Nitrogen transformations and fluxes in fish ponds: a modelling approach

Ricardo A. Jiménez-Montealegre

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Stellingen

1. It is evident that aquaculturists should feed their fish rather than their pond.
   This thesis

2. In aquaculture enterprises, the whole nitrogen cycle must be optimized to minimize nitrogen discharges into the environment.
   This thesis

3. Rate differences between the various parts of the nitrogen cycle result from environmental differences between aquaculture systems.

4. The concepts in the minds of scientists are easier to discuss and improve when they are presented as mathematical models.

5. Fish driven resuspension increases pond productivity.

6. In spite of growing computing power, computers will remain only tools to understand our environment better.

7. "Data become information if we know the processes involved in the system. Information becomes knowledge if we understand how the system is operating. But knowledge becomes wisdom only when we see how the system must change and deal with reality" (Peter Allen: Coherence, Chaos and Evolution in the Social Context. Futures 26: 597, 1994).

8. "A man with one watch knows what time it is. A man with two watches is never sure" (Segal's law).

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General Introduction

Background

Aquaculture represents one of the fastest growing food producing sectors. By 1998, the total production of cultured finfish, shellfish and aquatic plants reached 39.43 million tons (FAO 2000). World food production will have to increase to satisfy the increasing demands of the growing world population, which will have grown to 8 billion people by 2025. Fisheries production can not increase further, and therefore, any future growth in fish protein supply will have to come from aquaculture. The potential of aquaculture to meet the challenges of food security is clearly demonstrated by the rapid expansion of this sector, which has grown at an average annual rate of almost 10% since 1984 compared to 3% for livestock meat and 1.6% for capture fisheries production (FAO 1997).

Aquaculture uses natural resources like water, land, fertilisers and feed. Ground and surface freshwater resources are finite, while societal demands for these resources are growing. Considering the explosive growth of aquaculture and the limited availability of its resources, there is a need for more efficient resource use.

Aquaculture production systems can be characterised based on input/management levels, from extensive (low level of input/management) to intensive (high level of input/management). Growth in land-based aquatic production since 1984 was partly the result of intensification combining the use of high quality feeds, with increased stocking densities and water use. The high nutrient input levels applied in intensive culture may surpass the carrying capacity of the culture environment, and lead to water quality problems. By replacing the nutrient rich water with clean, nutrient poor water, culture problems due to bad water quality are avoided. However, the large amounts of water needed to maintain good water quality are not always available, and shortage leads to eutrophication of pond ecosystems. Dominance or frequent blooms of blue green algae (Sevrin-Reyssac and Pletikosic 1990), higher daily fluctuations in pH or dissolved oxygen concentrations (Smith 1985) and highly unbalanced C:N ratios (Avnimelech et al. 1992) are some of the problems related to eutrophication. In addition, discharging large amounts of nutrient rich water leads to eutrophication of the surrounding surface waters, where the above mentioned problems will also occur. With high levels of eutrophication, diseases occur more frequently, as reported for shrimp farms (Lightner et al. 1992). In regions with a high farm density, diseases easily spread among farms through the surrounding surface

waters. In all cases disease outbreaks leads to significant losses of farmed stocks and diminished financial returns.

Nitrogen and aquaculture

Nitrogen is an essential element in aquaculture. It is mainly present as protein, which is found in all life organisms. However, many inorganic forms of nitrogen are also present, and some forms can be toxic to aquatic organisms. Nitrogen inputs in the form of feeds/fertilisers enhance the aquatic production but simultaneously increase the potential of pollution of the surrounding environment. Control of nitrogen transformation processes in the pond combined with optimal feed utilisation in aquaculture systems are needed. On average, 30% of the nitrogen added to ponds as feed or fertiliser is recovered by the target organism, which means that 70% of the nitrogen input is excreted in a dissolved or particulate form (Edwards 1993). Nitrogen loading rates of aquaculture ponds are often limited by the capacity of the pond to assimilate nitrogenous excretion (Hargreaves 1998).

The principal end product of protein metabolism in fish is ammonia. After oxygen, ammonia is the second most common limiting factor for fish stocking density (Robinette 1976; Colt and Tchobanoglous 1978; Tomasso *et al.* 1979; Tomasso *et al.* 1980; Shilo and Rimon 1982; Schwedler and Tucker 1983; Palachek and Tomasso 1984; Meade 1985). Not all the effects of sub-lethal ammonia levels on growth are known, but ammonia may lead to proliferation of the gill epithelium, thus reducing the oxygen uptake capacity of the gills and affecting growth (Burrows 1964; Larmoyeux and Piper 1973). Production losses may be substantial (Meade 1985).

Just as in any other (intensive) agricultural practice, nitrogen discharge is one of the principal sources of pollution due to aquaculture (Jørgensen and Rasmussen 1991). The amount of nitrogen discharged from aquaculture farms is influenced by several factors such as the amount of feeds/fertilisers applied and their efficiency of use within the system. An important goal today is to maintain good water quality while improving the retention of the nutrient inputs into harvestable products. As a result, less nutrients will be discharged or lost.

The nitrogen cycle in ponds is a mixture of various biotic and abiotic processes. The complexity of the nitrogen cycle, with many different forms of nitrogen existing side

by side, and the numerous transformation processes are shown in Figure 1. Although the basic processes of the nitrogen cycle were described in detail, it remains difficult to understand the complexity of the whole nitrogen cycle.
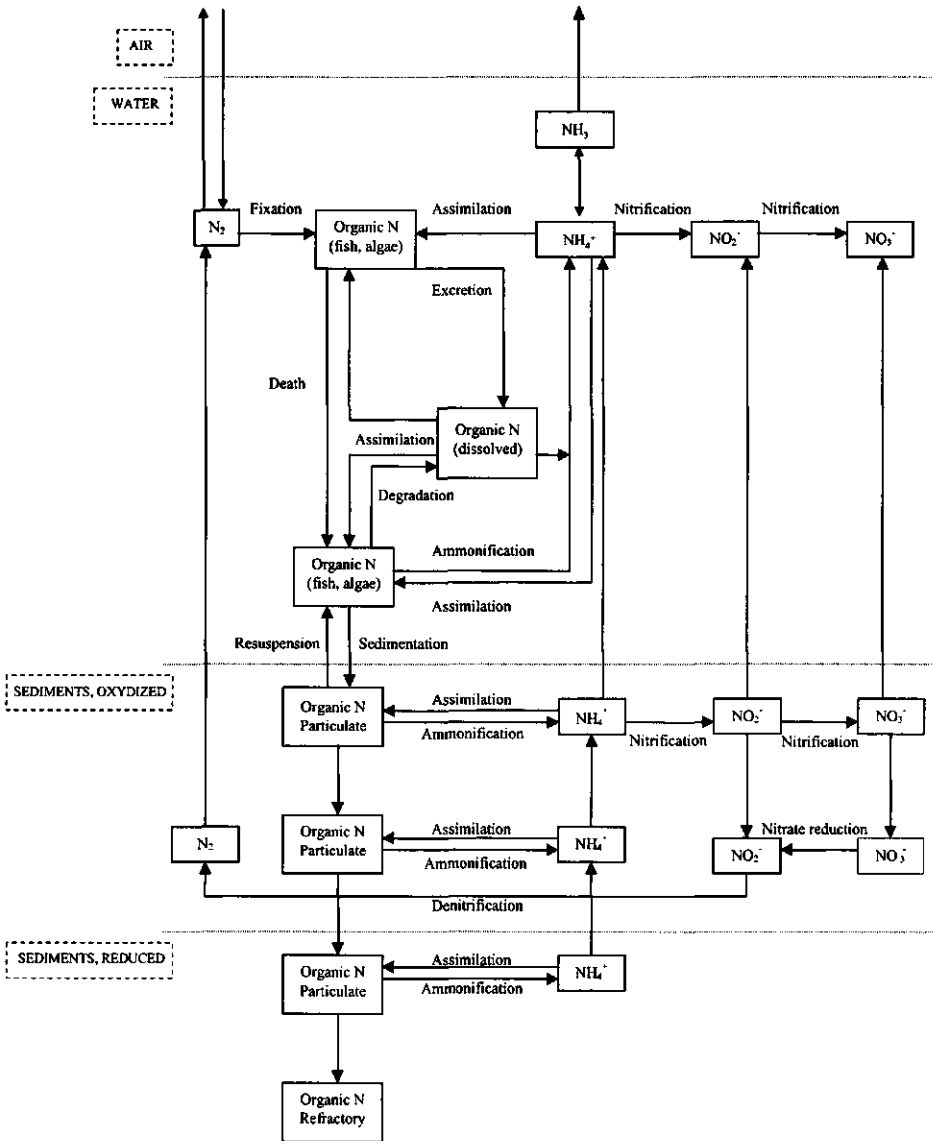
Figure 1. Principal nitrogen forms and processes within the nitrogen cycle

Most of the literature on nitrogen cycling in shallow aquatic systems has been directly applied to aquaculture ponds. Management strategies to control the deleterious effects of nitrogen accumulation in the system have been proposed, but their effectiveness is limited by our present understanding of the nitrogen cycle in aquaculture ponds. Studies of nitrogen cycling under the particular circumstances prevailing in earthen aquaculture ponds are scarce. A better understanding of the fluxes and transformations of nitrogen in aquaculture production systems is, therefore, needed.

Modelling of the nitrogen cycle

Modelling of the nitrogen cycle allows to explore, to understand, and to re-evaluate relationships between N-species in the system. In aquaculture, the use of computer models both to understand the basic structure and function of systems and to evaluate the impact of management practices has increased considerably. Still, application of mathematical modelling to aquaculture is a relatively new endeavour. General principles of modelling and simulation of aquaculture systems were reviewed (e.g. Bolte *et al.* 1986; Piedrahita 1988; Cuenco 1989; van Dam 1990). Most of the models, though, are related to particular aspects of the functioning of the aquaculture system (Colt and Orwicz 1991; Wheaton 1991; Piedrahita 1991; Kochba *et al.* 1994; Avnimelech *et al.* 1995; Hargreaves 1997; Montoya *et al.* 1999; Drapcho and Brune 2000; Verdegem *et al.* 2000). Models for nitrogen dynamics of whole farming systems have been proposed recently. Jamu (1999) proposed a model for integrated aquaculture/agriculture systems and Nielsen *et al.* (1999) proposed a model for the nitrogen cycle in a system for combined production of rice and crayfish.

The principal chemical and/or biological processes involved in the nitrogen cycle are: nitrification (some times divided into nitritification as the microbial oxidation of ammonia to nitrite, and nitratification, the oxidation of nitrite to nitrate, with a number of less important intermediates), denitrification (the dissimilatory reduction of oxidised nitrogen into gaseous oxides and nitrogen gas), nitrate reduction (another dissimilatory reaction, known also as nitrate respiration), ammonification (the bacterial conversion of organic nitrogen into NH_3 or NH_4^+), nitrogen utilisation (also known as assimilation or nitrogen immobilisation), fixation (the biological utilisation of N_2 by some prokaryotic

organisms) and excretion (by plants and animals). Besides, some physical processes are also involved in the cycle, namely volatilisation (a transport process from the water column to the air), and diffusion-flux (a passive physical process of movement of gases or dissolved nitrogen between the sediment-water interface, along a concentration gradient). Hargreaves (1998) presented an extensive review of nitrogen biogeochemistry in aquaculture ponds.

Nitrification

Nitrification has been generally modelled as a first-order process following one or two step oxidation processes (Zison *et al.* 1978). The Monod equation, analogous to Michaelis-Menten (single enzyme, single substrate) has been used and is normally written as:

$$\frac{dC}{dt} = k' \times C_M \times \frac{C}{C + Ks} \tag{1}$$

where k' is the specific substrate uptake rate, C is the substrate concentration, Ks is the Monod half-saturation constant, and C_M is the concentration of nitrifiers. If in a system the concentration of nitrifiers C_M is constant (e.g. in steady state conditions), the Monod equation can be simplified to:

$$\frac{dC}{dt} = k \times \left[\frac{C}{C + Ks} \right] \tag{2}$$

where k is the first-order rate constant. Stratton and McCarty (1967) applied Monod kinetics to nitritification and nitratification in ammonium or nitrite enriched water samples of a river, and calculated k' and Ks for measurements done under different temperatures and different initial substrate (NH_4^+ or NO_2^-) concentrations (Table 1). Typical values of kinetic constants for nitrification, following the Monod model, were reviewed by Painter (1977) and Sharma and Ahlert (1977).

Table 1. Some reported parameters for nitrogen processes in aquatic environments

Process/ Parameter	Name	Value	Source
Nitrification			
k'	Specific substrate uptake rate	0.75 to 2.52 d^{-1} [a] 3.44 to 7.56 d^{-1} [b]	Stratton and McCarty 1967
k_s	Monod constant	1.85 to 5.59 mg L^{-1} [a] 0.06 to 1.77 mg L^{-1} [b]	Stratton and McCarty 1967
θ	Temperature coefficient for nitrifying organisms	0.877 to 1.120	Jenkins 1969; Zanoni 1969; Smith 1977
Denitrification			
k	Kinetic parameters	12 to 143	Andersen 1977
b	Kinetic parameters	0.4 to 0.9	
k_d	First-order rate constant (nitrogen balance)	0.02 to 0.03 d^{-1}	Jørgensen et al. 1978
k_d	First-order rate constant (calibration)	0.039 to 0.044 d^{-1}	Jørgensen et al. 1978
k_d	First-order rate constant (curve fitting)	0 to 1.3 d^{-1}	Billén 1978
k_d	First-order rate constant	0.17 to 1.21 d^{-1}	Van der Borght and Billén 1977
k_d	First-order rate constant	0.406 to 1.175 d^{-1}	Madsen 1979
Ammonification			
k_{NH4}	First-order rate constant	0.021 to 0.120	Fallon and Brock 1979
k_{NH4}	First-order rate constant	0.1 d^{-1}	Avnimelech et al. 1995
Assimilation			
k_N	First-order rate constant	0.15 d^{-1}	Avnimelech et al. 1995
k_{NO3}	Michaelis-Menten constant for nitrate	0 to 0.035 mg L^{-1}	Scavia 1980
k_{NH4}	Michaelis-Menten constant for ammonium	0.025 mg L^{-1}	Scavia 1980
k_N	Michaelis-Menten constant	0.2 mg L^{-1}	Lehman et al. 1975; Chen and Orlob 1975
k_N	Michaelis-Menten constant	0.005 mg L^{-1}	Scavia and Park 1976
k_N	Michaelis-Menten constant	0.001 to 0.009 mg L^{-1}	Pasciak and Gavis 1974

[a] nitratification
[b] nitritification

Several models consider oxygen, temperature and/or pH as limiting factors for nitrification (see Box 1). According to Ohgaki and Wantawin (1989), the most crucial part of modelling nitrification is the accurate estimation of nitrifying bacterial growth, and the reliability of nitrification modelling will therefore depend on the quality of the bacterial growth model.

BOX 1. Factors affecting nitrification

Oxygen
In their review, Sharma and Ahlert (1977) presented an overview concerning the influence of oxygen concentration on the nitrification rate. According to these authors, oxygen becomes limiting at DO levels below 4 ppm (bulk water column).

Temperature
For the effect of temperature on nitrification, an exponential expression has been used (Ohgaki and Wantawin 1989):

$$\mu_m = \mu_{m, ref} \times \theta^{(T-Tref)}) \tag{3}$$

where μ_m and $\mu_{m,ref}$ are the maximum specific constants at temperature T and T_{ref} ($0°C$), respectively and θ is a constant for a specific temperature range referred to as the temperature coefficient. Some values for the temperature coefficient for nitrifying organisms are summarised in Table 1. For the temperature range of 15 to 25°C, Stratton and McCarty (1967) determined relations describing the temperature dependence of the specific uptake rates:

$$k_{NH4} = 1.47e^{0.084 \times (T\ 20)} \tag{4}$$

$$k_{NO2} = 4.90e^{0.056 \times (T-20)} \tag{5}$$

where k_{NH4} and k_{NO2} are the specific uptake rates of ammonium and nitrite, respectively. Similar relations were found by Knowles et al. (1965) for the temperature range of 8 to 30°C:

$$k_{NH4} = 2.54e^{0.095 \times (T-20)} \tag{6}$$

$$k_{NO2} = 12.3e^{0.059 \times (T-20)} \tag{7}$$

_pH_
Concerning pH limitations, nitrification seems to be pH-limited when pH is higher than 9 or lower than 7 (Sharma and Ahlert 1977); an optimum pH within that range with rapidly decreasing rates outside this range has been demonstrated (Grady and Lim 1980).

Bacterial population
To describe the growth of nitrifying bacteria, the empirical microbial growth equation after Monod has been employed. Watanabe et al. (1980) applied a zero-order reaction as the intrinsic growth rate of nitrifying organisms. Megee et al. (1972) proposed the growth model which uses a double substrate expression instead of the single substrate Michaelis-Menten expression. Müller et al. (1980) proposed double Monod kinetics for intrinsic growth rates in their mass transfer equations to evaluate nitrification in rotating biological reactors. Also Wanner and Gujer (1984) used the same double Monod function for growth rates when studying the competition between heterotrophs and nitrifying organisms.

In stratified or periodically mixed fish ponds, nitrification at the sediment-water interface is more important than nitrification in the water column (Hargreaves 1998). Although few direct measurements have been made, nitrification rates in aquaculture pond

sediments are in the order of 15 to 25 mg N m^{-2} d^{-1} (Henriksen and Kemp 1988). According to Hargreaves (1998), the magnitude of nitrification in aquaculture ponds is relatively small compared to the rate of other N transformations, being elevated only during periods between cropping cycles when pond bottoms are exposed to air.

Denitrification

A first-order model for denitrification in aquatic systems was proposed by Toms *et al.* (1975). Van der Borght and Billén (1977) and Billén (1978) found a first-order rate constant by curve fitting of the model to nitrate concentration profiles of sediment cores (Table 1).

A more complex approach was followed by Andersen (1977), who used two different models for the description of denitrification in sediments of six Danish fresh water lakes. The models were applied to the decrease of the nitrate concentration of the overlying water at 10°C under aerobic or anaerobic conditions. One of the models followed the Monod approach described above, and the other model was:

$$y = \left(\frac{V}{\Omega}\right) \times \left(\frac{dC}{dt}\right) = k \times C^b \tag{8}$$

where y is the nitrate flux in mg N m^{-2} d^{-1}, V is the volume of the overlying water, Ω is the surface of the sediment, k and b are the kinetic parameters, and C is the concentration of nitrate in the overlying water. The kinetic parameters are presented in Table 1. The value of b was almost equal to 1 under aerobic conditions, and this supports the first-order model; but under anaerobic conditions b was much lower, probably because of denitrification in the overlying water and the reduced importance of transport limitation. Jørgensen *et al.* (1978) used a denitrification coefficient based on nitrogen balance, and by automatic calibration they obtained similar values (Table 1).

Temperature has been frequently included in denitrification models (see Box 2). Madsen (1979) studied denitrification in sediment samples of fresh water lakes and a salt water fjord in Denmark using the $^{15}NO_3$-method and calculated first-order k-values; there was not much difference between results from salt and fresh water, and k_d presented a clear seasonal fluctuation due to differences in temperature and organic matter content.

The rate of denitrification in marine fish ponds was in the order of 45 to 80 mg N m^{-2} d^{-1} (Blackburn et al. 1988; Roos and Eriksen 1995). Since the concentration of nitrate in aquaculture ponds is typically below 0.5 mg N L^{-1}, denitrification is probably substrate limited.

BOX 2. Effect of temperature on denitrification

The dependence of denitrification on temperature was included in the model proposed by Toms et al. (1975). They determined the temperature dependence for their first-order rate constant in the range of 5 to $30^{\circ}C$ as:

$$k_{NH4} = 0.018e^{0.065 \times (T-20)} \tag{9}$$

Van Kessel (1976) also determined the temperature dependence of the overall denitrification rates for sediments. In the temperature range of 4 to $25^{\circ}C$ for nitrate and nitrite respectively, he found the following relations:

$$r_{NO3} = 4.4 \times T + 70 \, mg \, N \, m^{-2} \, d^{-1} \tag{10}$$

$$r_{NO2} = 7.2 \times T + 70 \, mg \, N \, m^{-2} \, d^{-1} \tag{11}$$

where r_{NO3} and r_{NO2} are the overall denitrification rates, and T is the temperature.

Stanford et al. (1975) studied temperature dependence of denitrification in soils. Optimum temperature seems to be between 60 and $65^{\circ}C$. They found mathematical relations in a nitrate concentration range of 4-100 ppm for the first-order rate constants (d^{-1}):

For $11^{\circ}C < T < 35^{\circ}C$:

$$k = 0.0318e^{0.070 \times (T-20)} \tag{12}$$

$$k = 0.0065e^{0.078 \times (T-20)} \tag{13}$$

For $T < 11^{\circ}C$:

$$k_{NH4} = 2.396e^{0.483 \times (T-20)} \tag{14}$$

$$k_{NH4} = 0.646e^{0.414 \times (T-20)} \tag{15}$$

where T is the temperature and k is the first-order rate constant.

Ammonification

Simple first-order models for the description of biodegradation of organic matter in aquatic systems have been used. The most simple equation for ammonification is the Streeper-Phelps first-order equation proposed by Stones (1979):

$$\frac{dC}{dt} = -k \times C \tag{16}$$

where C is the concentration of BOD, COD or particulate organic matter. However, this model appeared to match measurements only partially. As time proceeded during decomposition the value of k turned out to decrease, probably because the substrate consists of components with different degradability. The more easily degradable components are decomposed first, and the refractory part of organic matter degrades more slowly, or not at all. This latter fraction consists of humic acids and similar substances (Jewell and McCarty 1971; Sudo *et al.* 1978; Stones 1979). In order to make a correction for the refractory part (f), the first-order equation was written as (Stones 1979):

$$\frac{dC}{dt} = -k \times \left[C - \left(f \times Co \right) \right]$$ (17)

where C and Co are the substrate concentrations at time t and t_o, respectively, and k is the kinetic parameter. Although f is not a perfect constant in reality, this model was quite successfully used in a number of mineralization tests (e.g. De Pinto and Verhoff 1977; Sudo *et al.* 1978; Ulen 1978). Values for f usually ranged from 0.10 to 0.40, and the first-order rate constants (k-values) ranged from 0.021 to 0.120 d^{-1} (e.g. Fallon and Brock 1979).

Foree *et al.* (1970) constructed a model to estimate the refractory parts of nutrients in particulate organic matter originating from algae. They assumed that nutrient regeneration may take place in two ways: if a cell contains an excess of a particular nutrient, this nutrient will be regenerated at a relatively high rate (excess regeneration) and if the nutrient is present in the cell in its minimal concentration (3-5% for nitrogen) the nutrient regenerates with the same rate as organic-C mineralises (proportional regeneration). The model was fitted to the results of a large number of decomposition experiments, aerobic as well as anaerobic and in salt as well as in freshwater, by variation of the critical nutrient fraction. The model gave a satisfying fit for a wide range of values of refractory part.

The decomposition rate coefficient of organic matter in mud can also be modelled assuming a first-order reaction (Nakanishi *et al.* 1986):

$$k = \left(\frac{1}{t} \right) \times \ln \left(\frac{Sd - So}{Sd - St} \right)$$ (18)

where k is the decomposition rate coefficient (d^{-1}), t is the incubation period (days), Sd is the ultimate biodegradable nitrogen at 35°C after 30 days ($\mu g\ g^{-1}$ wet mud), So is the initial soluble nitrogen ($\mu g\ g^{-1}$ wet mud) and St the soluble nitrogen after t days with the same temperature as field conditions.

In aquaculture, the production of ammonia by the decomposition of organic matter has been modelled as a first-order process (Piedrahita *et al.* 1983). Avnimelech *et al.* (1995) considered mineralization to follow first-order kinetics with an average rate constant of 0.1 (d^{-1}).

Ammonia volatilisation

Volatilisation can be described mathematically by the use of the so-called two-film theory of mass transfer, in which the water phase is assumed to be well-mixed except near the interface. Ammonia is very soluble in water, but since the concentration in the atmosphere is low, volatilisation of ammonia always takes place, especially at high pH when the equilibrium between ammonia and ammonium is displaced towards the free form (Jørgensen 1989). Sherlock *et al.* (1989) estimated the ammonia flux into the atmosphere using the equation:

$$Fa = c \times \left(up - up_b\right) \tag{19}$$

where Fa is the ammonia flux at the height at which this flux to the atmosphere is measured, c is a constant calculated to be 0.11, up is the upwind, mean horizontal flux density of ammonia from the system, and up_b is the mean upwind horizontal flux density of ammonia for a background sampler.

The transfer rate can also be calculated as the re-aeration coefficient and as a function of wind velocity (Piedrahita *et al.* 1983; Boyd and Teichert-Coddington 1992). Reported rates of ammonia volatilisation in fish ponds are between 10 and 70 mg N $m^{-2}d^{-1}$ (Schroeder 1987; Gross *et al.* 1999).

Nitrogen utilisation

The microbial assimilation of nitrogen can be calculated assuming that degradation of the organic carbon present in the system follows first-order kinetics (e.g. Avnimelech *et al.* 1995). In most of the models, it is assumed that the concentration of bacteria

responsible for nitrogen compound transformations is invariable, and its biomass is included in the form of a rate constant. However, the concentration of micro-organisms that oxidise, for example, ammonium and nitrite may increase 2-3 orders of magnitude within a few days (Mijake and Wada 1968).

Nitrogen uptake by phytoplankton in fish ponds can be as high as 1500 mg N m^{-2} d^{-1} (Hargreaves 1998), and is the principal pathway of dissolved inorganic nitrogen removal in aquaculture ponds (Tucker et $al.$ 1984). Algae preferentially assimilate ammonia over nitrate (Syrett 1981) and the preference for ammonia uptake is generally modelled by a Michaelis-Menten expression in terms of ammonia concentration, the preference for ammonia being reflected in the value or the half-saturation constant (k_N). Several reported constants are presented in Table 1. Nitrogen uptake by algae can also be estimated assuming that uptake is equal to the daily primary production times the C:N ratio in algal cells (Kochba et $al.$ 1994).

Nitrogen uptake by phytoplankton occurs over a time scale of minutes to hours, and few models take this into account (Collins and Park 1989). The number of algal groups to be modelled is another aspect to consider. Scavia (1980) modelled five groups of algae, while other models include four (e.g. Canale et $al.$ 1976; Park et $al.$ 1985).

Photosynthesis is usually represented as a function of multiplicative factors for light, nutrients and temperature (Park et $al.$ 1985). The ecological models which take into account the impact of phytoplankton and zooplankton on the conversion of mineral nitrogenous matter describe the nitrogen compound transformation in comparatively simple mathematical formulations along with models which cover a more diverse composition of media components (e.g. Thomann et $al.$ 1974; Ikeda and Adachi 1976). Two principal ways of modelling nutrient limitation are used in current models: growth can be expressed as limited by external nutrient concentrations or by internally stored nutrients (Collins and Park 1989). Most comprehensive models use the minimum limiting nutrient construct. In the internal nutrient approach, nutrient limitation is assumed to be based on a threshold relationship: the most limiting internal nutrient determines the rate of photosynthesis.

Excretion

There are several forms of nitrogen excretion in water systems, but in fish ponds, the excretion by the cultivated organism (faeces and ammonia) is probably the most

important one. From the efficiency of fish nitrogen assimilation, it can be estimated that 60 to 70% of the N added in the feed is excreted. Faeces consist of a mixture of non-assimilated feed (settable, suspended and dissolved faeces) plus different non-reabsorbed residues of body origin from the intestine (mucosal cells, digestive enzymes, other excretions and microflora). Total nitrogen excretion (not considering faecal excretion) is the sum of endogenous excretion and exogenous excretion (Brett and Groves 1979). In fish, this excretion consists of NH_3, urea and negligible amounts of uric acid, amino acids, and other nitrogen containing compounds (Goldstein and Forster 1970; Fischer 1977). For the quantification and modelling of ammonia excretion by fish, approaches based on the intermediate metabolism and biochemical pathways were used (e.g. Machiels and Henken 1986; 1987).

Excretion of nitrogen by zooplankton and phytoplankton has also been studied. Paulson (1980) developed models of ammonia excretion that showed good agreement between actual and predicted values. The coefficient used, representing the inorganic nitrogen content of zooplankton excreta, was 0.08. A coefficient of 0.09 for nitrogen excretion by phytoplankton based on a carbon to nitrogen ratio of 5.5 was used by Canale *et al.* (1974). Excretion of ammonia by zooplankton and fish has been expressed also as function of respiration rate and population size (Piedrahita *et al.* 1983).

Diffusion-flux

Diffusion of nitrogen is important, since the different biotic and abiotic reactions that take place in the sediment cause a steep concentration gradient of dissolved components near the sediment-water interface.

The diffusion of dissolved components can be described according to the second law of Fick (Berner 1980):

$$\frac{\delta C}{\delta t} = Dm \times \left(\frac{\delta^2 C}{\delta z^2} \right) \tag{20}$$

where $\delta C / \delta t$ is the concentration gradient of a nitrogen form, C is the concentration of that nitrogen form, t the time, D_m the molecular diffusion coefficient, and z the depth coordinate. A correction factor related to porosity of the sediment can also be incorporated:

$$\frac{\delta C}{\delta t} = Dm \times \left(\frac{\delta^2 C}{\delta z^2} \right) \times k\pi \qquad (21)$$

where k is a diffusion resistance factor with a value of approximately 0.3, and π is the porosity.

The diffusion rates in the interstitial and overlying water differ markedly from each other (Davison 1985) since in the first the transport occurs mainly by molecular diffusion, while in the second convective processes are important too. The diffusive boundary layer is defined by Berner (1980) and Van Luijn (1997) as a thin layer of water adjacent to the sediment that is formed due to the decrease of the velocity towards the sediment surface. The exchange rate of dissolved species then depends on the thickness of the diffusive boundary layer (Revsbech *et al.* 1986).

Since ammonia accumulates in the reduced sediments of aquaculture ponds, this diffusion flux has received particular attention. However, estimates of ammonia flux in freshwater and marine aquaculture ponds are very variable, oscillating between less than 10 and more than 150 mg N m^{-2} d^{-1} (e.g. Avnimelech 1984; Hargreaves 1997; Riise and Roos 1997).

Sedimentation can also be considered as a diffusion flux of nitrogen. It is very important in ecological modelling as it represents a considerable mass transfer from the water column into the sediments. Suspended matter is removed from the water by sedimentation or settling. Settling rates depend on the viscosity of the water, the size and shape of the particles, the difference in density between the suspended matter and the water, the turbulence and velocity of the flow field. The removal of nitrogen by settling is most often described as a first-order reaction. A simulation model that partitioned the fate of nitrogen added to semi-intensive shrimp ponds predicted that 48 to 66% of the nitrogen will settle on the pond bottom in the form of phytoplankton (Lorenzen *et al.* 1997).

Objectives and outline of the thesis

If pond aquaculture is to satisfy the increasing demand for fish, more efficient and intensive fish production systems will be required. Such systems operate with high levels of nitrogen input. To minimise negative effects on water quality and prevent

environmental problems, effective management strategies for nitrogen will have to be developed. Although much is known about the basic processes of the nitrogen cycle, the ways in which separate processes are linked together and their relative importance under the unique conditions prevalent in earthen aquaculture ponds need further investigation. Models facilitate the understanding of complex systems and are essential for a quantitative approach that can lead to practical management recommendations. Thus, the principal objectives of this study were:

1. to integrate the information available on nitrogen processes in fish ponds into a predictive dynamic simulation model;

2. to identify and study processes on which information is lacking, and incorporate the results in the simulation model; and

3. to use the improved model to evaluate the effect of possible management strategies on the nitrogen dynamics in fish ponds .

This thesis is divided into five chapters. Chapter 1 deals with nitrogen budgets and fluxes in fish ponds. Most of the work on nitrogen balances in fish ponds is based on information obtained by comparing pond dynamics at the start and at the end of the growth cycle. In this work, the mass nitrogen balances were constructed for different periods during the growing cycle. Through this temporal approach, more information can be obtained. A complete overall recovery and good temporal recoveries were found for N accumulation in fish, accumulation in the sediment and for seepage. A first-order rate constant for decomposition of organic nitrogen for the whole pond was also obtained.

In Chapter 2, a simulation model for nitrogen dynamics and fluxes is proposed. To date, there are few integrative models for feed-driven fish ponds, and the model integrates existing knowledge about nitrogen transformations in fish ponds. The model is divided into three modules: fish, phytoplankton and sediment-water. After calibration and validation, the model was used for the simulation of nitrogen dynamics in fish ponds. Results show that the model needs improvement with regard to simulation of organic matter accumulation in the sediment. Therefore, two studies were done to investigate further the organic matter dynamics in the bottom.

In the first study (Chapter 3), the organic matter accumulation in the pond bottom is investigated. A simulation model that considers dead phytoplankton, uneaten feed and

faeces as the three principal nitrogen sources of organic matter in the pond bottom is constructed. First-order rate constants for mineralization of organic carbon and nitrogen are determined experimentally and used to parameterise the model.

In the second study (Chapter 4), sedimentation/resuspension is investigated as another process related to organic matter accumulation/decomposition in the pond bottom. The sedimentation and resuspension of organic nitrogen are determined over a growing cycle, in relation to nutrient input, water quality parameters, fish biomass/numbers and fish size. Using a dilution analysis method, it was possible to differentiate between sedimented and resuspended particles, so that sedimentation and resuspension rates could be calculated independently.

Finally, Chapter 5 integrates the information from Chapters 1, 3 and 4 into the model presented in Chapter 2. The improved model is validated with an independent set of data, and the output compared with the output of the initial model. At the end of the thesis, an overall discussion concerning nitrogen dynamics and fluxes in fish ponds is presented in the light of the initial objectives.

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Chapter 1

Nitrogen budget and fluxes in

Colossoma macropomum ponds

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Abstract

Following other studies in which nitrogen balances were obtained by comparing the pond at the beginning and at the end of a growth cycle, in this work nitrogen in the pond system was monitored during the growing cycle to obtain more information through this temporal approach. The accumulation of nitrogen in the water column, in the sediments, fish and seepage water was quantified during a production cycle of *Colossoma macropomum*. By combining estimates of the deposition rates of uneaten feed, faeces and dead phytoplankton with measurements of nitrogen accumulation in the sediment, the rate of decomposition of organic matter in the sediment was evaluated. The first order rate constant was 0.237 ± 0.019 d^{-1}, representing the decomposition rate of organic nitrogen in the pond as a whole. The total nitrogen recovery during the first 2 periods was about 65%. Later, during periods 3-5, the nitrogen recovery was close to 100%. The cumulative recovery at the end of the experiment was almost 100%, meaning that the nitrogen budget in the system studied can be fully explained without any consideration of nitrogen volatilisation, due to either denitrification or ammonia volatilisation. Feed conversion was positively correlated with nitrogen accumulation in sediment and water column, and inversely correlated with nitrogen accumulation in fish tissue. In the beginning of the growth cycle, the major flux of nitrogen was the sedimentation to the bottom soil. Intensive microbial degradation processes took place about 3-4 weeks later, leading to a release of inorganic nitrogen and an approach toward a steady state with regard to the accumulation of organic nitrogen. Feed was irregularly applied during the experiment but fish growth followed a smooth line, showing that the fish utilized detrital or planktonic feed during periods of low feeding. Nitrogen accumulated in the pond during periods of excessive feeding and was utilized by the fish during periods of low feeding. This cycling should be further studied and may be an important pond management technique.

