factor 2
Control	0.55 a ¹	-0.40 b
Chain	0.34 b	-0.22 b
Fish	-0.86 c	0.60 a
Catfish Feed	0.44 a	0.43 b
Distiller's Grain	0.33 a	-0.21 c
Chicken Manure	-0.11 b	0.76 a
Rice Bran	-0.62 c	-0.99 d
100 (JUN)	-0.93 c	-0.31 a
125 (JUL)	-0.06 b	0.04 a
150 (AUG)	0.44 ab	-0.06 a
175 (SEP)	0.46 a	0.15 a
150 (OCT)	0.02 ab	0.18 a

¹ Means with the same letter within a column not significantly different ($P>0.05$). Comparisons within treatment type only.

Temporal variation in whole-tank respiration between treatment types was similar and reflected dry matter enrichment rate (Figure 22, Figure 23). Whole-tank respiration in tanks with fish was lower than that of all other treatments throughout the experiment. Whole-tank respiration in undisturbed controls was greater than all other treatments from August until the end of the study (October).

Whole-tank respiration of tanks enriched with rice bran was lower than that of all other input types throughout the study. Temporal variation of whole-tank respiration was similar for other input types. Input characteristics (C:N, C, N) were relatively unimportant predictors of whole-tank respiration in the multiple regression model.

Oxidized nitrogen (NO_x) and fixed suspended solids (FSS) loaded most heavily into Factor 2, which explained 27% of the variance in water quality data (Table 22). Factor 2 was interpreted as the suspension of organic (VSS) and soil (FSS) particles by disturbance of the sediment by fish, which increased nitrification (NO_x) in the water column.

The C:N ratio of inputs was the most important predictor of nitrification associated with suspended particles (Table 23). The sign of the β -weight and regression analysis indicated that factor 2 was inversely related to C:N ratio (Figure 24). Mean factor 2 scores were greatest in tanks enriched with chicken manure (C:N=5.0) and lowest in tanks enriched with rice bran (C:N=17.8) (Table 24). The relative importance of nitrification associated with suspended particles increases when input C:N ratio is less than 10 (Figure 24).

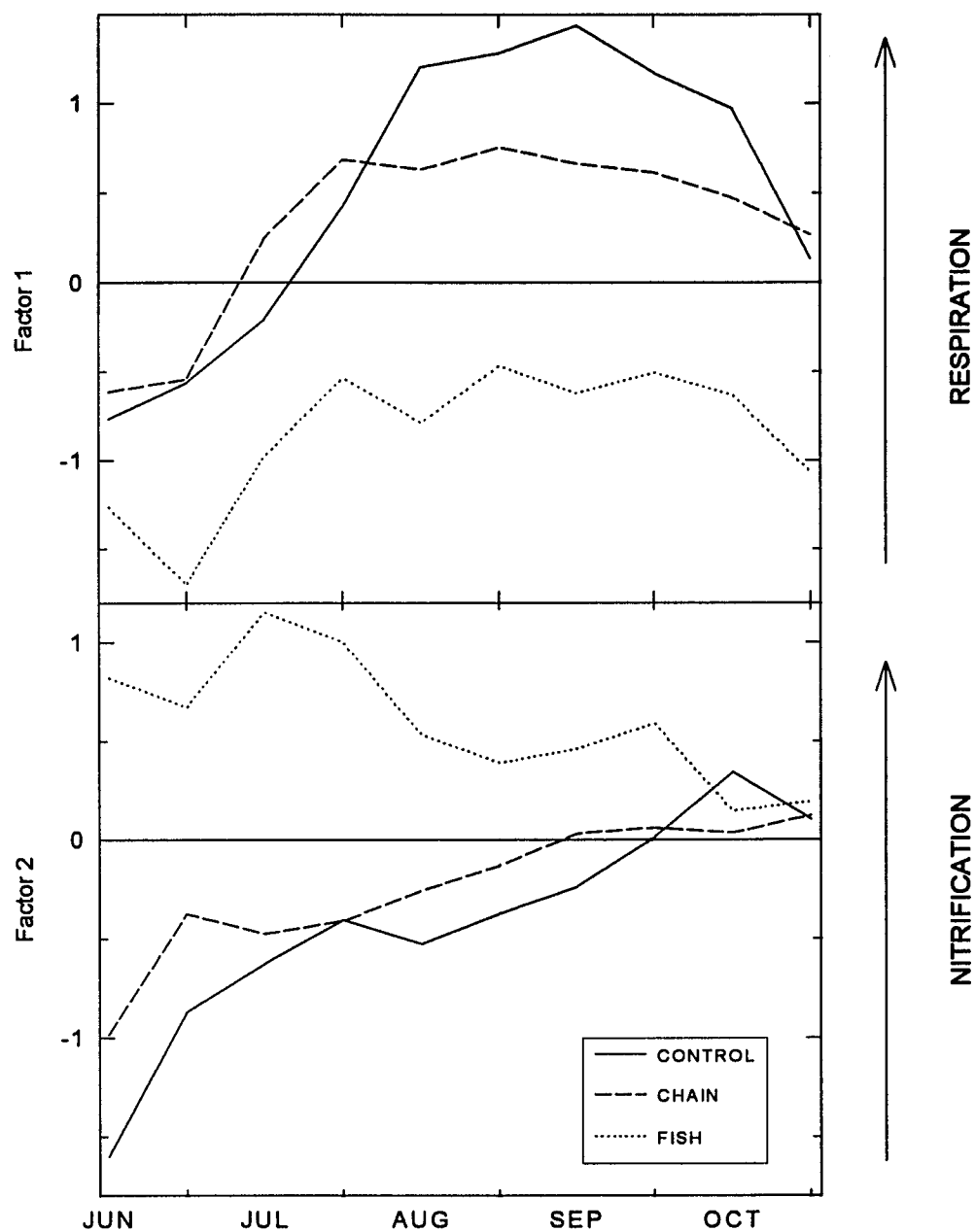


Figure 22. Mean scores extracted by factor analysis of important water quality processes in enriched aquatic mesocosms arranged by disturbance treatment with respect to time.

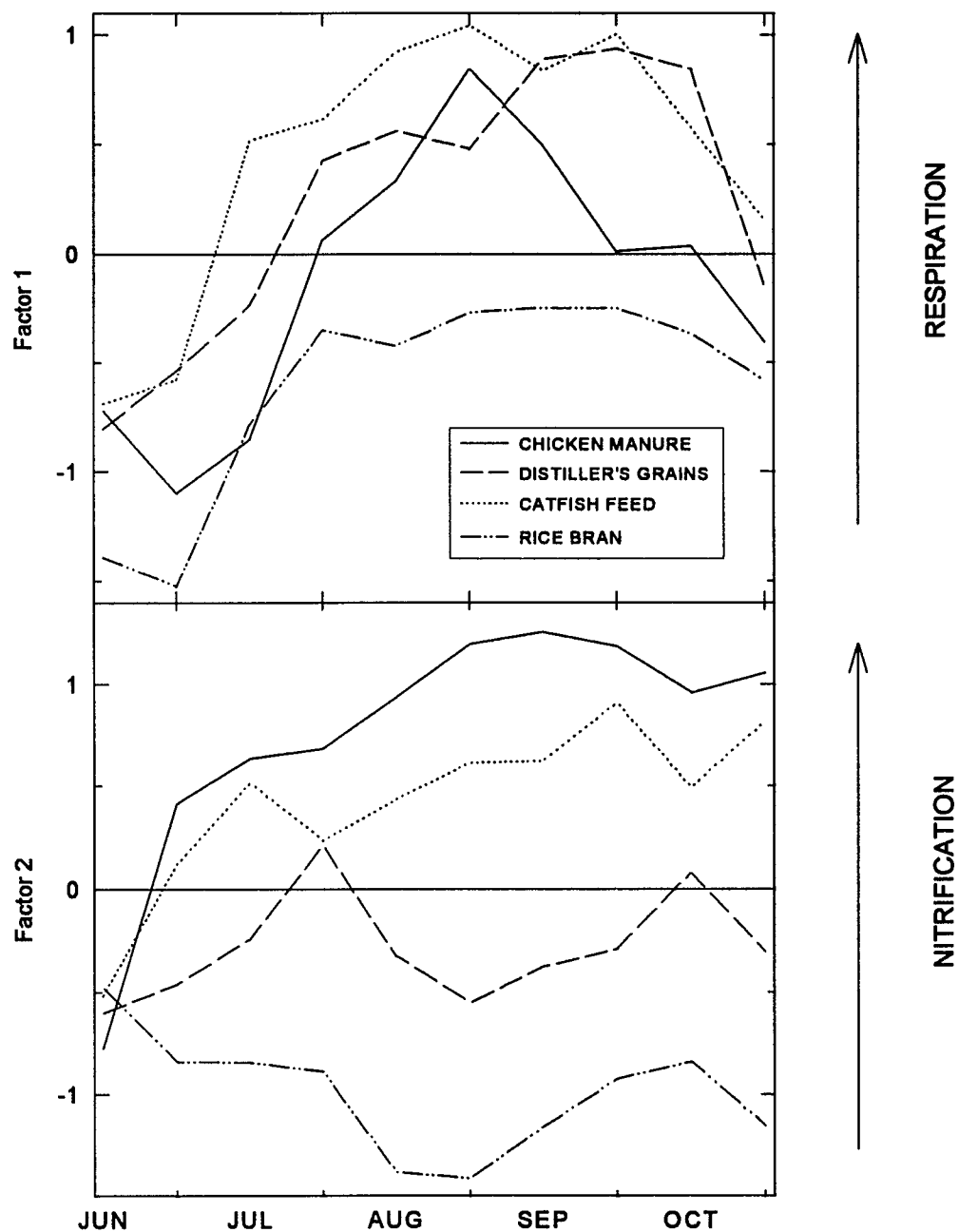


Figure 23. Mean scores extracted by factor analysis of important water quality processes in enriched aquatic mesocosms arranged by input type with respect to time.

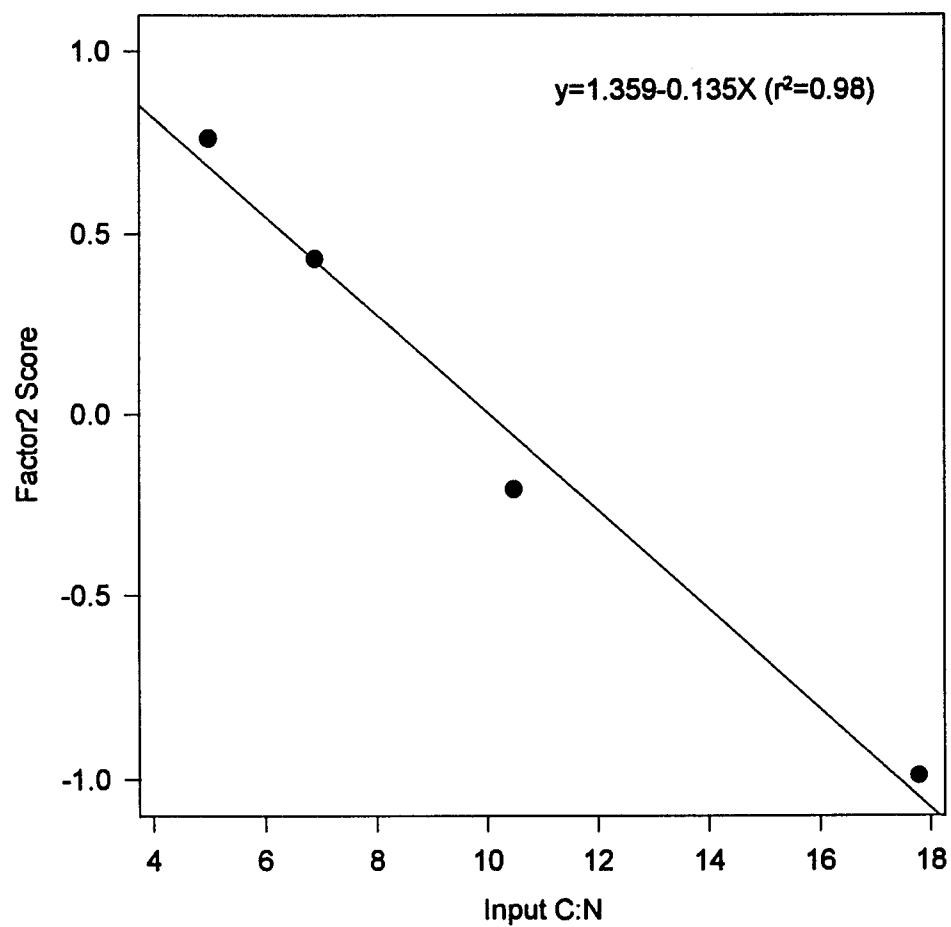


Figure 24. Regression of mean factor score for Factor 2 as a function of input C:N ratio.