Keywords: nitrogen budget; nitrogen balance; nitrogen flux; fish pond.

Introduction

In most aquaculture systems, fish retain only 20-30% of the nitrogen applied as feed (Avnimelech and Lacher 1979; Boyd 1985; Krom *et al.* 1985; Porter *et al.* 1987; Green and Boyd 1995). Inorganic nitrogen (ammonia and nitrite) may be harmful to fish, especially in intensive systems (Hargreaves 1995). Nitrogen not retained by fish is accumulating in the water column, and in the sediments, while smaller fractions are lost with discharged water (drainage, seepage) or lost through volatilisation of NH_3 and N_2 gas. There are reports that 30-95% of the nitrogen added to the ponds accumulates in the sediment (Avnimelech and Lacher 1979; Schroeder 1987; Myint *et al.* 1990; Oláh *et al.* 1994). Several processes affect the accumulation of nitrogen in the sediment, such as sedimentation (Schroeder *et al.* 1991), resuspension (Avnimelech and Wodka 1988; Avnimelech *et al.* 1999) and decomposition (Avnimelech 1984), all processes that are not well quantified. In previous works (Avnimelech and Lacher 1979; Boyd 1985; Krom *et al.* 1985; Porter *et al.* 1987; Green and Boyd 1995), nitrogen budgets were made by sampling the pond at the start and end of a growth cycle, without giving much attention to these processes. However, pond nitrogen budgets made at different points in time can help to quantify some of these processes.

Nitrogen fluxes are also related to feeding strategy. In ponds, nutrients provided through organic wastes stimulate the heterotrophic food chain and depending on fish species, a part of the fish growth is indirectly based on the exploitation of the heterotrophic food web (Schroeder 1983; Middendorp and Huisman 1995). Ekanem (1996) fed fish on alternating days. Here also, the feed provided, directly or indirectly, nutrients for the heterotrophic food chain. The fish exploited this food chain, thereby optimizing the overall food conversion efficiency.

This study quantified the accumulation of nitrogen during a production cycle in feed driven ponds, both in the water column and in the sediment. In addition, by combining estimates of the deposition rates of uneaten feed, faeces and dead phytoplankton with measurements of nitrogen accumulation in the sediment, the rate of decomposition of organic matter in the sediment was quantified.

Colossoma macropomum, a fast growing species (Van der Meer *et al.* 1995) which is commercially cultured in several South American countries (Goulding and Carvalho 1982) was used in this study. In ponds, *C. macropomum* grow best when

provided with a nutritionally balanced feed (Van der Meer and Martínez 1993), even when pond-feeding rates are high. The possibility of applying high feed loads to the ponds while maintaining good growth was considered advantageous for the present study.

Materials and Methods

Feeding and Fish Management

Four ponds of 65 m^2 and 1 m depth were stocked with *C. macropomum* of 30 g each at 1 fish m^{-2}. Fish were grown for 111 days. The growing cycle was divided into six periods of about 20 days each. Fish weight was estimated and samples of the sediment and water column were taken at the beginning of the experiment and on days 20, 34, 55, 76, 90 and 111. At least 60% of the fish population in each pond was sampled, ensuring a highly representative population sample.

Fish were fed with 3-mm dry floating pellets with 35% crude protein. The feeding levels applied are given in Table 1. The daily ration was divided into two equal portions applied at 0700 and 1500 hours, broadcasted evenly over the pond surface. During the first three weeks technical problems caused excessive feed administration of 14.3% body weight per day. Dissolved oxygen (DO) concentration just above the pond bottom was measured daily at 0630 hours. Fish were not fed when the early morning DO concentration was < 2 mg L^{-1}. Feeding was resumed when the early morning DO concentration increased to > 5 mg L^{-1}. The number of days fish were fed per sampling period is given in Table 1. Fish were not fed on sampling days.

Nitrogen Budgets

A nitrogen budget was prepared for each pond and for each sampling period. Nitrogen inputs considered were feed and in-flowing water. Nitrogen accumulations in fish, water column and sediment, as well as nitrogen losses through seepage were quantified.

The protein content on a wet weight basis of fed, pond-reared *Colossoma macropomum* is 15% (Van der Meer and Martínez 1993). The protein content of the fish was assumed to remain constant during the culture period. To estimate the amount of N storage at stocking and on sampling days in each pond, water and sediment samples

were collected from three locations (inlet, outlet and center) and pooled together. Composite water column samples were filtered through a GF/C Whatman glass fiber filter and the filtrate analyzed for NO_3^--N (cadmium reduction), NO_2^--N (diazotization) and NH_4^+-N (phenate method) (APHA 1989). Chlorophyll-a in non-filtered water column sample was performed using standard methods (APHA 1989). To measure particulate nitrogen, 500 mL of sample was filtered through a pre-washed 0.45-μm filter and the nitrogen retained on the filter was analyzed (Kjeldhal) according to AOAC (1980). Sediment samples were taken using a sampling tube of 6-cm diameter, 15-cm depth. In order to consider the patchy distribution of organic matter in sediments, the three soil samples were analyzed for organic matter content (dry ashing, Nelson and Sommers 1982); the uniformity of ignition loss between samples was used as the criterion permitting composite sampling. Only samples with < 5% difference in ignition loss were combined. Soil pore water was obtained by centrifugation (4000 rpm; 10 min) of the topmost 5-cm depth core, and dissolved nitrogen analyzed using standard methods (APHA 1989). The remaining soil was dried and the particulate nitrogen was determined after persulphate digestion of the samples (Raveh and Avnimelech 1979).

Nitrogen losses due to seepage were calculated. A staff gauge was mounted in each pond to measure water level. Water loss was measured daily, and seepage was calculated as:

$$S = L - E + P \tag{1}$$

where L is the measured water loss, E is the evaporation loss, and P is the precipitation. Daily-recorded evaporation and precipitation data (in mm per day) from a meteorological station located at a distance of 100 m from the experimental site were used. Water addition after filling was limited to replacement of losses due to seepage and evaporation. It was assumed that the seepage water had the same nitrogen concentration as the pore water collected from the sediment. The ponds were constructed in sandy soil and thus had a high hydraulic conductivity and extensive seepage water losses.

Decomposition Rate Estimation

The observed accumulation/decrease of organic matter (as nitrogen) in the pond sediments was used to estimate the decomposition rates of organic matter based on the following assumptions:

(a) chlorophyll-a concentration was converted to phytoplankton nitrogen by assuming a ratio of 5 mg N per mg chlorophyll-a (Laws and Bannister 1980);

(b) 50% of the phytoplankton biomass deposits daily (Schroeder *et al.* 1991);

(c) From the feed offered 15% is not eaten (Boyd and Tucker 1995);

(d) Overall feed digestibility is 70% (van Dam and Penning de Vries 1995); and

(e) 30% is excreted as faeces (Porter *et al.* 1987; Lovell 1988).

The rate of organic nitrogen decomposition was calculated from the changes of total organic nitrogen concentration in the sediment between the initial and final sampling dates. The first order decomposition rate constant (K_{decomp}) was obtained through nonlinear regression of the equation proposed by Avnimelech *et al.* (1995):

$$dS/dT = B - K(C) \qquad (2)$$

or its integrated form,

$$S = B/K_{decomp} - [(B - K_{decomp} * S_o) * e^{-K_{decomp} * T}] / K_{decomp} \qquad (3)$$

where S is the concentration of organic nitrogen, B is the daily addition of component S, T is the time and S_o is the concentration of organic nitrogen at T=0.

Statistical Analysis

A Pearson's correlation matrix was calculated for the principal parameters related to feed conversion, N input, accumulation (fish, sediment, water column) and seepage in the ponds. On each sampling date, the N input, accumulation and seepage were summed over all previous sampling periods. These periods are further referred to as 'pooled periods'.

Results and discussion

The principal flux in feed driven ponds is the feed. This flux was highly variable in the current experiment. Excessive feed was applied during the first period. Later on, low oxygen levels limited the application of feed. Details of feed application levels and timing are given in Table 1. The percentage of growing days during which feed was applied for each period varied from 100% to 29%.

Table 1. Feeding levels and nitrogen input (*n*=4 ponds, standard deviation given within parenthesis)

Period		Feeding				Fish	
Number	Time span (days)	Days fed	Feed per period (g pond^{-1})	N input (kg N ha^{-1})		Specific Growth Rate (% BW per day)	Feed Conversion Ratio
1	1-20	20 ($\pm$ 0.0)	9,828	84.7		3.78	5.05
	(20)		($\pm$ 498)	($\pm$ 4.2)		($\pm$ 0.22)	($\pm$ 0.33)
2	21-34	10 ($\pm$ 1.3)	5,558	47.9		4.39	1.90
	(14)		($\pm$ 766)	($\pm$ 6.6)		($\pm$ 0.17)	($\pm$0.27)
3	35-55	7 ($\pm$ 1.3)	4,447	38.3		2.33	1.11
	(21)		($\pm$ 802)	($\pm$ 6.9)		($\pm$ 0.07)	($\pm$ 0.21)
4	56-76	15 ($\pm$ 2.8)	15,292	131.7		2.93	2.13
	(21)		($\pm$ 3,095)	($\pm$ 26.7)		($\pm$ 0.06)	($\pm$ 0.62)
5	77-90	6 ($\pm$ 1.7)	6,573	56.6		2.05	1.39
	(14)		($\pm$ 2,003)	($\pm$ 17.3)		($\pm$ 0.05)	($\pm$0.42)
6	91-111	6 ($\pm$ 1.7)	8,666	74.7		1.89	0.76
	(21)		($\pm$ 2,469)	($\pm$ 21.3)		($\pm$ 0.18)	($\pm$0.14)
All	1-111	63 ($\pm$5.3)	50,363	433.9		2.89	2.06
			($\pm$ 4,231)	($\pm$ 36.5)		($\pm$ 0.95)	($\pm$ 1.48)

Fish growth, flux of nitrogen to the fish and Feed Conversion Ratio (FCR) for each period are given in Table 1 and fish growth in Figure 1. In 111 days, the fish grew from 30 to 698 g ($\pm$ 48.5, SD) realizing an average feed conversion ratio of 1.3 for the different culture periods. Fish growth followed a smooth line, regardless of the abrupt changes in feed application. Growth was not inhibited even in periods when feed was given only 29% of the time. Overall daily fish growth rate was 2.9% of body weight,

which is excellent compared to other results with the same fish (e.g., van der Meer *et al.* 1995). The fast growth is accompanied by an extensive use of nitrogen.
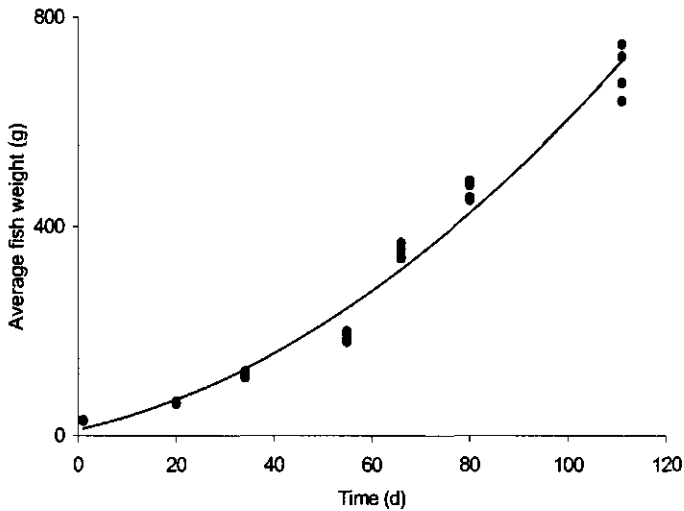
Figure 1. Fish growth of *Colossoma macropomum* over a 111 days growth period.

The smooth growth of the fish can be explained by assuming that the fish utilized detrital or planktonic feed during periods of low feeding. According to several works, fish store less than 25% of the feed protein (Avnimelech and Lacher 1979; Schroeder *et al.* 1990; Green and Boyd 1995). In certain experimental periods, protein retention was lower than the equivalent uptake of nitrogen by the fish (Table 2). The difference between the actual uptake of nitrogen and the potential supply from feed (last column, Table 2) is assumed to represent the protein uptake from the reserves built up in the pond. In period 6 protein uptake from the pond amounted to more than 50% of total protein uptake by the fish.

Table 2. Comparison of actual uptake of nitrogen and potential supply from feed in g nitrogen per day (mean ± SD, n = 4).

Period	Feed N	Theoretical feed N uptake[a]	N retained (g N d^{-1})	Actual N uptake from water[b]
1	26.66 (± 1.33)	6.66 (± 0.33)	2.27 (± 0.11)	- 4.40 (± 0.35)
2	21.54 (± 2.97)	5.38 (± 0.74)	4.90 (± 0.81)	- 0.48 (± 0.81)
3	11.49 (± 2.07)	2.87 (± 0.52)	4.43 (± 0.23)	1.56 (± 0.58)
4	39.50 (± 8.00)	9.88 (± 2.00)	8.26 (± 1.74)	- 1.62 (± 3.16)
5	25.47 (± 7.76)	6.37 (±1.94)	8.01 (± 1.43)	1.65 (± 2.33)
6	22.39 (± 6.38)	5.60 (± 1.59)	12.51 (± 1.66)	6.91 (± 0.57)

[a] 25% of feed N

[b] feed nitrogen uptake minus assumed nitrogen from feed. Negative values mean that the fish accumulate less than expected values. Positive values are indication of fish N uptake from planktonic or benthic sources.

Another major flux is the sedimentation flux. The experimental ponds were hardly used before the present experiment and thus, initially, the sediment had low levels of organic carbon and nitrogen (Table 3). Within the first 20 days, the total nitrogen concentrations in the sediment increased by a factor of 50, from about 6 ppm N to about 250 ppm N. The nitrogen accumulation in the sediment during this period comprised 54% of the added nitrogen (Figs. 2, 3). One possible reason for the very high accumulation is the fact that feeding during the first period was excessive, yet, since even at optimal feeding about 75% of the nitrogen is not used, similar accumulation should be expected under normal pond operation.

Looking into the composition of the total nitrogen in the sediment (Table 3), during the first period the increased total nitrogen concentration was not followed by an increase of adsorbed ammonium in the sediment and not by any increase of inorganic nitrogen in the interstitial water. The increase in adsorbed ammonium and of soluble inorganic nitrogen took place only later, reaching a peak at about the end of the 3^d or the 4th periods, i.e. after about 60 days. The increase of organic nitrogen accumulation in the sediment seemed to slow down with time.

The first obvious process to take place was the sedimentation of the residual organic nitrogen. The microbial degradation of the sedimented organic nitrogen was very slow in the beginning, thus an insignificant amount of inorganic nitrogen was released. Later, microbial degradation processes were taking place, leading to a build up

of high levels of inorganic nitrogen, both adsorbed and soluble. This accelerated decomposition is also reflected in the lowered accumulation of organic nitrogen in the sediment. This trend led to a lower percentage of sedimented nitrogen relative to the added nitrogen (Figs. 3, 4).

Table 3. Concentration of organic carbon and nitrogen in sediments and pore water (mean ± SD, n=4)

Period	Sediments			Porewater	
	$N-NH_4^+$	Kjeldhal N	Organic Carbon	$N-NO_3^-$	$N-NH_4^+$
	$\mu g \, g^{-1}$		$mg \, g^{-1}$	$mg \, L^{-1}$	
Initial	3.27	6.25	0.24	2.67	1.42
	(± 0.07)	(± 0.94)	(± 0.10)	(± 0.56)	(± 0.47)
1	2.14	254.51	2.75	3.60	2.29
	(± 0.05)	(± 15.51)	(± 1.79)	(± 3.41)	(± 2.65)
2	4.57	389.60	11.27	8.97	7.56
	(± 0.11)	(± 30.72)	(± 0.36)	(± 0.94)	(± 6.71)
3	7.13	301.04	6.73	17.99	23.64
	(± 0.08)	(± 30.42)	(± 0.54)	(± 3.80)	(± 16.00)
4	4.13	399.52	14.32	19.95	29.98
	(± 0.12)	(± 12.13)	(± 0.48)	(± 2.84)	(± 11.44)
5	5.09	137.74	13.70	24.01	57.95
	(± 0.06)	(± 93.99)	(± 0.40)	(± 8.77)	(± 57.39)
6	2.10	225.38	16.37	46.91	62.28
	(± 0.09)	(± 123.89)	(± 0.47)	(± 2.71)	(± 41.98)

Figure 2. Nitrogen input (as feed and supply water) and total nitrogen accumulation in sediments by period (n=4).
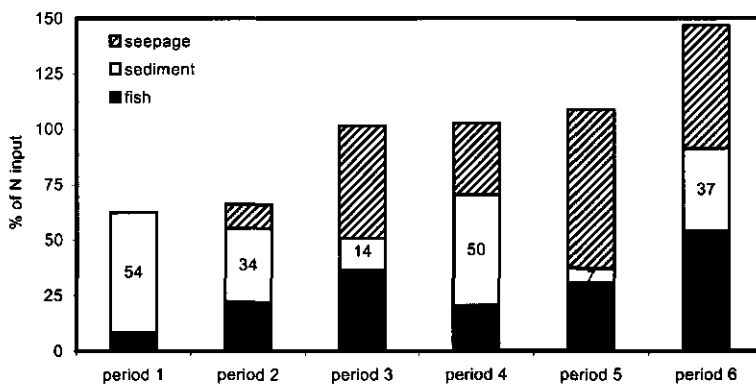
Figure 3. Percentage of N-input found in fish tissue and sediment, and calculated loss through seepage by period. The percentage of N input found in the sediment is listed by period. Nitrogen accumulated in the water was < 0.15% and is not shown.
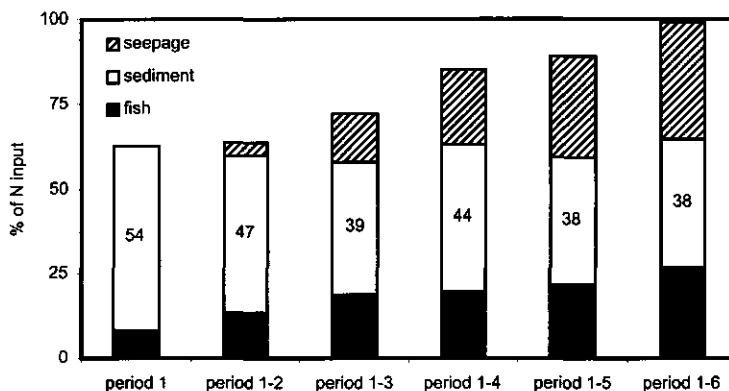
Figure 4. Percentage of N-input found in fish tissue and sediment, and calculated loss through seepage, pooled over time. The percentage of N input found in the sediment is listed by pooled period. Nitrogen accumulated in the water was < 0.10% and is not shown.

A relatively high percentage of accumulation was found in period 4, but this is probably due to the very high feed input in this period.

It is interesting to note that high nitrate levels were built up in the interstitial water. This indicates that aerobic conditions occurred in the sandy sediment in the experimental ponds. It is possible that some inorganic nitrogen diffused from the sediment into the water column, although nitrogen levels in the water remained. The heavy nitrogen loss through seepage (Fig. 3) is a clear implication of the elevated soluble nitrogen in the sediment. Such losses are not typical for ponds having an impermeable bottom and low seepage of water.

An important internal flux is the organic matter and organic nitrogen decomposition fluxes. The decomposition of organic nitrogen was evaluated by comparing the expected accumulation of organic nitrogen with the actual one (Table 4).

Table 4. Estimated deposition rate of organic nitrogen and decomposition rate based on periodic determinations of organic nitrogen in the sediments of four ponds.

Pond	Estimated organic nitrogen deposition rate (mg Nm^{-2} d^{-1})			Organic nitrogen accumulation rate (mg N m^{-2} d^{-1})	Decomposition rate (mg N m^{-2} d^{-1})	K_{decomp} (d^{-1}) [a]
	Uneaten feed	Faeces	Dead phytoplankton			
1	60.6	20.6	49.2	4.8	125.6	0.236
2	64.6	22.0	54.5	2.3	138.7	0.263
3	55.8	19.0	53.9	6.2	122.4	0.227
4	53.6	18.2	52.5	4.1	120.2	0.219
				Mean	126.7	0.237
				± SD	± 8.3	± 0.019

[a] First order rate constant for decomposition

Organic nitrogen sedimentation originated from uneaten feed, faeces and dead plankton. The amounts of these fluxes were calculated based on published results from the literature (Porter *et al.* 1987; Lovell 1988; Schroeder *et al.* 1991; Boyd and Tucker 1995). The difference between the expected sedimentation and the actual one is considered as the amount of decomposed nitrogen. The rate of nitrogen decomposition is represented by the first order rate constant, K, which was calculated. In the present study, the rate of nitrogen decomposition, as expressed by K was 0.237 ± 0.019 (CV = 8%). This value is higher than the values given by Avnimelech *et al.* (1995) for the decomposition rate constants in pond bottom sediments, yet, the decomposition processes considered here are taking place in the whole pond, i.e. sediment plus water.

In this regard, our results can be compared with those of Avnimelech *et al.* (1992), who also studied whole system decomposition in tanks and mixed ponds. Their rate constant, 0.06 d^{-1} is also lower than what was found here. The higher decomposition rate constant found in the present study could partly be explained by differences in temperature. It is obvious that sound quantitative data on decomposition of organic matter in fish ponds are lacking. The present data indicate that this process may be quite significant for understanding the pond dynamics.

In Table 5 a correlation matrix is given relating nitrogen input and utilization to N balance parameters. Feed conversion was positively correlated with N accumulation in sediment and water column, and inversely correlated with N accumulation in fish tissue. There was a strong positive correlation between N input and sediment N accumulation, and fish N accumulation was negatively correlated with water column N accumulation and with seepage N loss.

Table 5. Two-tailed Pearson correlation matrix based on six sampling periods and four ponds (24 data points). Bold values showed significant correlation ($P \leq 0.05$).

(unit)	N-input kg ha^{-1}	Fish-N accumulation kg ha^{-1}	Sediment-N accumulation kg ha^{-1}	Water column-N accumulation kg ha^{-1}	Seepage-N loss kg ha^{-1}
Feed conversion	0.3483	**-0.5815**	**0.4808**	**0.5624**	0.0440
N-input		0.3461	**0.8446**	-0.1786	-0.4274
Fish-N accumulation			0.1996	**-0.5626**	**-0.5688**
Sediment-N accumulation				0.1346	-0.3155
Water column-N accumulation					0.3368

The nitrogen budget for each separate period is given in Figure 3 and the cumulative budget in Figure 4. Three nitrogen fluxes are considered: fish uptake, sedimentation and seepage. Sedimentation was the major flux during the first two periods, followed by substantial seepage. The total nitrogen recovery during the first 2 periods was about 65%. Later, during periods 3-5, the nitrogen recovery was very close to 100% (104.0 ± 3.9). The recovery in the last period was almost 150% (Fig. 3). The cumulative recovery (Fig. 4) is lower than 100% all along the first 5 periods, affected by the incomplete recovery during the first 2 periods. However, the cumulative recovery at

the end of the experiment is 100%. These results lead to several interesting conclusions. It seems that the nitrogen budget in the system can be fully explained without any consideration of nitrogen volatilisation, due to either denitrification or ammonia volatilisation. Gross *et al.* (1999) found substantial volatilisation in catfish ponds in Alabama. However, in the present study ammonium concentrations in the water were low and it seems that the pond bottom was aerated. Possible denitrification in deep sediment layers may have been overlooked in the present study because they were integrated with the seepage losses. The fact that initially nitrogen recovery was incomplete and the fact that a complete recovery was found at the end of the experiment are puzzling. One possible explanation is that nitrogen sedimentation in the first period was patchy and thus the recovery of nitrogen in the sediments was incomplete (CV, the coefficient of variation of nitrogen in the sediment was in the order of 5% normally, but was much higher, 17%, during periods 1 and 2). It is possible, yet not proven, that the degradation of the patches of nitrogen led to formation of colloidal material, which was subsequently distributed more uniformly over the bottom.

Following other works where nitrogen balance was obtained by comparing the pond in the beginning and at the end of the growth cycle, in this work nitrogen in the pond system was followed along the growing cycle. More information can be obtained through this temporal approach. In the beginning of the growth cycle, the major flux of nitrogen was the sedimentation onto the bottom soil. Intensive microbial degradation took place about 3-4 weeks later, leading to a release of inorganic nitrogen and an approach toward a steady state with regard to the accumulation of organic nitrogen. The whole pond first order rate constant pertaining to organic nitrogen decomposition was 0.237 d^{-1}, a value that is important for pond simulation studies.

A complete overall recovery and fairly good temporal recoveries where found as fish N accumulation, accumulation in the sediment and seepage. The reasons for low recovery of nitrogen during the first few weeks and subsequent N release should be studied further.

An important scientific and practical conclusion is the storage of protein in the pond system and its subsequent utilization by fish. Nitrogen accumulated in the pond during periods of excessive feeding and was utilized by the fish during periods of low feeding, yielding an overall smooth growth and low FCR. This cycling should be further studied and may be an important pond management technique.

Acknowledgements

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Chapter 2

Conceptualization and validation of a dynamic model for the simulation of nitrogen transformations and fluxes in fish ponds

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Abstract

Nitrogen is a key element in aquatic environments and an important pond management variable. In some aquatic systems the accumulation of nitrogen leads to a deterioration of the system. The interactions between various N-species are complex and difficult to understand as a whole. Modelling can improve our understanding of the underlying complexity. This paper integrates existing knowledge about nitrogen transformations in fish ponds into a model that predicts the amounts of various N-compounds in the water column and in the sediment. The model is also used to gain insight into the relative importance of transformation processes between the various N-compounds.

The model was divided into three modules: fish, phytoplankton and sediment-water. The fish module is based on physiological and bio-energetic principles; the phytoplankton dynamics module is based on physico-chemical principles of algal growth. The water-sediment module is based on the bacterial transformations and chemical fluxes of N-species across the water-sediment interface. Relationships and parameter values were taken from the literature except for a few parameters that were estimated by fitting model predictions to observed data. The model was implemented in Turbo Pascal (7.0) using a fixed time step of one hour, and it was calibrated using a set of data from an earthen fish pond stocked with *Colossoma macropomum*. The validation was done using data from earthen ponds stocked with *Oreochromis niloticus*. The difference between the calibrated and validated model was related to the fish species. All concentrations of the various N-species present were simulated well except the N retained in organic matter in the sediment (average relative error –0.34).

Sensitivity analysis revealed that the concentrations of inorganic-N compounds both in the water column and in the sediment are more affected by changes in specific parameters included in the fish and phytoplankton modules, than by changes in other forms of nitrogen in the pond. The model works well, except for organic matter accumulation in the sediment. Further research should concentrate on a better understanding of the bottom organic matter dynamics, to make the model a powerful predictive tool.

Keywords: fish culture; nitrogen dynamics; modelling; fish pond; simulation

Introduction

Nitrogen is a key element in aquatic environments and aquaculture systems. Nitrogen input in the form of feeds or fertilizers is needed to enhance aquatic production of cultivated animals, and it is considered an important pond management variable. Nitrogen input in aquaculture systems also increases the potential of pollution to the surrounding environment. Although the basic processes of the nitrogen cycle are known, the ways in which they are linked together is poorly understood, and have to be investigated further.

In ponds, nitrogen is present in different forms. Wasted feed, dead plankton and faeces are mineralized and the resulting dissolved nitrogen species can be re-used by autotrophic organisms forming complex molecules again (Diab and Shilo 1986). Oxygen availability, water temperature, pH, light penetration and bacterial species composition in the system influence these transformations (Painter 1970; Otsuki and Hanya 1972; Stanford et al. 1975; Andersen 1977). The metabolic end products of nitrogen in feeds, as well as the microbial decomposition of organic matter in the surrounding water, lead to increased concentrations of free ammonia and nitrite, both toxic to fish even at low concentrations (Meade 1985).

Control of nitrogen transformation processes in the pond and optimal feed utilization in aquaculture systems are needed. A better understanding of the fluxes and transformations of nitrogen in aquacultural production systems is needed and the development of appropriate simulation models of pond aquaculture is recommended (Lannan et al. 1983). Models of varying degree of complexity were used for research of nitrogen compound transformations in aquaculture, and mathematical approximations of these processes can be found in the literature. General principles of computer modelling of aquaculture systems have been reviewed by Bolte et al. (1986), Piedrahita (1988) and van Dam (1990).

Numerous studies concentrated on N-transformation in aquaculture, and processes of the N-cycle were described mathematically. Paulson (1980) presented different models of ammonia excretion for trout; Lightfoot et al. (1993) presented a steady-state nitrogen model for a wetland rice field ecosystem with and without fish; Piedrahita et al. (1984) and Piedrahita (1991) simulated dissolved inorganic nitrogen concentrations in ponds; Kochba et al. (1994) developed a model to evaluate the effect of water exchange rate on dissolved inorganic nitrogen concentration in intensively

aerated fish ponds; Avnimelech *et al.* (1994) presented a model relating the concentration of any given residue to the water exchange rate in controlled intensive systems. Hargreaves (1997) developed a model for ammonium dynamics in channel catfish ponds. Lorenzen *et al.* (1997) used a mathematical model to investigate the impact of farming intensity and water management on nitrogen dynamics; Montoya *et al.* (1999) simulated nitrogen dynamics in intensive shrimp culture systems; Nielsen *et al.* (1999) simulated nitrogen dynamics in rice-crayfish systems; Jamu (1999) described the dynamics of nitrogen in integrated aquaculture-agriculture systems; and Verdegem *et al.* (2000) proposed a bio-energetic model of growth and waste production of tilapia in recirculation systems.

This paper integrates existing knowledge about nitrogen transformations in fish ponds into a predictive model for Tilapia (*Oreochromis niloticus*) and tambaquí (*Colossoma macropomum*) ponds in the tropics. Every hour, the model calculates the quantities of different N-compounds in the water column and in the sediment of ponds. After calibration and validation, the model can be used to (1) evaluate the sensitivity of the N-cycle to changes in individual processes; (2) pinpoint the principal sinks for N in the system; and (3) identify those processes that need further study.

Model description

General model description

The model contains three modules: the fish module, the phytoplankton module and the sediment-water module. The fish module is based on the model proposed by Machiels and Henken (1986, 1987), later adjusted for tilapia by van Dam and Penning de Vries (1995). The model developed by Jiménez-Montealegre *et al.* (1995) was used as a basis for the phytoplankton module. The sediment-water module was newly developed, and concentrates on transformations and fluxes between inorganic and organic N-compounds in both the water column and the sediment. Within this module, other biota normally found in sediments are included and are considered as organic nitrogen. The principal N-compounds, N-transformations and N-fluxes in the model are conceptualized in Figure 1. Table 1 presents the principal variables considered in each module; and in Annex 1 a description of the variables used and the related equations are presented.
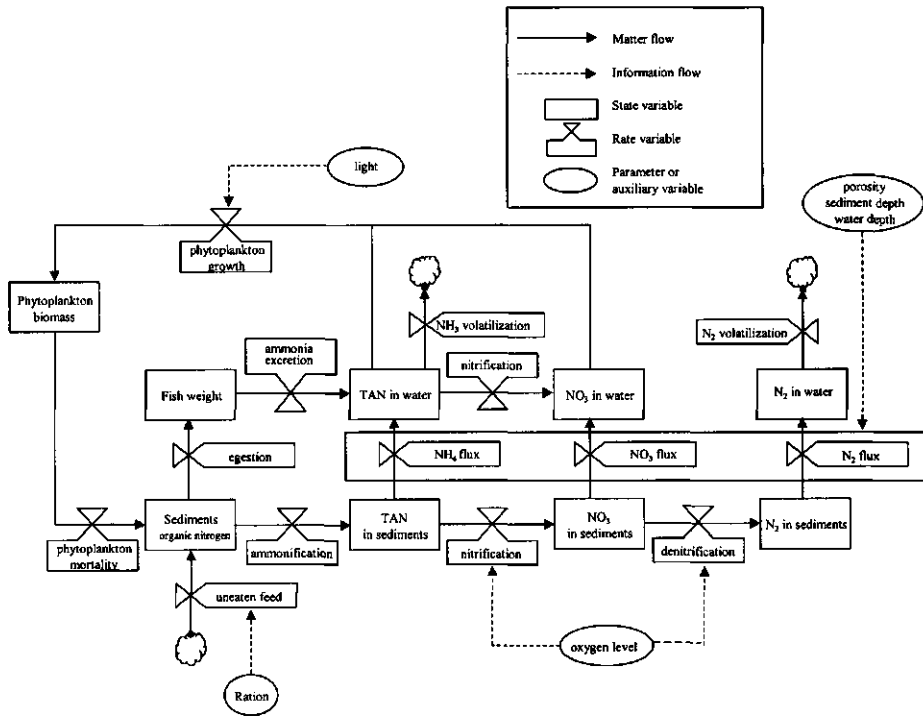
Figure 1. Conceptual model of nitrogen transformations and fluxes and relations between modules. All state variables have dimensions of mg N L^{-1}

The model analyses N-transformations and fluxes between compartments in stagnant water ponds. The only N-inputs considered are the protein in the feed and the dissolved N-compounds in the in-flow water. Nitrogen fixation is considered negligible in aquaculture ponds (El Samra and Oláh 1979; Lin *et al.* 1988) and was ignored in this model. In the fish module, a distinction is made between wasted (e.g., non-consumed) and consumed feed; the consumed feed is partly transformed into fish biomass, and the rest is excreted (as ammonium) or egested (as faeces). It is assumed that fish growth is only based on the feed input, and not on other sources from the pond biota. Phytoplankton assimilates both nitrate and ammonium ions for growth. In the sediment/water module, the N-transformations and N-fluxes between the various N-compounds in water and sediment are included.

Table 1. Principal states, rates and auxiliar variables used in the model

Water-sediment module		
State variables	Dimensions	Symbol
N_N$_2$ in sediments	mg N L^{-1}	s_n2sed
N_N$_2$ in water	mg N L^{-1}	s_n2wat
N_NH$_3$ in sediments	mg N L^{-1}	s_nh3sed
N_NH$_3$ in water	mg N L^{-1}	s_nh3wat
N_NH$_4^+$ in sediments	mg N L^{-1}	s_nh4sed
N_NH$_4^+$ in water	mg N L^{-1}	s_nh4wat
N_NO$_3^-$ in sediments	mg N L^{-1}	s_no3sed
N_NO$_3^-$ in water	mg N L^{-1}	s_no3wat
Organic matter (as N) in sediments	mg N L^{-1}	s_ornsed

Rate variables	Dimensions	Symbol
Ammonia excretion rate	mg N L^{-1} h^{-1}	r_ammnpro
Ammonification rate in sediments	mg N L^{-1} h^{-1}	r_ammonifsed
Ammonification rate in water	mg N L^{-1} h^{-1}	r_ammonifwat
Denitrification rate in sediments	mg N L^{-1} h^{-1}	r_denitrifsed
Egestion rate of protein	mg N L^{-1} h^{-1}	r_egspro
N$_2$ flux rate	mg N L^{-1} h^{-1}	r_n2flux
NH$_3$ flux rate	mg N L^{-1} h^{-1}	r_nh3flux
Refill rate for NH$_4^+$	mg N L^{-1} h^{-1}	r_nh4fill
NH$_4^+$ flux rate	mg N L^{-1} h^{-1}	r_nh4flux
Nitrification rate in sediments	mg N L^{-1} h^{-1}	r_nitrifsed
Nitrification rate in water	mg N L^{-1} h^{-1}	r_nitrifwat
Refill rate for NO$_3^-$	mg N L^{-1} h^{-1}	r_no3fill
NO$_3^-$ flux rate	mg N L^{-1} h^{-1}	r_no3flux
Phytoplankton dead rate	mg N L^{-1} h^{-1}	r_phytom
Uneaten feed, dm	mg N L^{-1} h^{-1}	r_uneadm
Volatilisation rate of N_N$_2$	mg N L^{-1} h^{-1}	r_volatn2
Volatilisation rate of N_NH$_3$	mg N L^{-1} h^{-1}	r_volatnh3

Phytoplankton module		
State variables	Dimensions	Symbol
Phytoplankton biomass	mg N L^{-1}	s_phytobiom
Dead phytoplankton biomass	mg N L^{-1}	s_phytodead

Rate variables	Dimensions	Symbol
Growth rate	mg N L^{-1}h^{-1}	r_phytogrowth
Death rate	mg N L^{-1}h^{-1}	r_phytom

Auxiliar variables	Dimensions	Symbol
Light limitation factor	dl	a_lighlim
Nutrient limitation factor	dl	a_nutrilim
Radiation per hour	ly h^{-1}	a_radhor
Water temperature	°C	a_temp

Table 1 (cont.)

Fish module		
State variables	Dimensions	Symbol
Amount of AA[*] converted into protein	gr amino acids	s_aapool1
Amount of AA converted into glucose	g amino acids	s_aapool2
Total pool of glucose available	g glucose	s_glpool
Total pool of body lipid	g lipids	s_libiom
Organic matter in sediments	mg N L^{-1}	s_ormsed
NH$_3$ in water	mg N L^{-1}	s_nh3wat
Individual fish weight	g (fresh)	s_wf

Rate variables	Dimensions	Symbol
Rate of gluconeogenesis	g amino acids d^{-1}	r_aagluc
Aminoacid oxidation rate	g amino acids d^{-1}	r_aaox
Nitrogen in ammonia excreted	mg N L^{-1} h^{-1}	r_ammnpro
Ammonium excretion rate	g NH$_4$$^{+}$ h^{-1}	r_ammpro
Rate of digestion of carbohydrates from feed	g carbohydrates d^{-1}	r_cardig
Egestion rate of protein as nitrogen, in faeces	mg N L^{-1} h^{-1}	r_egspron
Real feeding rate	g feed d^{-1} (dry)	r_fdrtdm
Lipid digestion rate	g lipid d^{-1}	r_lipdig
Lipid oxidation rate	g lipid d^{-1}	r_lipox
Lipid synthesis rate from glucose	g lipid d^{-1}	r_lipsyn2
AA production rate from digested feed	g amino acids d^{-1}	r_prodig
Protein synthesis rate	g protein d^{-1}	r_prosyn
Uneaten feed (dm)	g feed d^{-1} (dry)	r_uneadm
Nitrogen in uneaten feed	mg N L^{-1} h^{-1}	r_unean
Fish growth rate	g fish d^{-1} (fresh)	r_wfrate

Auxiliar variables	Dimensions	Symbol
Daily feed ration	g feed (fresh)	a_ration
Proportion of energy supplied by fat	dl	a_aalirat
Fraction of protein in feed (wet)	% dry matter	a_feedpr

[*] Aminoacids

Organic matter in the sediment is composed of wasted feed, dead phytoplankton and faeces. Decomposition of organic matter in the sediment results in ammonia-ammonium (N-NH$_3$ + N-NH$_4$$^{+}$), that may be transformed into NO$_3$$^{-}$ and possibly N$_2$ both in the water and in the sediment by nitrification-denitrification. Both seepage and biota that could escape from the system (flying insects) are considered not relevant for the nitrogen balance, and the emission of gaseous nitrogen (N-NH$_3$ and N-N$_2$) is assumed to be the only loss of nitrogen from the system.

Fish module:

The module (Fig. 2) was developed by Machiels and Henken (1986, 1987) on the basis of physiological and biochemical principles. Digestion of feed protein, carbohydrates and lipids results in amino acids, glucose, fatty acids and glycerol.

Figure 2. Fish module relational diagram

Amino acids are used for the synthesis of protein or glucose; fatty acids and glucose are used for the synthesis of body lipids. Van Dam and Penning de Vries (1995) assumed that the relative oxidation of lipids and protein for energy is determined by the protein feeding level and the protein/energy ratio of the feed. This approach allows the estimation of the ammonia excretion and faeces egestion rates of the fish:

$$r\_ammnpro = (14/17) * (0.16*(r\_aaox + r\_aagluc))$$ (1)

0.16	= % of nitrogen in protein
14/17	= nitrogen to ammonia ratio
r_ammnpro	= ammonium excretion rate (mg N $L^{-1}h^{-1}$)
r_aaox	= aminoacid oxidation rate (g amino acids d^{-1})
r_aagluc	= gluconeogenesis rate (g amino acids d^{-1})

$$s\_nh3wat(t) = s\_nh3wat\ (t\text{-}dt) + (r\_ammnpro - r\_volatnh3 - r\_nh3flux) * dt$$ (2)

s_nh3wat	= N_NH_3 in water (mg N L^{-1})
r_ammnpro	= ammonium excretion rate (mg h^{-1})
r_volatnh3	= NH_3 volatilisation rate (mg N $L^{-1} h^{-1}$)
r_nh3flux	= NH_3 flux rate (mg N $L^{-1}h^{-1}$)

$$r\_egspron = 0.16*(r\_egspro)$$ (3)

r_egspron	= faeces egestion rate (mg N $L^{-1}h^{-1}$)
r_egspro	= protein egestion rate (g protein h^{-1})

In the present model the uneaten feed was also included (Verdegem *et al.* 2000):

$$r\_unean = a\_ration * p\_mors$$ (4)

r_unean	= uneaten feed (mg N $L^{-1}h^{-1}$)
a_ration	= feed ration (g feed, dry weight)
p_mors	= fraction of feed not eaten (dl)

The excreted ammonia becomes part of the dissolved ammonia in the water, and both the faeces and the uneaten feed adds to the organic nitrogen pool in the sediments (Fig. 1, equation 9). Because in the model of van Dam and Penning de Vries (1995) all variables were expressed in g fresh weight, variables were converted to mg N L^{-1} to make the fish module compatible with the other modules. The model assumes a 12-hour

feeding period. For further details, reference is made to Machiels and Henken (1986, 1987) and van Dam and Penning de Vries (1995).

Phytoplankton module:

The module is based on a dynamic simulation model for the blooming of *Oscillatoria agardhii* (Jiménez-Montealegre *et al.* 1995). The biomass of phytoplankton is assumed to be affected by two different rates: growth and natural mortality (Fig. 3).

Figure 3. Phytoplankton module relational diagram

Light and nutrient availability directly control growth rate and limitations are assumed to be multiplicative:

$$r\_phytogrowth = p\_maxgrphyto * (a\_nutrilim * a\_lighlim) * s\_phytobiom \qquad (5)$$

r_phytogrowth	= phytoplankton growth rate (mg N $L^{-1}h^{-1}$)
p_maxgrphyto	= rate constant for phytoplankton growth (1 d^{-1})
a_nutrilim	= nutrients limitation (dl)
a_lighlim	= light limitation (dl)
s_phytobiom	= phytoplankton biomass (mg N L^{-1})

Light limitation is based on the formula proposed by Di Toro *et al.* (1971), taking into account the equation proposed originally by Steele (1965) which assumes an optimum radiation with a reduction of growth at intensities both above and below the saturation level. Light extinction is based on Beer's law with provision for self-shading due to suspended biomass, and photoperiod is used to estimate the actual radiation every hour. Nutrients (ammonium, nitrate, and phosphorus) come from refill water, fish excretion and organic matter decomposition (both in the water column and in the sediments); its availability is used to estimate the limitation for growth using the relation of Monod kinetics. It was assumed that the phytoplankton species use ammonium first and, only when this ion is depleted, nitrate seconds (McCarthy 1981; Syrett 1981). The phytoplankton natural mortality rate is a function of the water temperature:

$$r\_phytom = p\_mrphyto * exp(a\_tecorm) * s\_phytobiom \qquad (6)$$

r_phytom	= phytoplankton death rate (mg N $L^{-1}h^{-1}$)
p_mrphyto	= rate constant for phytoplankton mortality (1 d^{-1})
a_tecorm	= temperature correction for mortality (d^{-1})

For further details reference is made to Jimenez-Montealegre *et al.* (1995).

Water-Sediment module:

Water and sediment compartments are highly correlated in ponds, and are considered together as a module that holds several state variables (Fig. 4). Water pH and dissolved oxygen were inputs of the model; minimum values for these parameters were considered in the respective equations. Organic matter consists of wasted feed, dead phytoplankton and faeces from fish. The amount of wasted feed is difficult to assess in

ponds. Frier *et al.* (1995) proposed a model in which the fraction of feed eaten can be calculated by iteration knowing the actual fish growth for a given period and the amount of feed offered. Phillips *et al.* (1985) estimated that 15-30% of the feed in rainbow trout cage culture is wasted; Thorpe *et al.* (1990) found feed losses of 19% in salmon cage culture; and van der Meer *et al.* (1995) found for *Colossoma macropomum*, raised in recirculation units and fed at levels near satiation, feed losses fluctuating between 9 and 20% irrespective of the fish size.

Figure 4. Water-Sediment module relational diagram

In our model this value was set to 35% considering the system conditions (earthen fish pond, low fish density). Dead phytoplankton first decompose in the water column:

r_ammonifwat = if (a_phwat>2) and (a_dowat>2) then (s_phytodead * p_ammonifwat) else 0   (7)

r_ammonifwat	= ammonification rate in the water column (mg N $L^{-1}h^{-1}$)
a_phwat	= water pH
a_dowat	= dissolved oxygen in water (mg O_2 L^{-1})

s_phytodead	= dead phytoplankton biomass (mg N L^{-1})
p_ammonifwat	= rate constant of ammonification in water (d^{-1})

The rest of the organic matter (wasted feed, partly decomposed phytoplankton, faeces) is assumed to settle and decompose in the sediments:

$$r\_ammonifsed = \text{if } (a\_phsed > 2) \text{ and } (a\_dosed > 2) \text{ then } (s\_ornsed * p\_ammonifsed) \text{ else } 0 \quad (8)$$

r_ammonifsed	= ammonification rate in the sediments (mg N L^{-1}h^{-1})
a_phsed	= sediment pH
a_dosed	= dissolved oxygen in sediment (mg O$_2$ L^{-1})
s_ornsed	= organic matter (as nitrogen) in sediments (mg N L^{-1})
p_ammonifsed	= rate constant for ammonification in sediments (d^{-1})

$$s\_ornsed \ (t) = s\_ornsed \ (t\text{-}dt) + (r\_unean + r\_egspron + r\_phytom - r\_ammonifsed)*dt \quad (9)$$

r_unean	= rate of nitrogen input in uneaten feed (mg N L^{-1}h^{-1})
r_egspron	= egestion rate of protein (as nitrogen) in faeces (mg N L^{-1}h^{-1})

The compound resulting from ammonification/mineralization is TAN (total ammonia nitrogen, N_NH$_3$ + N_NH$_4$$^+$). Its concentration is influenced by pH and dissolved oxygen levels (Reddy and Patrick 1984; Oláh *et al.* 1987). In the model, the percentage of free ammonia is based on pH and temperature conditions (Delincé 1992). In the water column, ammonium can be used by phytoplankton for growth (see above), be transformed into nitrate:

$$r\_nitrifwat = \text{if } (a\_dowat > 2) \text{ then } (p\_nitrifwat * s\_nh4wat) \text{ else } 0 \quad (10)$$

r_nitrifwat	= nitrification rate in water (mg N L^{-1} h^{-1})
a_dowat	= dissolved oxygen in water (mg O$_2$ L^{-1})
p_nitrifwat	= rate constant for nitrification in water (d^{-1})
s_nh4wat	= dissolved N_NH$_4$$^+$ in the water column (mg N L^{-1})

or be diffused to the sediments based on a concentration gradient:

$$r\_nh4flux = (\text{-}p\_porosity * p\_nh4dif * (((s\_nh4sed\text{-}s\_nh4wat)/1000)/p\_seddepth)) \quad (11)$$

r_nh4flux	= flux of N_NH$_4$$^+$ (mg N L^{-1}h^{-1})
p_porosity	= soil porosity (dl)
p_nh4dif	= diffusion coefficient for N_NH$_4$$^+$ (m^2 d^{-1})

s_nh4sed   = dissolved $N\_NH_4^+$ in sediments (mg N L^{-1})

p_seddepth   = sediment depth (m)

The flux of nutrients is based on Fick's first law of diffusion, corrected for porosity and tortuosity of the sediments (Berner 1980). Due to the higher concentration of nutrients frequently found in the sediments (Boyd *et al.* 1994) the flux is most likely to occur from the sediments towards the water column and possibly transformed into nitrate and/or nitrogen gas:

$$\text{r\_nitrifsed} = \text{if (a\_dosed>2) then (p\_nitrifsed * s\_nh4sed) else 0} \tag{12}$$

     r_nitrifsed   = nitrification rate in sediments (mg N $L^{-1}h^{-1}$)

     a_dosed   = dissolved oxygen in water (mg O_2 L^{-1})

     p_nitrifsed   = rate constant for nitrification in sediments (d^{-1})

     s_nh4sed   = dissolved $N\_NH_4^+$ in the sediments (mg N L^{-1})

The latter process is oxygen dependent (Sharma and Ahlert 1977), and in the model this is taken into account. When oxygen is depleted in the sediments, nitrate can be transformed into nitrogen gas via denitrification:

$$\text{r\_denitrifsed} = \text{if (a\_dosed>2) then 0 else (p\_denitrifsed * s\_no3sed)} \tag{13}$$

     r_denitrifsed   = denitrification rate in sediments (mg N $L^{-1}h^{-1}$)

     a_dosed   = dissolved oxygen in water (mg O_2 L^{-1})

     p_denitrifsed   = rate constant for denitrification (d^{-1})

     s_no3sed   = dissolved NO_3^- in the sediments (mg N L^{-1})

Both ammonia and nitrogen gas (if any) can escape to the air via volatilisation depending on their concentrations in the water. The rate constants for volatilisation consider the diffusion coefficient of the respective gas, and the thickness of the water-air interface:

$$\text{r\_volatn2} = \text{p\_volatn2 * s\_n2wat} \tag{14}$$

     r_volatn2   = N_2 volatilisation (mg N $L^{-1}h^{-1}$)

     p_volatn2   = constant for N_2 volatilisation rate (h^{-1})

$$\text{r\_volatnh3} = \text{p\_volatnh3 * s\_nh3wat} \tag{15}$$

     r_volatnh3   = NH_3 volatilisation (mg N $L^{-1}h^{-1}$)

p_volatnh3   = constant for NH_3 volatilisation rate (h^{-1})

Model implementation and parameterization

The model was implemented in Turbo Pascal (7.0) using Euler's rectangular method for numerical integration. Because processes such as oxygen consumption and/or production could have high rates of change, the time coefficient for each state variable was calculated. The highest rate of increase (or decrease) during the simulation was identified, and the relative rate for that period calculated. Time coefficients, defined as the inverse of the relative rate of change, varied from 1.96 to 92.31 hours. De Wit and Goudriaan (1978) suggest that the time step for rectangular integration should be one tenth of the smallest time coefficient. However, a fixed time step of one hour was selected because a further reduction of the time step in the model did not improve the simulations substantially.

The program uses the following input data: water temperature, water pH, dissolved oxygen in both water and sediments, the rate of input of ammonium and nitrate via refill water, and the amount of feed offered daily. The general output of the model is the nitrogen concentration in water and sediments (as organic-N, $N-NH_4^+$ and $N-NO_3^-$), and the nitrogen stored in fish and phytoplankton biomass.

Material and methods

Experimental data collection for calibration

The data for the calibration (Table 2) were collected during an experiment designed for this purpose. A stagnant pond with an area of 65-m^2 and 1.2 m depth was used. The pond was stocked with *Colossoma macropomum* (Cuvier 1818) at a density of 1.1 fish per m^2. Fish were fed a 32% protein commercial feed; weekly average fish weight was used to estimate the amount of feed offered, considering a feeding rate of 2.5% body weight per day, and the amount of feed given was used as an input into the model. Dissolved oxygen, water pH and temperature in the middle of the water column were measured at 0600 and 1800 hours. The dissolved oxygen, and the temperature for each hour were estimated using linear interpolation assuming peak values at those hours. To estimate the hourly value of pH the concentration of H_3O^+ was also interpolated and the pH value obtained.

Table 2. Experimental data collected to perform the calibration of the model[*]

Day	State variable						
	s_nh4wat	s_nh4sed	s_no3wat	s_no3sed	a_fishw	s_phytobiom	s_ornsed
Initial	0.081	0.206	0.619	0.105	35.0	0.322	140.29
7	0.032	0.026	0.546	0.167	42.1	0.766	139.10
14	0.102	0.078	0.539	0.200	50.0	0.869	138.72
21	0.190	0.135	0.555	0.259	62.4	0.803	140.23
28	0.239	0.181	0.585	0.416	74.9	0.906	139.75
35	0.290	0.191	0.596	0.563	90.8	0.880	140.50
42	0.334	0.224	0.624	0.689	108.4	0.960	141.13
49	0.419	0.263	0.644	0.849	128.1	0.994	141.24
56	0.439	0.318	0.700	0.963	151.5	1.032	142.34

[*] All state variables are expressed in mg N per liter, except fish weight which is expressed in terms of grams per fish (individual fresh weight). For names explanation see Table 1.

The pond was sampled weekly for nitrogen contents in phytoplankton, sediments and water. The nitrogen cycle, especially the rates of transformation of dissolved inorganic compounds, is very dynamic and important changes in concentrations occur during the day (Meade 1985; Mires *et al.* 1990; Abdalla *et al.* 1996). For the comparison between simulated and observed values this fact was considered by comparing only values from similar hours of sampling.

Dissolved nitrogen (N-NO$_3^-$ and N-NH$_4^+$ + N-NH$_3$) in water and sediments was determined using traditional methods (APHA 1989). Organic nitrogen in water, sediments and phytoplankton was determined using the method of AOAC (1980). The nitrogen stored in fish biomass was calculated weekly based on average fish weight, and the determination of total nitrogen in fish flesh at harvest was determined using the micro-Kjeldahl method (AOAC 1980). After each weekly sampling, the volume of water lost via evaporation was replaced with water of known nitrogen (nitrate and ammonium ions) concentration. Soil porosity was also determined following the method described by Corredor and Morell (1985).

Calibration

The fish module was first calibrated for *Colossoma*. Field and laboratory determinations at the beginning of the experiment were used as initial values (Table 3).

Table 3. Initial conditions used in the simulations. Only parameters changed during calibration and validation runs are included

Variable	Symbol	Dimension	Calibration *Colossoma macropomum*	Validation *Oreochromis niloticus*
Fish number	a_fishnb	#	70	960
Fraction of protein in feed (wet)	a_feedpr	% in dry matter	0.32	0.30
Carbohydrate digestibility coefficient	p_digca	%	0.60	0.50
Lipids digestibility coefficient	p_digli	%	0.80	0.50
First factor for lipid oxidation rate	p_fac1	dl	6.456	4.300
Second factor for lipid oxidation rate	p_fac2	dl	0.9046	1.2000
Minimum fat % in fresh weight of fish	p_lipfin	%	3.5	3.0
Pond area	p_pondarea	m^2	65	800
Protein percentage in fish (fresh weight)	p_prperc	g prot /100 g fish	15.0	17.0
Reference temperature for routine metabolism	p_reftemp	°C	25	26
Routine metabolism coefficient	p_rmcf	mole ATP/ $g^{0.8}d^{-1}$	$1.05e^{-3}$	$1.21e^{-3}$
Total pool of body lipid	s_libiom	g lipid	2.800	2.808
NH_3 in sediments	s_nh3sed	mg N L^{-1}	0.105	0.002
NH_3 in water	s_nh3wat	mg N L^{-1}	0.002	0.005
NH_4^+ in sediments	s_nh4sed	mg N L^{-1}	0.206	0.105
NH_4^+ in water	s_nh4wat	mg N L^{-1}	0.081	0.240
NO_3^- in water	s_no3wat	mg N L^{-1}	0.619	0.114
Organic nitrogen in sediments	s_ornsed	mg N L^{-1}	140.29	38.18
Phytoplankton biomass	s_phytobiom	mg N L^{-1}	0.322	0.113
Total body protein biomass	s_prbiom	g protein (dm)	5.95	5.97
Individual fish weight (wet)	s_wf	g	35.0	35.1

Calibration was carried out by adjusting the equation coefficients in order to improve the fit between simulated and observed data. During the process, one selected coefficient value was changed at a time and the model was run for the whole simulation period. When the coefficient was previously reported in the literature that value was used as the initial value for the simulation.

Table 4. Coefficient values used in the model after calibration. Calibrated coefficients are in **boldface**.

Variable	Symbol	Units	Value	Initial source
g amino acids needed for 1 mole ATP	p_aaatp	g aa/mole ATP	4.76	v. Dam & Penning de Vries 1995
Fraction of digested protein used for gluconeogenesis	p_aafdgl	dl	0.05	Machiels & Henken 1986
Ammonification in sediments rate coefficient	**p_ammonifsed**	1 h^{-1}	5.04e^{-6}	Berner 1974
Ammonification in water rate coefficient	**p_ammonifwat**	1 h^{-1}	5.04e^{-3}	Otsuki & Hanya 1972
Ratio carbon/nitrogen in phytoplankton	p_cnratio	g C/g N	6.622	Redfield ratio
Constant for extinction	**p_constext**	dl	0.085	after Scavia 1980
Denitrification rate coefficient in sediments	p_denitrifsed	1d^{-1}	(0.0318* exp(0.078* (a_temp-20))	Stanford *et al.* 1975
Diffusion coefficient for NH_3	**p_diffcoefnh3**	$\text{cm}^2 \text{ h}^{-1}$	0.115	Broeker 1974
Carbohydrate digestibility coefficient	p_digca	%	0.50	v. Dam & Penning de Vries 1995
Lipid digestibility coefficient	p_digli	%	0.50	v. Dam & Penning de Vries 1995
Protein digestibility coefficient	p_digpr	%	0.80	v. Dam & Penning de Vries 1995
Light extinction due to water	**p_exwa**	1 m^{-1}	0.27	Scavia 1980
First factor for aalirate equation	**p_fac1**	dl	4.3	estimated after v.d. Meer & v. Dam 1998
Second factor for aalirate equation	**p_fac2**	dl	1.2	estimated after v.d. Meer & v. Dam 1998
Initial lipid	p_iliper	% fw	8.0	v. Dam & Penning de Vries 1995
Half saturation constant for Nitrogen	**p_kn**	mg N L^{-1}	0.3	Chen & Orlob 1975
Cost of lipid synthesis	p_liatco	mole ATP g^{-1}	0.015	v. Dam & Penning de Vries 1995
g lipids needed for 1 mole ATP	p_liatp	g lipid/mole ATP	1.96	v. Dam & Penning de Vries 1995
Minimum fat percentage in fresh weight of fish	p_lipfin	%	3.0	v. Dam & Pauly 1995
Phytoplankton growth rate coefficient	**p_maxgrphyto**	1 h^{-1}	0.104	Scavia 1980
Fraction of feed given to fish but not eaten	**p_mors**	dl	0.35	estimated
Phytoplankton mortality rate coefficient	**p_mrphyto**	1 h^{-1}	2.08e^{-4}	Jorgensen *et al.* 1978
Cost of gluconeogenesis	p_neoatco	mole ATP g^{-1}	-0.095	Li & Gregory 1974
Diffusion coefficient for NH_3	**p_nh3dif**	$\text{m}^2 \text{d}^{-1}$	1.0	Brezonik 1994
Diffusion coefficient for NH_4^+	**p_nh4dif**	$\text{m}^2 \text{d}^{-1}$	1.7107e-4	Li & Gregory 1974

(Table 4, cont.)

Variable	Symbol	Units	Value	Source
Nitrification in sediments rate coefficient	**p_nitrifsed**	$1\,h^{-1}$	0.010	Bansal 1976
Nitrification in water rate coefficient	**p_nitrifwat**	$1\,h^{-1}$	4.17e-4	Bansal 1976
Diffusion coefficient for NO_3^-	**p_no3dif**	$m^2\,h^{-1}$	6.84e-6	Li & Gregory 1974
Pond area	p_pondarea	m^2	70	own observation
Soil porosity	p_porosity	dl	0.84	own observation
Cost of protein synthesis	p_pratco	mole ATP g^{-1}	0.075	v. Dam & Penning de Vries 1995
Protein percentage in fresh weight	p_prperc	g prot (100 g)$^{-1}$ fish	17.00	v. Dam & Penning de Vries 1995
Q_{10} factor metabolism	p_q10	dl	2.0	Machiels & Henken 1986
Saturation light intensity	p_radop	$ly\,h^{-1}$	1.67	van Liere et al. 1978
Reference temperature for routine metabolism	p_reftemp	°C	26	Saint-Paul 1988
Routine metabolism coefficient	p_rmcf	mole ATP $g^{-0.8}\,h^{-1}$	5.04e-5	v.d. Meer & v. Dam 1998
Routine metabolism exponent	p_rmex	dl	0.8	v.d. Meer & v. Dam 1998
Maximum temperature for phytoplankton	p_temax	°C	35	Chen & Orlob 1975
Volatilisation of NH_3 rate coefficient	**p_volatnh3**	h^{-1}	3.19e-3	Wolfe et al. 1986
Water depth	p_waterdepth	m	1.2	own observation
Water/sediment film tickness	**p_zfilm**	cm	0.3	Wolfe et al. 1986

In some cases a range for the coefficient was reported in literature, so the calibration was performed changing the coefficient within that range. Calibrated rate coefficients are indicated in Table 4. The degree of agreement between simulated and observed data over the whole simulation was determined at every time when field data were available, calculating the relative error:

$$RE = (S_{sim} - S_{obs}) / \frac{1}{2}(S_{sim} + S_{obs}) \qquad (16)$$

in which RE is the relative error, and S_{sim} and S_{obs} are the simulated and observed values of the state variables at each moment. This way of calculating the RE takes into account the fact that both observed and simulated values are an approximation of the real value. Using a reiterative trial and error procedure, the coefficients were adjusted until the RE

was at least equal or lower than 0.25 for any sampling period (25% deviation from observed data); the same procedure was used for each coefficient. Final simulation was performed when all coefficients were set (Table 4) and the average relative error (ARE) for each state variable was used to assess the agreement for the whole experiment (van Dam *et al*. 1996).

Sensitivity analysis

Each time a selected rate coefficient was changed by +/- 10% of the value that gave the best fit between simulated and observed data, maintaining the other coefficients unchanged. The difference between the value of the state variable at each sampling date for the highest rate coefficient (+10%) and the value for the lowest rate coefficient (-10%) expressed as a percentage of the value using the coefficient obtained after calibration was used to quantify the sensitivity of the model to that particular coefficient (Piedrahita 1986).

Validation

For the validation of the model, independent data sets were used (Table 5). Two stagnant ponds of 800 m^2 and 1.2 m depth were stocked with 35.1 g ($\pm$ 7.0 SD) *Oreochromis niloticus* at a density of 1.2 fish m^{-2}. Fish were grown for 91 days and sampled every three weeks, sampling at least 50% of the entire pond population. Fish were fed a 3 mm 30% protein pellet. Average fish weight was used to estimate the amount of feed offered using a feeding rate of 2.5% body weight per day. The daily ration was divided into two equal portions and administered at 0700 and 1500 hours; fish were not fed on sampling days. Table 3 compares the initial conditions for the calibration and validation runs.

Dissolved oxygen, water pH and temperature in the water column were measured twice a day and interpolated to obtain hourly values as in the calibration experiment. Ponds were sampled weekly for nitrogen contents in phytoplankton, sediments and water using the methods described.

Table 5. Experimental data collected to perform the validation of the model[*]. Standard deviation is indicated between brackets

Day	s_nh4wat	s_nh4sed	s_no3wat	a_fishw	s_phytobiom	s_ornsed
Initial	0.240	0.105	0.114	35.1	0.113	38.18
	(0.027)	(0.011)	(0.012)	(7.0)	(0.028)	(1.4)
7	0.011	0.077	0.111		0.527	38.76
	(0.002)	(0.055)	(0.037)		(0.017)	(2.2)
14	0.087	0.067	0.076		0.752	38.02
	(0.031)	(0.041)	(0.010)		(0.036)	(1.3)
21	0.096	0.087	0.095	61.8	0.837	41.14
	(0.041)	(0.014)	(0.012)	(12.8)	(0.051)	(3.7)
28	0.192	0.162	0.114		0.854	45.53
	(0.061)	(0.014)	(0.011)		(0.043)	(2.3)
35	0.250	0.153	0.162		0.961	58.03
	(0.020)	(0.027)	(0.007)		(0.032)	(5.3)
42	0.261	0.174	0.189	102.4	1.085	54.54
	(0.016)	(0.027)	(0.024)	(12.2)	(0.089)	(7.8)
49	0.412	0.281	0.168		0.879	64.45
	(0.095)	(0.069)	(0.005)		(0.181)	(8.5)
56	0.327	0.211	0.192		0.923	82.23
	(0.042)	(0.027)	(0.007)		(0.135)	(3.8)
63	0.427	0.266	0.273	161.3	1.017	83.34
	(0.055)	(0.081)	(0.016)	(19.1)	(0.067)	(7.4)
70	0.344	0.388	0.401		1.076	85.54
	(0.210)	(0.040)	(0.064)		(0.087)	(9.5)
77	0.624	0.355	0.439		1.204	85.14
	(0.083)	(0.149)	(0.107)		(0.072)	(8.0)
84	0.621	0.504	0.406	236.1	1.191	81.52
	(0.212)	(0.013)	(0.048)	(19.8)	(0.080)	(12.9)
90	0.791	0.509	0.422		1.125	90.83
	(0.015)	(0.068)	(0.067)		(0.137)	(19.8)

[*] All state variables are expressed in mg N per liter, except fish weight which is expressed in terms of grams per fish (individual fresh weight). For names explanation see Table 1.

Results

Most of the coefficients used in the model were taken from literature. When information was not available, the coefficients were estimated by fitting model predictions to observed data for a time series of data and for all the state variables.

The calibrated model was run for the whole experimental period (56 days in this case) and simulated values were plotted against the observed values (Fig. 5). Ammonium and nitrate concentrations in the water column (s_nh4wat and s_no3wat) were well simulated, with predicted values randomly around the observed values. Simulated values for sediment organic and inorganic nitrogen compounds were higher than observed values most of the time, but average relative errors (AREs) remained

equal or below 0.11 (Table 6). The agreement between simulated and observed fish weight and phytoplankton biomass was good.
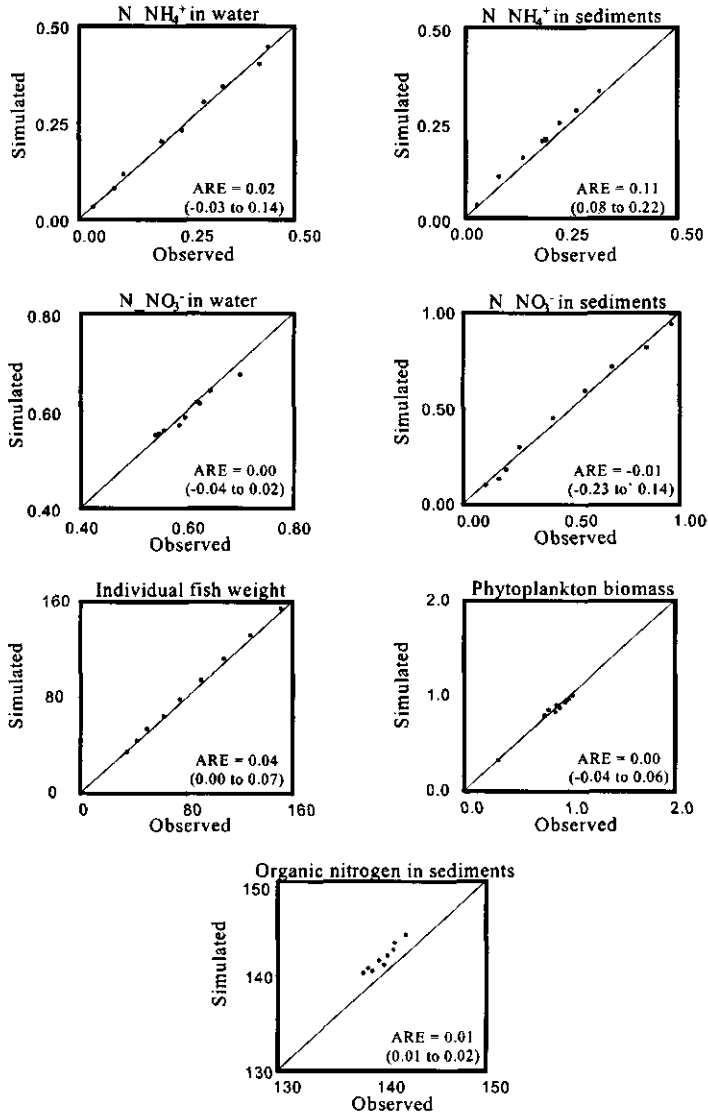

Figure 5. Calibration results for the model. The bisector represents perfect agreement between simulated and observed values (ARE = Average Relative Error, relative errors range is given within brackets)

The combination of all the coefficient values at the end gave relative errors oscillating between 0 and -0.23 (Table 6). The simulation of ammonium in the sediments (s_nh4sed) presented the highest positive deviation from observed values on days 7 and 21 (+0.22 and +0.16, respectively) while the highest negative deviation occurred on days 7 and 12 (-0.23 and -0.12, respectively) for the simulation of nitrate in the sediments (s_no3sed).

Table 6. Relative errors (RE*) of state variables after calibration

Day of simulation	N-NH$_4^+$ (water)	N-NH$_4^+$ (sediments)	N-NO$_3^-$ (water)	State variable N-NO$_3^-$ (sediments)	Fish weight	Phyto-plankton	Organic N (sediments)
1	0.00	0.08	0.00	-0.03	0.00	0.00	0.02
7	0.00	0.22	0.01	-0.23	0.05	0.03	0.01
14	0.14	0.10	0.02	-0.12	0.07	-0.04	0.02
21	0.07	0.16	0.01	0.14	0.04	0.06	0.01
28	-0.03	0.11	-0.02	0.09	0.05	-0.03	0.01
35	0.05	0.08	-0.01	0.06	0.04	0.03	0.01
42	0.03	0.11	-0.01	0.04	0.04	-0.02	0.01
49	-0.04	0.07	0.00	-0.03	0.03	-0.02	0.02
56	0.02	0.05	-0.04	-0.02	0.02	-0.02	0.01
ARE**	0.02	0.11	0.00	-0.01	0.04	0.00	0.01

* RE = $(S_{sim} - S_{obs}) / \frac{1}{2}(S_{sim} + S_{obs})$
  S_{sim} = Simulated state value
  S_{obs} = Observed state value
** ARE = $\Sigma(RE)/n$
  ARE = Average relative error
  n = Number of observations

The concentration of ammonium in the sediments also presented the higher average relative error (ARE) for the whole simulation period.