Sediment disturbance was the second most important predictor of nitrification associated with suspended particles. Differences in nitrification associated with suspended particles (Factor 2) among disturbance treatments were less important than differences among input types. Mean factor 2 scores were greatest in tanks stocked with fish. Factor 2 scores were greater in tanks in which sediment was disturbed with a chain than in tanks in which sediment was left undisturbed, although this difference was not significant.

Sediment Physical Characteristics

Organic matter accumulation on the surface of sediments in tanks with fish was negligible (<5 mm) over the course of the experiment (Figure 25). Organic matter accumulation on the surface of sediments disturbed with a chain or on undisturbed (control) sediments increased through time and was substantially greater than that on sediments in tanks with fish. The rate of increase in thickness of the flocculent layer of organic matter was lower in tanks in which sediment was disturbed by a chain when compared to undisturbed sediment.

Despite these apparent differences in organic matter accumulation, the distribution of organic and mineral matter and water in the upper 5 cm of sediment was similar among disturbance treatments (Figure 26). The proportion of organic matter was <3% in the upper 5 cm of sediments collected from tanks of all disturbance treatments and increased slightly with sediment depth. The proportion of mineral matter was approximately one order of magnitude greater than that of organic matter in the upper 5 cm of sediments and increased with depth, although it

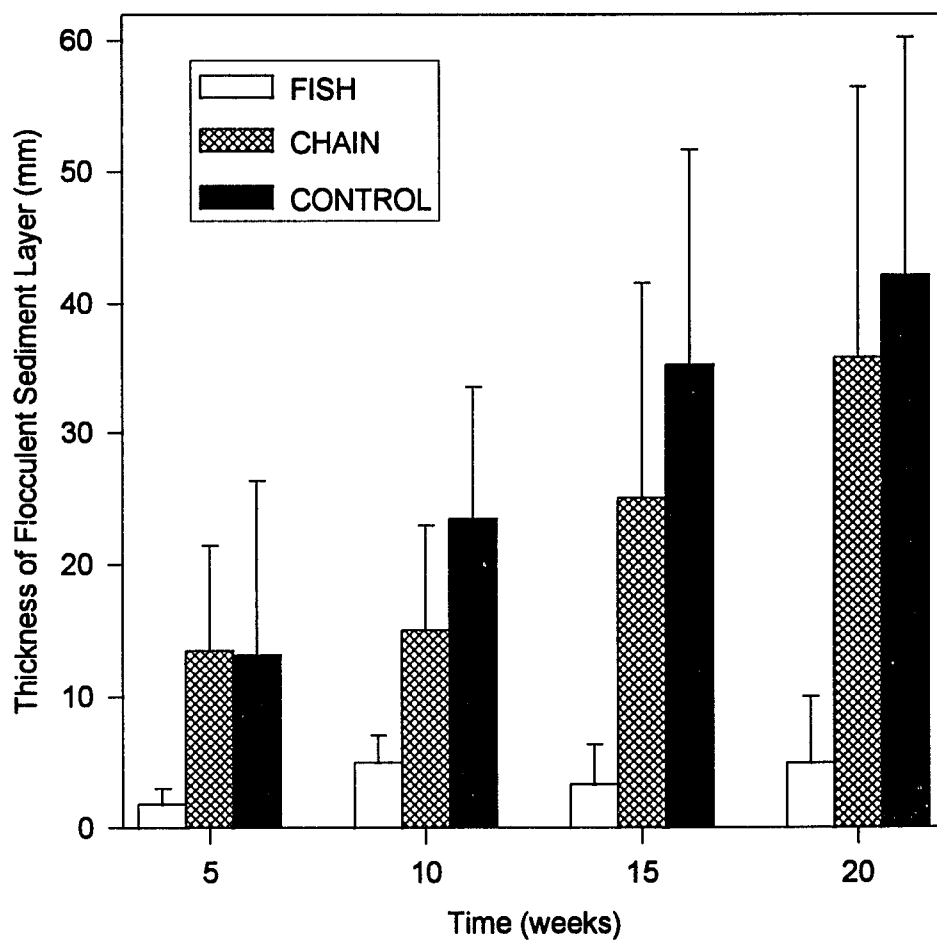


Figure 25. Mean (\pm SD) accumulation of flocculent organic layer on sediment according to disturbance treatment.

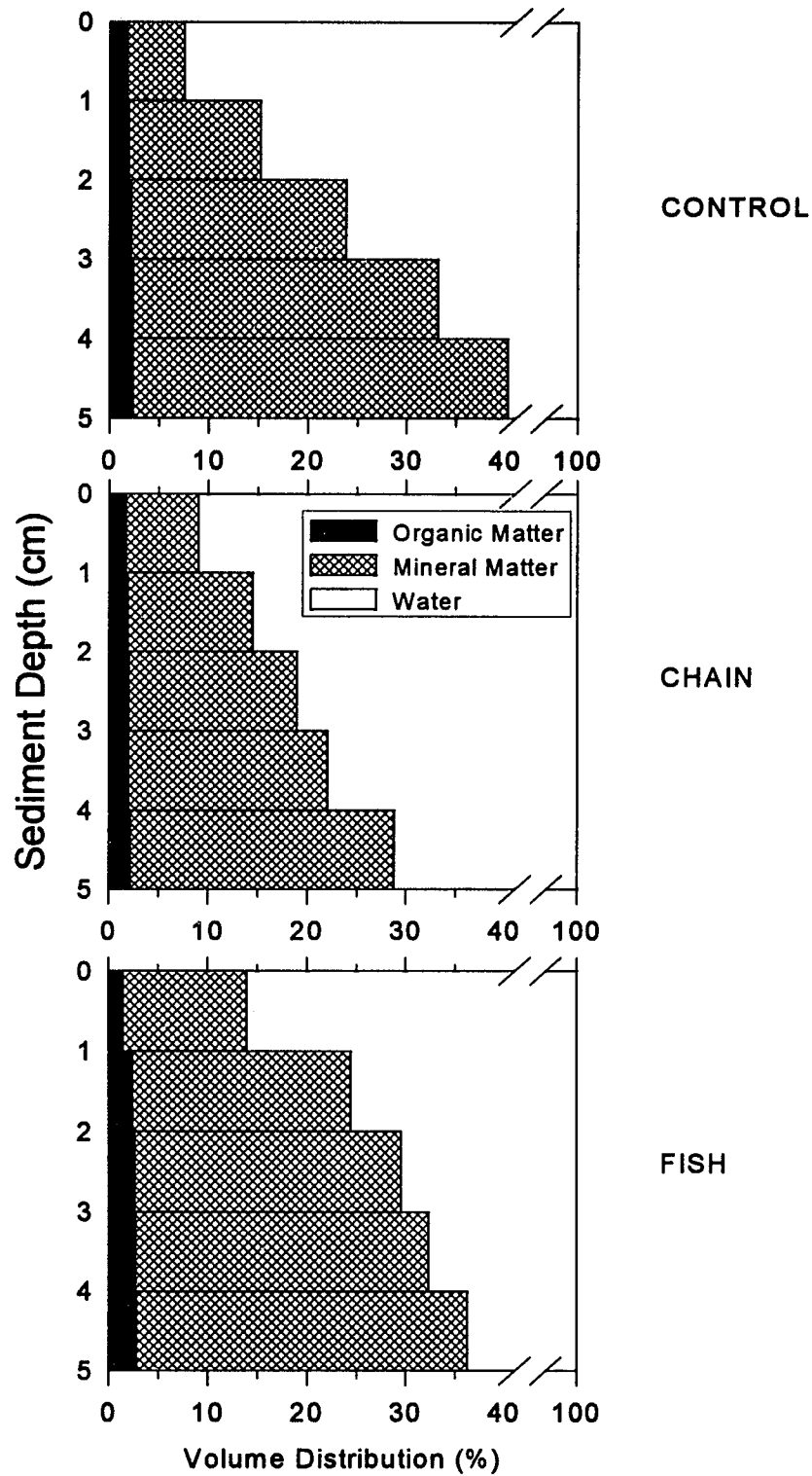


Figure 26. Volume distribution of organic matter, mineral matter, and water in the top 5 cm of sediment as a function of sediment disturbance.

accounted for <40% of sediment volume. The majority of sediment volume in the surface 5 cm was water and decreased from 90-95% in the surface 1 cm to 60-70% in the 4-5 cm layer. As the frequency of sediment disturbance increased the proportion of water in the surface 2 cm decreased.

Sediment Solute Flux

The sediment oxygen demand (SOD) in tanks with fish was approximately 50% that of control tanks (Table 25). The SOD of tanks disturbed by a chain was 10% lower than that of undisturbed control tanks. SOD was not significantly affected by organic input type, although SOD in tanks receiving chicken manure was 17-27% lower than in tanks receiving other inputs. Although disturbance treatment and input type did not have a significant effect on CO₂ flux, the same relative pattern described for SOD was upheld. TAN flux from the sediment to the water was identical for tanks disturbed with a chain and undisturbed control tanks. TAN flux in tanks with fish was 28% less than that in tanks of other disturbance treatments. TAN flux in tanks receiving rice bran was significantly lower than tanks receiving distiller's grains and catfish feed, which were nearly identical. The interaction between sediment disturbance treatment and input type was not significant for each solute for which flux rates were measured.

Fish Growth and Production

Although evaluation of fish growth was not the principal focus of this study, data collected at experiment termination (harvest) allowed an assessment of the

Table 25. Mean (\pm SD) solute (O_2 , CO_2 , and TAN) flux ($\text{mg m}^{-2} \text{h}^{-1}$) between water and sediment determined in intact sediment cores incubated for 4 hours at 30 C. Negative sign indicates flux from water to sediment; positive sign indicates flux from sediment to water.

Treatment	N	Solute		
		O_2	CO_2	TAN
Fish	12	-56 (10) b ¹	+109 (68) b	+29 (9) a
Chain	12	-91 (24) a	+134 (88) ab	+40 (23) a
Control	12	-103 (26) a	+191 (104) a	+41 (14) a
Catfish Feed	9	-86 (36) a	+164 (127) a	+45 (22) a
Distiller's Grains	9	-94 (31) a	+195 (73) a	+46 (15) a
Chicken Manure	9	-69 (15) b	+95 (58) a	+33 (9) ab
Rice Bran	9	-83 (28) ab	+126 (70) a	+23 (6) b

¹ Means in the same column with the same letter are not different ($P>0.05$); comparisons valid within treatment type only.

relative growth performance and fish production in tanks enriched with variable-quality organic inputs.

Growth performance and condition of 'koi' carp was superior to that of channel catfish across all input types (Table 26). Final biomass and net fish yield (NFY) of 'koi' carp exceeded that of channel catfish in tanks enriched by distiller's grains, rice bran and chicken manure and were similar for fish in tanks enriched by pelleted feed (Table 27). Survival was good across all treatments, although 'koi' survival was superior to that of channel catfish. Channel catfish survival was lowest in tanks enriched by distiller's grains.

Growth performance and production of fish in tanks enriched by catfish feed was greater ($P < 0.05$) than that of any other input type. Growth performance of 'koi' carp receiving distiller's grains was good ($SGR = 2.05\% \text{ d}^{-1}$) suggesting the suitability of this material as a direct pond input. However, utilization of distiller's grains by channel catfish was poor. Growth performance and NFY of 'koi' carp and channel catfish receiving chicken manure were extremely low. Channel catfish did not grow in tanks enriched with chicken manure, although there was no mortality.

DISCUSSION

Sediment disturbance improves water quality, although it was not possible to specifically evaluate the effect on fish growth and production in this study. In pond culture, DO is the water quality variable most likely to impact fish production. In this study, tanks with fish, specifically 'koi' carp, which are well-known for benthic feeding activity, improved the DO regime in organically-enriched aquatic mesocosms.

Table 26. Least squares means (\pm SE) final body weight, daily weight gain, specific growth rate and mean (\pm SD) condition factor (K) of 'koi' carp and channel catfish fed floating catfish feed (FD), distiller's grains (DG), rice bran (RB), or chicken manure for 20 weeks.

Fish	Input Type	Final Body Weight (g)	Daily Weight Gain (g)	Specific Growth Rate (%/d)	K ²
'Koi' Carp	FD	605 (20) a ¹	4.02 (0.14) a	2.22 (0.04) a	2.31 (0.71) a
	DG	458 (20) b	2.97 (0.14) b	2.05 (0.04) b	2.24 (0.19) ab
	RB	356 (20) c	2.25 (0.14) c	1.82 (0.04) c	2.05 (0.23) b
	CM	136 (21) d	0.67 (0.15) d	1.09 (0.04) d	1.77 (0.13) c
Channel Catfish	FD	707 (29) a	4.56 (0.20) a	1.60 (0.05) a	1.25 (0.12) a
	DG	167 (36) b	0.71 (0.26) b	0.69 (0.06) b	0.93 (0.06) c
	RB	154 (34) b	0.62 (0.24) b	0.53 (0.05) b	1.04 (0.07) b
	CM	68 (25) c	0.00 (0.18) c	0.00 (0.04) c	0.91 (0.06) c

¹ Least squares means with the same letter within the same column not different ($P>0.05$); comparisons valid within fish species only.