The response of the state variables to a 10% increment and 10% decrement in the value of selected parameters was used as a quantification of model sensitivity (Piedrahita 1986); sensitivity analysis was used to identify coefficients which have a strong effect over different state variables. Table 7 summarizes the sensitivity to selected parameters, and the corresponding state variable(s). The concentration of ammonium in the water column (s_nh4wat) was strongly affected by the percentage of protein in the feed and by the routine metabolism exponent; this last coefficient also had an important effect on the concentration of ammonium in the sediments (s_nh4sed) and in the fish weight (s_fw). Fish weight was also strongly affected by the protein digestibility coefficient.

The validation was performed using two independent data sets from earthen ponds. Fourteen field data points collected weekly in both ponds were used to evaluate model performance. Most of the coefficients obtained during calibration (Table 4) were maintained unchanged, whereas the only parameters being changed were the initial conditions of the system, and the fish species-specific coefficients (see Table 3). Because the fish species was changed, the corresponding coefficients that characterize the species were also changed accordingly and the fish module was first calibrated for this species. Fish density and initial weight were maintained equal. The pond area, and the initial conditions, specially the amount of organic nitrogen in the sediments, were also different.

Table 7. Sensitivity analysis. Effect of increasing or decreasing by 10% the indicated coefficient

Coefficient	Module	State variable affected	± 10% [*]
g amino acids needed for 1 mole ATP	Fish	$N-NH_4^+$ in water	18.3
		$N-NH_4^+$ in sediments	16.7
		Fish weight	-15.9
Protein digestibility coefficient	Fish	Fish weight	22.6
Routine metabolism exponent	Fish	$N-NH_4^+$ in water	25.4
		$N-NH_4^+$ in sediments	22.3
		$N-NO_3^-$ in sediments	18.0
		Fish weight	-20.9
Fraction of protein in feed	Fish	$N-NH_4^+$ in water	21.6
		$N-NH_4^+$ in sediments	19.7
		$N-NO_3^-$ in sediments	16.6
Carbon/nitrogen ratio	Phytoplankton	$N-NO_3^-$ in water	15.5
		Phytoplankton	-18.4
Constant for light extinction	Phytoplankton	$N-NO_3^-$ in water	15.5
		Phytoplankton	-18.4

[*] Numbers indicate the difference between the final value of the state variable when the respective coefficient was increased by 10% and the value of the state variable when the coefficient was decreased by 10% as a percentage of the state variable value obtained using the coefficient after calibration. The sign represents under- or over-estimation depending whether the sign is negative or positive. Table includes only coefficients whose change affected by more than 15% the value of any state variable.

Figure 6 presents the comparison between the predicted output from the simulation model and the experimental data used during the validation, and Table 8 summarizes the relative errors for the principal state variables. For all state variables there were no significant differences between the two ponds (paired t-test, p>0.05), so data sets were averaged. The organic nitrogen in the sediments (s_orgnsed) was poorly simulated, especially after day 28; the average relative error (ARE) was also the highest (-0.34) indicating that predicted values were lower than observed values.
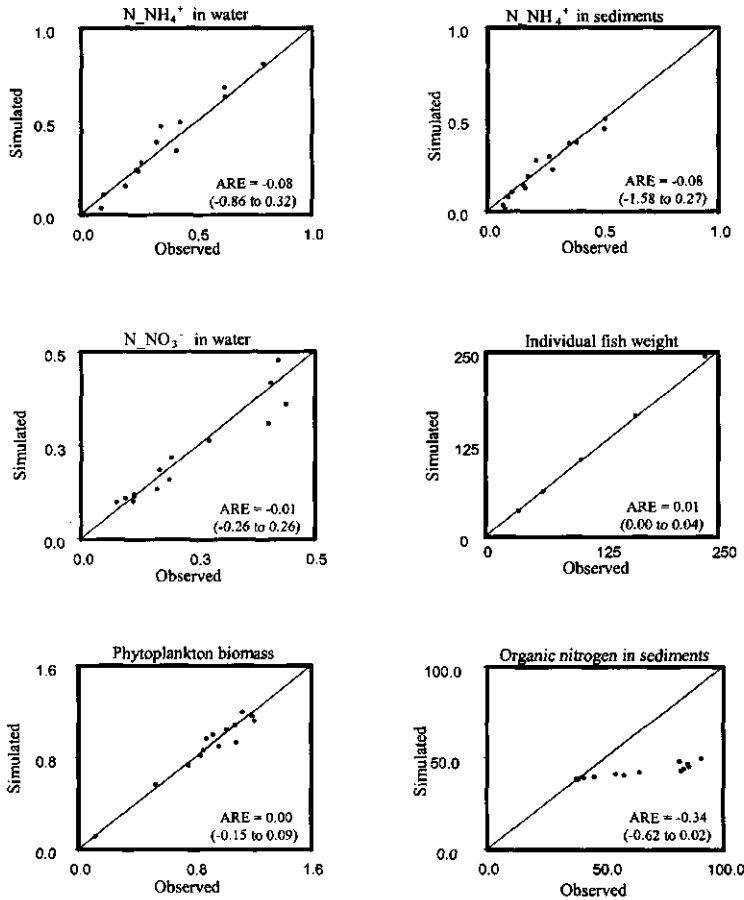

Figure 6. Predicted output from the simulation model and experimental data (mean, n = 2) used during validation. The bisector represents perfect agreement between simulated and observed (ARE = Average Relative Error, relative errors range is given within brackets)

Table 8. Relative errors (RE*) of state variables obtained during validation. REs are calculated for the average of the two ponds used

Day of simulation	N-NH$_4^+$ (water)	N-NH$_4^+$ (sediments)	N-NO$_3^-$ (water)	Fish weight	Phytoplankton	Organic N (sediments)
1	0.00	0.00	0.00	0.00	0.00	0.00
7	-0.76	-1.58	-0.10		0.07	-0.01
14	-0.86	-0.74	0.26		-0.02	0.02
21	0.11	-0.15	0.14	0.00	-0.02	-0.04
28	-0.23	-0.24	0.03		0.01	-0.13
35	-0.07	-0.06	-0.20		-0.07	-0.35
42	0.07	0.10	-0.17	0.02	-0.15	-0.28
49	-0.18	-0.21	0.10		0.09	-0.42
56	0.17	0.27	0.13		0.08	-0.62
63	0.15	0.12	-0.04	0.02	0.02	-0.61
70	0.32	-0.03	-0.26		0.00	-0.61
77	0.02	0.04	-0.20		-0.07	-0.58
84	0.10	-0.11	0.03	0.04	-0.03	-0.51
90	0.03	0.00	0.12		0.06	-0.59
ARE**	-0.08	-0.19	-0.01	0.01	0.00	-0.34

* RE = (S$_{sim}$ - S$_{obs}$) / ½(S$_{sim}$ + S$_{obs}$)
   S$_{sim}$ = Simulated state value
   S$_{obs}$ = Observed state value

** ARE = Σ(RE)/n
   ARE   = Average relative error
   n   = Number of observations

Simulated values for ammonium in the sediments (s_nh4sed) were lower than observed values most of the time, and presented a high ARE (-0.19); nitrate concentration in the water column (s_no3wat) was simulated better than ammonium (s_nh4wat). The fish weight (s_wf) and the phytoplankton biomass (s_phytobiom) were well simulated by the model, presenting the lowest ARE. In the case of fish, this was expected as we calibrate the fish module first for this species.

Discussion

A comprehensive simulation model, covering the fish, phytoplankton and the pond physical environment was developed, calibrated and validated. Most of the initial values of the coefficients used in the model were found in the literature and were set after calibration within reported ranges (Table 4). Data used for calibration and

validation originated from ponds of different area (800 m^2 vrs 65 m^2) and length/surface area ratio (0.15 vrs 0.50); this could affect the ecological conditions of the systems, but the model simulated most of the nitrogen allocation sources in the pond fairly well.

Simulation of inorganic nitrogen species in the water column

Total ammonia nitrogen (TAN) and nitrate in the water column were fairly well simulated. In the model it was considered that TAN originates from mineralization *in situ* of organic nitrogen, fish excretion, or diffusion from the sediments, and that mineralization was influenced by pH and dissolved oxygen (Reddy and Patrick 1984; Oláh *et al.* 1987). This approach led to reasonably accurate predictions.

The nitrate concentration in the water was also predicted with reasonable accuracy. According to Sharma and Ahlert (1977), nitrification is a function of dissolved oxygen, temperature, pH, substrate concentration, light and number of nitrifying bacteria. Numbers of nitrifying bacteria were not included in the present work, assuming that the pond is in a steady state with regard to microbial population. Hargreaves (1997) assumed that the nitrification rate could be described by a first-order reaction. The first order constant is then temperature dependent (p_nitrifwat=0.002+0.0025*a_temp). In our model the nitrification coefficient (p_nitrifwat) was set constant (see Table 4), and it was assumed that nitrification did not proceed when dissolved oxygen was lower than 2 ppm (Knowles *et al.* 1965; Wuhrmann 1968); the rate of nitrification was determined by substrate concentration when oxygen was above this value (equation 10). Both approaches gave similar results with differences between simulated and observed dissolved nitrate and ammonium concentrations not higher than 15%.

During the validation, positive and negative differences between simulated and observed values of N-NH$_4^+$ or N-NO$_3^-$ were negatively correlated to each other (Table 8). This is possibly related to the nitrification rate. However, when changing the value used for the rate constant of nitrification (4.17 x 10^{-4} h^{-1}; Bansal 1976), the simulations were not improved. Considering the "preference" of phytoplankton for ammonium instead of nitrate (McCarthy 1981), another explanation could be the uptake rate of TAN by the phytoplankton and TAN's concentration: at the higher negative N-NH$_4^+$ difference (day 14) the phytoplankton uptake rate was 39.28 mg N m^{-2} d^{-1}, while at the

higher positive difference (day 70) the uptake rate was 20.24 mg N m^{-2} d^{-1}. If the uptake rate of TAN is higher than estimated it causes a negative difference of its concentration.

Phytoplankton

Phytoplankton biomass increased from an initial concentration of 0.322 to 1.032 mg of nitrogen per liter. When the nitrogen content in the dead phytoplankton was also taken into account, the total produced biomass of algae was estimated to be 1.16 mg L^{-1} on day 56. The importance of phytoplankton as a regulator of ammonia concentrations in fish ponds is well known (Tucker *et al.* 1984; Krom *et al.* 1989), and uptake of dissolved inorganic nitrogen from the water column is the primary pathway of nitrogen removal (Barica 1974; Boyd 1974). The uptake rate of nitrogen by phytoplankton was 264.4 mg N m^{-2} d^{-1} at the beginning of the experiment, and decreased during the growing cycle partly due to nutrient and light limitations, the last caused by algal self-shading.

To facilitate practical use of the model, phytoplankton biomass was measured as chlorophyll-a concentration. This does not permit separation of phytoplankton biomass into species or groups of algae. Still, the proposed model was accurate enough to predict phytoplankton concentrations. During calibration, phytoplankton was very well simulated with an ARE of 0 and REs not greater than 0.06. Validation, using different initial conditions for phytoplankton such as initial biomass and pond area (Table 3) also resulted in good agreement of simulated and observed data.

Simulation of nitrogen species in the sediments

The concentration of inorganic nitrogen in the sediments was less well simulated than in the water column. The ammonium concentration in the sediments presented the higher ARE after calibration (0.11, see Table 6) with always positive REs. Several factors can cause model estimations to exceed observed values. Ammonium can be adsorbed by negatively charged soil colloids (Boyd 1995). Soil pH was assumed to follow a daily fluctuating pattern based on field determinations and, if actual pH values were lower than assumed, then part of the N-NH$_4^+$ would not be accounted for. Since laboratory measurements of TAN included both dissolved and adsorbed nitrogen in sediments, cation exchange capacity (CEC) was not considered. Sediment samples used for the laboratory analysis were taken from the upper 5 cm layer. According to Diab and

Shilo (1986), the concentration of free and bound ammonia is lower in the upper layer, increases with depth up to 15-20 cm and decreases in deeper layers. The profile of N-NH_4^+ seems important to consider, and a separation of layers could result in a better simulation of nitrogen compounds in the sediments. Finally, if part of the dissolved N-NH_4^+ seeps through the pond bottom, the model will overestimate N-NH_4^+. Although seepage data were not available, the ponds used were rather old and seepage in earthen ponds is reduced over time (Boyd 1990). Another possible pathway for N-NH_4^+ is diffusion towards the water column. According to Hargreaves (1998), sediments are a source of ammonium to the water column. Within the equations for diffusion rate, both the concentration gradient between water and sediments and a fixed soil porosity (p_porosity = 0.84) were included. As stated before, N-NH_4^+ in the water column was simulated well and, again, any increase of the coefficients of the flux rate equation did not improve the simulations any further. The nitrate concentration in the sediments was fairly well simulated although it can be noticed that the highest positive difference of N-NH_4^+ and the highest negative difference of N-NO_3^- also occurred on the same day (day 7).

Organic matter accumulated in the observed system. The calibrated organic nitrogen in the sediments was well simulated (Fig. 5). However, validation showed that this state variable is not simulated well. The organic nitrogen in the sediment might be partly incorporated in biota, as insect larvae that fly out of the system. Reports on insect larval abundance in shallow ponds are highly variable, in the order of < 0.5 up to >100 g m^{-2} (e.g., Merla and Müller 1970; Drake and Arias 1995; Stagliano *et al.* 1998). Information on this aspect is scarse and it should be investigated further.

To keep the model simple, the ammonification rate was modeled as a first-order reaction with respect to organic matter concentration, taking into account a minimum pH and dissolved oxygen to proceed. The dynamics of organic matter decomposition are much more complex. Anaerobic decomposition of organic matter was not considered in the model. However, anaerobic bacteria in sediments can produce ammonium at the same rate (or even higher) as aerobic ones (Jacobsen and Jörgensen 1975). The anaerobic ammonia production is dependent on the C:N ratio of the organic material, soil texture, pH, temperature, and nutrient availability (Reddy and Patrick 1984). Furthermore, higher oxygen concentrations affect the microbial transformations of nitrogen or organic carbon degradation (Avnimelech *et al.* 1992). Therefore, it might

be useful to include the sedimentation and resuspension of organic matter in future studies. Avnimelech and Wodka (1988) found that resuspended material accounted for 50% of the total sedimentation flux in a reservoir of 8 m deep, and this value is much higher in aquaculture ponds (Avnimelech *et al.* 1999).

Simulation of fish growth

Well documented and reported values of parameters from the literature were directly used for the growth simulations of tilapia and *Colossoma* (Table 3). Simulation for the size used (35 to 155 g in calibration runs, 35 to 270 g in validation runs) resulted in REs not greater than 0.07. Because of the good agreement between simulated and observed values, any further assessment of these coefficients is not a priority for model improvement.

Feed sources are difficult to assess in fish ponds, particularly in extensive or semi-intensive systems. In manured fish ponds, 50-80% of the fish yield originates from algal-based food webs harvested primarily after the algae had been processed within the detritus of the pond bottom (Schroeder 1987). Depending on the fish species, phytoplankton could be an important natural food source for fish cultured in extensive culture systems (Yusoff and McNabb 1997). In our case, it was assumed that fish preferred artificial feed to natural feed, and that fish take artificial feed independent of the concentration of natural feed sources (Schroeder 1978; Brummett and Noble 1994; Milstein *et al.* 1995; Jamu 1999). The amount of feed not eaten is also difficult to assess (Nijhof 1994; van der Meer *et al.* 1995), but the assumption that 35% of the feed offered was not eaten (p_mors = 0.35) proved adequate. The effect of this parameter on the simulation of fish growth is presented in Figure 7. Sensitivity analysis (see below) demonstrated that changing this value by +/- 10% did not result in relevant effects on the simulations. Only when this coefficient was changed by more than 30% (that is increasing or decreasing p_mors to 0.45 or 0.25), the simulation of state variables such as TAN in water or sediments was affected.

The fish growth module, in general, had a strong impact on the N budget in water and in sediments. This is logical since feed nitrogen is the largest N-input, and feed utilization and metabolism should have a large influence on the sinks of nitrogen in the system. The fact that the model simulates most N-variables well also means that such dynamic modelling of metabolic processes is a good approach.
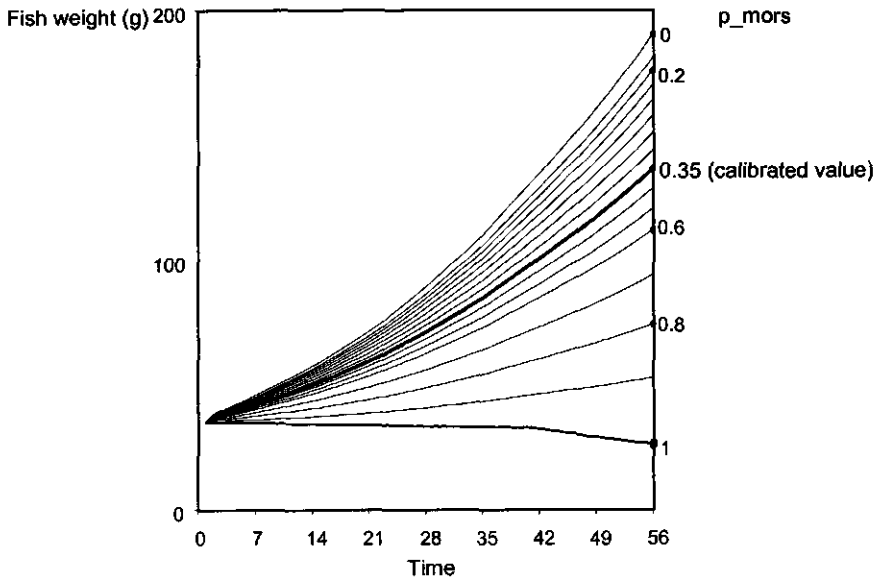
Figure 7. Effect of feed spill parameter (p_mors) on fish growth

Sensitivity analysis

Sensitivity analysis resulted in the identification of coefficients with important effects on the model simulations. The principal state variables affected by changing the specific coefficients were $N-NH_4^+$ and $N-NO_3^-$ both in water and sediments (Table 7). The C:N ratio in phytoplankton presented an important effect on the phytoplankton module. In the model this parameter was used to convert phytoplankton biomass into organic carbon, so it influences the rate of degradation of organic matter. With a large C:N ratio, the substrate does not supply enough nitrogen to satisfy microbial requirements (Boyd 1990). Hence, nitrogen will be immobilized rather than mineralized.

In summary, the present model simulates fairly well most of the nitrogen allocation sources in the pond. Simulated organic nitrogen in sediments was higher than observed values most of the time. Special attention should be given to soil organic matter equations. The dynamics of organic matter accumulating in the sediments,

especially the processes of deposition/accumulation and decomposition should be studied. All coefficients that presented high sensitivity in the model belong to the fish and phytoplankton modules, and as stated before, the simulation of these two state variables were good after calibration and during validation, so changes in their coefficients would not be a priority for model improvement.

Acknowledgements

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Annex 1. Variables used in the model during calibration, and respective equation involved.

Variable	Equation involved
p_aaatp	r_aaox = a_aaswtch*r_atpmet*p_aaatp
p_aafdgl	s_aapool1(t) = s_aapool1(t - dt) + ((1-p_aafdgl)*r_prodig - r_prosyn) * dt
	s_aapool2(t) = s_aapool2(t - dt) + (p_aafdgl*r_prodig - r_aagluc) * dt
p_ammonifsed	r_ammonifsed = if (a_phsed > 2) and (a_dosed >2) then (s_ornsed*p_ammonifsed) else 0
p_ammonifwat	r_ammonifwat = if (a_phwat>2) and (a_dowat>2) then (s_phytodead*p_ammonifwat) else 0
p_cnratio	a_poc = s_phytobiom*p_cnratio
p_constext	a_totlext = p_exwa+p_constext*a_poc
p_denitrifsed	p_denitrifsed = (0.0318*EXP(0.078*(a_temp-20)))/24
p_diffcoefnh3	p_volatnh3 = p_diffcoefnh3/(p_waterdepth*100*p_zfilm)
p_digca	r_cardig = r_fdrtdm*(a_feedca)*p_digca*1.11
p_digli	r_lipdig = r_fdrtdm*(a_feedli)*p_digli*0.96
p_digpr	r_prodig = r_fdrtdm*(a_feedpr)*p_digpr*1.18
	r_egspro = ((1-p_digpr)*a_feedpr*r_fdrtdm)
p_exwa	a_totlext = p_exwa+p_constext*a_poc
p_fac1, p_fac2	a_aalirat =MAX(0, 0.95-(p_fac1/100)*a_prolev-(p_fac2/100)*(a_pe* SQRT(SQRT(a_prolev))))
p_iliper	Initial lipid
p_kn	a_nutrilim = if (a_nleft<=0) then 0 else (nleft/(p_kn+a_nleft)) *(a_pleft/(p_kp+a_pleft))
p_liatco	r_atplip = p_liatco*r_lipsyn1
p_liatp	r_lipox = a_liswtch*r_atpmet*p_liatp
p_lipfin	a_aaswtch = if a_liperc>p_lipfin then (1-a_aalirat) else 1
	a_liswtch = if a_liperc>p_lipfin then a_aalirat else 0
p_maxgrphyto	r_phytogrowth = p_maxgrphyto*(a_nutrilim*a_lighlim)*s_phytobiom
p_mors	r_fdrtdm = a_ration*a_feeddm*(1-p_mors)
	r_fdrtfw = a_ration*(1-p_mors)
	r_uneadm = a_ration*a_feeddm*p_mors
	r_uneafw = a_ration*p_mors
p_mrphyto	r_phytom = p_mrphyto*EXP(a_tecorm)*s_phytobiom
p_neoatco	r_atpneo = p_neoatco*r_aagluc
p_nh3dif	r_nh3flux =(-p_porosity*p_nh3dif*(((s_nh3sed-s_nh3wat)/1000)/ p_seddepth)) *(1000/(24*p_waterdepth))
p_nh4dif	r_nh4flux =(-p_porosity*p_nh4dif*(((s_nh4sed-s_nh4wat)/1000)/ p_seddepth)) *(1000/(24*p_waterdepth))
p_nitrifsed	r_nitrifsed = if a_dosed >4 then (p_nitrifsed*s_nh4sed) else 0
p_nitrifwat	r_nitrifwat = if (a_dowat>2) then (p_nitrifwat*s_nh4wat) else 0
p_no3dif	r_no3flux = (-p_porosity*p_no3dif*(((s_no3sed-s_no3wat)/1000)/ p_seddepth)) *(1000/(24*p_waterdepth))
p_pondarea	a_pondvol = (p_pondarea*p_waterdepth)*1000
p_porosity	r_n2flux = (-p_porosity*p_n2dif*(((s_n2sed-s_n2wat)/1000)/p_seddepth)) *(1000/(24*p_waterdepth))
	r_nh3flux = (-p_porosity*p_nh3dif*(((s_nh3sed-s_nh3wat)/1000)/ p_seddepth)) *(1000/(24*p_waterdepth))
	r_nh4flux = (-p_porosity*p_nh4dif*(((s_nh4sed-s_nh4wat)/1000)/ p_seddepth)) *(1000/(24*p_waterdepth))
	r_no3flux = (-p_porosity*p_no3dif*(((s_no3sed-s_no3wat)/1000)/ p_seddepth)) *(1000/(24*p_waterdepth))
p_pratco	r_atppro = p_pratco*r_prosyn
p_prperc	r_wfrate = (r_prosyn-r_aaox)/(p_prperc/100)
	r_wfraten = 0.16*r_wfrate*(p_prperc/100) *(1000/a_pondvol) *a_fishnb
p_q10	r_roumet = p_q10^((a_temp-p_reftemp)/10)*p_rmcf* (s_wf^p_rmex)

p_radop	a_lighlim = ((2.178*a_phoper)/(a_totlext*p_waterdepth))*(EXP (-(a_decisor/p_radop)*EXP(-a_totlext*p_waterdepth))) - EXP(-(a_decisor/p_radop)*EXP(-a_totlext*p_waterdepth))
p_reftemp	r_roumet = p_q10^((a_temp-p_reftemp)/10)*p_rmcf* (s_wf^p_rmex)
p_rmcf	r_roumet = p_q10^((a_temp-p_reftemp)/10)*p_rmcf* (s_wf^p_rmex)
p_rmex	r_roumet = p_q10^((a_temp-p_reftemp)/10)*p_rmcf* (s_wf^p_rmex)
p_seddepth	r_n2flux = (-p_porosity*p_n2dif*(((s_n2sed-s_n2wat)/1000)/p_seddepth))*(1000/(24*p_waterdepth))
	r_nh3flux = (-p_porosity*p_nh3dif*(((s_nh3sed-s_nh3wat)/1000)/ p_seddepth))*(1000/(24*p_waterdepth))
	r_nh4flux = (-p_porosity*p_nh4dif*(((s_nh4sed-s_nh4wat)/1000)/ p_seddepth))*(1000/(24*p_waterdepth))
	r_no3flux = (-p_porosity*p_no3dif*(((s_no3sed-s_no3wat)/1000)/ p_seddepth))*(1000/(24*p_waterdepth))
p_temax	a_tecorm = IF a_temp>p_temax then (a_temp-p_temax) else 0
p_volatnh3	p_volatnh3 = p_diffcoefnh3/(p_waterdepth*100*p_zfilm)
	r_volatnh3 = p_volatnh3*s_nh3wat
p_waterdepth	r_n2flux = (-p_porosity*p_n2dif*(((s_n2sed-s_n2wat)/1000)/p_seddepth))*(1000/(24*p_waterdepth))
	r_nh3flux = (-p_porosity*p_nh3dif*(((s_nh3sed-s_nh3wat)/1000)/ p_seddepth))*(1000/(24*p_waterdepth))
	r_nh4flux = (-p_porosity*p_nh4dif*(((s_nh4sed-s_nh4wat)/1000)/ p_seddepth))*(1000/(24*p_waterdepth))
	r_no3flux = (-p_porosity*p_no3dif*(((s_no3sed-s_no3wat)/1000)/ p_seddepth))*(1000/(24*p_waterdepth))
	a_lighlim = ((2.178*a_phoper)/(a_totlex*p_waterdepth))*(EXP (-(a_decisor/p_radop)*EXP(-a_totlext*p_waterdepth))) - EXP(-(a_decisor/p_radop)*EXP(-a_totlext*p_waterdepth))
	a_pondvol = (p_pondarea*p_waterdepth)*1000
	p_volatn2 = p_diffcoefn2/(p_waterdepth*100*p_zfilm)
	p_volatnh3 = p_diffcoefnh3/(p_waterdepth*100*p_zfilm)
p_zfilm	p_volatn2 = p_diffcoefn2/(p_waterdepth*100*p_zfilm)
	p_volatnh3 = p_diffcoefnh3/(p_waterdepth*100*p_zfilm)

Parameters

p_aaatp, g amino acids needed for 1 mole ATP; p_aafdgl, fraction of digested protein used for gluconeogenesis; p_ammonifsed, instant rate of ammonification in sediments; p_ammonifwat, instant rate of ammonification in water; p_cnratio, ratio carbon/nitrogen in phytoplankton; p_constext, constant for extinction; p_denitrifsed, instant rate of denitrification; p_diffcoefn2, diffusion coefficient for N2; p_diffcoefnh3, diffusion coefficient for NH3; p_digca, carbohydrate digestibility coefficient; p_digli, lipid digestibility coefficient; p_digpr, protein digestibility coefficient; p_exwa, light extinction due to water; p_fac1, first factor for aalirat equation; p_fac2, second factor for aalirat equation; p_iliper, initial lipid; p_kn, half saturation constant for Nitrogen; p_kp, half saturation constant for phosphorus; p_liatco, cost of lipid synthesis; p_liatp, g lipids needed for 1 mole ATP; p_lipfin, minimum fat percentage in fresh weight of fish; p_maxgrphyto, instant rate of phytoplankton growth; p_mors, fraction of feed given to fish but not eaten; p_mrphyto, instant rate of phytoplankton mortality; p_neoatco, cost of neoglucogenesis; p_nitrifsed, instant rate of nitrification in sediments; p_nitrifwat, instant rate of nitrification in water; p_n2dif, diffusion coefficient for N2; p_nh3dif, diffusion coefficient for NH3; p_nh4dif, diffusion coefficient for NH4; p_no3dif, diffusion coefficient for NO3; p_pondarea, pond area; p_porosity, soil porosity; p_pratco, cost of protein systhesis; p_prperc, protein percentage in fresh weight; p_q10, Q10 factor metabolism; p_radop, saturation light intensity; p_reftemp, ref. temp. routine metabolism; p_rmcf, routine metabolism coefficient; p_rmex, routine metabolism exponent; p_seddepth, sediment depth; p_temax, maximum temperature for phytoplankton; p_volatnh3, instant rate of volatilisation of NH3; p_volatn2, instant rate of volatilisation of N2; p_waterdepth, water depth; p_zfilm, water/sediment film tickness.

States

s_aapool1, amount of AA converted into protein; s_aapool2, amount of AA converted into glucose (gluconeogenesis); s_n2sed, N2 in sediments; s_n2wat N2 in water; s_nh3sed, NH3 in sediments; s_nh3wat, NH3 in water; s_nh4sed, NH4 in sediments; s_nh4wat, NH4 in water; s_no3sed, NO3 in sediments; s_no3wat, NO3 in water; s_omsed, amount of organic nitrogen matter in sediments; s_phytodead, organic N (as dead phytoplankton) in water; s_phytobiom, phytoplankton biomass; s_wf, individual fish weight(wet weight).

Auxiliars

a_aalitrat, proportion of energy sypplied by fat; a_aaswtch, switch, becomes 1 when fat percentage of fish falls below p_lipfin, thus switching energy to 100% protein oxidation; a_decisor; a_dosed, dissolved oxygen in sediment; a_dowat, dissolved oxygen in water; a_feedca, fraction of carbohydrate in feed (wet); a_feeddm, fraction of dry matter in feed; a_feedli, fraction of lipid in feed (wet); a_feedpr, fraction of protein in feed (wet); a_fishnb, number of fish present

initially; a_lighlim, light limitation factor; a_liperc, fat percentage of the fish; a_liswtch, switch, becomes 0 when fat percentage of fish falls below p_lipfin, thus switching off lipid oxidation; a_nleft, N that remains in water; a_nutrilim, nutrients limitation; a_pe, feed protein/gross energy ratio; a_phoper, photoperiod; a_phsed, sediments pH; a_phwat, water pH; a_pleft, P that remains in water; a_poc, organic carbon in phytoplankton; a_pondvol, pond volume; a_prolev, actual protein feeding level; a_ration, daily feed ration; a_tecorm, temperature correction for mortality; a_temp, water temperature; a_totlext, total light extinction.

Rates

r_aagluc, rate of gluconeogenesis; r_aaox, amino acid oxidation rate; r_ammonifsed, ammonification in sediments; r_ammonifwat, ammonification in water; r_atplip, rate of energy use for lipid synthesis from lipid; r_atpmet, rate of energy use for total metabolism; r_atpneo, rate of energy use for gluconeogenesis; r_atppro, rate of energy use for protein systhesis; r_cardig, rate of digestion of carbohydrates from feed; r_egspro, egestion rate protein; r_fdrtdm, real feeding rate; r_fdrtfw, feed consumption rate; r_lipdig, lipid digestion rate; r_lipox, lipid oxidation rate; r_lipsyn1, lipid synthesis rate from digested lipids; r_n2flux, flux of N2; r_nh3flux, flux of NH3; r_nh4flux, flux of NH4; r_nitrifsed, nitrification in sediments; r_nitrifwat, nitrification in water; r_no3flux, flux of NO3; r_phytogrowth, phytoplankton growth; r_phytom, phytoplantion death; r_prodig, amino acid production rate from digested feed; r_prosyn, protein synthesis rate; r_roument, routine metabolic rate; r_uneadm, uneaten feed (dw); r_uneafw, uneaten feed (fw); r_volatnh3, ammonia volatilisation; r_wfrate, fish growth rate; r_wfraten, fish growth rate in terms of nitrogen.

Chapter 3

The effects of organic nitrogen and carbon mineralization and sediment-water inorganic nitrogen fluxes on bottom organic matter accumulation in fish ponds

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Abstract

In aquaculture ponds with high loads of organic inputs, organic matter accumulates at the bottom over time. Uneaten feed, senescent phytoplankton and faeces are the principal sources of accumulated material, but quantifications are scarce. The sedimented organic matter develops into a flocculent layer in which different processes transform the material into inorganic forms. A better understanding of factors influencing organic matter accumulation/decomposition in the sediment is needed to better understand and manage the dynamics of nitrogen in fish ponds. In this study, the rate of mineralization of organic nitrogen and the nitrogen flux between the sediment and the water column were measured. Organic matter accumulation in fish ponds was quantified, and the data were used to construct, calibrate and validate a dynamic simulation model of organic matter deposition/decomposition in fish ponds. The accumulating material consisted of dead phytoplankton, fish faeces and uneaten feed. Through model calibration, the proportion of these materials in the total accumulated organic matter was determined. In the model, gross photosynthetic rate was estimated from an empirical relationship with feed input. After calibration, the model was validated using independent data. The model simulated well the concentrations of organic carbon and nitrogen in the sediments but it may be developed further, especially by considering the effects of resuspension.

Keywords: organic matter; accumulation; nitrogen model; modelling; nitrogen flux.

Introduction

Fish production in aquaculture is characterized by high loads of organic matter in the form of feed and/or organic fertilizers. As a result, organic matter accumulates in pond soils over time (Tucker 1985; Ayub *et al.* 1993). In channel catfish ponds, sediment organic matter increased by 0.23% year[-1] (Tucker 1985). In sediments of ponds enriched with chicken manure, Ayub *et al.* (1993) reported an increase of organic carbon in the order of 0.1% month[-1]. At the bottom surface, settled particulate matter develops into a dynamic, flocculent, organic layer (Visscher and Duerr 1991; Hopkins *et al.* 1994). With the accumulation of organic matter, the oxygen demand increases and oxygen depletion in the sediments may occur. Aerobic and anaerobic decomposition result in a thin aerobic top layer above a gradually more reduced sediment (Brown *et al.* 1987). The micro-organisms in anaerobic soils produce reduced substances such as nitrite, hydrogen sulfide, ferrous iron, and manganese. Ammonia also accumulates in the reduced sediment layer because the biochemical pathway of ammonia transformation requires oxygen. When anaerobic conditions develop in the pond bottom, fish growth is adversely affected because of the formation of toxic substances such as ammonia, nitrite and hydrogen sulfide (Boyd 1990) and because fish avoid grazing in the anaerobic sediments (Avnimelech *et al.* 1981).

The nitrogen concentration in the sediment is the net result of the deposition of organic nitrogen, the decomposition of the organic matter, and the flux between the water and the sediments. The main sources of organic nitrogen are uneaten feed, faeces and dead phytoplankton. The amount of uneaten feed is difficult to assess in ponds. For fish cage culture, Phillips *et al.* (1985) and Thorpe *et al.* (1990) estimated feed spills of 15-30%. In aquarium systems, van der Meer *et al.* (1997) found that 21% of the feed offered to *Colossoma macropomum* remained uneaten. Boyd (1995) stated that uneaten feed usually is less than 5-10%, but more conclusive measurements are scarce. Faeces generally account for 5-15% of the nitrogen ingested by fish (30-40% being incorporated into fish biomass, 25-80% excreted as ammonia or dissolved organic nitrogen; Guerin-Ancey 1976; Kaushik 1980; Krom *et al.* 1985; Porter *et al.* 1987; Lovell 1988). Phytoplankton is considered the major source of organic matter in aquaculture ponds (Boyd 1995). Schroeder *et al.* (1991) found that as much as 50% of the algal standing crop settles on the sediment surface each day.