² K=Weight (g) x 100 / Total Length (cm)³

Table 27. Least squares means (\pm SE) final biomass, net fish yield (NFY), and mean survival (\pm SD) of 'koi' carp and channel catfish cultured in tanks receiving floating catfish feed (FD), distiller's grains (DG), rice bran (RB), or chicken manure (CM) for 20 weeks.

Fish	Input Type	Biomass (kg ha ⁻¹)	NFY (kg ha ⁻¹ d ⁻¹)	Survival (%)
'Koi' Carp	FD	5803 (16) a ¹	38.7 (0.1) a	100 (0) a
	DG	4386 (15) b	28.5 (0.1) b	100 (0) a
	RB	3339 (16) c	21.1 (0.1) c	100 (0) a
	CM	1224 (16) d	5.9 (0.1) d	97.5 (5) b
Channel Catfish	FD	6100 (67) a	39.0 (0.5) a	98 (4) ab
	DG	1821 (66) b	8.5 (0.5) b	91 (20) b
	RB	1256 (67) c	4.4 (0.5) c	97 (5) b
	CM	516 (62) d	0.0 (0.4) d	100 (0) a

¹ Least squares means with the same letter within a column not different ($P>0.05$); comparisons within fish species only.

Concentrations of early morning DO rarely fell to critical levels ($<2 \text{ mg L}^{-1}$). In addition, the amplitude of diel fluctuations in DO (an index of net primary productivity) were minimal in tanks with fish. Reduced whole-tank respiration relative to other disturbance treatments was the principal mechanism for elevated early morning DO and reduced diel DO fluctuation in tanks with fish. A similar suppression of diel changes in DO due to sediment suspension by benthivorous fishes (*Ictalurus nebulosus*, *Cyprinus carpio*) was measured by Cline *et al.* (1994). The amplitude of whole-tank respiration was likely minimized by reduced phytoplankton density and more complete mineralization of organic matter. Sediment suspension by the feeding activity of fish increased non-algal turbidity that reduced light penetration and thereby limited phytoplankton density. Results of a dissolved oxygen simulation model (Smith and Piedrahita 1988) suggest that an intermediate phytoplankton density ($100\text{-}250 \text{ } \mu\text{g L}^{-1}$ chlorophyll *a*) will result in higher dissolved oxygen at dawn. However, these investigators caution that shading will decrease the oxygen production from phytoplankton and that oxygen production will be maximized by maximizing algal growth rate.

In tilapia ponds stirred twice weekly (Tuesday and Friday) by dragging the bottom with a weighted rake, DO was lower but not significantly different than that in un-stirred ponds (Costa-Pierce *et al.* 1993). In Indian carp fingerling ponds, daily dragging of a weighted nylon rope ("hora") increased turbidity, but did not significantly affect other water quality parameters measured when compared to control ponds (Beveridge *et al.*, in press). In shrimp ponds in which sediments were

suspended by daily repositioning of aerators, DO at dawn was lower, but not significantly different than that in ponds in which sediments were deposited in the center of the pond bottom (Hopkins *et al.* 1994). These results suggest that water quality improvement from sediment resuspension is variable, transitory, and strongly dependent on disturbance frequency and pond fertilization history. Water quality may be improved by frequent sediment disturbance, but infrequent disturbance may have a deleterious effect due to a transitory "spike" of reduced dissolved oxygen following suspension of organic-rich sediments. The potential improvement of water quality by sediment disturbance is less likely in newly-renovated ponds (Beveridge *et al.*, in press) when compared to ponds with a history of organic fertilization. In addition, the respiratory demand of the water column often exceeds that of the sediment in high productivity waters (Oviatt *et al.* 1986), so reduction of sediment oxygen demand would be expected to have a minimal effect on water column DO.

In research on the nutrient dynamics of nearshore marine sediments, disturbance by burrowing worms (bioturbation) increased nutrient (ammonium) flux from the sediment by 50% (Blackburn and Henriksen 1983). Carbon and nitrogen budgets and sediment-water solute flux rates were evaluated in experimental cores to which polychaete worms were added (Kristensen and Blackburn 1987, Hansen and Blackburn 1992). The presence of macrofauna increased the flux of O₂, CO₂ and TAN when compared to control (defaunated) cores, presumably by the increase in effective oxidized surface area in the sediment and by irrigation of polychaete burrows. The concept of bioturbation has been extended to describe the foraging

activities of fish, which periodically suspend sediments into the water column. Blackburn *et al.* (1988) estimated that 30% of the flux of O_2 , CO_2 and TAN can be attributed to fish bioturbation in marine aquaculture ponds. In this study, flux rates of O_2 , CO_2 and TAN in tanks with fish were reduced by 54, 58 and 70% respectively, when compared to those of sediments in control (undisturbed) tanks. Presumably continuous suspension of sediments by fish prevented the accumulation of organic matter and thereby reduced sediment oxygen demand. In addition, the flocculent layer of undisturbed sediments was observed to contain large numbers of macrofaunal burrows that undoubtedly increased solute flux rates relative to sediments in tanks with fish.

Intensive Israeli and Taiwanese fish ponds are aerated and mixed continuously. The suspension of sediments and aerobic conditions produced by paddlewheel aeration are favorable for water column (planktonic) nitrification. Nitrate accumulation has been measured in such systems (Avnimelech *et al.* 1986, Avnimelech *et al.* 1992). Similarly, sediment suspension by fish (*Ictalurus nebulosus* and *Cyprinus carpio*) enhanced nitrite+nitrate concentrations in a mesocosm experiment (Cline *et al.* 1994) and in this study. Although lower TAN concentration in tanks with fish was most likely related to nitrification associated with suspended particles, to some extent direct consumption and incorporation of nutrients contained in inputs (particularly feed) by fish may have reduced the amount of nitrogen released to the water column.

Factor analysis was used to reduce the dimensionality of the data set consisting of a number (7) of interrelated water quality variables (Jolliffe 1986, Milstein 1993). New variables (principal components, factors) were linear functions of the original variables and retain most of the variation present in the original data set. Application of factor analysis to aquaculture water quality data sets has been limited (Milstein *et al.* 1989, Milstein 1990, Milstein *et al.* 1991), despite the utility and interpretive power of this multivariate statistical technique. In this study, whole-tank respiration (Factor 1) explained 39% of the variance in water quality data and nitrification associated with suspended sediment (Factor 2) explained an additional 27% of the variance. In intensive, mechanically stirred ponds, the impact of feed (Factor 1), algal biomass (Factor 2) and decomposition rate (Factor 3) explained 59, 15 and 12% of variance, respectively (Milstein 1990). In manured polyculture ponds, autotrophic activity (Factor 1), nitrification and algal uptake of nitrogen (Factor 2), and respiration (Factor 3) explained 32%, 18% and 16% of the variance in water quality data, respectively (Milstein *et al.* 1991). In reservoirs used for tilapia cage culture and free-swimming carp culture, algae activity (Factor 1) and water column oxygenation (Factor 2) explained 37% and 21% of the variance in water quality data, respectively (Milstein *et al.* 1989). The results of these studies point to the overwhelming influence of processes related to phytoplankton as primary determinants of water quality in fish ponds. Processes occurring at the sediment-water interface are important, but secondary.

Factor analysis extracted a combination of water quality variables that described the amplitude of diurnal whole-tank respiratory cycles, primarily due to phytoplankton, and thus reflected water quality related to respiration on a relatively short (diel) time scale. Organic matter mineralization (respiration) on a time scale relating to the duration of the experiment can be estimated by the rate of accumulation of alkalinity. Such increases have been observed when organic matter is added to fish culture systems (Piedrahita *et al.* 1987).

Organic matter decomposition results in an increase in total alkalinity (Stumm and Morgan 1981). The oxidation of organic matter by oxygen or nitrate as terminal electron acceptors produces carbon dioxide that reacts with water to produce carbonic acid (H_2CO_3). By exchange reaction with cations in the sediment, H^+ ions replace Ca^{2+} ions and release bicarbonate (Boyd 1990). The summary reaction produces 1 mole of bicarbonate for each mole of carbon dioxide released during respiration or fermentation. Alkalinity accumulation represents the net rate of carbon dioxide production (respiration, fermentation) and removal (phytoplankton uptake, methanogenesis) and thus indicates the trophic status of the culture system. An accumulation of alkalinity suggests net heterotrophy; a depletion suggests net autotrophy.

Although the amplitude of daily whole-tank production/respiration, as extracted by factor analysis, was lower in tanks disturbed by fish, estimates of cumulative organic matter decomposition from alkalinity accumulation over the time-scale of months indicated no differences among disturbance treatments. Thus, the

quantity of organic matter mineralized was independent of sediment disturbance. However, alkalinity increases were measured in ponds disturbed twice weekly by a weighted rake (Costa-Pierce *et al.* 1993, Chikafumbwa *et al.* 1993).

Differences in alkalinity accumulation rate were apparent among input types and can be related to input characteristics. The respiratory mineralization of chicken manure, as measured by the accumulation of alkalinity, was significantly greater than that of all other input types (Table 28). The particle size distribution of chicken manure was the most broad of all input types and consisted predominately of small particles. These small particles likely remained in suspension in the water column, rendering them more susceptible to aerobic mineralization than in the sediment.

The following model of chicken manure mineralization is hypothesized in which small particles of chicken manure function as nuclei of microbial activity. First, particles are colonized by heterotrophic microbes that begin mineralization of labile organic constituents. Later, particles are colonized by nitrifying bacteria. Two aspects of organic matter are favorable for colonization by nitrifying bacteria. First, negatively-charged carboxyl groups on partially-decomposed organic matter attracts cations, such as ammonium, to the particle surface. Thus, organic particles serve as loci of attraction for nitrifying bacteria by concentrating substrate from the dilute bulk water phase into energetically-rich patches. Secondly, nitrifying bacteria require surfaces for attachment. Small particles of chicken manure thus represent a near perfect surface.

Table 28. Estimated carbon flux rates ($\text{g C m}^{-2} \text{ d}^{-1}$) in aquatic mesocosms enriched with organic inputs of variable quality.

Input Type ¹	Gains		Losses		Δ^6
	Input ²	Net Photosynthesis ³	Fish ⁴	RM ⁵	
CM	4.24	2.07	0.06	1.77	4.48
RB	6.11	1.55	0.26	1.20	6.20
FD	5.79	2.19	0.78	1.15	6.05
DG	6.35	1.96	0.37	1.02	6.92

¹ CM=chicken manure, RB=rice bran, FD=catfish feed, DG=distiller's grains.

² Calculated from CHN analysis of input types multiplied by average daily dry matter enrichment rate.

³ Estimated from difference between dissolved oxygen at dawn and dusk; assumes one mole of CO_2 fixed for each mole of O_2 released.

⁴ Assumes 0.1 g C fixed per g fish multiplied by net fish yield (Table 13).

⁵ RM=respiratory mineralization; estimated from alkalinity accumulation; assumes 1 mole HCO_3^- released from sediment for every mole CO_2 respired ($\text{H}^+/\text{Ca}^{2+}$ sediment exchange).

⁶ Δ =difference.

Nitrification was relatively more important than phytoplankton uptake as a mechanism of ammonium transformation in tanks enriched by chicken manure as indicated by the relatively higher nitrate concentration than that in tanks enriched by other inputs. It is also possible that fish appetite was not satisfied by chicken manure and thus sediment disturbance by fish was more vigorous than in tanks enriched by other inputs. This is supported by the poor fish growth and production in tanks enriched by chicken manure.

The rate of respiratory mineralization of rice bran was second to that of chicken manure. It is likely that a similar mechanism for mineralization related to particle size was operable in tanks enriched with rice bran. However, a primary difference was related to nutrient quality. Nitrate did not accumulate in tanks with rice bran and was the lowest of all input types. The C:N ratio of rice bran was the highest of all input types (17.8), a ratio that can be considered typical of "stable" organic matter. Thus, nutrients released during mineralization were most likely assimilated into microbial biomass. This is supported by sediment-water solute flux measurements for TAN, which were significantly lower in tanks enriched by rice bran than in tanks enriched by catfish feed or distiller's grains.

The respiratory mineralization rate of distiller's grains was the lowest measured of the input types, suggesting the generally refractory nature of this input type. Sediment-water solute flux rates were highest and DO at dawn lowest of the input types evaluated. The C content and thus daily C loading of distiller's grains was highest of all input types.

Fish growth and production in tanks enriched by distiller's grains was second only to that in tanks enriched by catfish feed. The suitability of distiller's grains as a protein source in diets for channel catfish (Webster *et al.* 1991) and freshwater prawn (Tidwell *et al.* 1993) has been demonstrated, although results of this study suggest that distiller's grains may be suitable as a direct input for carp production.

The proportion of carbon not accounted for in the budget is approximately equivalent to daily loading of input carbon. Assuming negligible accumulation in sediment (a questionable assumption), the difference would thus represent mineralization of organic matter through fermentative pathways. If this is the case, then anaerobic mineralization occurs at 4-6x the magnitude of aerobic mineralization. This is not surprising given the fact that oxygen penetration into the sediment is minimal and thus the bulk of sediment volume is anaerobic, despite the fact that aerobic mineralization is energetically more efficient. Anaerobic pathways (sulfate reduction) accounted for 50-70% of carbon mineralization in eutrophic marine mesocosms (Sampou and Oviatt 1991).

An additional source of error in the budget may be related to estimation of net photosynthesis. Net photosynthesis may have been underestimated due to aeration and mixing of tanks that undoubtedly led to outward diffusion of oxygen during late-afternoon episodes of DO exceeding saturation and inward diffusion during episodes of low DO. In any event, anaerobic processes predominate in the sediment and may account for the bulk of carbon losses estimated for these mesocosms.

Sediment disturbance is a practical method of water quality improvement in aquatic mesocosms, and by extension, aquaculture ponds in which phytoplankton biomass is excessive. An increase in non-algal turbidity is an inevitable consequence of paddlewheel aeration. This may have the unintended effect of reducing whole-pond respiration by limiting algal biomass through reduced light penetration. However, the consequences of sediment disturbance with respect to the potential for off-flavor, disease due to stress associated with the potential functional impairment of fish gills and the costs of sediment disturbance require evaluation relative to potential benefits of improved dissolved oxygen regime and lower total ammonia-nitrogen concentration. In addition, sediment disturbance may reduce fish production in ponds in which phytoplankton-based food chains predominate (Chikafumbwa *et al.* 1993).

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Table 29. Half-matrix of Pearson correlation coefficients (R) among log₁₀-transformed water quality variables (N=120).

Variable ¹	C+P	VSS	FSS	TAN	NO _x	CO ₂	DO_AM
C+P	-						
VSS	0.719	-					
FSS	-0.024	-0.015	-				
TAN	0.522	0.330	-0.269	-			
NO _x	0.317	0.311	0.497	0.008	-		
CO ₂	0.388	0.231	-0.169	0.412	-0.089	-	
DO_AM	-0.393	-0.159	0.435	-0.399	0.241	-0.394	-

¹ C+P=chlorophyll + phaeophytin; VSS=volatile suspended solids; FSS=fixed suspended solids; TAN=total ammonia nitrogen; NO_x=oxidized nitrogen (nitrite+nitrate); CO₂=early morning carbon dioxide; DO_AM=early morning dissolved oxygen.

Table 30. Partial co-efficients of determination ($R^2 \times 100$) and sign of the standardized estimate (β -weight) of predictor variables used in a multiple regression model for water quality parameters.

Predictor Variable	Water Quality Parameter								
	DO_AM	DO_PM	DEL_DO	CO ₂	TAN	NO _x	FSS	VSS	C+P
DISTURB	33.0 (+)	0.5 (-)	23.0 (-)	8.3 (-)	14.6 (-)	7.8 (+)	56.3 (+)	5.3 (-)	29.8 (-)
FD_RT	2.9 (-)	5.0 (-)	2.6 (-)	23.2 (+)	10.8 (+)		0.8 (-)	24.1 (+)	15.7 (+)
C:N			5.9 (-)	1.8 (+)	5.6 (-)	47.8 (-)	1.9 (-)		29.8 (-)
C	3.8 (-)	0.4 (-)	0.6 (+)	13.5 (+)	3.6 (+)				4.9 (+)
N	1.5 (-)	8.5 (+)		2.8 (+)	1.3 (-)			26.6 (+)	
T	25.8 (-)	4.5 (-)		2.7 (-)			3.4 (+)		
PAR		8.5 (+)	7.5 (+)	5.1 (+)					
AV_PAR	0.3 (+)			1.9 (+)	2.3 (+)				
Total Explained	67.4	27.5	39.7	59.2	39.2	56.4	63.1	56.6	67.6
n	720	720	720	120	120	120	120	120	120

CHAPTER 5

THE EFFECT OF SUPPLEMENTAL NITRATE ON WATER QUALITY IN STATIC AND MIXED AQUATIC MESOCOSMS

INTRODUCTION

Organic matter accumulates at a variable rate in the sediment of aquaculture ponds, depending largely on input nutrient composition. Sediment organic matter increased by $0.23\% \text{ yr}^{-1}$ in channel catfish ponds over 8 years (Tucker 1985). Approximately 65% of input N accumulated in the sediment of tropical fish ponds, leading to an increase in organic N of 0.06% after 4 months (Acosta-Nassar *et al.* 1994). In general, the accumulation rate of organic matter in the sediment of channel catfish ponds is low.

Many commercial channel catfish ponds are operated by a multiple-batch cropping system, in which several cohorts of fish are present simultaneously, and harvesting and re-stocking ("topping off") are accomplished without removing water (Tucker *et al.* 1992). As these ponds may not be drained for 5 years or more, opportunities for sediment management of organic matter content are limited.

The current study was conducted to evaluate the effect of nitrate supplementation on sediment organic matter decomposition. By oxidizing sediment organic matter it may be possible to reduce sediment oxygen demand and therefore increase dissolved oxygen concentration in aquaculture ponds.

The widely-accepted model of organic matter decomposition in aquatic sediments describes the distribution of terminal electron acceptors in discrete layers of decreasing redox potential (E_h) with increasing sediment depth (Reddy and Patrick

1984). These electron acceptors are assumed to function sequentially, that is, complete consumption of oxygen must occur before E_h declines sufficiently to allow nitrate respiring bacteria to mediate the oxidation of organic matter. E_h may be poised at a particular redox potential by maintaining a sufficient concentration of a particular electron acceptor, thereby limiting further decline in E_h .

However, recent evidence suggests that different terminal electron acceptors may function simultaneously to oxidize organic matter. The sediment has been described as a heterogeneous assemblage of variable- E_h microzones that only on average decreases with depth (Jørgensen 1977, Jenkins and Kemp 1984, Kerner 1993). These microzones consist of particles with oxidized exteriors grading to reduced interiors. The magnitude of the dimensional gradient will depend upon particle geometry, density and organic composition, but is typically on the order of 100-200 μm (Jørgensen 1977, Paerl 1984). In any case, nitrate supplementation may accelerate the decomposition of sediment organic matter, and thereby reduce sediment oxygen demand.

Nitrate supplementation has also been proposed as a means of reducing eutrophication in lakes and estuaries (Ripl 1976). The mechanism by which this occurs is by maintaining sediment E_h above that ($> +220 \text{ mV}$) limiting the solubilization and subsequent release of iron-bound phosphorus (Andersen 1985). In water bodies in which primary production is limited by phosphorus, nitrate supplementation may therefore reduce eutrophication. Lower primary production

may reduce the risk of oxygen depletion in aquaculture ponds from excessive phytoplankton respiration.

Avnimelech and Zohar (1986) measured the depletion rate of nitrate in fish ponds enriched with KNO_3 to estimate the extent of anaerobic conditions on the pond bottom. These investigators suggested that nitrate enrichment offers a practical method for the control of E_h in pond sediment. However, products of nitrate dissimilation (nitrite and ammonia) are potentially toxic to fish.

In addition to effects on organic matter decomposition, nitrate enrichment was evaluated with respect to water quality in aquatic mesocosms stocked with channel catfish. More specifically, the effect of nitrate supplementation on those water quality parameters important to fish culture, particularly dissolved oxygen, total ammonia-nitrogen, and nitrite was evaluated. Secondly, the effect of nitrate supplementation on soluble phosphorus concentration was evaluated.

MATERIALS AND METHODS

Experimental Design

A 3x2 factorial experiment was designed to evaluate the effect of nitrate enrichment and mixing regime on water quality in aquatic mesocosms (fiberglass tanks). Water quality in tanks enriched to two target nitrate concentrations (10 and 20 mg L^{-1}) was compared to that of unenriched (control) tanks. Industrial-grade NaNO_3 (98% pure, 16.5% N) was dissolved in a plastic bucket of tank water and dispersed evenly over the tank surface weekly (Wednesday). Nitrate concentration was determined 6 days after addition (Tuesday). The quantity of chemical added to

each tank was calculated by the difference between target and measured nitrate concentration multiplied by tank volume.

In addition, CaCl_2 was added on two occasions to mitigate potential nitrite toxicity to fish. The first addition was made to raise Cl^- concentration to 30 mg L^{-1} and the second addition was made to raise Cl^- concentration to 50 mg L^{-1} . The proportion of methemoglobin in catfish blood was estimated by the empirical method of Schwedler and Tucker (1983), which predicted methemoglobin concentration as a function of the molar ratio between nitrite and chloride.

The effect of mixing on water quality was also evaluated in this study. Water was either circulated by placing an air diffuser at the center of the tank near the bottom (MIX) or not circulated and allowed to stratify (STR).

Each treatment combination (total=6) was randomly assigned to two fiberglass tanks (experimental units). Fiberglass tanks (3.66 m D x 1.22 m H) were located at a facility consisting of four double rows of 26 tanks each. Each double row of tanks was serviced by an independent water and air supply system. Sandy clay-loam soil (58% sand, 26% clay, 16% silt) was added to fiberglass tanks to a depth of 15-30 cm. Tanks were filled with surface water from a reservoir (total alkalinity= 158 mg L^{-1} as CaCO_3) to a depth of 98 cm to provide a total water volume of 10.3 m^3 . Water was added only as needed to replace evaporative loss and was maintained at a level 4-5 cm below the top of the standpipe to minimize overflow during rainfall events. Tanks had been used the previous summer and fall in a study to evaluate the effect of sediment disturbance and organic input type on water quality.

Tanks were stocked with channel catfish (*Ictalurus punctatus*) at a density of 2 m⁻² (20 per tank) on 31 May 1994. Initial weight of channel catfish averaged 125 g. Channel catfish were fed a 32%-protein diet consisting of floating, 4 mm pellets formulated primarily from cereal grain meals (soybean, corn) and fish meal and fortified with vitamins and minerals. Fish were fed once daily, usually between 0800 and 1000 h. Feeding rate was equivalent to 125 kg DM ha⁻¹ d⁻¹ in MIX tanks and 50 kg ha⁻¹ d⁻¹ in STR tanks for the duration of the study.

Water Quality

Water samples (125 mL) were collected from each tank with a 90-cm, integrated water column sampler, transferred to clean polyethylene bottles, and transported immediately to the laboratory. Samples were filtered (Whatman No. 42) prior to analysis (Table 31).

Solute Flux

One sediment core (5 cm diameter) was collected from each tank after 5, 10 and 15 weeks. Cores were transported to the laboratory and placed in an incubator at the sediment temperature at the time of collection. Overlying water in sediment cores was carefully replaced by filtered (0.45 µm) water collected from the water supply reservoir that had been aerated overnight. After 4 hours, dissolved oxygen of the overlying water was measured with a polarographic probe. Water was removed by siphon, the volume measured and pH determined with a combination probe. TAN and NO₂⁻-N was measured in a filtered (Whatman No. 42) water sample. Free carbon dioxide was calculated from equilibrium relationships between alkalinity

Table 31. Frequency and methods used for measurement of water quality parameters in a study evaluating the effect of nitrate enrichment and water mixing regime on water quality in tank mesocosms.

Parameter	Frequency	Method ¹
temperature	weekly	minimum-maximum thermometer
dissolved oxygen (DO)	weekly (dawn)	polarographic probe
nitrite-N	weekly	diazotization
nitrate-N	weekly	Cd reduction followed by diazotization
total ammonia-N (TAN)	every 2 weeks	phenol-hypochlorite
soluble reactive-P (SRP)	every 2 weeks	ascorbic acid method
pH	every 2 weeks	combination probe
total alkalinity	every 2 weeks	titration with dilute HCl to the methyl orange endpoint
total suspended solids (TSS)	every 2 weeks	filtration through pre-ashed GF/C glass fiber filters; dry at 105 °C for 2 hours
volatile/fixed solids (VSS/FSS)	every 2 weeks	loss-on-ignition of dried GF/C glass fiber filters at 550 °C
chlorophyll <i>a</i> and phaeophytin	every 2 weeks	acetone-methanol extraction; acidification of extract
chloride	monthly	titration with HgNO ₃

¹ All methods after Boyd and Tucker (1992) and APHA *et al.* (1992), except chlorophyll *a* and phaeophytin after Pechar (1987).

components and pH. Solute flux was calculated from the difference between initial and final concentrations relative to the surface area of the core.

Denitrification

Denitrification rate was estimated by the acetylene blockage technique (Sørensen 1978, Chan and Knowles 1979). Nitrous oxide is produced as an intermediary of the denitrification process. Acetylene blocks the reduction of nitrous oxide to dinitrogen gas. The accumulation rate of nitrous oxide was therefore assumed to be equivalent to the denitrification rate.

On two occasions during the study (week 6 and week 13) one sediment core was collected from each tank. An additional sediment core from one tank of each treatment combination was collected as a control. Cores were transported to the laboratory and placed in aerated 18.9-L plastic buckets of reservoir water amended with KNO_3 to a nitrate concentration corresponding to nitrate enrichment treatment. Buckets were placed in an incubator at 30 C overnight.