A wide range of values for decomposition rates are reported in the literature. Rate constants for decomposition for different algae and aquatic plants varied between 0.03 d^{-1} and 0.20 d^{-1} (Sudo *et al.* 1978; Fallon and Brock 1979). For the decomposition of organic matter in the water column, values in the order of 0.001 to 0.340 mg N L^{-1} d^{-1} are reported (e.g. Harrison 1978; Barat and Jana 1987). Decomposition rates of organic matter in the sediments varied threefold, ranging from 0.011 to 0.032 d^{-1} (Foree and McCarty 1970). The flux of nutrients from the sediment into the water column depends on factors such as concentration gradient and bioperturbation, and fluctuate between 0.07 and 6.72 mg N L^{-1} d^{-1} (e.g. Krom and Berner 1980; Schroeder 1987; Blackburn and Henriksen 1983; Seitzinger 1988; Valdés and Real 1994). Estimates of total ammonia nitrogen (TAN, NH_3-N + NH_4^+-N) flux from marine and freshwater sediments into the overlying water vary between 0.03 and 22 mg N m^{-2} d^{-1} (Boynton *et al.* 1980; Brannon *et al.* 1985).

The present study is part of a larger project with the objective to gain more insight into the nitrogen dynamics of feed driven fish ponds by constructing a dynamic simulation model. Changes in concentrations of N-species in the water column and sediments in earthen fish ponds were modeled (Chapter 2, this thesis), but the model was not accurate in predicting the concentration of organic matter in the sediments. A better understanding of the factors that affect organic matter accumulation in the sediment is needed. Therefore, the objectives of the present study were: (1) to estimate the rate of mineralization of organic nitrogen; (2) to measure the flux of inorganic nitrogen forms between sediments and water; (3) to quantify the amount of organic matter accumulating in the sediment of semi-intensively managed fish ponds, and (4) to use that information to construct, calibrate and validate a dynamic simulation model of organic matter deposition and decomposition in fish ponds.

Material and Methods

General

Three pond experiments were done at the 28 Millas Research Station of the Universidad Nacional (UNA) in Limón, Costa Rica, between February and September 1998. At this site, fish ponds have sandy-soils, and water is collected from a nearby wetland. In Experiment 1, organic nitrogen mineralization rate was measured. In

Experiment 2 the flux of organic nitrogen species between water and sediment, and the accumulation of organic matter in pond sediment were quantified. A simulation model for organic matter accumulation and mineralization was then formulated and the data from Experiment 2 were used for calibration. An independent set of data (Experiment 3) was used for model validation.

Experiment 1

Four earthen fish ponds (Ponds 7-10) of 800 m^2 were stocked with 47.2 ± 0.1 g (mean ± SD) *Colossoma macropomum* at a density of 1 fish m^{-2}. Fish were fed 32% protein pellets at 2% of the average individual body weight per day, for 90 days. Every fortnight, primary productivity using the light-dark method was measured in each pond. Samples where incubated at two depths (20 cm and 60 cm) for two hours, and the average gross photosynthetic rate (GPR, g C m^{-2} d^{-1}) calculated. A relationship between gross photosynthesis rate and feed input was estimated.

To determine mineralization of organic nitrogen, soil samples from the four ponds were taken using a soil corer of 8 cm diameter and the cores were transported carefully to the laboratory. After dissection of the soil, the undisturbed samples from the top layer (0-5 cm) were placed in tubes of the same diameter. Water samples taken from three different parts of the pond were mixed, and the mixture poured carefully over the soil samples for incubation. During incubation, the water was sampled for TAN every 9 hours and analyzed using standard methods (APHA 1989). The initial and final concentrations of organic matter in the soil were determined using the method proposed by Raveh and Avnimelech (1972). The amount of ammonia-nitrogen produced and the loss of organic matter during incubation were used to calculate the mineralization rate constants for nitrogen and carbon.

Experiment 2

Two 1-m deep 800-m^2 ponds (Ponds 1 and 2) were stocked with *Oreochromis niloticus* at a density of 1.25 fish per m^2 for 84 days (Table 2). The initial fish weight was 35.3 ± 8.6 g for one pond, and 74.9 ± 20.5 g for the other. Fish were fed a commercial 30.5% protein floating pellet at a feeding rate of 2.2% of the average body weight per day. No water flow-through was allowed in the ponds, and evaporation and seepage losses were replenished weekly.

Every day at 0600 and 1700 hours, dissolved oxygen, water pH and temperature were measured. Water samples were taken every Tuesday (0900) at different locations in the ponds, the samples of each pond mixed, and the composite water sample was filtered through a Whatman GF/C glass fibre filter and analyzed for dissolved NO_3^--N, NO_2^--N, TAN and alkalinity. Another sample without filtration was analyzed for chlorophyll-a in the water column using standard methods (APHA 1989). Each pond was divided into 8 sectors of 100 m^2 and three soil samples of 6 cm diameter and 15 cm deep from each sector were taken every three weeks using a soil corer. One sample was divided into three sub-samples (0-5 cm, 6-10 cm and 11-15 cm depth) and analyzed for soil porosity, TAN (trapped and absorbed) and total nitrogen (Kjeldahl) (APHA 1989).

For the determination of inorganic nitrogen fluxes, the other two soil samples were used. The water above the undisturbed soil samples was removed and replaced carefully with 300 mL of filtered (0.45 μm) pond water. Cores were incubated in the dark for 6 hours and the difference between initial and final concentrations in the water column was used for the determination of flux rates of TAN, NO_2^--N and NO_3^--N.

At the end of the trial, the ponds were emptied and the final fish weight and fish mortality determined. Proximate whole body composition was determined at the beginning and at the end of the experiment.

Experiment 3

Four small stagnant ponds (65 m^2) were stocked with *Colossoma macropomum* of 30-g (±0.5 SD) (Ponds 3-6) at a density of 1 m^{-2}. Fish were fed a 3-mm dry floating pellets with 35% crude protein, at a ration of 2.5% of the average body weight per day. In every pond, accumulated soil organic nitrogen was measured five times during 76 days.

Statistical analysis

In Experiment 1, a linear regression between TAN and time was estimated. From the initial and final concentration of soil organic matter a linear relationship was calculated. The mineralization rate was estimated from the slopes of the two lines.

For Experiment 2, mean values of soil porosity, soil TAN and total nitrogen were compared by repeated measures Analysis of Variance (ANOVA) with three depths and two ponds as main factors and five sampling periods as sub-factor (Gomez and

Gomez 1984), with eight cores per pond (n=240). Mean values of fluxes of TAN, NO_2^- and NO_3^- were compared also by repeated measures ANOVA with pond as the main factor and sampling period as the sub-factor. When a main effect was significant, pair-wise comparison of treatment means was done by Tukey HSD test ($\alpha = 0.05$). All analyses were done using ANOVA procedure of SAS version 6.12 (SAS Institute Inc., Cary NC 27513, USA).

Model formulation

The model includes four state variables: (1) sediment organic nitrogen (s_organic_N); (2) sediment organic carbon (s_organic_C); (3) total ammonia nitrogen in the sediment (s_TAN_sediment); and (4) total ammonia nitrogen in the water column (s_TAN_water).

Table 1. Parameters settings after model calibration.

Parameter[*]	Value used	Source
P protein digestibility (%)	80	van Dam and Penning de Vries 1995
P carbohydrate digestibility (%)	50	van Dam and Penning de Vries 1995
P C mineralization rate constant (d^{-1})	$3.83 - 2.68 \times 10^{-5}$	Experiment 1 (this study)
P N mineralization rate constant (d^{-1})	$5.20 - 3.64 \times 10^{-4}$	Experiment 1 (this study)
P % protein in diet (%)	30.5	Experiment 1/2 (this study)
P % carbohydrate in diet (%)	46.6	Experiment 1/2 (this study)
P maximum gross photosynthesis ($g\,C\,m^{-2}\,d^{-1}$)	7.70	Experiment 1 (feed input vs. chlorophyll-a)
P TAN flux rate constant (d^{-1})	0.10-0.25	Experiment 2 (this study)
I initial C:N ratio	106/16	Redfield ratio
P phytoplankton sedimentation rate constant (-)	0.20	calibration
P faeces sedimentation rate constant (-)	0.70	calibration
P feed sedimentation rate constant (-)	0.90	calibration
P fraction uneaten (%, from feed offered)	30	calibration

[*] for symbols explanation see text

Table 2. Total ammonia nitrogen (TAN-N) and Total nitrogen (Total N) in sediments at three different depths (mean ± SD, n=8). Samples were collected in ponds 1 and 2 at 5 different periods during the growing cycle

Period	Pond	TAN-N (μg g^{-1})			Total N (mg 100g^{-1})		
		0-5 cm	6-10 cm	11-15 cm	0-5 cm	6-10 cm	11-15 cm
1	1	1.15 ± 0.29	1.59 ± 0.25	2.28 ± 0.49	46.25 ± 5.52	28.68 ± 7.19	21.01 ± 4.29
	2	1.65 ± 0.37	2.06 ± 0.52	2.66 ± 0.60	42.56 ± 9.41	27.41 ± 6.27	18.34 ± 3.83
2	1	1.30 ± 0.41	1.67 ± 0.45	2.53 ± 0.41	60.18 ± 5.12	36.84 ± 9.81	28.00 ± 3.68
	2	1.76 ± 0.77	2.35 ± 0.89	3.02 ± 0.84	64.02 ± 5.75	39.35 ± 10.05	30.36 ± 5.25
3	1	3.27 ± 1.00	4.89 ± 1.81	7.28 ± 2.08	65.82 ± 7.63	39.52 ± 9.21	30.47 ± 8.57
	2	3.66 ± 2.25	6.57 ± 1.76	9.33 ± 1.52	61.59 ± 7.71	38.91 ± 8.98	28.62 ± 6.31
4	1	2.62 ± 1.76	4.38 ± 2.29	5.73 ± 2.65	72.95 ± 7.42	44.70 ± 14.08	34.46 ± 11.40
	2	3.05 ± 2.15	5.14 ± 2.33	7.27 ± 2.34	75.07 ± 8.79	44.65 ± 13.25	34.65 ± 9.91
5	1	2.37 ± 0.73	3.34 ± 0.71	4.29 ± 0.91	88.55 ± 14.80	60.73 ± 19.16	53.64 ± 16.08
	2	2.80 ± 1.30	3.87 ± 1.79	6.22 ± 2.56	83.86 ± 20.51	64.94 ± 18.14	50.64 ± 7.55

The sources of bottom organic carbon and nitrogen were dead phytoplankton, fish faeces, and uneaten feed. Sedimentation rates were related to the production rates of these three sources:

$$r\_c\_sedimentation = a\_c\_gross\_photosynthesis * p\_phyto\_sedimentation\_rate\ constant \quad (1)$$

where

r_c_sedimentation	= sedimentation rate for carbon (mg C m^{-2} d^{-1})
a_c_gross_photosynthesis	= gross photosynthesis rate (mg C m^{-2} d^{-1})
p_phyto_sedimentation_rate_constant	= phytoplankton sedimentation rate constant (-)

Similar equations were defined for the sedimentation rates of nitrogen from phytoplankton and of carbon and nitrogen from faeces and uneaten feed.

The rates of mineralization of organic carbon and nitrogen were modeled as first-order functions based on organic matter concentration:

r_c_mineralization = s_organic_c_soil * p_c_mineralization_rate constant (2)

where

 r_c_mineralization   = mineralization rate for carbon (mg C $m^{-2}d^{-1}$)

 s_organic_c_soil   = organic carbon in soil (mg C m^{-2})

 p_c_mineralization_rate constant   = carbon mineralization rate constant (d^{-1}, Table 1),

and

r_n_mineralization = s_organic_n_soil * p_n_mineralization_rate constant (3)

where

 r_n_mineralization   = mineralization rate of nitrogen (mg N $m^{-2}d^{-1}$)

 s_organic_n_soil   = organic nitrogen in soil (mg N m^{-2})

 p_n_mineralization_rate constant   = nitrogen mineralization rate constant (d^{-1}, Table 1).

The rate of mineralization of organic matter decreases with increasing C:N ratio (Alexander 1961). The initial C:N ratio was assumed to be 6.625 (the Redfield ratio):

p_n_mineralization rate constant = if a_CN_ratio > 6.625 then A else B (4)

where

 p_n_mineralization rate constant   = nitrogen mineralization rate constant (d^{-1})

 a_CN_ratio   = carbon to nitrogen ratio (-)

 6.625   = the Redfield ratio (-)

 A, B   = possible values for nitrogen mineralization rate constant,

and

p_c_mineralization rate constant = if a_CN_ratio > 6.625 then C else D (5)

where

 p_c_mineralization rate constant   = carbon mineralization rate constant (instant rate, d^{-1})

 C, D   = possible values for carbon mineralization rate constant

In the model, a_CN_ratio was calculated from the sediment organic carbon and nitrogen concentrations.

The resulting TAN in the sediments diffuses into the water column if a concentration gradient is formed:

r_tan_flux = if (s_tan_soil < s_tan_water) then 0 else (s_tan_soil * p_tan flux_rate constant) (6)

where

r_tan_flux	= rate of TAN flux or diffusion (mg N m^{-2} d^{-1})
s_tan_soil	= TAN soil concentration (mg N m^{-2})
s_tan_water	= TAN water column concentration (mg N m^{-2})
p_tan__flux_rate constant	= TAN flux rate constant (s_tan_soil, after calibration, Table 1).

Gross photosynthesis rate was estimated empirically in the model by establishing a relationship between feed input and photosynthesis in Experiment 1. A relational diagram of the model is presented in Figure 1. Principal parameters used in the model are presented in Table 1. The model was implemented in Stella® version 5.1.1. (High Performance Systems Inc., Hanover NH 03755, USA)
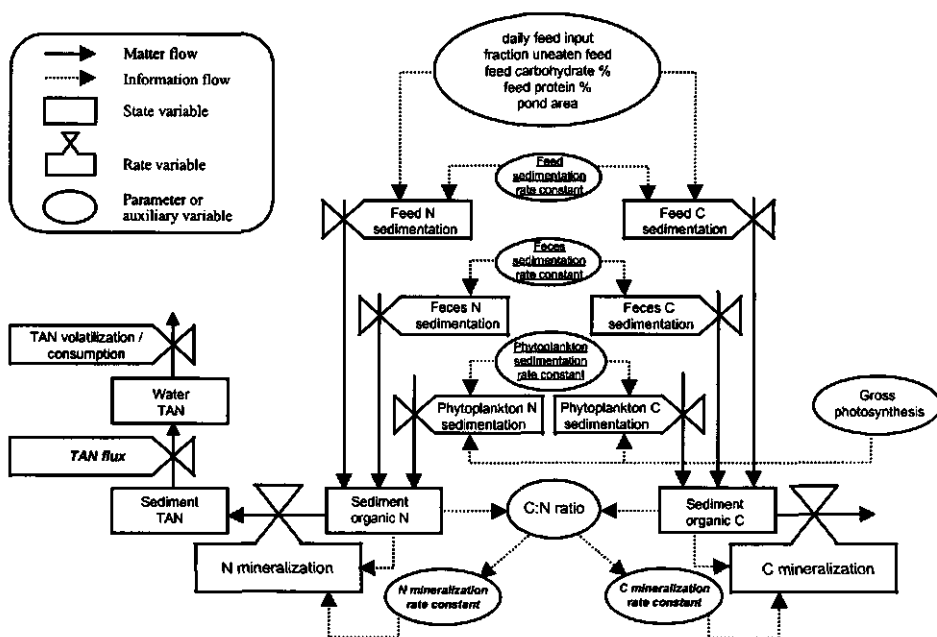

Figure 1. Principal variables and their relations in the model. Calibrated parameters are underlined; measured parameters are *italicized*. For symbols explanation see text.

Model Calibration

Data from Ponds 1 and 2 (Experiment 2) were used for model calibration. The actual feed input, feed composition (percentage of protein, carbohydrates, dry matter), pond dimensions and fish density used in Experiment 2 were input parameters in the

model. Digestibility for protein and carbohydrates were set at 80% and 50%, respectively (van Dam and Penning de Vries 1995). Since no data are available on the sedimentation rate constants of each of the sources of organic matter (dead phytoplankton, fish faeces and uneaten feed), a range of most probable values was evaluated during calibration. Values for the mineralization of organic carbon and nitrogen (A, B, C and D in equations 4 and 5) were also derived through calibration. Using a trial and error procedure, each parameter was changed until the simulated values for sediment organic nitrogen and carbon were within 15% of the field data.

To assess the agreement between simulated and observed data, the relative error was calculated for each sampling date:

$$RE = \frac{(S_{Sim} - S_{Obs})}{0.5 * (S_{Sim} + S_{Obs})} \qquad (7)$$

where

RE	= relative error
S_{sim}	= simulated value of the state variable
S_{obs}	= observed value of the state variable,

and the average relative error for the whole culture period:

$$ARE = \frac{\sum RE}{n} \qquad (8)$$

where

ARE	= average relative error
n	= number of observations

Sensitivity analysis

For selected state variables and parameters, sensitivity analysis was performed by changing the value of the parameter by ± 10% of the calibrated value (while maintaining the other parameters unchanged) and looking at the effect on the state variable. Sensitivity was calculated as the difference between the values of the state variable at the high and low parameter values divided by the calibrated value of the state variable (expressed as a percentage; Piedrahita 1986).

Model validation

The calibrated model was run with the input parameters from Experiment 3 and model output was compared with the observed field data.

Results

Organic nitrogen mineralization rate (Experiment 1)

Figure 2 shows the results of the soil incubation experiments. The regression equation of TAN concentration (y) and time (x) was $y = 0.00104x + 0.31892$ ($R^2 = 0.97$, n=32, P<0.01). From the difference between final and initial TAN concentration and the volume of the water in the cylinder, it was calculated that 0.0061 mg N were produced in 63 h. Assuming that the soil sample contained 4.5 mg N (45 mg 100g^{-1} dry soil as determined in Experiment 2, sample size in cylinder 10 g), this leads to an ammonification rate constant of 5.20×10^{-4} d^{-1}.

The equation for the decrease in organic matter was $y = -0.00083x + 521.61$. The difference between initial and final organic matter content was 0.052 mg 100g^{-1}. With the mean organic matter content of 521.59 mg 100g^{-1}, this resulted in a mineralization rate constant of 3.83×10^{-5} d^{-1}.

Accumulation of organic matter in the sediments and flux of organic nitrogen species between water and sediment (Experiment 2)

During the 84 days of Experiment 2, the fish in Pond 1 grew to 238.7 ± 63.5 g with an average growth rate of 2.27% individual body weight per day and a feed conversion ratio of 2.1; the fish in Pond 2 grew to 330.3 ± 103.0 g with an average growth rate of 1.77% individual body weight per day, realizing a feed conversion ratio of 2.3. In both ponds, there were recruits of tilapia that accounted for 33.1% and 25.9% of the total final biomass, respectively. These recruits were included in estimating feed conversion ratio. The mortality of stocked fishes over the whole culture period was 32% and 27% for ponds 1 and 2, respectively.
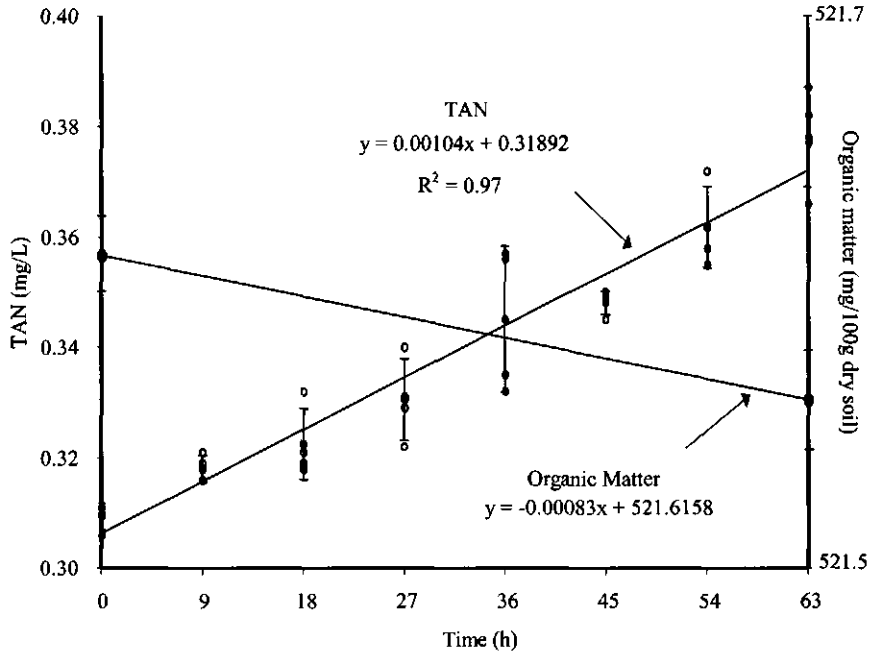
Figure 2. Organic matter concentration in soil and TAN concentrations in water during 63 h of incubation of sediment samples in Experiment 1 (n=4).

The average concentration of nitrogen species in the water column showed values of 0.6 ± 0.2 mg L^{-1} (NO_3^--N), 0.04 ± 0.03 mg L^{-1} (NO_2^--N) and 0.06 ± 0.05 mg L^{-1} (TAN) (mean $\pm$ SD, n=24) for the whole period. Overtime, these parameters did not show clear increase/decrease. Total alkalinity (in meq L^{-1}) decreased with time from 1.2 $\pm$ 0.3 to 0.4 $\pm$ 0.6, while chlorophyll-a increased steadily from 51.9 ± 16.5 to $190.9 \pm$ 54.4 mg m^{-3} at the end of the experiment. Secchi disk visibility decreased in both ponds accordingly, with values from >100 cm at the beginning of the experiment to 45 cm at the end.

For both ponds, porosity of the sediment was always higher in the top layer and lower in the deeper layer (ANOVA, P = 0.0001); there was an increase with time in porosity at each depth but the difference between depths did not change over time. TAN in the sediments ranged from 1.15 ± 0.29 to 9.33 ± 1.52 μg g^{-1} dry sediment (Table 2), being always higher in lower layers (P = 0.0001). There was a significant difference between ponds (P = 0.0046) and periods (P = 0.0001). Total nitrogen (Kjeldahl-N) was

significantly different at different depths (P= 0.0001) and increased over time in both ponds, with values ranging from 18.34 ± 3.83 at the beginning of the experiment to 88.55 ± 14.80 mg N $(100 \text{ g})^{-1}$ dry soil at the end; ponds were not different (P = 0.7459). All periods, except 2 and 3, were significantly different (P<0.05, Table 2).

NO_2^--N and NO_3^--N flux rates were always negative (net flux from the water column into the sediments) and ranged from 0.02 to 0.46 mg N m^{-2} d^{-1} for nitrite, and from 0.07 to 0.39 mg N m^{-2} d^{-1} for nitrate. For the NO_2^--N flux rate, the difference between ponds was marginally significant (P = 0.0569), and for NO_3^--N was not significant (P = 0.2557). TAN flux rate was always positive (net flux from the soil to the water column) and ranged from 1.15 to 7.42 mg N m^{-2} d^{-1} (Table 3).

Table 3. Flux rate of total ammonia nitrogen (TAN), nitrite (NO_2^-) and nitrate (NO_3^-) estimated after laboratory incubations (mean ± SD, n=8). Negative sign indicate flux from the water column to the sediment.

Period	Pond	TAN	NO_2^-	NO_3^-
			Flux rate (mg N m^{-2} d^{-1})	
1	1	7.42 ± 3.41	-0.02 ± 0.01	-0.21 ± 0.06
	2	7.20 ± 3.44	-0.11 ± 0.04	-0.23 ± 0.11
2	1	4.26 ± 2.24	-0.10 ± 0.01	-0.22 ± 0.15
	2	1.85 ± 1.05	-0.03 ± 0.01	-0.27 ± 0.15
3	1	4.93 ± 1.63	-0.24 ± 0.03	-0.14 ± 0.06
	2	5.76 ± 1.46	-0.46 ± 0.22	-0.15 ± 0.04
4	1	3.40 ± 1.61	-0.16 ± 0.13	-0.17 ± 0.11
	2	1.89 ± 1.18	-0.23 ± 0.07	-0.07 ± 0.05
5	1	5.91 ± 3.00	-0.23 ± 0.11	-0.39 ± 0.19
	2	1.15 ± 0.78	-0.26 ± 0.13	-0.18 ± 0.08

The estimated first-order rate of ammonium diffusion was in the range of 0.10 to 0.25 d^{-1}. TAN flux rate was significantly different, both between periods (P = 0.0001) and ponds (P = 0.0215).

Data collection for validation (Experiment 3)

During the 76 days of Experiment 3, fish grew from 30 g to 390 g (±23.5, SD) realizing an average growth rate of 3.35% individual body weight per day and a feed conversion ratio of 2.1. Fish mortality remained below 10% in all ponds. Mean sediment organic nitrogen concentration increased from 0.3 g N m^{-2} to about 13.9 g N m^{-2} at the end of the experiment (Table 4).

Table 4. Organic nitrogen in sediments (g m^{-2}) in ponds 3 to 6 used for the validation of the model (mean ± SD, n=3) and overall mean (n=12).

Period	Pond 3	Pond 4	Pond 5	Pond 6	Mean
1	0.274 ± 0.018	0.542 ± 0.073	0.149 ± 0.048	0.355 ± 0.032	0.330 ± 0.154
2	4.733 ± 0.418	4.588 ± 0.220	4.649 ± 0.292	6.034 ± 0.224	5.001 ± 0.675
3	7.170 ± 0.085	7.920 ± 0.509	8.922 ± 0.343	8.312 ± 0.254	8.081 ± 0.722
4	9.433 ± 0.644	9.319 ± 0.594	11.723 ± 0.375	12.118 ± 0.097	10.648 ± 1.398
5	15.042 ± 1.487	13.077 ± 0.924	13.652 ± 0.836	13.737 ± 0.498	13.877 ± 1.137

Model calibration

The regression equation between gross photosynthesis rate and feed input in Experiment 1 was (Fig. 3):

$$GPR = 0.4780 * F + 2.2742 \ (R^2=0.95, P<0.01) \quad \text{for } 0 < F < 11.5$$
$$7.70 \quad \text{for } F > 11.5 \quad (9)$$

where

GPR = gross photosynthesis rate (g C m^{-2} d^{-1})
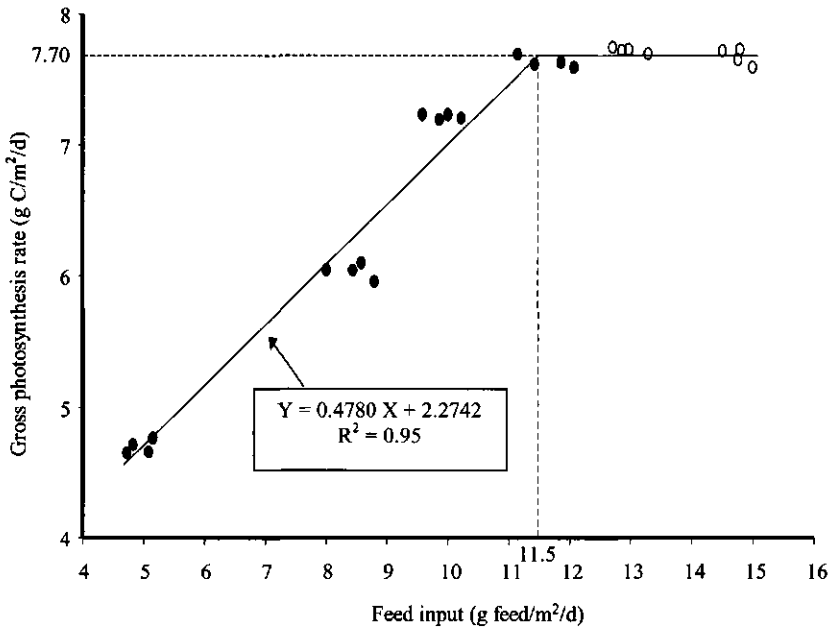
F = feed input (g feed m^{-2} d^{-1})

Figure 3. Relation between gross photosynthesis rate (GPR) and feed input (n=4 ponds) in Experiment 1. The continuous line represents the equation used in the model (see text). Data points used for calculating the sloping line are filled, points for the plateau are open.

Best results were achieved when the instant ammonification rate constants were reduced by 30% when the C:N ratio was higher than the Redfield ratio. Equations 4 and 5 can then be re-written as:

$$\text{p\_n\_mineralization rate constant} = \text{if a\_CN\_ratio} > 6.625 \text{ then } 3.64 \times 10^{-4} \text{ else } 5.20 \times 10^{-4} \quad (10)$$

and

$$\text{p\_c\_mineralization rate constant} = \text{if a\_CN\_ratio} > 6.625 \text{ then } 2.68 \times 10^{-5} \text{ else } 3.83 \times 10^{-5} \quad (11)$$

Calibration of the sedimentation rate constants of phytoplankton, faeces and uneaten feed resulted in values of 0.20, 0.70 and 0.90, respectively. Calibration results are presented in Figure 4. Relative errors for sediment organic nitrogen were between

+0.01 and +0.08, and for sediment organic carbon between +0.02 and +0.17, while the average relative errors were +0.06 and +0.10, respectively.
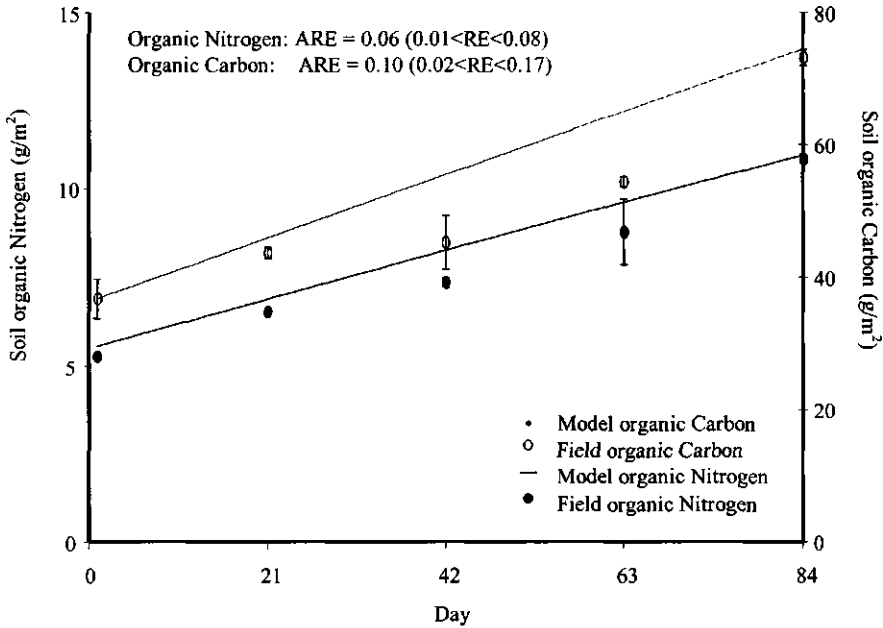

Figure 4. Simulated values and field data (average of Ponds 1 and 2, Experiment 2) of organic nitrogen and carbon (after calibration).

Sensitivity analysis

Table 5 shows the results of the sensitivity analysis. The mineralization rate constants had the strongest effect on sediment organic nitrogen and carbon (9.30 and - 9.62%, respectively), while the sedimentation rate constant of phytoplankton also had an important effect on both nitrogen and carbon (-6.22 and -6.20, respectively).

Table 5. Sensitivity analysis. Effect of increasing or decreasing by 10% the indicated parameter on soil organic nitrogen and soil organic carbon *.

Parameter	State variable	
	S organic N soil*	S organic C soil*
P % carbohydrate (%)	-0.96	-1.92
P % protein (%)	-1.92	-0.77
P faeces sedimentation rate constant (d^{-1})	-1.92	-0.76
P feed sedimentation rate constant (%, daily basis)	0.13	0.12
P phytoplankton sedimentation rate constant (%, daily basis)	-6.22	-6.20
P fraction uneaten (%, from feed offered)	-0.13	0.12
P C mineralization rate constant (d^{-1})	2.09	-9.62
P N mineralization rate constant (d^{-1})	9.30	-2.13

* Numbers indicate the difference between the value of the state variable for the highest parameter and the value for the lowest parameter as a percentage of the calibrated parameter. The sign of the value used as sensitivity represents under- or over-estimation depending whether the sign is negative or positive.
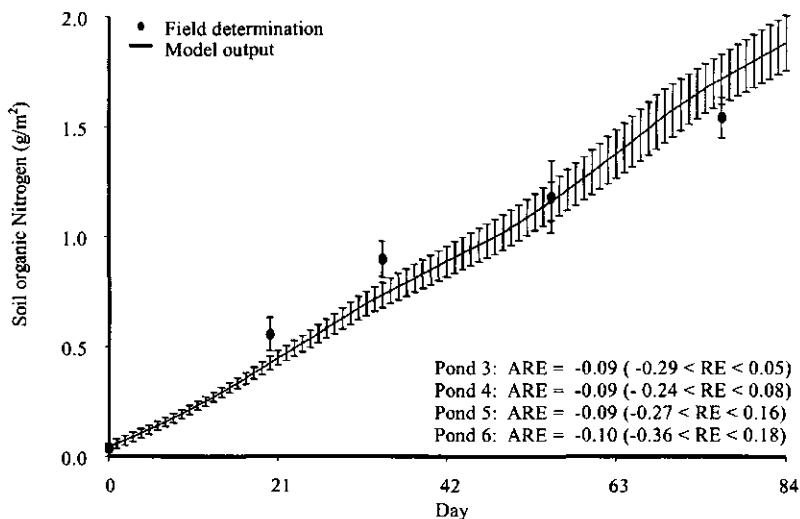

Figure 5. Field data of soil organic nitrogen and model output in stagnant *Colossoma macropomum*. Bars represent the standard deviation of field determinations in the ponds (n=4) and the confidence limits of model simulations (n=4).

Model validation

The validation of the model for sediment organic nitrogen using data from Experiment 3 (Ponds 3-6) is presented in Figure 5. The ARE's for the four ponds were all 0.09-0.10, with RE's ranging from -0.36 to +0.18.

Discussion

The first objective of this study was to estimate the rate of mineralization of organic nitrogen in semi-intensive fish ponds. The values for the mineralization rate constants of nitrogen and carbon obtained from the incubation of pond sediment were 5.2×10^{-4} d^{-1} (0.190 y^{-1}) measuring the increase of ammonium concentration, and 3.83×10^{-5} d^{-1} (0.014 y^{-1}) measuring the decrease of organic matter. Relating those values to the actual organic nitrogen concentrations resulted in a rate of ammonification of 7.21 mg N m^{-2} d^{-1}. For natural systems, Billén (1978) reported an ammonification rate of 25.4 mg N m^{-2} d^{-1}. Similarly, Blackburn and Henriksen (1983) reported rates of 24.2 and 1.45 mg N m^{-2} d^{-1} for aerobic and anaerobic ammonification, respectively. For fish ponds, Avnimelech (1984) also reported values for not freshly deposited material, with first order rate constant close to 0.213 y^{-1}. The rate of organic nitrogen decomposition in our fish ponds was comparable to values reported in these other studies.