After approximately 12 hours, cores were removed from buckets and 120-130 ml of water overlying sediments was removed. Each core was capped with a rubber stopper into which a serum stopper had been affixed. Acid-washed ($0.1 \text{ N H}_3\text{PO}_4$) acetylene was bubbled into the remaining overlying water for approximately 5 minutes. Each treated core was then pre-incubated for 3 hours to allow diffusion of acetylene into the sediment. Control cores were similarly incubated without acetylene.

After 3 hours, each core was purged with air for 3 minutes. Then, a volume of acetylene equivalent to 10% of the head space volume was injected into treated core head space. Control cores were not amended with acetylene. A head space gas sample was collected from each core and injected into a pre-evacuated, 3-mL serum tube (Vacutainer®) that was further sealed with silicone adhesive. Head space gas samples were collected every 2 hours for 6 hours.

Nitrous oxide was measured with a gas chromatograph (Tremetrics 9001) equipped with a 1.2 m Porapak Q (80/100 mesh) pre-column, a 4.2 m Porapak Q analytical column and a ^{63}Ni electron capture detector. The carrier gas consisted of 5% methane in ultra-high purity argon (36 ml min^{-1}). Temperatures were 35 C in the column, 80 C in the injection port and 300 C in the detector. Changes in headspace N_2O concentrations were used to calculate the rate of N_2O production ($\text{mg N m}^{-2} \text{ h}^{-1}$), which was then used to estimate denitrification rate.

The rate of nitrate depletion was calculated by difference between target nitrate concentration and measured concentration after 6 days adjusted for tank volume and sediment surface area. Nitrate depletion was a measure of dissimilatory nitrate reduction, denitrification, and assimilatory nitrate reduction by phytoplankton and bacteria.

Fish Survival

Fish mortalities were removed as they appeared on the tank surface and replaced within 3 days. At the termination of the experiment, tanks were drained of water and remaining fish were counted and removed.

Statistical Analysis

Water quality parameter means were compared by least significant difference (LSD) tests following analysis of variance (ANOVA) using PROC GLM (SAS Institute 1989b) to evaluate the effects of nitrate enrichment and mixing regime. Additional models were prepared to evaluate the effect of tank history (previous disturbance treatment and organic input type) on water quality parameters. Differences were declared significant at $\alpha < 0.05$.

The rate of alkalinity accumulation in each tank was evaluated by linear regression of total alkalinity with respect to time. Slope parameters were compared as described above to evaluate nitrate enrichment and mixing effects.

Under the assumption that processes affecting water quality were different and dependent upon mixing regime, water quality data were sorted by mixing regime and subjected to factor analysis (PROC FACTOR) to determine the most important processes acting upon the system (SAS Institute, Inc. 1989a). Distributional characteristics of each parameter were evaluated by examination of parameter skewness, kurtosis, and the W statistic of the Shapiro-Wilk test (PROC UNIVARIATE) and suitable transformations were made to normalize the distribution of parameters. A correlation matrix was used to determine the loadings from each water quality parameter into the three common factors explaining the most variance. Factor scores determined for each observation were extracted and compared by ANOVA (PROC GLM) to evaluate the effect of temporal and treatment (nitrate

enrichment) effects for each mixing regime. Mean factor scores were further compared by LSD tests.

RESULTS

Mixing Effects

Water Quality. Mixing had a more profound effect than nitrate enrichment on water quality parameters important to fish culture. Despite a feeding rate in MIX tanks that was 2.5x that of STR tanks, mean dissolved oxygen at dawn was more than 2x higher in MIX tanks (Table 32). DO was too low to support fish in STR tanks during much of the study.

TAN was significantly lower in MIX tanks than in STR tanks (Table 33). Mixing by airstones caused sediment suspension (FSS) into the water column. Increased FSS was associated with lower TAN ($R=0.298$, $P=0.021$) and higher NO_3^- -N ($R=0.261$, $P=0.044$) in MIX tanks.

SRP of STR tanks was approximately 2x greater than that of MIX tanks (Table 33). SRP was inversely correlated with DO concentration in STR tanks ($R=-0.391$, $P=0.002$), but not in MIX tanks, suggesting that DO controls SRP solubility only at low DO concentrations ($<2 \text{ mg L}^{-1}$).

Primary production (C+P) and organic matter (VSS) in mixed tanks ($646 \mu\text{g L}^{-1}$ and 80 mg L^{-1}) were higher than in stratified tanks ($552 \mu\text{g L}^{-1}$ and 73 mg L^{-1}). Algal scums typically dominated the surface of stratified tanks, thereby limiting light penetration and primary production.

Table 32. Mean (\pm SD) early morning dissolved oxygen (mg L^{-1} and % saturation) in aquatic mesocosms enriched with NaNO_3 at three target nitrate-N concentrations and two mixing regimes.

Treatment	N	Dissolved Oxygen	
		mg L^{-1}	saturation (%)
0	80	3.45 (2.26) a	43 (28)
10	80	3.63 (2.44) a	46 (31)
20	80	3.80 (2.42) a	48 (30)
STR	120	2.27 (2.56) b	29 (33)
MIX	120	4.99 (0.99) a	62 (10)

¹ Means within a column followed by the same letter not significantly different ($P>0.05$); comparisons valid within treatment type only.

Table 33. Mean (\pm SD) total ammonia-nitrogen (n=120), nitrite-nitrogen (n=240) and soluble reactive phosphorus (n=120) in aquatic mesocosms enriched with NaNO₃ at three target nitrate-N concentrations and two mixing regimes.

Treatment	TAN	NO ₂ ⁻ -N	SRP
0	0.59 (0.63) a	0.15 (0.44) c	0.53 (0.39) a
10	0.64 (0.59) a	0.58 (0.50) b	0.53 (0.36) a
20	0.71 (0.43) a	1.03 (0.73) a	0.29 (0.24) b
STR	0.78 (0.58) a	0.61 (0.69) a	0.62 (0.34) a
MIX	0.52 (0.49) b	0.56 (0.66) a	0.29 (0.27) b

¹Means within a column followed by the same letter not significantly different ($P>0.005$); comparisons valid within treatment type only.

Sediment Solute Flux. Mean sediment oxygen demand (SOD) in MIX tanks was 43% greater than that in STR tanks (Table 34). Similarly, CO₂ flux from sediment was significantly greater in MIX tanks than that in STR tanks. In STR tanks, TAN flux was slightly greater than, but not significantly different from that of MIX tanks. The molar ratio of oxygen consumed to ammonia released was significantly greater in MIX tanks (2.5) than in STR tanks (1.6) suggesting that sediment nitrification was a more important mechanism of ammonia removal in MIX tanks.

Denitrification. Mixing did not affect denitrification rate as measured by the acetylene blockage technique (Table 35). However, the denitrification rate in MIX tanks (32 mg N m⁻² d⁻¹) was slightly greater than that measured in STR tanks (28 mg N m⁻² d⁻¹) suggesting that coupled nitrification-denitrification was slightly greater in mixed tanks or that denitrification was stimulated by the greater concentration of nitrate in the overlying water of mixed tanks.

Nitrate depletion in STR tanks was approximately 2x that in MIX tanks, most likely a function of the lower DO concentration in STR tanks. DO concentration was inversely correlated with nitrate depletion ($R=-0.255$, $P=0.0001$).

Fish Survival. Although the feeding rate in STR tanks was not excessively high in relation to those considered to minimize the requirement for supplemental aeration (Tucker *et al.* 1979), fish survival in STR tanks was poor (Table 36). At the conclusion of the experiment, no fish were recovered from stratified tanks. Fish survival was greater in MIX tanks although mortality was substantial.

Table 34. Mean (\pm SD) solute (O_2 , CO_2 , TAN, and NO_2^- -N) flux ($mg\ m^{-2}\ h^{-1}$) between water and sediment determined in intact sediment cores collected during week 5, 10 and 15, and incubated for 4 hours at 30 C. Negative sign indicates flux from water to sediment; positive sign indicates flux from sediment to water.

Treatment	N	Solute			
		O_2	CO_2	TAN	NO_2^- -N
0	12	-62 (23)a ¹	+127 (72) a	+15 (10) a	+0.008 (0.054) c
10	12	-56 (16) a	+67 (62) b	+13 (5) a	+0.214 (0.088) b
20	12	-53 (24) a	+62 (47) b	+17 (5) a	+0.329 (0.202) a
STR	18	-47 (19) b	+60 (73) b	+16 (9) a	+0.168 (0.165) a
MIX	18	-67 (18) a	+111 (49) a	+14 (5) a	+0.199 (0.207) a

¹ Means in the same column with the same letter are not different ($P>0.05$); comparisons valid within treatment type only.

Table 35. Nitrate reduction ($\text{mg N m}^{-2} \text{ d}^{-1}$) as measured by weekly depletion (WD) of NaNO_3 and denitrification rate ($\text{mg N m}^{-2} \text{ d}^{-1}$) as measured by the acetylene blockage (AB) technique.

Treatment	Treatment					
	Mixed		Stratified		Mean	
	WD	AB	WD	AB	WD	AB
0	NA ¹	1.2	NA	0.6	NA	0.9
10	370	37.9	1004	20.0	687	28.9
20	743	57.8	1268	64.0	1006	60.8
Mean	556	32.3	1136	28.2		

¹ NA=not applicable.

Nitrate Enrichment Effects

Water Quality. Mean water column nitrite concentration increased in direct relation to nitrate enrichment ($R=0.529$, $P=0.0001$) and was significantly different among nitrate enrichment treatments (Table 33). Apparently nitrite was produced during dissimilatory nitrate reduction (DNR) in tank sediments and released to the water column. Mean nitrite concentration was significantly different ($P<0.05$) among nitrate enrichment treatments.

TAN concentration increased with increasing nitrate enrichment, but was not different among nitrate enrichment treatments ($P>0.05$). TAN concentration in the water column represented a balance between ammonium production by organic matter mineralization and dissimilatory nitrate reduction and uptake by phytoplankton or oxidation by nitrifying bacteria. There was a significant interaction between nitrate enrichment treatment and mixing regime.

Nitrate enrichment reduced SRP concentration in the water column only at the highest target nitrate concentration, but this effect was not as great as that of mixing. SRP concentration was inversely correlated with nitrate concentration in both MIX ($R=-0.327$, $P=0.011$) and STR ($R=-0.396$, $P=0.002$) treatments.

Enrichment of tanks with nitrate increased DO at dawn only marginally, approximately 0.2 mg L^{-1} for each 10 mg L^{-1} of target nitrate concentration. Differences in mean dissolved oxygen at dawn were significant only when tank history was taken into account. In ANOVA models that included tank history effects (previous disturbance treatment, previous organic input type), nitrate enrichment was

only the third most important effect after mixing regime and previous disturbance treatment.

The rate of accumulation of alkalinity was directly proportional to the quantity of NaNO_3 added ($R=0.965$, $P=0.0001$) (Figure 27). Nitrate respiration increased the amount of CO_2 released relative to unenriched systems. This CO_2 reacted with water to produce carbonic acid (H_2CO_3). By exchange reactions with cations in the sediment, H^+ replaces Ca^{2+} and releases bicarbonate (Boyd 1990). In addition, nitrate respiration consumes H^+ , thereby increasing pH. Consequently, carbon dioxide at dawn (calculated from equilibrium relationships between alkalinity components and pH, compensated by temperature) was inversely related to nitrate enrichment.

Chlorophyll plus phaeophytin (C+P) concentrations in this study were very high, reflecting the hypereutrophic nature of these tank mesocosms. Chlorophyll + phaeophytin concentrations were more than 30% higher in tanks enriched with nitrate, indicating that exogenous nitrate was an important source of N for phytoplankton growth. C+P was inversely correlated with NO_2^- -N concentration ($R=-0.257$, $P=0.047$) in the MIX treatment, suggesting algal uptake of NO_2^- was of some importance when uptake was not limited by light.