The second objective was to measure the flux of inorganic nitrogen forms between sediments and water. TAN fluxes measured (Table 3) were similar to the values found in manured polyculture ponds (4.2 mg N m^{-2} d^{-1}; Schroeder 1987) and for intensive fish pond systems (11 mg N m^{-2} d^{-1}; Avnimelech 1984). The directions of the fluxes show that aquaculture pond sediments are a source of reduced inorganic nitrogen (ammonium) and a sink for oxidized inorganic nitrogen (nitrate and nitrite) (Hargreaves 1998). Concentrations of dissolved nitrogen species in the water column (NO_3^--N, NO_2^--N and TAN) were low, and did not increase or decrease much during Experiment 2. The low concentrations were probably related to phytoplankton biomass. Throughout Experiment 2, chlorophyll-a concentration increased, Secchi disk depth decreased and alkalinity decreased simultaneously. Dissolved inorganic nitrogen uptake by phytoplankton in ponds is the primary pathway of nitrogen removal, and in semi-intensive aquaculture ponds a dense phytoplankton population often develops (Hargreaves 1998).

The third objective was to quantify the amount of organic matter accumulating in the sediment. Total nitrogen (Kjeldahl-N) in the sediment indeed increased with time (Table 2). Nitrogen recovery (% of N input found in the bottom sediments) was 15.5 ± 2.9 % in Pond 1 and 14.6 ± 5.2 % in Pond 2. Similar results were found in an intensive eel pond (8-13%; Chiba 1986) and in semi-intensive shrimp ponds (15-22%; Hopkins *et al.* 1994).

Soil porosity was always higher in the top layers and increased with time due to the looseness of fresh organic matter that accumulates in the bottom during the growing cycle (Tucker 1985). For systems with high biological activity (such as fish ponds), the high porosity is particularly important because resuspension enhances the aeration of the upper layers of soil. This facilitates the exchange of pore water with pond water (Boyd 1995), stimulates the decomposition of organic matter and increases the flux of nutrients towards the water column (Henriksen *et al.* 1980; Blackburn and Henriksen 1983).

TAN in sediments ranged from 1.15 to 9.33 μg g^{-1} dry sediment. Ammonium concentration was low at the sediment-water interface (0-5 sediment layer) and increased with depth. Total nitrogen (consisting mainly of organic nitrogen forms) was higher in the surface layer and decreased with depth. This opposite trend of TAN and organic matter with depth was found in other studies of fish pond sediments (e.g. Avnimelech and Lacher 1979; Shilo and Rimon 1982) and is caused by the constant addition of organic matter by sedimentation from the water column and the simultaneous ammonification with a flux of ammonia to the water column.

With regard to the fourth objective to construct a simulation model of organic matter deposition and decomposition in fish ponds, a good fit between simulated and observed concentrations of sediment organic carbon and nitrogen was achieved in the calibrated model with relative errors lower than 0.17 and average relative errors of 6% for organic nitrogen and 10% for organic carbon (Fig. 4). During validation with the independent data of Experiment 3, the average relative errors for the simulation of organic nitrogen remained below 10% (Fig. 5). Although the datasets for calibration and validation were substantially different (notably with regard to pond size and fish species), the model performs well under different conditions.

The sedimentation rate constants of the three organic matter sources were estimated through model calibration, and gave good results in the validation. 90% from

the uneaten feed, 70% of the faeces and 20% of the phytoplankton standing crop settles to the pond bottom every day. Sinking rates, calculated using Stock's equation, take into consideration the volume and density of the particles, the density and the viscosity of the medium, and the acceleration due to gravity (Jørgensen 1989). Because faeces and feed have higher densities, volume and weight than planktonic cells, higher sinking rates are expected. For the sedimentation rate constant of phytoplankton, Schroeder *et al.* (1991) reported that as much as 50% of the algal standing crop settles to the sediment surface each day. Our lower estimate of 20% is in accordance with Larocque *et al.* (1996) who reported a daily settlement of 2-16% of the epilimnetic algal biomass for a temperate lake.

The sensitivity analysis showed that both soil organic nitrogen and soil organic carbon are strongly affected by its respective mineralization rate. In the original model, the mineralization rate of settled organic nitrogen and carbon only depended on its own concentration and did not take into account the proportion of carbon and nitrogen in the organic matter (C:N ratio). In the present model, nitrogen and carbon mineralization rates were dependent on the C:N ratio. The assumption that the C:N-ratio of the organic matter plays a key role in its decomposition rate has been documented earlier (e.g. van der Borght *et al.* 1977; Almazán and Boyd 1978; Blackburn and Henriksen 1983; Boyd 1995), but little quantitative information on the relationship between C:N ratio and mineralization exists. The 30% reduction in mineralization of both organic C and organic N with a C:N ratio above the Redfield ratio was derived by calibration of the model. More research is needed before a better description of this process can be incorporated into the model.

Although not considered in the present work, the suspension/resuspension of sediments plays an important role in the transfer of chemical components between the water column and the sediment. Resuspension is an important process in fish ponds (Avnimelech *et al.* 1999), and probably has an effect on the amount of organic matter that accumulates in the sediments. Avnimelech *et al.* (1999) found that resuspended material accounted for approximately 60 to 90% of the total sedimentation flux. Because in this study resuspension was not taken into account, the apparent rate of decomposition was calculated. Incorporation of suspension/resuspension in the model could represent a major change, and therefore will be the subject of further studies.
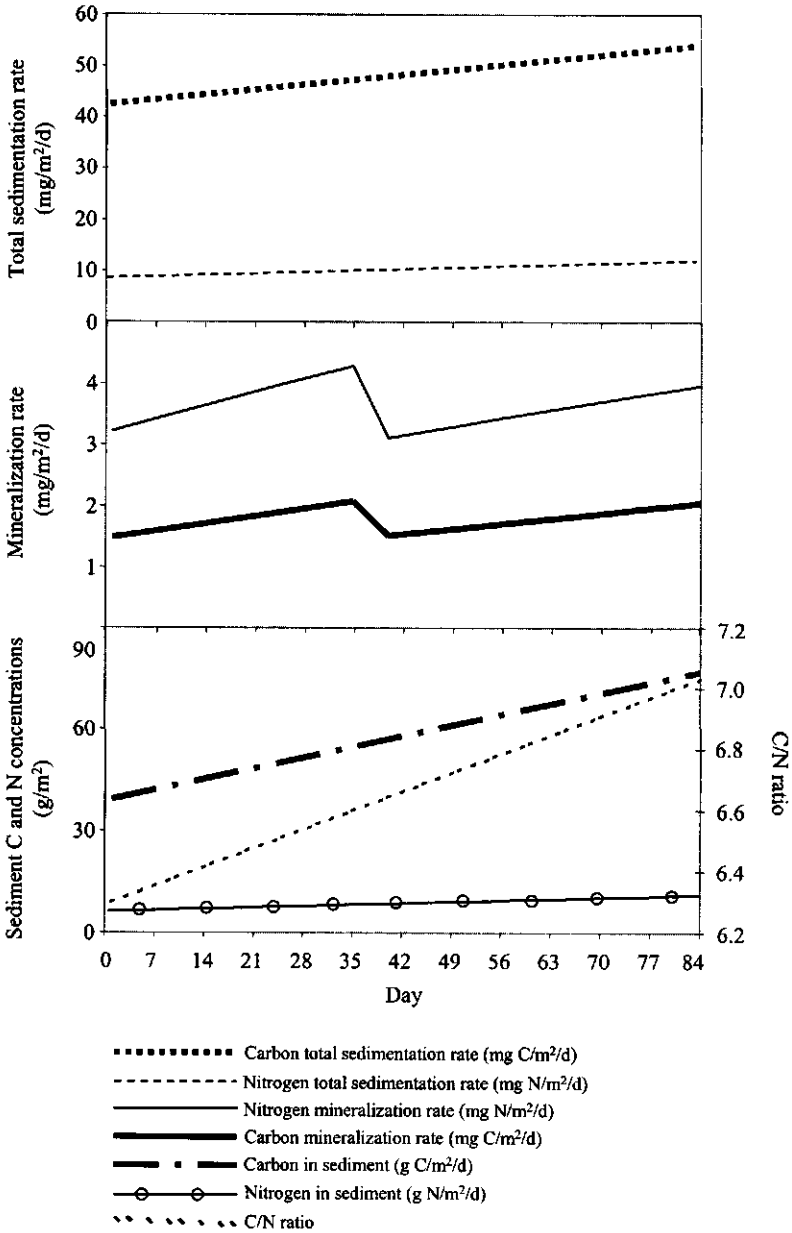
Figure 6. Model output. Comparison of total sedimentation and mineralization rates for Carbon and Nitrogen, C and N concentrations in sediments and C/N ratio

With the information from this study, the comprehensive pond model (Chapter 2, this thesis) can be improved. The sedimentation rate of phytoplankton and the mineralization rates were the most important ones in determining the accumulation of organic nitrogen and carbon in the pond bottom. Sedimentation increased steadily, as it was related to the feeding rate and faeces production (both increasing with increasing fish biomass) and primary production (also increasing with time). Decomposition of organic matter is governed by the decomposition rates of organic nitrogen and carbon. Because organic nitrogen is decomposed more rapidly than organic carbon, as shown by the different values of the decomposition rate constants, the C:N ratio of the sediment increases with time. This leads to even slower decomposition rates and enhances accumulation of (more and more refractory) organic matter in the sediment (Fig. 6).

Management measures to avoid accumulation would include the reduction of sedimentation of organic matter, e.g. by prevention of feed losses and increasing the digestibility of diets. Another possibility would be to harvest primary production before it can settle to the sediment using a herbivorous fish species (although this would create another flux of faecal matter to the sediments). Results from studies on the use of substrates in aquaculture ponds show that particulate organic matter may be trapped by periphyton (Keshavanath *et al.* 2001). It may also be possible to manipulate the C:N ratio of sediments by changing the composition of feeds (Avnimelech *et al.* 1999), thus preventing the decomposition process from slowing down. The mechanisms explored in the current model will be incorporated in a more comprehensive simulation model that includes primary productivity, fish growth, sedimentation and resuspension, and decomposition processes, and will be used to evaluate different management alternatives.

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Chapter 4

Organic matter sedimentation and resuspension in Tilapia (*Oreochromis niloticus*) fish ponds during a growing cycle

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Abstract

Pond bottom soils are subjected to the continuous deposition and decomposition of organic matter. Deposited organic matter accumulates or mineralizes *in situ*, or is resuspended into the water column. In this study the rates of sedimentation and resuspension were measured during a tilapia (*Oreochromis niloticus*) production cycle, while considering nutrient input, water quality and fish size and biomass. Fish were fed for 50 days, after which feeding was stopped until day 65, when feeding was resumed. Starting on days 7 and 9, two sediment traps were placed on the bottom of the pond every fortnight. Material collected in the traps and undisturbed soil samples collected at the same time as the trap material, were dried, weighed and analyzed for organic carbon, total nitrogen, and iron and aluminum concentrations. Concurrently with trap placement, water samples were taken to measure chlorophyll-a and total suspended solids. Primary productivity was measured using light/dark bottles. Measuring the dilution of iron or aluminum to differentiate between sedimented and resuspended particles, sedimentation and resuspension rates were calculated. The rate of material collected in the traps increased from 88.5 ± 7.1 g m^{-2} d^{-1} initially to 330 g m^{-2} d^{-1} on day 50. Although the fish biomass increased over time, while fish density remained nearly constant, the relative rate of resuspension did not change significantly, being always in the range of 42 to 47% of the total amount of collected material in the traps. Comparing measured and expected organic carbon sedimentation rates, the measured sedimentation rate was on average 10.9 times higher. When comparing measured and expected organic carbon resuspension rates, the measured resuspension rate was on average 8 times higher. Total solids sedimentation and resuspension rates were both highly correlated ($P \leq 0.01$) to fish weight/biomass, chlorophyll-a, water suspended solids, total feed input and Secchi depth.

Keywords: fish pond sediment; sedimentation rate; resuspension rate; total Nitrogen; organic Carbon.

Introduction

In aquatic systems, the increase or decrease of organic matter in the sediments is the difference between the *in situ* produced and allochtonous material that reaches the bottom before being mineralized, and the amount of organic matter that is mineralized in the sediment. The rate of sedimentation (deposition) of particulate matter is determined by factors such as the particle size, shape, density relative to water density, and viscosity (Jørgensen 1989). In pond aquaculture, the allochthonous part is considerable due to the addition of feed and/or organic fertilizer. For fish ponds, Avnimelech *et al.* (1999) reported sedimentation rates of total nitrogen and organic carbon in the order of 1-3 g m^{-2} d^{-1} and 15-30 g m^{-2} d^{-1}, respectively.

Settled particles can be resuspended. Organic matter decomposition in fish pond bottoms leads to anoxic conditions, and resuspension would favor the aerobic decomposition of organic matter. Considering that the various biotic and abiotic reactions in the sediments result in large concentration differences between the sediments and the overlying water (Boyd 1995), resuspension would also increase the material fluxes between water and sediments.

Resuspension has not been considered in most works of organic matter accumulation and decomposition. Information regarding the significance of resuspension in fish ponds as well as in other aquatic systems is rather new. Avnimelech and Wodka (1988) found that resuspended material accounted for 50% of the total sedimentation flux in a reservoir of 8 m deep. In fish ponds, resuspension of organic matter accounted for 60 to 90% of the total solids flux (Avnimelech *et al.* 1999). Scheffer (1998) reviewed and developed quantitative approaches regarding resuspension in shallow lakes. Resuspension depends upon the hydromechanics of the pond bottom, the less consolidated soils being more sensitive (Lee 1970). Water turbulence also causes resuspension, transport and resettling of sediments at places with less activity (Peterson 1999). The size of the lake, its depth and wind speed are very important factors in resuspension (Scheffer 1998), although for fish ponds, having a relatively small size and short wind fetch, the wind effect is probably limited. Sediment resuspension by fish was found to be of importance also in lakes, having an approximate linear relationship between benthivorous fish biomass (carp or bream) and

resuspension. The resuspension (kg resuspended per day) was found to be approximately 5 times the fish biomass (Breukelaar *et al.* 1994; Scheffer 1998).

Of special interest in fish ponds is the bio-turbulence created by fish. Fish move around actively while searching for food, causing water turbulence (Havens 1991; Tátrai *et al.* 1997). Resuspension is largely a fish-driven process, whereas sedimentation is a function of primary productivity and nutrient input. Direct measurements of resuspension and sedimentation are scarce, as is information about factors that influence the rates of sedimentation and resuspension. Several studies have identified fish weight and/or fish biomass, together with the fish species, as the main determinants of resuspension rate in fish ponds (e.g. Tátrai *et al.* 1997; Avnimelech *et al.* 1999). Primary productivity and nutrient input, that partly determine the sedimentation rate, were not considered concurrently. The objectives of this study were to compare two methods to measure sedimentation and resuspension rates during a growing cycle of *Oreochromis niloticus* in ponds, and to identify and quantify the principal factors (e.g. feed input, fish weight and number, and water parameters such as chlorophyll-a concentration) that contribute to resuspension or to sedimentation.

Material and methods

Sedimentation was measured by placing sediment traps on the pond bottom. Because resuspended material can contribute to trap yield (Fig. 1), the measured sedimentation will be the sum of primarily organic matter that settles down from the water column plus the material that is resuspended from the bottom:

$$ST = Sed + Res \qquad (1)$$

where ST is the total material trapped, Sed is the trapped material from sedimentation and Res is the trapped material from resuspension. The material sedimenting from the water is mainly organic, while resuspended material contains a larger fraction of inorganic matter. The amount of organic matter caught in the trap equals:

$$ST\_OM = (Sed\_OM * Sed) + (Res\_OM * Res) \qquad (2)$$

where Sed_OM and Res_OM are the fractions of organic matter in the sedimented (Sed) and resuspended (Res) material, respectively. A similar equation can be written for the inorganic material trapped:

$$ST\_Inorg = (Sed\_Inorg * Sed) + (Res\_Inorg * Res) \tag{3}$$

where ST_Inorg is the total inorganic matter trapped, Sed_Inorg is the fraction inorganic matter in the sedimented material, and Res_Inorg is the fraction of inorganic matter in the resuspended material. Equivalent equations can be formulated for other fractions collected in the sediment traps.

When organic matter is considered, one method to distinguish between the sedimented material ("first-time" settled) and the resuspended organic matter, is by comparison between the concentrations of tracers in seston, trap catch and the upper part of the sediment. This enables assessment of resuspension because the elemental composition of resuspended particles is different from particles originating from the water column. Avnimelech *et al.* (1999) proposed a dilution analysis to evaluate the magnitude of sedimentation and resuspension based on the assumption that in most fish ponds resuspended material contains elements (Fe, Al, Si) abundant in the soil. The method requires that these elements be at very low concentrations in the particles originating from the water. The concentration of an element in the material collected is the weighed average of the concentration in the sedimentation and the resuspension fluxes. If the concentration of the relevant ions (Fe, Al or Si) in the resuspension flux is assumed to be identical to the composition of the upper layer of the pond bottom, and the concentration of that soil-derived element in the sedimentation flux is assumed to be zero, then the calculation of the total resuspension and sedimentation flux rates in terms of g m^{-2} d^{-1} is possible, if the trap cross-sectional area and the retention time are considered (Avnimelech *et al.* 1999):

$$Wr/Wt = Ct/Cr \tag{4}$$

where Wr is the dry weight of material collected from resuspension, Wt is the total mass collected in the trap, and Ct and Cr are the concentrations of Fe, Al or Si in the material collected, and in the resuspension flux, respectively. This approach was followed in the

present study, and the different components used for the calculations are shown in Figure 1.
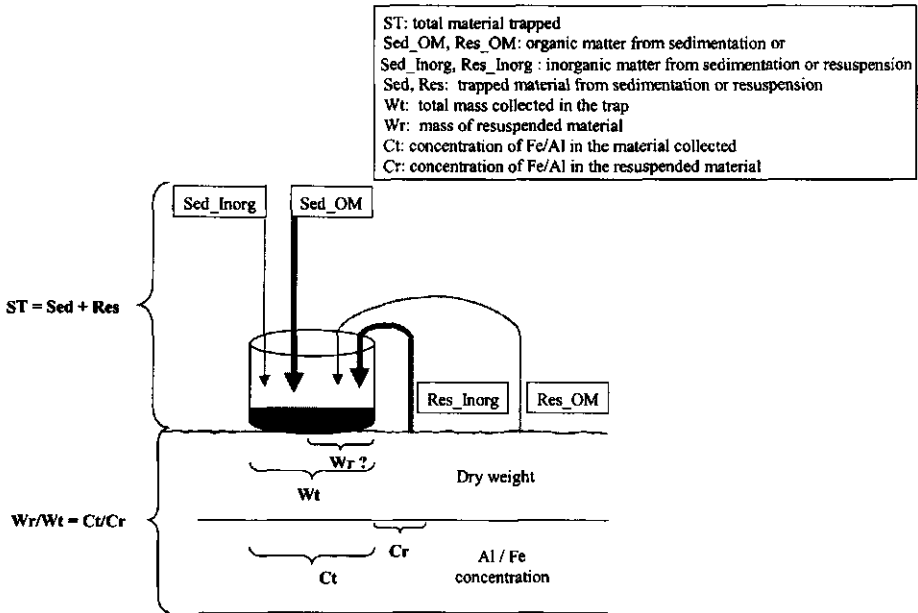

Figure 1. Components considered for the determination of the sedimentation and resuspension rates based on Al and/or Fe analysis. Wr is calculated using the equation; all other terms of the equation are obtained from laboratoy determinations.

An earthen fish pond of 800-m^2 and 1-m depth was stocked with 87-g *Oreochromis niloticus* at 1.5 fish m^{-2}. Fish were fed a 5-mm dry floating pellets with 30% crude protein, at a feeding rate of 2% body weight per day at the beginning of the experiment, decreasing towards 1.2% at the end of the experiment. The daily ration was divided into three equal portions applied at 0700, 1100 and 1500 hours, broadcasted as evenly as possible over the pond surface. After 50 days, feeding was stopped for a fortnight to get insight into the influence of feeding on the parameters measured. Feeding was resumed after day 65.

Starting on days 7 and 9, two sediment traps were placed 20 meters apart at the pond bottom. Depending on the amount of sample collected by the traps, the tubes were

removed from the pond after 24-48 hours. Until day 77, traps were replaced every fortnight. The sediment traps were made from PVC pipe with a diameter of 7 cm. Traps were placed firmly in the pond bottom and trap heights were always close to 30 cm with no difference between samplings. All the material collected was transferred to plastic bags. Soil immediately next to the trap was also sampled using a soil corer of 6 cm diameter.

The top 2-cm layer of the pond bottom soil cores, and the material collected in the traps was dried. The weight of the material collected was determined following drying at 60°C to constant weight. Soil and collected material were analyzed potentiometrically for organic carbon following a dichromate oxidation (Raveh and Avnimelech 1972), and for total Kjeldahl nitrogen (AOAC 1980). Fe and Al concentrations in the material collected in the soil core samples and in the traps were determined using atomic absorption spectroscopy after acid digestion (Lim and Jackson 1982).

Pond water was sampled, on the same days the sediment traps were placed, using a 10 cm diameter, 60 cm height sampling tube. Samples were collected from three locations (near the inlet, outlet and center) and thoroughly mixed before analysis. Chlorophyll-a concentration (acetone extraction) and total suspended solids (gravimetric and volumetric bases) in unfiltered samples were determined (APHA 1989). The water samples were filtered through a GF/C Whatman glass fiber filter and the filtrate analyzed for total alkalinity, NO_3-N (cadmium reduction), NO_2-N (diazotization) and TAN (NH_4^+-N+NH_3-N, phenate method) (APHA 1989). Also during trap placement, primary productivity (light/dark bottles) was also measured at two depths (20 cm and 50 cm below water level), and the primary production values from both depths were averaged.

Sedimentation and resuspension rates were calculated for total solids, organic carbon and total nitrogen. The measured organic carbon sedimentation was compared to the estimated organic carbon sedimentation assuming that all the carbon in the feed not retained in the fish sediments, and that the amount of algae that settles on the bottom is equal to the primary production. The last assumption yields an upper limit for algal sedimentation, since part of the dead algae is probably degraded in the water prior to sedimentation.

To assess the possible correlation between the sedimentation/resuspension rates and other parameters, a Pearson correlation matrix was constructed. The parameters included were chlorophyll-a in the water column, Secchi disk depth, fish weight/biomass, total feed offered, and water column suspended solids.

Results

The fish grew from an average weight of 86.6 ± 17.9 g to 210.0 ± 64.0 g in 77 days, realizing a specific growth rate of 1.15% body weight, and a feed conversion ratio of 1.75 over the culture period. The feed input at the beginning of the experiment was 3.24 g m^{-2} d^{-1}, and reached 6.19 g m^{-2} d^{-1} on day 77. The overall fish biomass reached 2729 kg ha^{-1} and the total mortality was 13% (Table 1). Both water suspended solids and chlorophyll-a increased steadily until day 63, and decreased drastically between day 63 and 65, indicating that an algae crash occurred during that time interval (Table 1). On day 77, water suspended solids and chlorophyll-a concentrations increased again.

The rate of material collected (dry basis) during the experiment increased from 88.5 ± 7.1 g m^{-2} d^{-1} (first week) to 330.7 g m^{-2} d^{-1} before day 50 when feeding was stopped (Table 2). As soon as feeding was stopped, the total amount of material collected in the traps started to decrease. Between day 63 and 65, the rate of deposition increased to the highest value during the experiment (377.7 ± 21.0 g m^{-2} d^{-1}), coinciding with the decrease in total suspended solids and chlorophyll-a concentration in the water column.

The mean concentrations of organic carbon and total nitrogen, Fe and Al, both in soil and material collected are presented in Table 2. In soil samples, organic carbon was 9-10 times higher than total nitrogen, representing an average C:N ratio of 9.8 ± 1.5 (mean $\pm$ SD) for the whole experiment. A similar C:N ratio was found in the trapped material (9.7 ± 1.8). The concentrations of organic carbon and total nitrogen in the trapped material were on average 8 times higher than in the top 2-cm sediment layer (Table 2, Fig. 2).

Table 1. Fish weight and biomass, feed input and related water quality parameters.

	Day	Average fish weight (g fish^{-1})	Fish biomass (kg ha^{-1})	Total feed input (g m^{-2} d^{-1})	Water total suspended solids (g m^{-2})*	Chlorophyll-a concentration (mg m^{-3})**
	7	93.8	1392	3.24	105.8 ± 9.9	109.3 ± 17.7
	9	96.3	1424	3.31	116.5 ± 8.3	123.8 ± 13.7
	21	112.2	1624	3.76	133.4 ± 7.2	121.0 ± 3.8
	23	115.0	1659	3.83	134.6 ± 9.9	141.5 ± 6.8
	35	132.9	1875	4.31	148.5 ± 8.3	181.6 ± 71.7
	37	136.1	1912	4.39	156.8 ± 4.2	141.0 ± 84.6
	49	156.0	2143	4.90	176.5 ± 9.2	206.9 ± 144.7
Feeding stopped (day 50)	51	159.5	2183	0	175.9 ± 6.0	207.3 ± 40.9
	63	181.7	2428	0	184.6 ± 4.1	205.2 ± 7.7
Feeding resumed	65	185.5	2470	0	22.6 ± 3.2	18.0 ± 2.8
	77	210.0	2729	6.19	97.7 ± 9.3	90.1 ± 8.5

* 1 meter depth; mean ± SD, n = 4

Table 2. Mean concentration (± SD, n=3) of organic carbon, total nitrogen, iron and aluminum in soil and collected material (dry basis).

| Day	Collected material (g m^{-2} d^{-1})	SOIL				TRAP			
		Organic carbon (g 100g^{-1})	Total nitrogen (g 100g^{-1})	Fe (mg g^{-1})	Al (mg g^{-1})	Organic carbon (g 100g^{-1})	Total nitrogen (g 100g^{-1})	Fe (mg g^{-1})	Al (mg g^{-1})
7	88.5 ± 7.1	3.99 ± 2.06	0.42 ± 0.20	23.03 ± 1.82	13.13 ± 0.18	46.77[*]	6.10 ± 0.88	9.67 ± 1.10	6.05 ± 0.14
9	104.3 ± 1.4	7.61 ± 1.82	0.83 ± 0.19	21.40 ± 0.27		51.49[*]	5.23 ± 0.56	10.02 ± 0.08	
21	179.6 ± 12.0	10.04 ± 0.13	0.98 ± 0.01	27.13 ± 0.21	33.69 ± 0.33	52.95 ± 2.68	5.81 ± 0.56	11.59 ± 0.19	14.98 ± 0.19
23	276.0 ± 0.9	6.54 ± 2.09	0.62 ± 0.20	30.68 ± 0.34		52.35 ± 1.12	4.98 ± 0.25	14.47 ± 0.24	
35	298.9 ± 16.1	10.99 ± 0.80	1.08 ± 0.03	35.68 ± 0.68	39.18 ± 0.18	52.27 ± 1.12	5.25 ± 0.63	14.78 ± 0.26	16.97 ± 0.16
37	297.9 ± 14.6	4.03 ± 1.04	0.45 ± 0.08	31.24 ± 0.42		51.61 ± 7.89	5.58 ± 0.82	14.73 ± 0.07	
49	330.7 ± 7.5	8.23 ± 0.87	0.88 ± 0.14	33.79 ± 0.85	39.98 ± 0.52	45.40 ± 4.30	5.08 ± 0.31	15.02 ± 0.17	17.77 ± 0.03
Feeding stopped (day 50) 51	223.2 ± 13.0	6.78 ± 1.23	0.66 ± 0.09	36.52 ± 0.30		52.63 ± 4.36	5.11 ± 0.77	15.97 ± 0.68	
63	163.5 ± 3.7	5.70 ± 0.86	0.62 ± 0.24	38.76 ± 1.54	42.79 ± 0.85	55.41 ± 6.06	4.12 ± 0.14	16.34 ± 0.20	18.63 ± 0.11
Feeding resumed 65	377.7 ± 21.0	12.01 ± 0.58	1.69 ± 0.21	37.67 ± 1.31		67.85 ± 4.01	9.01 ± 0.34	15.97 ± 0.98	
77	291.9 ± 23.4	3.23 ± 0.17	0.33 ± 0.03	43.41 ± 0.78	44.34 ± 0.59	35.50 ± 3.08	4.19 ± 0.37	16.59 ± 0.81	20.17 ± 0.45

[*] No replicates
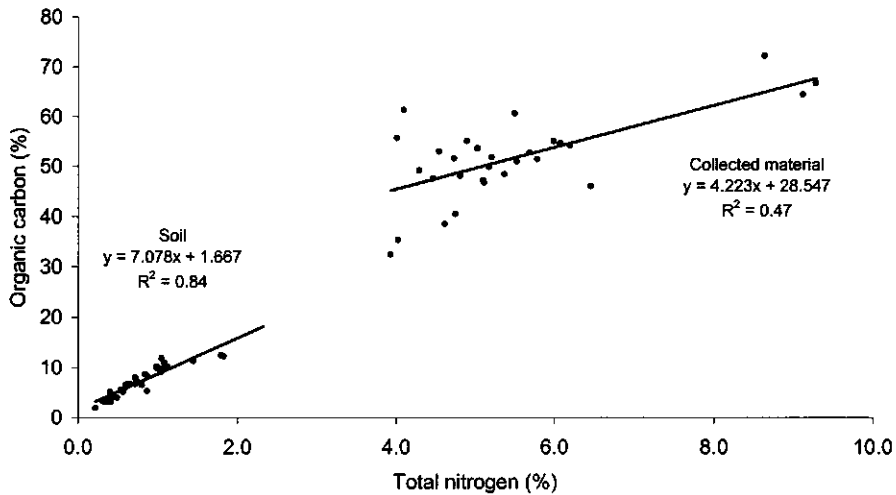
Figure 2. Relation between total nitrogen and organic carbon in soil and collected material.

The use of either iron or aluminum as traces yielded similar results (P > 0.05). Therefore, the obtained values were averaged. The rate of sedimentation for total solids, organic carbon and total nitrogen, expressed as g m^{-2} d^{-1} (Table 3) and based on the Fe and Al concentration differences between trapped material and the top 2-cm sediment layer were calculated (equation 3). At the beginning of the experiment, total solids sedimentation rate was close to 50 g m^{-2} d^{-1} and increased almost by 3 folds over six weeks. After day 50, the rate decreased, increasing again when feeding was resumed. Organic carbon and total nitrogen presented the same pattern of total solids. The fluxes of organic carbon were 25 to 90 g m^{-2} d^{-1} and for total nitrogen 3 to 9 g m^{-2} d^{-1}. Only on day 65 the sedimentation rates were higher, due to the algal die-off. The percentage of sedimented material in the trapped material ranged between 50 to 60% during the whole experiment. The rates of resuspension of total solids, organic carbon and total nitrogen, and the percentage of resuspended matter collected in the trapped material are given in Table 4. Resuspension was considerably high during the experiment, accounting for 40 to 50% of the material collected in the traps. When feeding was stopped, both sedimentation and resuspension rates decreased.

Table 3. Sedimentation rates, and percentage of total collected material, estimated from total solids, organic carbon and total nitrogen. Determination based on iron and aluminum concentrations in the soil and the material collected were not different (P > 0.05) and were averaged (mean ± SD, n=6).

	Day	Sedimentation rate (g m^{-2} d^{-1})			Percentage of total collected
		Total solids	Organic carbon	Total nitrogen	
	7	49.8 ± 3.2	23.2 ± 0.6	3.0 ± 0.3	56.0 ± 2.6
	9	55.5 ± 0.1	28.8 *	2.9 ± 0.3	53.2 ± 0.8
	21	101.4 ± 5.8	53.7 ± 1.2	5.9 ± 0.2	56.4 ± 1.2
	23	146.0 ± 0.8	76.5 ± 1.4	7.3 ± 0.3	52.8 ± 0.4
	35	172.5 ± 11.2	90.2 ± 7.0	9.1 ± 0.5	57.6 ± 1.4
	37	157.6 ± 7.2	81.3 ± 8.4	8.8 ± 1.5	52.8 ± 0.4
	49	183.8 ± 2.9	83.5 ± 6.2	9.3 ± 0.4	55.6 ± 0.6
Feeding stopped (day 50)	51	125.6 ± 4.8	66.1 ± 5.7	6.4 ± 1.2	56.3 ± 1.5
	63	93.5 ± 1.8	51.8 ± 5.7	3.9 ± 0.1	57.6 ± 1.4
Feeding resumed	65	218.1 ± 17.1	148.0 ± 23.9	19.7 ± 2.2	57.6 ± 1.3
	77	170.2 ± 20.7	60.4 ± 8.6	7.1 ± 0.9	58.1 ± 4.3

* No replicates

Table 4.: Resuspension rates, and percentage of total material collected, estimated from total solids, organic carbon, and total nitrogen. Determination based on iron and aluminum concentrations in the soil and the material collected were not different (P > 0.05) and were averaged (mean ± SD, n=6).

| | Day | Resuspension rate (g m^{-2} d^{-1}) | | | Percentage of total collected |
		Total solids	Organic carbon	Total nitrogen	
	7	39.2 ± 4.3	18.1 ± 0.6	2.4 ± 0.2	44.0 ± 1.3
	9	48.9 ± 1.5	25.3*	2.6 ± 0.2	46.8 ± 0.6
	21	78.3 ± 5.6	41.5 ± 1.4	4.5 ± 0.2	43.6 ± 1.2
	23	130.4 ± 1.5	68.2 ± 1.8	6.5 ± 0.4	47.2 ± 0.4
	35	126.8 ± 5.5	66.2 ± 3.5	6.7 ± 0.5	42.4 ± 1.4
	37	140.6 ± 7.6	72.6 ± 7.1	7.8 ± 1.4	47.2 ± 0.4
	49	147.1 ± 4.4	66.8 ± 3.8	7.5 ± 0.2	44.4 ± 0.6
Feeding stopped (day 50)	51	97.8 ± 8.8	51.5 ± 7.6	5.0 ± 1.2	43.7 ± 1.5
	63	70.2 ± 3.4	38.9 ± 5.3	2.9 ± 0.2	42.4 ± 1.4
Feeding resumed	65	160.0 ± 4.2	108.6 ± 16.8	14.4 ± 0.9	42.4 ± 1.3
	77	122.0 ± 12.6	43.3 ± 5.2	5.1 ± 0.6	41.9 ± 4.3

* No replicates

Comparing measured and expected organic carbon sedimentation rates, the measured sedimentation rate was on average 10.9 times higher than the theoretical (expected) one (Table 5). When comparing measured and expected organic carbon resuspension rates, the measured resuspension rate was on average 8 times higher.