Sediment Solute Flux. Sediment oxygen demand was inversely related to nitrate enrichment, although differences were not significant (Table 34). However, CO_2 flux was significantly lower in tanks enriched with nitrate, suggesting nitrate-mediated sediment consumption of CO_2 . TAN flux from sediment was not affected

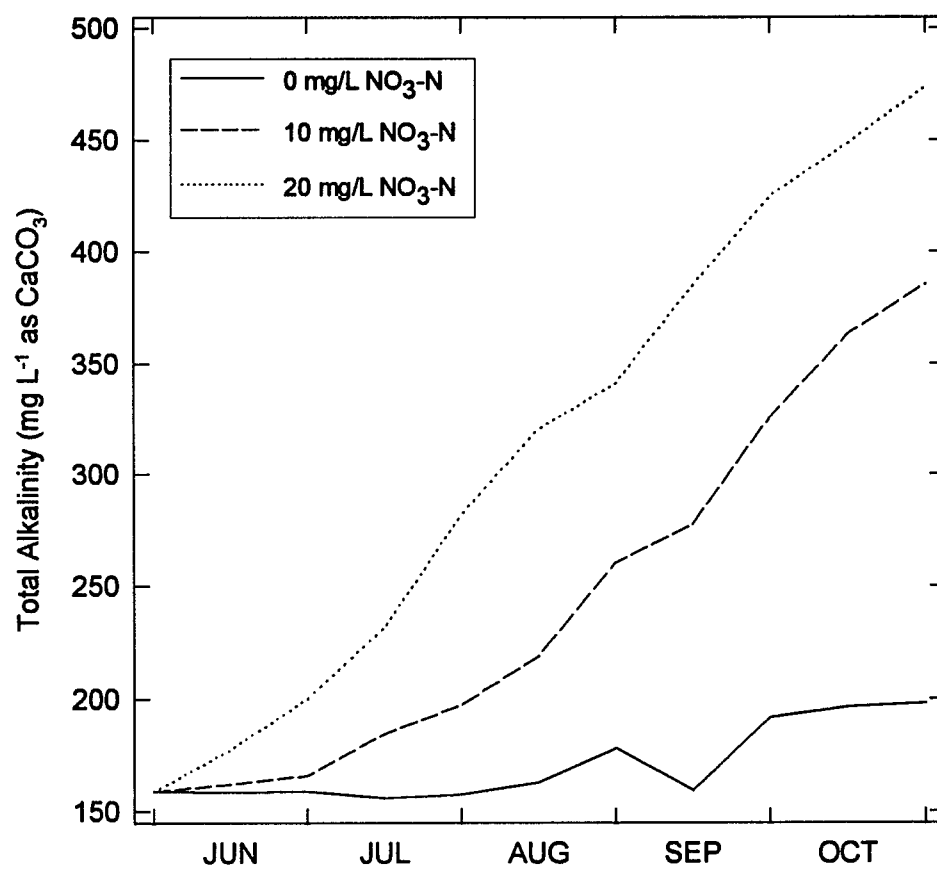


Figure 27. Accumulation of alkalinity with respect to time in aquatic mesocosms enriched with nitrate to 0, 10 or 20 mg N L⁻¹.

by nitrate enrichment although the greatest mean TAN flux occurred at the highest level of nitrate enrichment. NO_2^- -N flux increased in direct response to target nitrate concentration and was significantly different between all nitrate enrichment treatments.

Denitrification. Denitrification rate increased directly in proportion to nitrate enrichment level and was significantly different between nitrate enrichment treatments (Table 35). Denitrification as measured by the acetylene blockage technique accounted for a relatively small proportion (<10%) of nitrate loss as measured by nitrate depletion. The potential for nitrate reduction in soils of enriched aquatic mesocosms was very high (Figure 28). Nitrate depletion rates increased with nitrate enrichment level.

Fish Survival. Fish survival at the 10 mg L⁻¹ enrichment level was similar to that in tanks receiving no supplementation (Table 36). Survival was reduced at the highest nitrate enrichment level. Fish survival was likely affected by a combination of elevated nitrite concentration leading to elevated methemoglobin concentration in channel catfish blood and chronic low dissolved oxygen. The maximum estimated methemoglobin proportion of channel catfish blood increased with nitrate enrichment level and was approximately 10% at 0 mg L⁻¹, 30% at 10 mg L⁻¹, and 80% at 20 mg L⁻¹ (Figure 29). The proportion of methemoglobin increased rapidly in fish in tanks enriched at the highest nitrate concentration due to chloride depletion, resulting in a decrease in the molar ratio of nitrite:chloride. Chloride depletion was most likely a result of co-precipitation with Na⁺ derived from NaNO₃ additions.

Table 36. Mean number of fish mortalities and mortality factor (MF)¹ in aquatic mesocosms.

Treatment	Treatment				Mean	
	Mixed		Stratified			
	no.	MF	no.	MF	no.	MF
0	47	2.35	77	3.85	61.8	3.09
10	47	2.35	71	3.55	59.0	2.95
20	67	3.35	110	5.50	88.5	4.43
Mean	62.0	3.10	85.8	4.29		

¹Mortality Factor= Σ Mortality÷Stocking Density (20/tank)

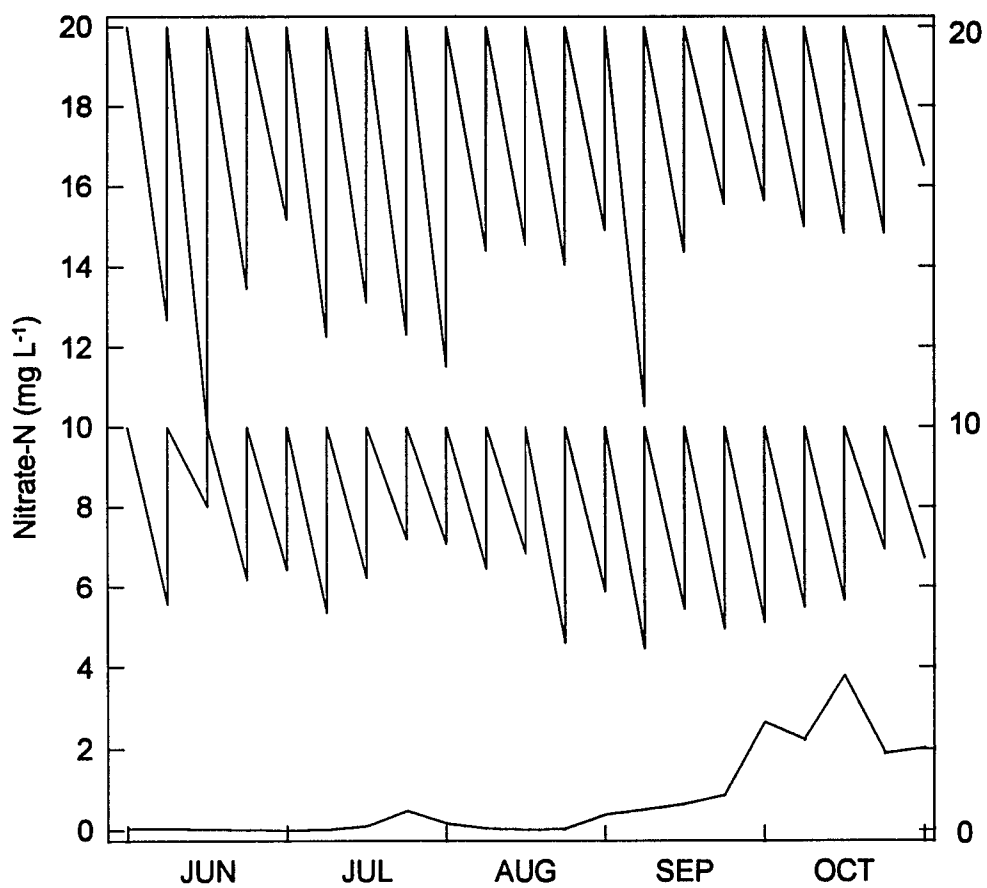


Figure 28. Nitrate-N concentration in aquatic mesocosms enriched to 0, 10 or 20 mg L⁻¹ NO₃⁻-N.

Factor Analysis

Factor analysis performed on data (Table 41) sorted by mixing regime identified different processes having important effects on water quality. In the MIX treatment, three common factors explained 62% of the variation in water quality data. Factor 1 was associated with negative loadings by C+P and positive loadings by TAN and NO_2^- -N. Factor 1 explained 29% of the variance in water quality data and was interpreted with respect to common factor loadings by water quality variables (Table 37) as the balance between dissimilatory nitrate reduction (DNR) and phytoplankton-mediated uptake of TAN and oxidation of NO_2^- -N. When phytoplankton biomass is low, TAN and NO_2^- -N are relatively high. Positive values of factor 1 were associated with a shift towards DNR and negative values were associated with a balance shift towards nitrogen uptake and removal mechanisms.

Factor 1 was greatest at the highest nitrate enrichment level indicating that DNR was more important than phytoplankton uptake as nitrate concentration increased. The mean score of Factor 1 for tanks that were not enriched with nitrate was not different from that of the highest nitrate enrichment level (Table 39). However, the mean score of Factor 1 for mesocosms enriched at 10 mg L^{-1} was significantly lower than that of the other two nitrate enrichment treatments, possibly related to the greater mean phytoplankton density in this treatment.

Temporal variation of factor 1 (not shown) suggested that DNR was more important than phytoplankton uptake at the very beginning of the experiment but, as phytoplankton biomass increased, the relative importance of DNR declined until the

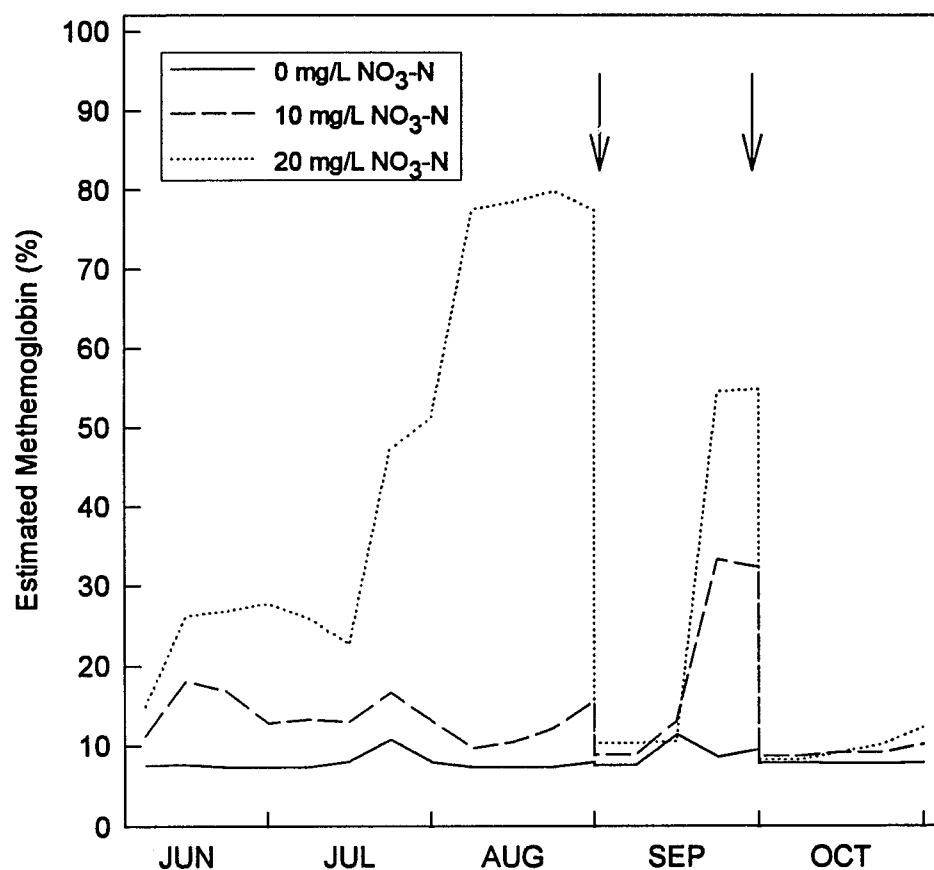


Figure 29. Estimated proportion (%) of methemoglobin in blood of channel catfish cultured in tanks enriched with NaNO_3 to 0, 10 or 20 mg N L^{-1} . Methemoglobin estimated as a function of molar chloride:nitrite ratio (Schwedler and Tucker 1983). Arrows indicate CaCl_2 additions.

Table 37. Loadings of water quality variables on, and variance explained by, common factors describing important processes in MIX mesocosms.

Variable	Factor 1	Factor 2	Factor 3
TAN	0.65¹	0.25	-0.33
NO ₂ ⁻ -N	0.62	0.19	-0.45
SRP	0.35	-0.08	0.19
DO_AM	0.33	0.19	0.73
CO ₂	0.51	0.28	0.56
C+P	-0.73	0.42	-0.09
VSS	-0.63	0.70	0.01
FSS	0.32	0.72	-0.10
Variance Explained (%)	29	18	15

¹ Bold face indicates most important factor loadings ($\geq 75\%$ of maximum loading).

Table 38. Loadings of water quality variables on, and variance explained by, common factors describing important processes in STR mesocosms.

Variable	Factor 1	Factor 2
TAN	-0.71¹	-0.15
NO ₂ ⁻ -N	-0.00	-0.55
SRP	-0.66	0.48
DO	0.64	-0.40
CO ₂	-0.83	0.40
C+P	0.68	0.53
VSS	0.66	0.57
FSS	0.62	0.08
Variance Explained (%)	42	19

¹ Bold face indicates most important factor loadings ($\geq 75\%$ of maximum loading).

Table 39. Mean factor scores by mixing regime and target nitrate-N concentration (mg L⁻¹) and results of multicomparison (LSD) test.

Mixing Regime	Factor	Target Nitrate Concentration		
		0	10	20
MIX	1	0.06 a	-0.52 b	-0.45 a
	2	-0.49 b	-0.33 a	-0.17 a
	3	0.57 a	-0.09 b	-0.49 c
STR	1	-0.28 b	-0.04 ab	0.31 a
	2	0.75 a	-0.28 b	-0.47 b

¹ Means with the same letter within a row not significantly different (P>0.05). Comparisons are valid within a row only.

last two months of the study when DNR once again was relatively more important than phytoplankton uptake.

Factor 2 was associated with positive loadings by organic (VSS, C+P) and inorganic (FSS) solids. Factor 2 explained an additional 18% of variance in water quality data and was interpreted to be related to the nutrient enrichment effect of nitrate supplementation. Nitrate enrichment significantly increased levels of Factor 2, although the mean factor score was higher at 10 mg L⁻¹ than at 20 mg L⁻¹ (Table 37). Factor 2 was not significantly different through time (not shown), although it was lower in the beginning of the study, oscillated slightly, and tended to follow temperature trends.

Factor 3 explained an additional 15% of variance in water quality data. Factor 3 was associated with positive loadings by DO and CO₂, and negative loadings by NO₂⁻-N and TAN (Table 37). Mean factor scores were significantly different among all three nitrate enrichment treatments and were inversely related to nitrate enrichment. As target nitrate concentration increased, DO and CO₂ concentrations decreased and NO₂⁻-N and TAN concentrations increased. Although the mean DO concentration of mixed tanks was highest in tanks receiving no supplementation, differences between other nitrate treatments were very small. However, CO₂ concentration was reduced by nitrate supplementation through increases in pH. Mean factor scores of Factor 3 became more negative through time (data not shown), following a temporal trend reflecting a cumulative process, e.g. alkalinity accumulation related to nitrate respiration.

Whereas nitrate addition effects were the third most important process/factor in the MIX treatment, it was the most important determinant of water quality in the STR treatment (Table 38). Factor 1 explained 42% of the variance in water quality in stratified mesocosms and, similar to Factor 3 in the MIX treatment, reflected a cumulative process through time. CO_2 was the water quality variable that loaded most heavily into Factor 1. As Factor 1 increased, concentrations of CO_2 decreased. TAN was the second most important water quality variable loading into Factor 1, suggesting that DNR to ammonium was a more important process than phytoplankton uptake or nitrification in STR than in MIX mesocosms. Increased levels of Factor 1 were associated with increased phytoplankton biomass (C+P, VSS), indicating the effect of nitrate as an important nutrient for primary production in stratified mesocosms. Increasing levels of Factor 1 were also associated with reduced SRP, indicating the effect of nitrate on sediment redox potential and the solubility of phosphorus. Finally, increasing levels of Factor 1 were associated with increased DO at dawn, suggesting that the DO sparing effect of nitrate was more important at the lower concentrations of DO at dawn measured in the STR treatment. Factor 1 increased in response to nitrate enrichment and was lowest in the control (no enrichment) treatment and highest in the 20 mg L⁻¹ treatment (Table 39). Mean factor scores of Factor 1 of the 20 mg L⁻¹ treatment were significantly greater than that of the control treatment, but neither were significantly different from the 10 mg L⁻¹ treatment.

Factor 2 accounted for an additional 18% of variance in water quality data in STR mesocosms. NO_2^- -N and organic matter (C+P, VSS) loaded most heavily into Factor 2 (Table 38). Thus, factor 2 represented the balance between nitrite production by DNR and oxidation or uptake in the water column. Increasing levels of factor 2 were inversely related to nitrate enrichment (Table 39). Mean factor scores of mesocosms enriched with nitrate were significantly lower than that of unenriched mesocosms. Temporally, DNR was more important than nitrification/uptake for the first 6 weeks of the study (data not shown), possibly representing a period of acclimation by nitrifying bacteria. For the remainder of the study nitrification/uptake were more important determinants of nitrite concentration than DNR.

DISCUSSION

Nitrate supplementation of aquaculture pond mesocosms did not improve water quality for fish production. Average early morning DO increased only marginally in tanks receiving nitrate supplementation. It was hypothesized that nitrate would spare oxygen consumed during sediment organic matter decomposition. Nitrate is invoked as an electron acceptor over a range of redox potential only slightly lower than that of oxygen. Complete consumption of oxygen was assumed to be necessary before nitrate is utilized as an electron acceptor. However, the sediment consists of a heterogeneous assemblage of microsites of variable redox potential, suggesting that nitrate may be active in decomposition as a terminal electron acceptor despite the presence of oxygen. Nitrate supplementation did not

significantly improve early morning DO in tanks because tanks were hypereutrophic and water column respiration was the predominant consumer of oxygen. Results of factor analysis suggest that DO sparing by nitrate was important only at low concentrations.

Although sediment oxygen demand declined in inverse relation to target nitrate concentration, differences between nitrate supplementation treatments were not significant. Andersen (1977a) measured no effect of nitrate on sediment oxygen demand up to a nitrate concentration of 10 mg N L⁻¹. Reduction in sediment oxygen demand has only a minor effect on water column DO. The difference between sediment oxygen demand at the highest target nitrate concentration and the control was equivalent to a difference in reduction in water column DO of approximately 0.1 mg L⁻¹ over a 12-h night.

Nitrate supplementation is not a practical technique for water quality improvement in aquaculture ponds due primarily to increased concentrations of potentially-toxic forms of dissolved inorganic nitrogen (ammonia and nitrite). Apparently, products of nitrate dissimilation were produced in abundance. In general, concentrations and sediment flux rates of both total ammonia-nitrogen and nitrite increased directly in relation to nitrate supplementation. Total ammonia-nitrogen and nitrite-nitrogen concentrations represent the balance between source and sink processes. Source processes include fish excretion, organic matter mineralization, and in tanks enriched with nitrate, dissimilatory nitrate reduction. Sink processes include phytoplankton uptake and nitrification. The balance between

dissimilatory nitrate reduction and phytoplankton uptake was the most important process related to nitrate supplementation.

Nitrite concentrations were sufficiently elevated to increase the proportion of methemoglobin in channel catfish blood to levels sufficient to induce anoxia and mortality. A high proportion of methemoglobin coupled with low average DO concentration led to high mortality in STR tanks. Interestingly, a baseline proportion of approximately 10% methemoglobin was estimated to be present in the blood of channel catfish cultured in tanks not supplemented with nitrate. Temporal variation in the estimated proportion of methemoglobin indicated the importance of chloride supplementation. Chloride was depleted from tanks supplemented with nitrate to an extent reflecting nitrate supplementation rate. The depletion of chloride may be related to the form of nitrate (sodium salt) added. Although not measured, sodium undoubtedly accumulated in solution, as the rate of nitrate depletion was high. Sodium may have co-precipitated with chloride and possibly other anions in order to maintain ionic balance in solution.

The rate of nitrate depletion was high and was a function of mixing regime and target nitrate concentration. The difference in nitrate depletion between mixing treatments at a given nitrate concentration was greater than the difference in nitrate depletion between nitrate concentrations within a mixing regime. Nitrate was depleted by assimilatory and dissimilatory pathways, although the relative magnitude of each can only be estimated.

Phytoplankton density was approximately 30-40% greater in tanks enriched with nitrate compared to that of control (no enrichment) tanks suggesting that phytoplankton assimilation of nitrate may have been important in tanks enriched with nitrate. However, the relative magnitude of this process is probably small because ammonium concentration controls assimilation. Ammonium is the preferred form of nitrogen for assimilation and the presence of ammonium at low concentrations will inhibit assimilatory nitrate reduction.

Two dissimilatory pathways of nitrate reduction in sediments have been identified. Nitrate may be reduced to ammonium (DNRA=dissimilatory nitrate reduction to ammonium) or to dinitrogen gas (denitrification). The proportion of nitrate reduced by each mechanism is variable. In Japanese coastal bays, DNRA can account for 20-70% (Koike and Hattori 1978) or 43-73% (Nishio *et al.* 1982) of sediment nitrate uptake. In an estuarine soil, Buresh and Patrick (1981) recovered 15-28% of added nitrate as ammonium and organic nitrogen, and concluded that DNRA generally accounts for approximately 15% of nitrate reduction.

DNRA is favored by intensely reducing conditions. At a redox potential less than -200mV, the proportion of ammonium and organic nitrogen recovered increased to 35-42% of added nitrate (Buresh and Patrick 1981). Similarly, Sørensen (1978) determined that DNRA increased with sediment depth such that 50% or more of nitrate may be dissimilated by this pathway. DNRA can account for 40% of nitrate reduction near the sediment surface but almost 100% at a sediment depth corresponding to the zone of maximum sulfate reduction (Sørensen 1978). Although

redox potential was not measured in STR tanks, average DO at dawn was low (2.27) and sediments were undoubtedly highly reduced. Further evidence reinforcing this assertion can be derived from measurements of gas collected from sediments in this study (Crozier¹¹, unpublished data). The volume of gas collected from sediments in STR tanks was approximately twice that of MIX tanks and the composition of gas in STR tanks was dominated by methane, indicating the highly reduced condition of sediment in STR tanks. The greater nitrate reduction rate in STR tanks implicates DNRA as the predominant dissimilatory pathway.

Organic matter can stimulate the DNRA pathway. Buresh and Patrick (1978) recovered 28% of added nitrate as ammonium and organic nitrogen in sediment cores amended with glucose. After a 1-day pre-incubation with glucose, this proportion increased to 70%. In general, DNRA is favored by high organic carbon to electron acceptor ratios (Tiedge 1988, D'Angelo and Reddy 1993). Kaspar *et al.* (1988) measured greater DNRA in sediments directly beneath a chinook salmon cage culture site than at an adjacent (control) site unaffected by the facility. In this study, sediments were covered by a layer of flocculent organic matter consisting of sedimented algae and uneaten feed. This organic layer may have stimulated DNRA.

Although potentially less important than the effects of redox potential and organic matter, the proportion of nitrate reduced by DNRA and denitrification is also a function of nitrate concentration. As the concentration of nitrate increases, the proportion of nitrate reduced to ammonium decreases (King and Nedwell 1985,

¹¹ Wetland Biogeochemistry Institute, Louisiana State University.

D'Angelo and Reddy 1993). Tiedge (1988) explains this phenomenon as a result of the higher energy yield to microbes from denitrification than from DNRA. In the relatively carbon-rich, electron-acceptor poor (low nitrate concentration) environments typical of aquatic sediments, DNRA may be a way to conserve nitrogen within the sediment (King and Nedwell 1985).

In this study it was difficult to partition the proportion of the two dissimilatory pathways. Denitrification rates determined by the acetylene blockage technique were 8-10% of nitrate depletion in MIX tanks and 2-5% of nitrate depletion in STR tanks, suggesting DNRA was the predominant nitrate removal mechanism. Alternatively, denitrification estimates may have been underestimated by as much as an order of magnitude. The acetylene blockage method inhibits nitrification as well as denitrification (Sloth *et al.* 1992). Nitrification and denitrification are tightly coupled in aquatic sediments. Thus, denitrification at low ($<0.5 \text{ mg N L}^{-1}$) *in situ* nitrate concentrations in the overlying water may be limited by the lack of substrate produced during nitrification rather than by inhibition of denitrification *per se*. In addition, inhibition of denitrification may be limited by the diffusion of acetylene to sites of denitrification. In addition, low estimations of denitrification may reflect a nitrate concentration effect. D'Angelo and Reddy (1993) calculated that denitrification accounts for an increasing proportion (60-93%) of nitrate reduction as nitrate concentration increases from 1-100 mg N L^{-1} .

A number of studies have measured denitrification rates at elevated nitrate concentrations. Andersen (1977b) measured nitrate reduction rates of 100-500 mg N

$\text{m}^{-2} \text{d}^{-1}$ in cores incubated over a range of nitrate concentration (0-10 mg N L^{-1}) and temperature (5-22 C). Van Kessel (1977) measured denitrification rates $<200 \text{ mg N m}^{-2} \text{d}^{-1}$ in sediment cores enriched to 20 mg N L^{-1} . Kaspar (1982) measured a denitrification potential of $281 \text{ mg N m}^{-2} \text{d}^{-1}$ in anaerobic, nitrate-saturated marine sediment slurries. Seitzinger and Nixon (1985) measured denitrification rates of approximately $300 \text{ mg N m}^{-2} \text{d}^{-1}$ in marine mesocosms at the highest level of N enrichment ($1.3 \text{ g N m}^{-2} \text{d}^{-1}$). Binnerup *et al.* (1992) measured denitrification rates of $420\text{-}490 \text{ mg N m}^{-2} \text{d}^{-1}$ in an estuarine sediment. Clearly, higher denitrification rates at elevated nitrate concentrations are possible than indicated by the data of this study, suggesting that denitrification rates as measured by the acetylene blockage technique were underestimated by as much as an order of magnitude.

Regardless of which dissimilatory pathway was most important in the present study, nitrate mediated the decomposition of substantial quantities of organic matter. Although sediment organic matter content before and after the experiment was not measured, the accumulation of alkalinity reflected the extent of net or cumulative respiration.