Table 5. : Comparison between expected and measured organic carbon sedimentation and resuspension rates

Day	Carbon in feed (g C m⁻²d⁻¹)	Carbon retained by fish (g C m⁻² d⁻¹)	Net primary productivity (g C m⁻²d⁻¹)	Expected organic carbon that sediments [1] (g C m⁻² d⁻¹)	Expected organic carbon that resuspends [2] (g C m⁻² d⁻¹)	Expected Total organic carbon (g C m⁻² d⁻¹)	Measured organic carbon that resuspends [3]	Measured / Expected (Resuspension)	Measured total organic carbon [4] (g C m⁻² d⁻¹)	Measured / Expected (Total)
7	1.62	0.420	1.24	2.44	1.57	4.0	18.3	11.7	41.64	10.4
9	1.66	0.425	1.38	2.61	3.72	6.3	25.2	6.8	53.78	8.5
21	1.88	0.461	1.45	2.87	7.86	10.7	41.5	5.3	95.17	8.9
23	1.92	0.468	2.02	3.47	8.52	12.0	68.3	8.0	144.71	12.1
35	2.16	0.503	2.82	4.48	13.92	18.4	66.3	4.8	156.47	8.5
37	2.20	0.509	3.03	4.71	5.66	10.4	72.5	12.8	153.86	14.8
49	2.45	0.545	3.08	4.98	12.10	17.1	66.8	5.5	150.19	8.8
Feeding stopped (day 50)										
51	1.22	0.552	3.81	4.48	6.63	11.1	49.1	7.4	112.40	10.1
63	0	0.587	3.81	3.22	4.00	7.2	38.1	9.5	89.93	12.5
Feeding resumed										
65	0	0.594	0.55	-0.05	19.22	19.2	106.6	5.5	251.42	13.1
77	3.10	0.629	1.72	4.18	3.94	8.1	41.0	10.4	97.79	12.0
								Mean = 8.0		Mean = 10.9

[1]: Sedimentation (expected) = {(feed carbon – carbon retained by fish) + net primary productivity]; [2]: Resuspension (expected): [carbon in trapped material * (% of total material collected after resuspension/100)]; [3]: Resuspension (measured): [Resuspension (measured) * (% resuspension/100)]; [4]: Collected from sedimentation + resuspension

Total solids sedimentation and resuspension rate were both highly correlated (P ≤ 0.01) to fish weight/biomass, chlorophyll-a, water suspended solids, total feed and Secchi disk depth (Table 6). Fish weight and fish biomass were highly correlated to total solid sedimentation rates, whereas resuspension was also correlated to other water-related parameters such as chlorophyll-a, total suspended solids and Secchi disk depth.

Table 6: Two-tailed Pearson correlation matrix based on seven sampling periods (days 7 to 49) (significant correlation, P ≤ 0.01)

	TSSR	TSRR	CHL-A	SD	FW	FB	TF
Sedimentation rate, total solids (TSSR)	1.00						
Resuspension rate, total solids (TSRR)	0.98	1.00					
Chlorophyll-a concentration (CHL-A)	0.86	0.84	1.00				
Secchi depth (SD)	-0.79	-0.77	-0.90	1.00			
Fish weight (FW)	0.78	0.73	0.97	-0.83	1.00		
Fish biomass (FB)	0.79	0.74	0.97	-0.83	1.00	1.00	
Total feed (TF)	0.80	0.75	0.97	-0.83	1.00	1.00	1.00
Water suspended solids	0.80	0.76	0.97	-0.86	0.99	0.99	0.99

Discussion

With increasing feed input, the amount of suspended solids (including phytoplankton biomass) and the concentration of inorganic dissolved nutrients (ammonium, nitrate, nitrate) in the water column increased (data not shown). When feeding was stopped, the phytoplankton biomass (expressed as chlorophyll-a concentration) stayed constant at 205-208 mg m^{-3} up to day 63. This was followed by a sudden die-off of phytoplankton, and on day 65 the chlorophyll-a concentration dropped to 18 mg m^{-3}. Studies on phytoplankton populations have not lead to conclusive results to explain the phytoplankton dynamics in fish ponds (Sevrin-Reyssac 1997). In this study too, it was not possible to identify the cause(s) of this die-off. A possible explanation is that considering that feed was the only nutrient input in the system, when feeding was suspended a severe nutrient limitation developed leading to the collapse of the phytoplankton population. The fact that, once the feeding was restarted phytoplankton biomass increased again, supports this conclusion.

The C:N ratio of the sediment was comparable to the C:N ratio of the material collected. When organic matter is mineralized, nitrogen is used faster than carbon and

the resultant material is characterized by a lower carbon to nitrogen ratio (Nixon and Pilson 1983). Hamilton and Mitchell (1997) mentioned that particle settling velocities could be in the order of 1 mm sec^{-1}. Considering the trap height of 30 cm, the total water column residence time would be 300 sec, a limited time for slow chemical reactions such as microbial degradation to occur (Avnimelech *et al.* 1999). The similarity in the C:N ratios of both the sediment and the material collected confirms this. Carbon and nitrogen concentrations in the trapped material were on average 8 times higher than the concentrations found in the top 2-cm sediment cores. Most likely, relatively more organic carbon was resuspended than inorganic soil particles.

The use of aluminum or iron as tracers for estimating resuspension gave similar results, and the use of one tracer is sufficient for future studies. The amount of material collected in the traps ranged between 90 and 400 g m^{-2} d^{-1}. Avnimelech *et al.* (1999) reported flux rates of 622 and 1331 g m^{-2} d^{-1} for two ponds stocked with similarly sized tilapias at a comparable density. Breukelaar *et al.* (1994) reported a resuspension rate equaling ± 5 times the fish biomass per m^2 per day, which is also higher than our values. One possible explanation is the characteristic of the pond bottom. When comparing the rates at which organic carbon was collected in the traps to the expected sedimentation of algae and suspended solids from the water column, 11 times more organic carbon was trapped than expected. This suggests that the flux per day of organic carbon through resuspension is much higher than the calculated flux of 42-47% (Table 5). This is possible if we consider that the difference represents an estimate of the proportion of trap contents derived from sediment resuspension. Similarly, when comparing the expected and measured resuspension without taking into account the sedimentation of fresh material that settles down for the first time, the measured resuspension was 8 times higher. Resuspended material was most likely organic matter with a low density, while the inorganic soil was hardly resuspended, because the size and density was too large to be lifted higher than 30 cm into the water column. The fact that the pond soil was sandy confirms this result. If all the soil particles would had the same density as the organic matter then the amount of trapped material would also have been 8-10 times higher. Although the studies by Avnimelech *et al.* (1999) reported higher resuspension rates, the important question is how much organic matter was resuspended, as this drives the food web in the pond. In this study, 45-68% of the trapped material was carbon.

The values for the sedimentation rate for organic carbon and total nitrogen (but not for total solids) are comparable to other reports for similar fish size. In Avnimelech et al. (1999) increased fish size and increased nutrient input, as time progressed, were positively correlated to increased sedimentation rate. Both the fish weight increase and the increased feed input led to higher suspended solids in the water column. The characteristics of the pond management (e.g. feed input) are important to consider when reporting sedimentation rates because the absolute value of the sedimentation found in this experiment was 2-3 times higher after 50 days. Another important result is that, although fish weight increases over time, the relative resuspension did not change significantly during the experiment, being always in the range of 42 to 47% of the total collected material and irrespective of the component measured (total solids, organic carbon or total nitrogen). The constant relative resuspension can be explained by the fact that, during the experiment, the primary productivity (so the phytoplankton biomass) and the feed input increased at the same time as the fish increased its weight (see Table 5). It should be noticed also that the number of fish did not change significantly during the experiment, so this effect was not considered. It is probably more convenient to relate the resuspension rate to the fish weight and the number of fishes and not the fish biomass only. It is also not convenient to relate fish weight (and number) to the percentage of resuspension without giving information on the total amount of material collected in the traps. Therefore, the use of absolute values seems to be more convenient.

For the evaluation of sedimentation and resuspension rates, the pause of fish feeding could have two important consequences. Firstly, organic matter and nutrients were not introduced any longer in the system, and dissolved nutrients in the water column decreased to the level that could even limit the phytoplankton growth. Moreover, the exhaustion of nutrients could cause phytoplankton populations to collapse. Secondly, feeding causes fish to move more actively, so if no feed is applied less turbulence is caused and less resuspension is expected (Havens 1991). Following data of Table 4, it seems that fish always searched in the sediments, inducing a little less disturbance when no feed was found. Resuspension (absolute and relative values) decreased during feed cessation, although not significantly.

In conclusion, real resuspension rates were 8-11 times higher than expected. The dilution analysis to evaluate the magnitude of sedimentation and resuspension based on

Al/Fe as tracers assumes that soil particles and organic particles have similar density and are resuspended in the same proportion. This is not always the case, as presented here. What is important in estimating sedimentation/resuspension fluxes in fish ponds is to evaluate the dynamics of the organic matter that accumulates in the pond bottom, and the effect of those processes on the mobilization of inorganic forms such as nitrogen or phosphorus. Further research on this respect is recommended.

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Chapter 5

The role of sedimentation and resupension in the nitrogen dynamics in fish ponds: a modelling approach

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Abstract

Simulation models of nitrogen dynamics in fish ponds are needed for proper pond management. A previous model (Pond Nitrogen Simulator, PNS) for the dynamics of nitrogen transformations and fluxes in fish ponds did not simulate the organic nitrogen in the sediments well. To improve the understanding of the dynamics of nitrogen accumulating in the pond bottom of earthen fish ponds, this work integrates information on organic matter accumulation, sedimentation and resuspension into a predictive model. The proportion of three principal sources of organic matter which accumulate in the pond bottom (senescent phytoplankton, faeces, and uneaten feed) were included as parameters of the sedimentation process. A logistic equation relating the rate of resuspension and the fish biomass was calculated. Also seepage, as a potential loss of nitrogen from the system, was considered. After calibration and validation, the model simulated well the concentrations of organic and inorganic nitrogen in the sediments. When compared to PNS, model predictions were 5-fold improved by including submodules for sedimentation, fish driven resuspension and seepage. The model was also used to evaluate the effect of different pond management strategies on the dynamics of nitrogen in fish ponds.

Keywords: nitrogen balance; modelling; organic matter accumulation; sedimentation; resuspension.

Introduction

Feed and fertilizers enhance the production of cultivated animals in aquatic systems, but they are also a major source of pollutants in aquaculture operations since they influence the type and quantity of organic matter and nutrients in pond water and effluents (Cowey and Cho 1991). In stagnant water ponds, feeding above 55-60 kg ha^{-1} d^{-1} will lead to an excessive accumulation of ammonia concentrations in the water column (Tucker *et al.* 1979).

When the oxygen supply to either the whole system or the pond bottom is lower than the input rate of organic matter, systems become anoxic. Under these conditions the potentially toxic nitrogenous compounds are produced. One solution for this problem is to exposed to the air the pond bottom between cropping cycles to mineralize the accumulated organic matter. Few reports on sediment management while the pond is filled has been published. Mixing of the sediments was evaluated by Beveridge *et al.* (1994) without any significant effects on water and sediment nutrient levels after sediment mixing.

Hargreaves (1998) reviewed the processes of the N- cycle occurring in the pond bottom, and the processes related to the interaction between the sediment and the water column in aquaculture ponds. Much of the information is derived from research in estuarine or lacustrine environments, and few controlled studies on nitrogen cycling under the particular conditions prevalent in earthen aquaculture ponds have been reported. Our understanding of sedimentation, resuspension and inorganic nitrogen flux is still limited. These processes are interrelated; for instance, the flux of inorganic nitrogen compounds between the sediment and the water column is influenced by sedimentation, and its mobility is accelerated by resuspension. Seepage, another process occurring in earthen aquaculture ponds, has not been investigated either.

One way to study the nitrogen dynamics in fish ponds is through modelling. A previous model (Pond Nitrogen Simulator, PNS) for the dynamics of nitrogen transformations and fluxes in fish ponds was developed (Chapter 2, this thesis), but the amounts of organic nitrogen accumulating in the sediments were not well simulated. In Chapter 3 (this thesis) the proportion of the principal sources of organic nitrogen in pond bottoms (senescent phytoplankton, faeces and uneaten feed) were quantified through model calibration. Organic nitrogen accumulation and the rate of mineralization

of organic matter in pond soils were also measured. Further, flux rates between the sediments and the water column were measured for TAN, $N-NO_2^-$ and $N-NO_3^-$ (Chapter 3, this thesis). Finally, sedimentation and resuspension in fish ponds, and its relation to other parameters such as fish and phytoplankton biomass were measured in Chapter 4 (this thesis).

The objective of the present work was to integrate the information on organic matter accumulation and mineralization, inorganic nitrogen fluxes between the water column and the sediment, organic nitrogen sedimentation, and nitrogen resuspension, into a dynamic model for the simulation of nitrogen transformations and fluxes in fish ponds. The model was used to quantify the effect of different pond management strategies.

Material and Methods

Model description

The present model is based on the model proposed in Chapter 2 of this thesis (referred onwards as PNS). It comprises three modules (fish, phytoplankton and sediment-water). For details on the principal N-compounds, N-transformations and N-fluxes included in PNS refer to Tables 1 and 2 of Chapter 2 (this thesis).

The new additions to PNS are depicted in Figure 1. In PNS it was assumed that the principal sources of organic matter accumulating in the sediments were uneaten feed, dead phytoplankton and faeces. Only dead phytoplankton was partly decomposed in the water column, and the remaining settled on the pond bottom along with the other components. It is likely that also the faeces and the uneaten feed are partly decomposed in the water column. The amount of organic nitrogen present in the water column (*s_orntosed*), expressed in terms of mg N L^{-1}, is the sum of the three sources:

$$s\_orntosed = s\_phytom + s\_unean + s\_egspron \qquad (1)$$

where *s_phytom*, *s_unean* and *s_egspron* are the amounts of dead phytoplankton, uneaten feed and faeces, respectively. After calibration, it was found that the proportion of phytoplankton sedimenting (daily basis) was 0.20, and for faeces and uneaten feed the proportions were 0.70 and 0.90, respectively. These values were included in the

present model. The relative sedimentation rate parameter (*p_relsed*) oscillates between 0 and 1, and is expressed as:

$$p\_relsed = \left[\left(\frac{s\_phytom}{s\_orntosed}\right) \times p\_phytomsed\right] + \left[\left(\frac{s\_unean}{s\_orntosed}\right) \times p\_uneansed\right] +$$

$$\left[\left(\frac{s\_egspron}{s\_orntosed}\right) \times p\_egssed\right] \tag{2}$$

where *p_phytomsed*, *p_uneansed* and *p_egssed* are the proportion of phytoplankton, uneaten feed and faeces settling daily to the sediments.
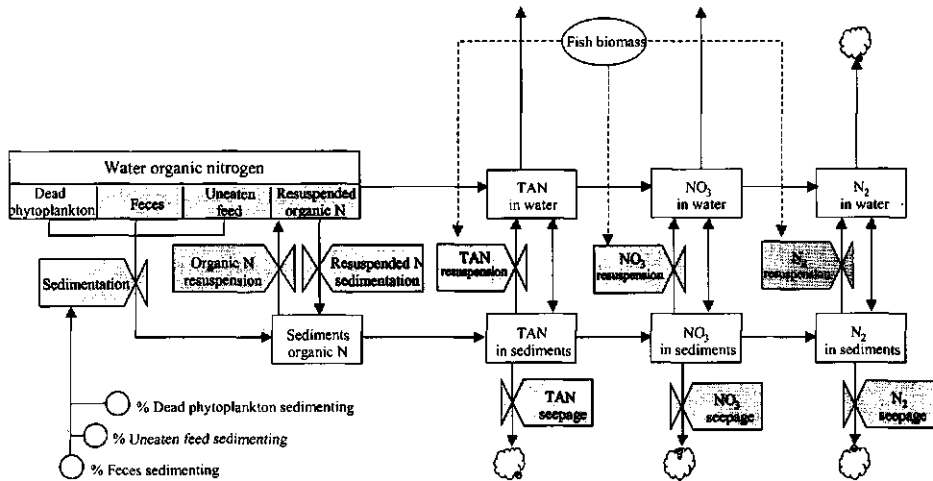
Figure 1. Relational diagram showing the new state variables and processes added (gray shading) to the Pond Nitrogen Simulator (PNS) model. The detailed diagrams for the different modules are presented in Chapter 2.

The rate of sedimentation of organic nitrogen to the sediments (*r_sedimon*, in mg N L^{-1} h^{-1}) is then expressed as:

$$r\_sedimon = \left(\frac{p\_relsed \times s\_orntosed}{24}\right) \tag{3}$$

Once the organic matter is sedimented, it can be further decomposed. In PNS, dissolved oxygen in the sediments was used as a constraint for ammonification to proceed. This was not considered in the present model since ammonification can also proceed under anaerobic conditions (Reddy and Patrick 1984). The organic nitrogen that accumulates in the sediments is now calculated as the difference between the organic nitrogen that deposits (equations 1 to 3) minus its ammonification (Table 1).

Table 1. Differential equations of the principal state variables of the model. Changes to the Pond Nitrogen Simulator (PNS) are indicated in *italics*.

Sediments

d(organic nitrogen)/dt	=	*sedimentation* – ammonification – *resuspension*
d(NH$_4^+$)/dt	=	ammonification + diffusion from/to sediments – nitrification – *seepage* – *resuspension*
d(NH$_3$)/dt	=	diffusion from/to sediments – *resuspension*
d(NO$_3^-$)/dt	=	nitrification + diffusion from/to sediments – denitrification - *seepage* – *resuspension*

Water

d(organic nitrogen)/dt	=	*resuspension* – ammonification – *sedimentation*
d(NH$_4^+$)/dt	=	fill + ammonification + *resuspension* – diffusion from/to sediments – phytoplankton uptake
d(NH$_3$)/dt	=	fish excretion + *resuspension* – flux from/to sediments – volatilisation
d(NO$_3^-$)/dt	=	fill + nitrification + *resuspension* – diffusion from/to sediments – phytoplankton uptake

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Another addition to PNS is the resuspension of nitrogen from the sediments. Resuspension is an important process in fish ponds, and resuspension rates of total nitrogen in the order of 2 to 14 g N m^{-2} d^{-1} were reported in Chapter 4 (this thesis). It was also found that, from the collected material in sediment traps, between 40 and 50% originated from resuspension. An important conclusion was that fish biomass has an strong effect on this resuspension. Data on fish biomass and the rate of resuspension of

total nitrogen (organic and inorganic forms) from that experiment was fitted to a logistic equation (Fig. 2):

$$r\_resuspension = \frac{31.95}{1 + 71148.53\exp(-0.0922 \times s\_fishbiom)} \tag{4}$$

where *r_resuspension* is the rate of nitrogen resuspension (in mg N L^{-1} h^{-1}), and *s_fishbiom* is the total fish biomass in the system (in kg of fish per 1000 m^2). Resuspension of the different nitrogen species is calculated as:

$$r\_resX = \left(\frac{s\_Xsed}{s\_totnsed}\right) \times r\_resuspension \tag{5}$$

where *r_resX* is the resuspension rate (or the accelerated mobility, in the case of inorganic nitrogen forms), with *X* being $N-NH_3$, $N-NH_4^+$, $N-NO_3^-$ or organic nitrogen. *S_Xsed* is the respective concentration (mg L^{-1}), and *s_totnsed* is the sum of all nitrogen forms in the sediments (mg N L^{-1}).
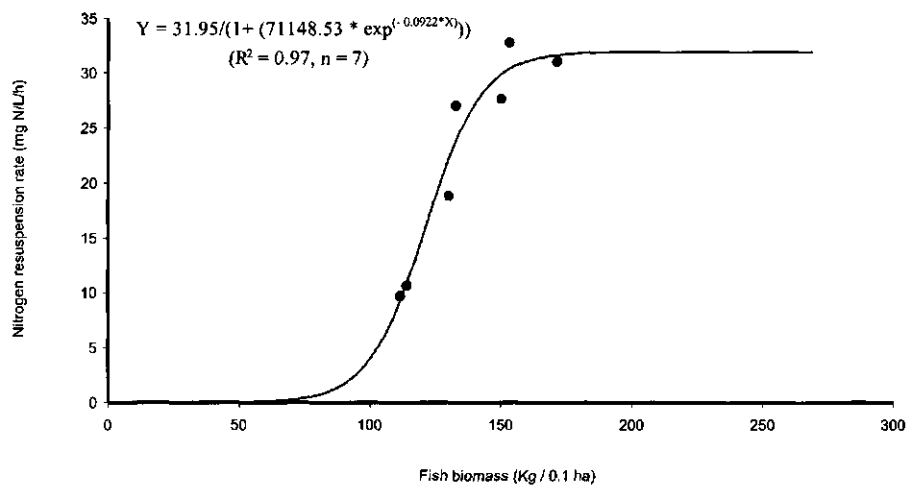
Figure 2. Logistic relation between Nitrogen resuspension rate and fish biomass.

Resuspended organic nitrogen was added to the total organic nitrogen in the water column. Its sedimentation rate (*r_sedreson*) is calculated as:

$$r\_sedreson = p\_sedreson \times s\_reson \qquad (6)$$

where *p_sedreson* is the first order resuspended organic nitrogen sedimentation rate parameter and *s_reson* is the concentration of this material (in mg N L^{-1}). *P_sedreson* is assumed to be different to *p_relsed* (equation 2) because during deposition the organic nitrogen is chemically and physically transformed by processes such as ammonification and particle aggregation.

A final consideration was the seepage. Seepage could have a profound effect on the nitrogen budget of fish ponds (Chapter 1 of this thesis). In the model, seepage is considered as a loss of dissolved nitrogen in the sediments. Since data for seepage were available (see below) this rate was entered in the model as an external variable.

The state variables both for the water column and for the sediments were modified according to the new additions (Table 1).

Model implementation and parameterization

The model was implemented in Turbo Pascal (7.0) using the Euler's rectangular method for numerical integration, and a fixed time step of one hour. The input data included water temperature, pH and dissolved oxygen concentration in both water and sediment, and the rates of input of ammonium and nitrate via refill water, the seepage rate, and the feeding rate.

Calibration

The same data used for the validation of PNS were used to calibrate the present model. Two ponds of 800 m^2 and 1.2 m depth were stocked with 35.1 g (± 7.0 SD) *Oreochromis niloticus* at a density of 1.2 fish m^{-2}. For further details on experimental conditions, feeding, and field measurements, reference is made to Chapter 2.

Calibration was carried out by adjusting equation coefficients to improve the fit between simulated and observed data. Most of the initial values used for the calibrated rate coefficients were taken from PNS (Table 2).

Table 2: Rate coefficient. Only calibrated coefficients, or coefficients not included in Pond Nitrogen Simulator (PNS) model (in **bold**) are presented[*]

Rate coefficient	Symbol	Units	Initial value	Calibrated value
Ammonification in sediments	p_ammonifsed	h^{-1}	2.17×10^{-5}	8.55×10^{-2}
Ammonification in water	p_ammonifwat	h^{-1}	5.04×10^{-3}	2.10×10^{-3}
Volatilisation of NH_3	p_diffcoefnh3	$cm^2\ h^{-1}$	10	0.115
Diffusion of NH_3 (*water-sediment interface*)	p_nh3dif	$m^2\ d^{-1}$	0.1	1.0
Nitrification in water	p_nitrifwat	h^{-1}	4.17×10^{-4}	1.00×10^{-3}
Diffusion of NO_3^- (water-sediment interface)	p_no3dif	$m^2\ h^{-1}$	6.84×10^{-6}	1.642×10^{-5}
Sedimentation of resuspended organic nitrogen	**p_sedreson**	h^{-1}		0.50
Proportion of daily faeces that is added to sediments	**p_egssed**	0 to 1	0.70	0.70
Proportion of phytoplankton daily added to sediments	**p_phytomsed**	0 to 1	0.20	0.20
Proportion of daily uneaten feed that is added to sediments	**p_uneansed**	0 to 1	0.90	0.90

[*] for a complete list of parameters refer to Tables 1 and 3, Chapter 2

The degree of agreement between simulated and observed data over the whole simulation was determined at every time when field data were available, calculating the relative error:

$$RE = \frac{Ssim - Sobs}{\frac{1}{2} * \left(Ssim + Sobs \right)} \tag{7}$$

in which *RE* is the relative error, and *Ssim* and *Sobs* are the simulated and observed values of the state variables at each moment. The coefficients were adjusted until the RE was at least equal to or lower than 0.25 for any sampling period. Final simulation

was performed when all coefficients were set (Table 2). For each state variable, the average relative error (*ARE*), defined as:

$$ARE = \frac{\sum RE}{n} \qquad (8)$$

were calculated, in which *RE* is the relative error and *n* is the number of observations used to assess the agreement between simulated and observed values for the whole experiment.

Sensitivity analysis

One by one, the rate coefficients were changed by +/- 10% of the value that gave the best fit between simulated and observed data, maintaining the other coefficients unchanged. The difference between the value of the state variable at each sampling date for the highest rate coefficient (+10%) and the value for the lowest rate coefficient (-10%) expressed as a percentage of the value for the coefficient obtained after calibration was used to quantify the sensitivity of the model to changes of that particular coefficient (Piedrahita 1986).

Validation

The model was validated using two independent data sets, one from each pond. Two stagnant ponds (800 m², 1.2 m depth) were stocked with 5.0 g *Oreochromis niloticus* at a density of 1.2 fish m⁻². The principal differences between the data used for the calibration and the validation were the initial conditions of the pond (nitrogen concentrations) and the fish size (5 g vrs 35 g). Fish were grown for 210 days, and were sampled six times during this period. Fish were fed a 3 mm 30% protein pellet. The amount of feed offered was 2.5% of the individual body weight per day, and the daily ration was divided into two equal portions and administrated at 0700 and 1500 hours. Ponds were sampled every three weeks for nitrogen contents in phytoplankton, sediments and water. Methods used were previously described in Chapter 2.

Seepage losses were calculated using evaporation and precipitation data collected in a nearby meteorological station. A marked stick to measure the water level was mounted in each pond, water loss was measured daily, and seepage was calculated

as the difference between the water loss, the evaporation and the precipitation. It was assumed that the nitrogen concentration in the seepage water was the same as the concentration of the sediment pore water.

To test if the added equations improved PNS, the outputs of the PNS model and present model, using the same field data were compared. Finally, the effect of different pond management (changing the feeding rate or the protein percentage in the feed, the dissolved oxygen concentration, or applying water exchange) on the concentrations of selected state variables were calculated using the model.

Results

Six parameters used in PNS and four new parameters were calibrated (Table 2). After calibration, $N-NH_4^+$ in the water column presented the higher deviations between observed and simulated values, but for the whole simulation period ARE for all state variables remained equal or below to 0.11 (Table 3).

Table 3: Relative errors (RE) and average (ARE) of the principal state variables after calibration

Time (days)	N-NH$_4^+$ (water)	N-NH$_4^+$ (sediments)	N-NO$_3^-$ (water)	Fish weight	Phytoplankton	Organic N (sediments)
1	0.00	0.00	0.00	0.00	0.00	0.00
7	1.54	-0.02	0.04		-0.08	-0.05
14	-0.40	-0.35	0.42		-0.05	-0.16
21	0.08	-0.17	0.20	-0.03	-0.03	-0.13
28	-0.15	-0.10	0.02		0.00	0.16
35	-0.10	-0.05	0.02		-0.07	-0.02
42	0.11	-0.06	-0.07	-0.01	-0.14	0.10
49	-0.22	-0.03	0.04		0.11	0.07
56	0.11	-0.01	-0.01		0.10	-0.02
63	0.06	-0.02	-0.02	-0.02	0.04	0.04
70	0.47	-0.01	-0.03		0.02	-0.06
77	-0.12	-0.03	0.00		-0.07	0.09
84	-0.11	-0.02	0.11	0.00	-0.03	-0.03
90	0.13	-0.02	0.13		0.04	-0.04
ARE	0.11	-0.06	0.06	-0.01	-0.01	-0.00

Sensitivity analysis was conducted for the calibrated parameters. TAN both in the sediments and in the water column were strongly affected by several parameters, but

in particular the proportion of daily uneaten feed that is added to sediment (p_uneansed) caused a drastic effect on the simulation of these two state variables (Table 4).

Table 4: Sensitivity analysis. Effect of increasing or decreasing by 10% the indicated coefficient

Coefficient	Process involved	State affected	± 10% *
p_ammonifsed	Ammonification in sediments	TAN in water	40.14
		TAN in sediments	32.62
p_egssed	Sedimentation	TAN in water	26.59
		TAN in sediments	32.52
p_uneansed	Sedimentation	TAN in water	89.72
		TAN in sediments	109.50

* Table includes only coefficients whose change affected by more than 15% the value of any state variable.

Table 5: Initial conditions used in the simulations during validation runs. V1 refers to the data used for the first validation, and V2 was used during the second validation

Variable	Symbol	Dimension	V1	V2
Fish species			*Oreochromis niloticus*	
Fish number	a_fishnb	#	1000	
Individual fish weight	s_wf	g (fresh weight)	5.0	
Pond area	p_pondarea	m^2	800	800
NO_3^- in sediments	s_no3sed	$mg\ N\ L^{-1}$	0.100	0.100
NO_3^- in water	s_no3wat	$mg\ N\ L^{-1}$	0.007	0.005
Organic N in sediments	s_ornsed	$mg\ N\ L^{-1}$	22.94	28.16
Organic N in water	s_ornwat	$mg\ N\ L^{-1}$	0.140	0.125
NH_3 in sediments	s_nh3sed	$mg\ N\ L^{-1}$	0.05	0.03
NH_3 in water	s_nh3wat	$mg\ N\ L^{-1}$	0	0
NH_4^+ in sediments	s_nh4sed	$mg\ N\ L^{-1}$	11.71	13.48
NH_4^+ in water	s_nh4wat	$mg\ N\ L^{-1}$	0.006	0.009
Total N in sediments	s_totnsed	$mg\ N\ L^{-1}$	34.67	41.67
Phytoplankton biomass	s_phytobiom	$mg\ N\ L^{-1}$	0.140	0.125

The experimental data collected for the calibration is presented in Table 5 of Chapter 2

Initial conditions for validation are given in Table 5. For both validations, and for every state variable, average relative errors remained within −0.37 and +0.57 (Table 6).

Table 6: Average relative errors (ARE) of the principal state variables after validation. Observed range is indicated between brackets. Last column present the ARE and range using PNS model.

	Validation 1	Validation 2	PNS model
$N-NH_4^+$ in water	0.38	0.13	1.91
	$(-0.06 < RE < 1.37)$	$(-0.11 < RE < 0.42)$	$(1.72 < RE < 1.83)$
$N-NH_4^+$ in sediments	-0.32	-0.16	-1.04
	$(-1.01 < RE < 0.27)$	$(-0.43 < RE < 0.09)$	$(-1.32 < RE < -0.53)$
$N-NO_3^-$ in water	0.57	0.36	1.50
	$(-0.06 < RE < 1.13)$	$(-0.08 < RE < 0.73)$	$(0.07 < RE < 1.82)$
Fish weight	-0.31	-0.35	-0.39
	$(-0.82 < RE < 0.00)$	$(-0.77 < RE < 0.00)$	$(-0.87 < RE < 0.00)$
Phytoplankton	-0.12	-0.12	0.89
	$(-0.43 < RE < 0.24)$	$(-0.41 < RE < 0.22)$	$(0.00 < RE < 1.37)$
Organic N in sediments	-0.23	-0.37	-1.22
	$(-0.46 < RE < 0.11)$	$(-1.14 < RE < 0.13)$	$(-1.62 < RE < 0.00)$

To get an idea of the magnitude of model improvement, the simulations of PNS model and the present model after calibration were compared using the same data set. The simulations of all state variables were improved using the present model (Figs. 3 and 4). Relative errors and their averages are summarized in the last column of Table 6.

Discussion

Three important processes not included in previous models of nitrogen dynamics in fish ponds (seepage, sedimentation and resuspension) were included here. Seepage is important in ponds with sandy soils, having a high hydraulic conductivity. In a previous work (Chapter 1, this thesis) nitrogen losses through seepage represented around 30% of the nitrogen loss in the system; those ponds were particularly sandy, but taken into account that nitrogen concentrations in pond bottom soil solution are normally one to two orders of magnitude higher than in water (Ram *et al.* 1982; Boyd 1995) this loss should be considered when constructing nitrogen budgets in fish ponds. On the other hand, the concentration of the different nitrogen compounds in the seepage water was not determined. The assumption that the concentration of the different inorganic

nitrogen forms in the water lost through seepage was equal to the concentration in the porewater may have resulted in a small difference between simulated and observed data.
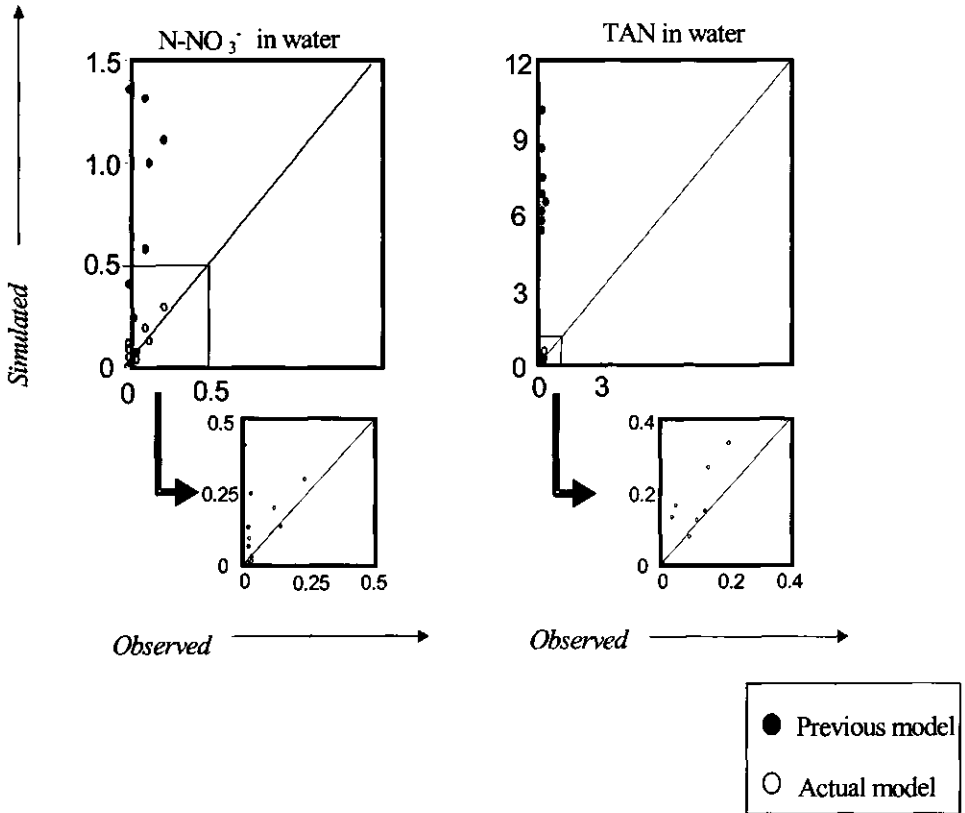
Figure 3. Dissolved nitrogen in the water column using PNS model (Chapter 2) and the actual model (this chapter). The bisector represents perfect agreement between simulated and observed values.
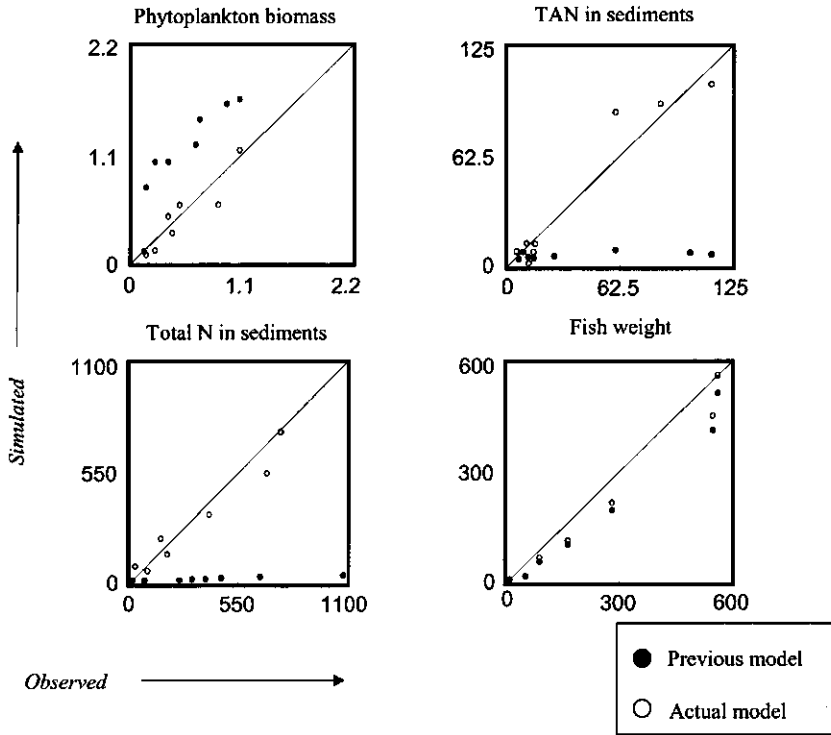
Figure 4. Validation results for phytoplankton biomass, fish weight, TAN and total nitrogen in the sediments, using PNS model (Chapter 2) and the actual model (this chapter). The bisector represents perfect agreement between simulated and observed values.