Carbon accumulation in control tanks as estimated by alkalinity accumulation was relatively low ($0.37 \text{ g C m}^{-2} \text{d}^{-1}$) suggesting autotrophic and heterotrophic processes were approximately in balance. However, net heterotrophy was indicated by an accumulation of alkalinity in tanks enriched with nitrate at 10 and 20 mg N L^{-1} equivalent to net mineralization of 2.86 and $3.97 \text{ g C m}^{-2} \text{d}^{-1}$, respectively. The rate of carbon accumulation can be related to rates of organic matter decomposition.

Linear regression of net carbon mineralized with respect to nitrogen added (as nitrate) yielded the following relationship: $\text{net C mineralized} = 0.141 + 4.25 * \text{N added}$ ($r^2=0.93$). Thus, approximately 4.25 g C were mineralized for each g of NO_3^- -N supplemented.

Nitrate supplementation was proposed as a method of limiting P release from sediment by controlling redox potential (Ripl 1976, Andersen 1985). Nitrate supplementation was not effective at reducing eutrophication in these mesocosms receiving elevated quantities of nutrient and organic inputs. Most semi-intensive aquaculture ponds are hypereutrophic, particularly in the summer, and phytoplankton growth is likely light-limited. However, in ponds in which phytoplankton growth is limited by P, nitrate supplementation may control eutrophication. In this study, SRP concentration was controlled predominately by DO concentration in the overlying water. SRP concentrations in STR tanks were more than 2x those of MIX tanks. In aerobic waters, nitrate supplementation may enhance the effect of oxygen and extend the depth of sediment redox potential above +220 mV. The thickness of such a layer is directly related to its effectiveness as a barrier to the diffusion of reduced substances, such as iron-associated P, to overlying water.

Whole pond additions of nitrate are costly, impractical and increase the risk of water quality deterioration due to the production of ammonia and nitrite from nitrate dissimilation. The potential benefits of improved dissolved oxygen and reduced soluble phosphorus are likely outweighed by the risks of increased stress associated with potentially sublethal concentrations of ammonia and nitrite. The

relative importance of processes associated with nitrate supplementation is a function of mixing regime (Table 40).

Table 40. Ranking of importance of processes related to nitrate supplementation suggested by factor analysis.

Process	Mixing Regime	
	STR	MIX
DO sparing	5	
DNR ¹ /phytoplankton uptake balance	2	1
fertilizer effect	3	2
organic matter mineralization	1	3
P solubility	4	

¹ DNR=dissimilatory nitrate reduction

It may be possible to obtain some benefit with respect to organic matter decomposition at lower target nitrate concentrations. For example, the concentration of nitrate equivalent (equimolar) to that of oxygen at saturation in freshwater at 25 C at sea level (8.24 mg DO L⁻¹) is 3.6 mg NO₃⁻-N L⁻¹. In this study, target nitrate concentrations were equivalent to DO concentrations of 22.8 and 45.7 mg L⁻¹. Further research is required to evaluate the efficacy of nitrate concentrations <10 mg N L⁻¹ as a sediment management technique.

It may also be possible to obtain some benefit from nitrate supplementation with respect to organic matter decomposition by alteration of the application technique. In this study, nitrate was spread over the tank surface as a concentrated solution. It may be more practical to broadcast the salt over the tanks surface and allow the chemical to settle undissolved to the pond bottom. Decomposition in the sediment may be enhanced by directed application of nitrate. Direct nitrate supplementation of fish pond sediments may hasten organic matter decomposition, reduce sediment oxygen demand and phosphorus solubilization, and minimize environmental impact by reducing the organic matter concentration of discharged water, particularly during the seining phase of pond harvest.

While whole pond additions of nitrate are not recommended as a practical treatment, nitrate supplementation may be useful as an indicator of the reducing power of fish pond sediment. A fairly rapid evaluation of sediment condition may be accomplished by inserting a plastic pipe into the sediment, supplementing water enclosed in the pipe with nitrate and measuring the rate of nitrate depletion. Temporal or spatial variation in the depletion rate may offer insights into changes in the condition of fish pond sediment.

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Table 41. Half-matrix of Pearson correlation coefficients (R) among water quality variables (N=120).

Variable ¹	DO	NO ₂ ⁻ -N	TAN	SRP	VSS	FSS	C+P	CO ₂
DO	-							
NO ₂ ⁻ -N	0.010	-						
TAN	-0.351	0.365	-					
SRP	-0.534	-0.189	0.297	-				
VSS	0.180	-0.110	-0.290	-0.083	-			
FSS	0.693	-0.004	-0.296	-0.501	0.327	-		
C+P	0.168	-0.069	-0.400	-0.137	0.775	0.198	-	
CO ₂	-0.143	-0.175	0.334	0.162	-0.220	0.034	-0.261	-

¹ DO=dissolved oxygen at dawn; NO₂⁻-N=nitrite-nitrogen; TAN=total ammonia-nitrogen; SRP=soluble reactive phosphorus; VSS=volatile suspended solids; FSS=fixed suspended solids; C+P=chlorophyll + phaeophytin; CO₂=carbon dioxide.

CONCLUSIONS

This research demonstrates the importance of sediment in the flow of nitrogen in aquaculture ponds. Sediments are the locus of organic matter decomposition in aquaculture ponds and thus are a source of mineralized (reduced) nitrogen. Organic matter settling to sediment consists largely of senescent phytoplankton, which is labile and therefore subject to rapid mineralization. The majority of ammonium produced during mineralization diffuses to the overlying water. A modeling study indicates that sediments contribute from 25-33% of the ammonium supplied to fish ponds.

Coupled nitrification-denitrification reactions are relatively unimportant pathways of nitrogen transformation in sediment. However, sediment nitrification is important during spring and fall when conditions are favorable. Sediment nitrification is controlled by the depth of oxygen penetration during the growing season and by temperature during winter. Seasonal peaks of nitrite in semi-intensive channel catfish ponds can be attributed, in part, to sediment nitrification. Although sediments may be a seasonal source of oxidized nitrogen (nitrite and nitrate), in general, sediments are anaerobic and therefore sinks for nitrite and nitrate.

Sediment nitrogen transformations are interconnected with those of carbon and oxygen. The C:N ratio of organic inputs to aquaculture ponds has a strong effect on the fate of nutrients. Nitrogen mineralization is greater from inputs of low C:N ratio as microbial metabolic requirements for nitrogen are exceeded; nitrogen is conserved or sequestered by microbial assimilation when C:N ratio is high. For

similar reasons, planktonic nitrification is inversely related to input C:N ratio. Low C:N ratio organic matter is a locus for microbial activity, particularly mineralization and nitrification.

Sediments are sinks for oxygen. Oxygen is usually present in a thin (1-5 mm) layer at the sediment-water interface. Oxygen is consumed during organic matter decomposition, chemautotrophic nitrification and in the oxidation of reduced substances diffusing from anaerobic sediment. Thus, the ratio of oxygen consumed to nitrogen released from sediment is variable. Higher ratios are characteristic of highly reduced sediment or sediment with high nitrification rates; lower ratios indicate that most oxygen is consumed for organic matter mineralization and nitrification is relatively unimportant.

Although oxygen is the most important terminal electron acceptor involved in organic matter decomposition, large quantities of organic matter are mineralized or fermented under anaerobic conditions. Although nitrate can function as a terminal electron acceptor, nitrate concentrations are usually insufficient to be of any importance as a mediator of organic matter decomposition. However, if nitrate concentrations are elevated, substantial quantities of organic matter decomposition mediated by nitrate will occur. This was inferred from accumulation rates of alkalinity, which increased in response to carbon dioxide production. Alkalinity accumulation rate is a useful indicator of the trophic status of the production system.

The goal of sediment management is to improve water quality for fish production. Secondary goals may be related to nutrient conservation or removal. In

fish production systems based on the manipulation of detrital or phytoplankton-based food webs, it is important to conserve nutrients within the pond. Nutrients are supplied to intensive or semi-intensive fish production systems in excess of the physiological capacity for assimilation by fish and are released in a form that can not be utilized by the target organism. Mechanisms of nutrient removal are required in such systems.

The sediment management methods evaluated in this research demonstrate that sediment disturbance can increase the mineralization of nutrients sequestered in sediment organic matter. Light penetration is limited by the suspension of solids (primarily clay turbidity) that reduces primary production and therefore respiration. Continuous suspension of sediment in culture systems based on phytoplankton-based food webs is therefore undesirable. However, in semi-intensive productions systems, such reductions in phytoplankton and respiration are desirable. In addition, the enhancement of planktonic nitrification by solids suspension can increase nitrogen removal by increasing water column nitrate concentration. Although sediment denitrification rates in aquaculture ponds are low, increasing nitrate concentration in the overlying water will increase nitrogen removal by denitrification.

The effect of additional sediment management methods on nitrogen biogeochemistry requires evaluation. Drying the pond bottom is the most important and effective sediment management method to improve water quality. Clearly, this method suffers the disadvantage of requiring that the pond be drained, thereby

limiting the length of the production cycle. *In situ* methods of sediment management require further development and testing.

Further refinements in research methodology relating to sediment nitrogen transformations are required. Although short-term incubations provide a rapid assessment of solute flux rates across the sediment-water interface and fairly large numbers of cores can be incubated simultaneously, the accuracy of this method is open to question. Flow-through systems allow the maintenance of stable conditions and thus may be more accurate, but require relatively lengthy incubations to establish steady-state conditions. Ideally, measurements will be made *in situ*, perhaps using benthic chambers, although the management of a large number of simultaneous incubations is logistically difficult. In addition, sediment perturbation upon placement of the chamber may bias results. The sensitivity of methods used to measure sediment nitrification is low. Recent refinements in microelectrode technology coupled with modeling of sediment porewater profiles and stable isotope enrichment offers the possibility of accurate determination of the distribution and rates of sediment nitrification and denitrification.

Model sediment systems can be developed for process isolation in the laboratory or for whole-system analysis in the field. Such field-based mesocosms are useful for fairly low-cost determination of the relative effect of a treatment across a practical range. Results from studies conducted in aquatic mesocosms should not be extrapolated directly to full-scale (commercial) production systems. Differences

across the range of a particular treatment effect are more important than actual values.

Additional research is required regarding sediment-water column interactions in aquaculture ponds. In particular, the factors affecting seasonal maxima of nitrite concentration require more accurate elaboration. A better understanding of the dynamics of sediment oxygen demand with respect to organic matter mineralization and nitrification may provide insights with potential management application. The competition for nutrients and other interactions between phytoplankton and planktonic bacteria in aquaculture ponds are not well known. The nutrient uptake rates of natural assemblages of phytoplankton in aquaculture ponds have not been thoroughly investigated.

This research has focused on nitrogen biogeochemistry of aquaculture pond sediments and how nitrogen interacts with carbon and oxygen. However, the capacity of sediments to adsorb substantial quantities of phosphorus under aerobic conditions and desorb phosphorus under anaerobic conditions, and the importance of phosphorus as a limiting factor of phytoplankton growth point to the need to include this nutrient in a comprehensive research program of sediment dynamics.

VITA

John Hargreaves was born in Hartford, CT on 24 July 1958. He attended public schools in Connecticut and Pennsylvania before moving to Brazil in 1974, where he attended private school until 1976. He attended Amherst College in Amherst, MA from 1976-1980, and graduated with a B.A. in anthropology (*magna cum laude*) and biology (*rite*). Upon graduation, he worked for two years as an inland fisheries extension agent for the U.S. Peace Corps in Sierra Leone, West Africa. After returning to the U.S., he worked at a shellfish hatchery and an experimental farm before accepting a position as a research technician with the College of the Virgin Islands Agricultural Experiment Station aquaculture research program. In 1985, he entered Department of Fisheries and Allied Aquacultures at Auburn University, Alabama. After receiving the M.S. degree in 1986, he returned to the University of the Virgin Islands as a research specialist in the aquaculture research program. In 1991, he was awarded a Board of Regents Fellowship to support research toward the Ph.D. in Wildlife and Fisheries Science through the School of Forestry, Wildlife and Fisheries of Louisiana State University.

DOCTORAL EXAMINATION AND DISSERTATION REPORT

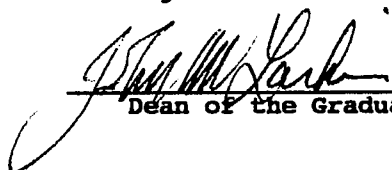
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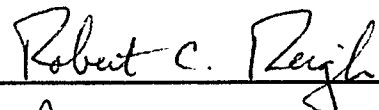
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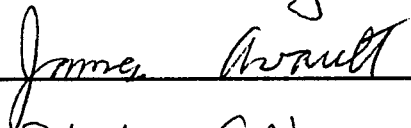
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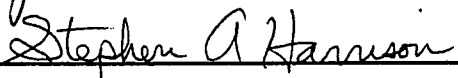

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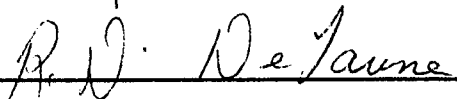

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EXAMINING COMMITTEE:











Date of Examination:

January 25, 1995