Sedimentation represents an important process when considering nutrient balances since organic material and nutrients are removed from the water by this process. Several forms of organic matter accumulate in fish ponds: uneaten feed, faeces and dead phytoplankton. A quantification of the proportion of offered feed that is not eaten has been reported for several aquaculture systems, being in the order of 5 to 30% (e.g. Thorpe *et al.* 1990; Boyd 1995; van der Meer *et al.* 1997) but conclusive values for pond aquaculture are scarce. Another possible source of organic matter accumulating in the pond bottom are the faeces produced by fish. Based on energy balance studies, on average 40% of the nitrogen ingested by fish is incorporated into fish biomass, 25 up to 80% is excreted as ammonia or dissolved organic nitrogen, and 5-15% is excreted as

particulate organic matter in the form of faeces (e.g. Kaushik 1980; Krom *et al.* 1985; Porter *et al.* 1987; Lovell 1988). Finally, phytoplankton that settles down is also an important source to consider. According to Schroeder *et al.* (1991) in ponds as much as 50% of the algal standing crop settles to the sediment surface each day. Avnimelech *et al.* (1999) used a maximal sedimentation rate as equal to daily primary productivity.

Resuspension was another addition to PNS. Using the information collected in Chapter 4 (this thesis), a correlation between total nitrogen resuspension rate and fish biomass was calculated, based upon experimental data (equation 4); the equation shows that when fish biomass is higher than 500 kg ha^{-1}, bioperturbation will have an effect on resuspension. A similar correlation between fish density (kg ha^{-1}) and the percentage of total dry matter collected in sediment traps was presented by Avnimelech *et al.* (1999); they reported a steep increase in the percentage of resuspended material when average fish weight was increasing towards 200 g, or when fish densities were higher than 1000 kg ha^{-1}. In this work we found that even at lower fish density (500 kg ha^{-1}) resuspension is also important to consider. In a shallow lake, Meijer *et al.* (1989) found an almost instantaneous increase in water transparency after the density of benthivorous fish was decreased from 600 kg ha^{-1} to 200 kg ha^{-1}. Sondergaard *et al.* (1990) reported for a shallow eutrophic lake, that a reduction of the fish stock from 300 kg ha^{-1} to 150 kg ha^{-1} changed the biological structure of the system markedly. For a similar system, van Donk *et al.* (1994) found that reduction from 150 to 57 kg ha^{-1} did not lead to an increase in water transparency. A positive linear relation between the total biomass of fish in ponds and the amount of material found in sediment traps was also reported by Tátrai *et al.* (1997).

Resuspension is partly responsible to the nutrient enrichment of the water column due to the accelerated mobility of inorganic forms. Resuspension caused by fish has been identified as a source of nitrogen and phosphorus in the water column by many authors in lakes (e.g. Stenson *et al.* 1978; Henrikson *et al.* 1980; Smeltzer 1980; Shapiro *et al.* 1982; Shapiro and Wright 1984; Wright and Shapiro 1984; Tátrai *et al.* 1985; Hambright *et al.* 1986; Tátrai and Istvánovics 1986; Meijer *et al.* 1990; Benndorf 1995; Tátrai *et al.* 1997). Little information is available for aquacultural ponds. Through our model, we evaluated the importance of this process by comparing the simulations when resuspension was included and when this process was not included. From our estimations, inorganic nitrogen concentrations in the sediments decreased on average by

25% due to resuspension/accelerated mobility, and at the same time the concentration of inorganic nitrogen forms in the water column, specially in the form of TAN, increase on average by 15%. These results are similar to previous reports by Blackburn *et al.* (1988) who attributed 30% of the solute flux from the sediment of a marine fish pond to disturbance by fish. This demonstrates the importance of resuspension in aquaculture ponds. The estimated TAN flux rate from the sediments to the water column due to resuspension was 18.7 mg N m^{-2} d^{-1}. This value is higher than previously reported (2.6 to 7.3 mg N m^{-2} d^{-1}, Chapter 3 of this thesis). Considering that TAN is mainly derived from the mineralization of settled organic matter at the sediment-water interface (Hargreaves 1998), the difference between our previously reported flux and the model estimations are probably due to the difference on organic matter concentration between the systems. The value is also in the range of –18 to 276 mg NH$_4^+$ m^{-2} d^{-1} reported by Cerco (1989).

Another parameter included in the present model is the sedimentation of resuspended organic matter (p_sedreson, Table 2). The range of particle settling velocities of different origins have been reported to be in the order of 36 to 1730 m d^{-1} (e.g. Vinogradov 1961; Smayda 1969; Fowler and Small 1972; Hamilton and Mitchell 1997). Sinking rates are calculated using Stock's equation which takes into consideration the volume and density of the particles, the density and the viscosity of the medium, and the acceleration due to gravity (Jørgensen 1989). The model approach does not permit to evaluate the speed at which resuspended material settles back; there is no information on the physical characteristics of the resuspended material. However, the calibrated first-order value of 0.5 d^{-1} gave good simulation results. Further research on the type and forms of the organic matter that accumulate in the pond bottom of fish ponds is needed.

The four new parameters were added to PNS model based upon conventional approach (Table 2). Compared to PNS, an increase of four orders of magnitude was obtained to the first-order rate coefficient for ammonification of sedimented organic matter. Most probably, this is caused by the effect of resuspension. As sediment are resuspended, anaerobic prevailing conditions are changed to aerobic conditions, leading to accelerated microbial reactions. The calibrated value (8.55 x 10^{-2} d^{-1}) gave the best simulations, and the value used is comparable with previous reports for aerobic algae decomposition rates of Otsuki and Hanya (1972, 6.8 x 10^{-2} d^{-1}) and Ulen (1978, 1.0-8.0

x 10^{-2} d^{-1}). Another important change was the value of the coefficient of mobility for volatilisation of N-NH$_3$. This process is strongly affected by atmospheric conditions such as temperature, wind speed and solar radiation (Jayaweera and Mikkelsen 1991), factors not considered in the present model. Contrasting results about the importance of volatilisation of N-NH$_3$ in fish ponds have been reported. Following a nitrogen budget approach (Chapter 1, this thesis) volatilisation seemed to be not important. According to model prediction, the volatilisation rate of N-NH$_3$ was in the order of 0.20 to 13.55 mg N-NH$_3$ m^{-2} d^{-1}. Higher values (8.8 to 71.0 mg N-NH$_3$ m^{-2} d^{-1}) were reported by Gross *et al.* (1999).

After calibration, the average relative errors (ARE) were below 0.11 (Table 3), which is considered good. Sensitivity analysis (Table 4) showed that the concentration of TAN both in the water column and in the sediments are the two state variables more affected by changes in model parameters. The concentration of those species is difficult to predict in fish ponds (Boyd 1990), and the parameters that control its concentration need further investigation. Particular attention should be given to the proportion of uneaten feed in fish ponds; changing this parameter by 10% resulted in a change on the simulation of TAN concentrations in the order of 89.72 to 109.5% (Table 4).

With the validation data set, the ARE for all parameters were between –0.37 and +0.57 (Table 6). PNS was also run with the same data set. Table 6 present the ARE for both models, and figures 3 and 4 compare the performance of the two models. When comparing the ARE of PNS with the mean of the ARE for the two validations (Table 6), it can be concluded that the changes made to PNS improved the simulations by 500%.

In aquaculture, modelling helps to understand the basic structure and function of the system, but can also be used to develop better management practices. Model predictions of the relative effect of different pond management on the pond are presented in Table 7. Using model estimations we can conclude that when feed protein level is increased from 30% to 35%, the final fish weight will be increased from 493 g to 554 g (individual fish weight). Increasing the feeding rate from 2.5% to 2.8% body weight per day the final fish weight will be increased to 541g (individual fish weight). This would probably have a profound impact on the profitability of the enterprise. But besides the effect on the fish production, these two parameters have also an important effect on the water and sediment concentration of TAN (Fig. 5).

Table 7: Model prediction of the relative effect (% of change) on selected parameters after different pond management at the end of the growing cycle. Positive/negative signs are used to indicate the increase/decrease of the parameter value.

	Feed protein (%)		Feeding rate (% body weight h^{-1})		Dissolved Oxygen (mg L^{-1})		Water exchange rate (% d^{-1})	
	- 10%	+ 10%	- 10%	+ 10%	- 10%	+ 10%	+ 5%	+ 10%
Water NO$_3^-$	-9.1	+36.4	- 27.3	+9.1	- 9.1	+9.1	-63.6	-90.9
Water TAN	-23.3	+688.4	-47.7	+31.4	+31.4	-22.1	-36.0	-60.5
Phytoplankton biomass	-0.6	+1.3	-1.3	+7.4	+0.6	-0.1	-15.7	-32.5
Sediments TAN	-41.3	+39.0	-12.6	+17.1	+9.7	-9.5	-2.9	-4.0
Sediments Total N	-25.4	+33.0	-10.6	+15.4	+2.2	-7.3	-0.4	-0.9
Fish weight	-28.6	+18.8	-9.5	+9.7	0.0	0.0	0.0	0.0

Increasing the feeding rate will cause an increase on the accumulated organic nitrogen in the sediments from 860 mg N L^{-1} to more than 1000 mg N L^{-1}. The TAN in the water column will also increase from 0.09 to 0.12 mg N L^{-1}. A similar 10% change of the protein content (from 30% to 40%) will boost this concentration to 0.68 mg N L^{-1}. Considering that TAN is potentially toxic to fish, the model would allow to predict under which conditions the concentration of ammonia will be toxic for the cultured organisms.

Another possible management practice is aeration. According to model predictions a decrease of dissolved oxygen from 7 ppm to 5.5 ppm would have an effect on processes such as nitrification. This is evident when looking to the decrease of nitrate concentration (from 0.011 to 0.009), and the accumulation of TAN in the water column (increased value from 0.09 to 0.33 mg N L^{-1}, Table 7, Fig. 5).
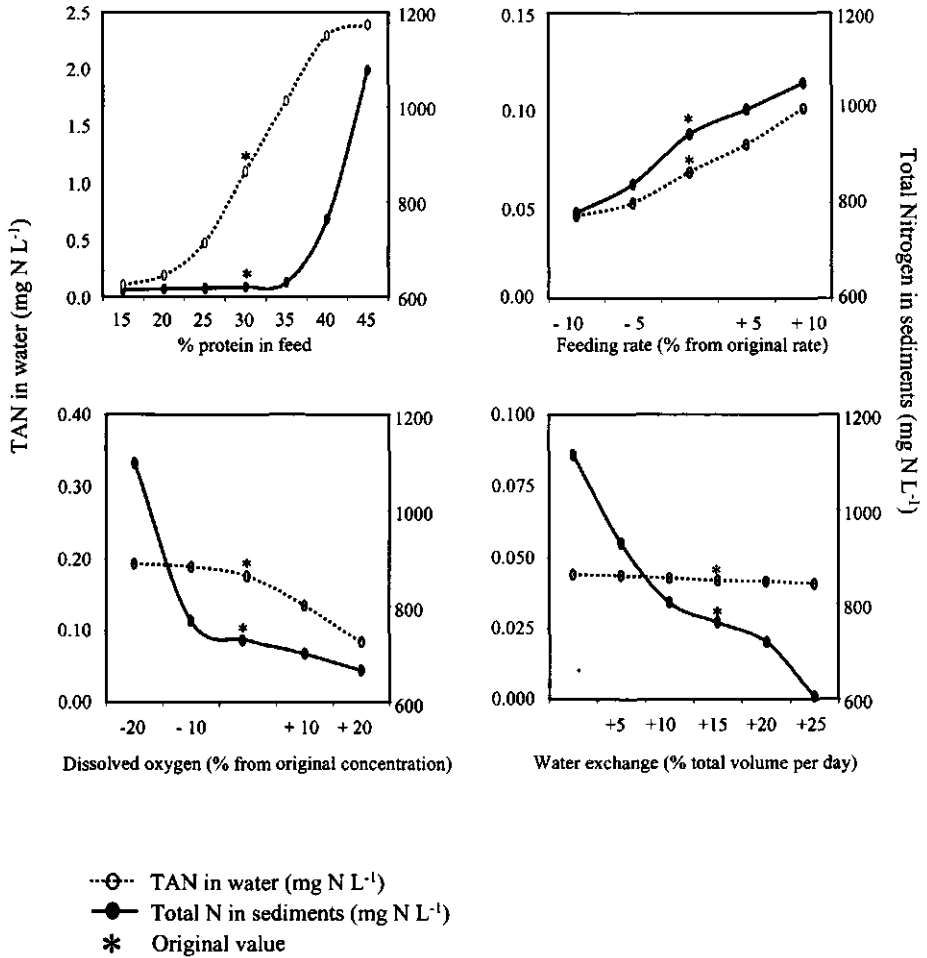
Figure 5. Change in water TAN and Total-N concentration (mg N L^{-1}) with different management practices.

Although water exchange should be optimized to avoid nutrient discharge, a water exchange rate of 10% per day would cause a large decrease of inorganic nitrogen forms in the water column, and also in the phytoplankton biomass (Table 7). According to model predictions, a water exchange rate of 10% per day will cause a decrease of in the concentration of N-NO$_3^-$ from 0.011 to 0.001 mg L^{-1}, and also a decrease on the concentration of TAN in the water column (from 0.086 to 0.034 mg L^{-1}). Phytoplankton biomass is also decreased from 1.06 to 0.71 mg N L^{-1}. For our pond (800 m^2, 1 m

depth), the total amount of nitrogen that is removed from the system when the 10% of the water volume is replaced would be in the order of 27.5 g N d^{-1} at the end of the growing cycle (Fig. 6).
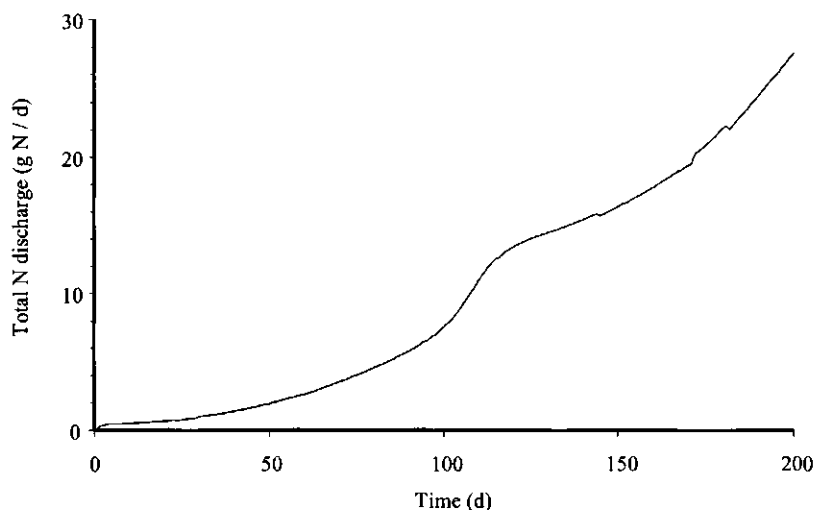
Figure 6. Estimated daily nitrogen discharge when applying 10% water exchange per day.

Considering that daily nitrogen input by feed at the end on the growing cycle was 530 g N d^{-1}, and assuming that fish retain 30% of that nitrogen in the body, the total daily addition of nitrogen in the pond system (water and sediment) will be 371 g N. A 10% daily water exchange will then represent a discharge of about 37 g N d^{-1}. The difference between the estimated concentration and resultant discharge (37-27.5) is explained considering both the organic matter accumulating in the pond bottom, and the volatilisation. In a similar way, the model can be used to quantify the discharge of nutrients to the surrounding environment under different system conditions.

In summary, it was demonstrated that sedimentation, and especially the enhanced mobility of inorganic nitrogen compounds are important to consider when modelling nitrogen dynamics in fish ponds, since the last process increase the nitrogen flux from the sediments to the water column by more than 15%. Research on the dynamics of TAN in the water-sediment interface is needed, but the results presented here suggest that further modelling efforts should also take into consideration the

sedimentation of organic nitrogen and the accelerated mobility of inorganic nitrogen compounds due to resuspension.

Acknowledgement

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Chapter 6

Conclusions and Recommendations

Introduction

As in any aquatic ecosystem, nitrogen is a key element in fish ponds. In natural aquatic systems, it forms part of organic molecules, and is converted and used for growth by autotrophic organisms. In aquaculture systems, nitrogen is added in the form of feeds and/or fertilisers to enhance the production of the cultured organisms that utilise it either directly (feeds) or indirectly (fertilisers). However, apart from the beneficial effects, nitrogen can also lead to eutrophication of surface waters, and pollute the culture system. In fact, nitrogen pollution is one of the principal causes of sub-optimal water quality in fish ponds (Jørgensen and Rasmussen 1991). The amount of nitrogen discharged from the system is influenced by both the amount of feeds (protein) and the efficiency of nutrient utilisation inside the system. Thus, nitrogen in aquaculture is both a need and a nuisance.

The principal end product of protein metabolism in fish is ammonia. After oxygen, ammonia is the second most common factor affecting fish stocking density (Knud-Hansen *et al.* 1991). Although the effects of ammonia on growth are unknown for most cultured animals, growth reduction may be the most important sub-lethal effect (Burrows 1964; Colt and Armstrong 1979; Meade 1985).

The general objective of this thesis was to investigate the dynamics of nitrogen in earthen fish ponds, integrating the available information into a predictive model. Some key mechanisms that were not well understood were investigated experimentally, especially the mechanisms related to the organic matter dynamics in pond bottoms. The results of these studies were used to improve the model. With the improved model, the effects of possible management practices on the nitrogen dynamics in fish ponds were evaluated.

In Chapter 2, the model (Pond Nitrogen Simulator or PNS) was used to gain insight into the relative importance of the fluxes and the transformation processes involved in the nitrogen cycle in earthen fish ponds. The results of simulated N-concentrations during calibration and validation were compared with the N-concentrations observed in experimental ponds using the relative error (RE), calculated as $(S_{sim}-S_{obs})/[(S_{sim}+S_{obs})/2]$, where S_{sim} and S_{obs} are the simulated and observed N-concentrations, respectively. All nitrogen concentrations were simulated well (average

RE's ranging from -0.19 to +0.01), with the exception of the nitrogen retained in organic matter that accumulates in the sediments (average RE's -0.34).

Consequently, the dynamics of organic nitrogen in the pond bottom were investigated in the next two chapters. In Chapter 3, the factors that affect organic matter accumulation in the sediments were studied. In Chapter 4, sedimentation and resuspension rates of organic carbon, organic nitrogen, and total solids were determined. In Chapter 5, the information obtained in the two previous chapters was integrated in the PNS model developed in Chapter 2. The model predictions were improved 5-fold (average RE's were between -1.22 and +1.91). The model was then used to evaluate the effect of different pond management strategies on the dynamics of nitrogen in fish ponds.

In view of the extensive discussion in Chapter 5, this last chapter discusses briefly the scope and limitations of the modelling approach followed. It also addresses some practical implications for pond management with regard to nitrogen management in earthen fish ponds. General conclusions and research needs conclude this chapter.

Modelling as a tool for the study of nitrogen dynamics

Frequently, the aquaculturist faces two nitrogen-related problems in his production system. High concentrations of total ammonia nitrogen (TAN) in the water column have to be prevented because of the deleterious effects on the culture organisms. At the same time, the organic nitrogen accumulation in the pond bottom should be avoided since mineralization of organic matter and the subsequent regeneration of nutrients at the sediment-water interface causes an important emission of ammonia into the water column (Hargreaves 1998).

The model developed in this thesis can be used as a research tool to assess the importance of the different processes on the concentration of TAN in the water column and on the accumulation of organic nitrogen in fish ponds. Figure 1 presents the relevance of the different processes directly involved in the TAN dynamics in the water column. Ammonification in the water column mobilises (converts) nearly 1 g of organic nitrogen into ammonia/ammonium (TAN) in a period of 24 hours, increasing steadily during the growing cycle. For the decomposition of organic matter in the water column, values up to 0.34 g N m^{-2} d^{-1} are reported (e.g. Harrison 1978; Barat and Jana 1987).

The higher values found are probably related to the high concentration of TAN in our system. The TAN is further transformed into nitrate, but at a lower rate when compared with the ammonification rate. Another process that increases the concentration of TAN in the water column is the flux and mobilisation by fish-induced suspension/resuspension. This process becomes very important when organic matter accumulates over time in the pond bottom, increasing up to 79 mg m^{-2} d^{-1} at the end of the growing cycle.

	7-8 1	35-36 2	63-64 3	91-92 4	119-120 5	147-148 6	175-176 7
■ TAN input by refill water	4.95	4.95	4.95	5.32	4.95	4.95	4.78
□ Ammonification in water	6.35	7.63	8.17	8.51	8.85	8.93	9.04
▨ TAN flux and mobilization	0.00	0.00	4.65	6.85	7.94	7.97	7.98
■ N removed by phytoplankton	8.34	8.40	8.46	8.49	8.52	8.55	8.57
▤ Fish N excretion as ammonia	7.02	7.85	7.91	7.83	8.05	8.15	8.25
□ Volatilization of ammonia	6.65	6.72	6.93	7.33	7.61	7.70	7.89
▨ Nitrification in water	5.48	5.38	6.35	6.67	7.13	7.46	7.65

Figure 1. Model prediction of nitrogen flux for different processes influencing the concentration of TAN in the water column. Data represent seven periods of 24 hours along a growing cycle. All values expressed in terms of mg N m^{-2} were transformed after multiplying the actual values by 1000 and taking the logarithm.

Estimates of ammonia flux for similar systems are reported to be in the range of 9 to 185 mg N m^{-2} d^{-1} (e.g. Avnimelech 1984; Blackburn *et al.* 1988; Hargreaves 1997; Riise and Roos 1997). Yet another process that contributes to the potential accumulation of TAN in the water column is the ammonia excreted by fish, which increases steadily from 8.7 to 149.4 mg N m^{-2} d^{-1} with increasing fish biomass and feed uptake. All these processes result into the final accumulation of TAN in the water column. However, the concentration of TAN does not reach very high levels since phytoplankton uses both

nitrate and TAN for growth. Uptake of dissolved inorganic nitrogen from the water column by phytoplankton is mostly the primary pathway of nitrogen removal (Hargreaves 1998). According to model output, phytoplankton removes up to 300 mg N m^{-2} d^{-1}, thereby being in fact the principal source of ammonia removal from the water column. Volatilisation of ammonia is another important source of ammonia removal (Lorenzen *et al.* 1997; Fig. 1) with increasing values from 3.7 up to 64.8 mg N m^{-2} d^{-1}. Similar values were found by Gross *et al.* (1999) in channel catfish ponds.

	7-8	35-36	63-64	91-92	119-120	147-148	175-176
	1	2	3	4	5	6	7
■ Organic N by sedimentation	4.80	5.73	6.28	6.49	6.70	6.88	7.04
▨ Ammonification in sediments	4.32	4.41	4.78	5.10	5.20	5.50	5.74
☐ Fish N excretion by faeces	3.67	4.41	4.49	4.51	4.71	4.83	4.93
☐ N in uneaten feed	5.10	5.84	5.92	5.94	6.14	6.26	6.33
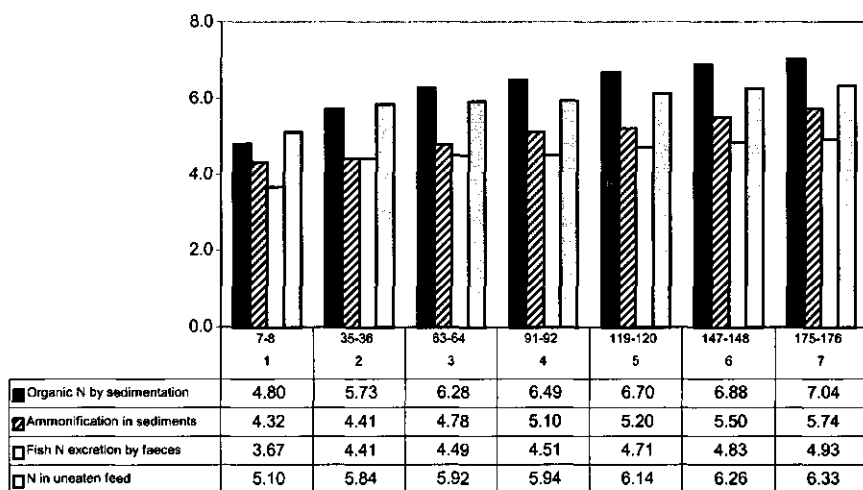
Figure 2. Model prediction of nitrogen flux for different processes influencing the accumulation of organic Nitrogen in the sediments. Data represent seven periods of 24 hours along a growing cycle. Data in terms of mg N m^{-2} were transformed by taking the logarithm of the actual values.

Organic nitrogen in the pond bottom increases over time by sedimentation (Fig. 2), as does the ammonification rate in the sediments. Ammonification rate in the sediment is on average 50% lower than in the water column (about 0.5 g N m^{-2} is mineralized daily), leading to the accumulation of organic nitrogen in the pond sediments. Fish excretion by faeces increases up to 70.9 mg N m^{-2} d^{-1} while uneaten nitrogen in feed reach a value of up to 1.7 g N m^{-2} d^{-1} at the end of the growing cycle. Both the fish excretion and the uneaten feed contribute to this accumulation, increasing

slowly but steadily along the growing cycle. Daily organic N sedimentation is close to 9 $g N m^{-2} d^{-1}$ at the end of the culture period.

In Chapter 5, the model was used to evaluate the effect of different pond management strategies on the dynamics of nitrogen in fish ponds. It was demonstrated that feed protein level has a profound effect on the concentration of TAN, and that the model predicts under which conditions the concentration of ammonia would reach toxic levels for the cultivated organisms. Using aeration as a management strategy would promote the nitrification and consequently would decrease TAN concentrations in the water column.

Finally, an important management strategy is water exchange, which will cause a large decrease of inorganic nitrogen forms as well as of phytoplankton biomass. The model could be used to quantify the discharge of nutrients to the surrounding environment under different system conditions. For instance, under the conditions studied in Chapter 5, a 10% daily exchange of water will cause a total nitrogen discharge of 340 g N per hectare of pond area at the end of the growing cycle. The primary environmental concern with fish pond effluents is the possibility of eutrophication of receiving stream waters by feed-derived nutrients. However, it is only a small percentage of the total nutrient input that is eventually discharged into the environment. This low level of waste discharged from fish ponds is due to two factors: (1) natural processes within the ponds remove wastes from the water resulting in a very diluted discharge of nutrients relative to the total amount of feed added to ponds and (2) ponds are managed to minimize water discharge.

Although these practical applications clearly indicate the efficacy of the model, it should be mentioned that the model was not validated with data from a wide range of environments. Therefore, more work is needed to assess whether the model also works well outside the range tested originally.

Practical implications for pond management

Without effective control of nitrogen transformation processes in the fish pond and optimal feed utilisation, nitrogen accumulates in the pond system. This accumulation occurs both in the water column and in the pond bottom. The nitrogen that accumulates in the water column is principally in inorganic forms. Inorganic nitrogen is

used by phytoplankton for growth, and, therefore, stimulation of phytoplankton growth could be a way to reduce TAN concentrations in the water. However, the development of phytoplankton populations is not always desired, especially when nuisance species, such as blue-green algae, develop. In practice, the control of nuisance phytoplankton species is difficult, and aquaculturists prefer to avoid excessive phytoplankton development by management strategies such as water exchange. Yet, this is not environmentally desirable since the pollution problem is conveyed to adjacent water bodies, and may lead to eutrophication problems there.

Most of the nitrogen not used by the cultivated organism settles onto the pond bottom, where it accumulates. Senescent phytoplankton, uneaten feed, and faeces are the principal sources of organic nitrogen in pond bottoms. This organic nitrogen is transformed into ammonia by bacteria. Under aerobic conditions, ammonia can be further transformed into nitrate, which is toxic to organisms, but only under high concentrations (Boyd 1990). However, pond bottoms frequently become anoxic, and then ammonia accumulates in the reduced sediment since the biochemical pathway of ammonia transformation requires oxygen. Furthermore, sediments become a source of ammonia emission into the water column (Riise and Roos 1997). One way to prevent or reduce nitrogen accumulation is optimising feeds and feeding practices (Cole and Boyd 1986; Li and Lovell 1992). The most obvious management measure in this area is preventing the feed from settling onto the pond bottom in uneaten form. The model suggestss that the amount of uneaten feed has a strong effect on TAN concentration of both the water and the sediment. Increasing the feeding rate from 2.5% to 2.8% of the body weight per day will cause an increase of the accumulated organic nitrogen in the sediments of nearly 20% (Chapter 5). Together with the fact that feeding costs represent a major part of the operational cost of feed-based aquaculture operations, this should be a strong incentive for good feeding management.

Another strategy is to aerate the pond and increase circulation within the system. Aeration will positively affect TAN removal by nitrification in the water column. Increased circulation will cause resuspension of settled organic matter and stimulate its mineralization (Thomforde and Boyd 1991). Avnimelech et al. (1986) proposed a system in which water is circulated continuously by paddlewheel aeration, so that settled organic matter is concentrated and removed continuously, but for semi-intensive earthen ponds this technique has not been applied frequently.

General conclusions and suggestions for further research

The approach followed in this thesis resulted in a better understanding of the nitrogen dynamics in fish ponds. The available information on nitrogen processes in fish ponds was integrated in the dynamic simulation model (PNS - Pond Nitrogen Simulator). This model can be used as a research tool for earthen ponds stocked with tilapia or tambaquí. The model was used to identify and study processes on which information is scarce. Newly obtained knowledge about organic nitrogen accumulation in fish pond bottoms and on sedimentation and resuspension of organic and inorganic nitrogen in fish ponds were incorporated in the model and improved the simulations considerably. With the model, several conclusions about pond management could be made in reference to the dynamics of nitrogen in fish ponds.

An important addition that should be incorporated in future versions of the model is the dynamics of the microbial biomass as a source of feed for the cultivated organisms. Research is needed to study how bacteria can incorporate ammonia and how bacterial biomass can be utilised by fish. Avnimelech *et al.* (1989) and Avnimelech (1999) proposed the manipulation of the C:N ratio to promote the formation of microbial biomass. Manipulation of the C:N ratio of sediments can be done by changing the composition of the feed, thus preventing the decomposition process from slowing down as the culture cycle progresses. If quantitative data on the production of bacterial protein were available, these mechanisms could be incorporated in the model to improve it further.

Application of the model is still limited by its rather high data requirements. Data sets of experiments that include all the necessary measurements along a growing cycle are not available, but badly needed for further improvement of the model. Also, data sets from other pond environments are needed to make the model applicable to a wider range of culture environments. The fish growth compartment can be calibrated for different fish species but could also be adapted for the simulation of shrimp growth. The model may then also be applied to shrimp culture systems, where water quality deterioration and negative environmental impacts are still widespread problems.

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SUMMARY

Nitrogen transformations and fluxes in fish ponds:
a modelling approach

Aquaculture is one of the fastest growing food producing sectors, with an average annual growth of almost 10%. By 1990, the total production of cultured animal and plants was close to 14 million tons while in 1996 the total production was 34 million tons. Since oceanic fisheries are not increasing substantially, in the future fish protein supply will have to come from aquaculture.

Aquaculture in ponds was established originally in regions where water resources were readily available but in some areas supplies are becoming limiting due to increased demand from households, industry and agriculture. Methods must be developed to use water more efficiently.

Intensification of aquaculture should lead to systems with a high resource use efficiency that are socio-economicly feasible. Environmental impact should be low, for continuous, sustainable production within any particular system as well as to avoid any impacts downstream of the culture system. Development of techniques for intensive, low- impact use of water requires considerable research for improved management of individual aquatic systems.

One prerequisite for improving the efficiency of water use, decreasing/ minimising the potential pollution, and optimising the utilisation of resources is a better understanding of the nutrient dynamics within the production unit. In this thesis the dynamics of nitrogen in earthen aquaculture ponds were investigated, aiming to increase our understanding of nitrogen transformations and fluxes with the goal to optimise the overall nitrogen utilisation.

Nitrogen is an essential element in aquaculture since it forms parts of proteins, which are essential for growth. However, nitrogen is also an important pollutant, not only for the surrounding environment but also because some forms are toxic to the cultured animals. Management strategies to control the deleterious effects of nitrogen accumulation in the system and to minimise the discharge to the surrounding environment have been proposed. However, their effectiveness is limited by the knowledge of the nitrogen cycle in aquaculture ponds.

In Chapter 1, nitrogen balances in four earthen fish ponds stocked with *Colossoma macropomum* were elaborated for a complete growing cycle. In contrast with previous nitrogen balances elaborated for fish ponds, in which only initial and final concentrations of nitrogen were considered, the temporal approach followed here allowed tracing the allocation of nitrogen during the growing cycle. Nitrogen was quantified in the water column, in the fish and in the sediments. Nitrogen loss via seepage was estimated. The information collected was also used to estimate the rate of decomposition of organic nitrogen in the pond. Nitrogen recovery during the first two periods of 20 and 14 days respectively was about 65%, while in the rest of the periods nitrogen recovery was nearly 100%. An important conclusion was that in these particular systems the relevance of nitrogen volatilisation in the form of ammonia or N_2 was limited. Feed was irregularly applied, with excessive feeding at the beginning of the experiment. This led to the accumulation of organic nitrogen in the sediments. Three to four weeks later, an intensive microbial degradation process had developed, leading to a release of inorganic nitrogen. Despite the irregularity in feeding, fish growth followed a smooth line, showing that the fish utilised detrital or planktonic feed during periods of low feeding. The first order rate constant for decomposition (0.237 ± 0.019 d^{-1}) was comparable to other studies, and represents the decomposition rate of organic nitrogen in the pond as a whole.

In Chapter 2, knowledge of the nitrogen transformations in fish ponds was used to construct, calibrate and validate a dynamic simulation model for nitrogen transformations and fluxes in earthen fish ponds. The Pond Nitrogen Simulator (PNS) model calculates the concentrations of the different nitrogen forms in the water column, in the fish and in the sediments. The model includes three modules: fish, phytoplankton and sediment-water. The fish module is based on bio-energetic and physiological principles, and is used to calculate the nitrogen excreted by the fish in the form of ammonia and faeces, along with the nitrogen incorporated in fish biomass. The potential feed that is not eaten was also considered. The phytoplankton module is based on physico-chemical principles of algae growth. It is also used to estimate the utilization of inorganic nitrogen species by phytoplankton, and the deposition of senescent phytoplankton to the pond bottom. The water-sediment module takes into consideration the passive flux of nitrogen between the water column and the sediments, and the

microbiological transformations of organic and inorganic nitrogen forms both in the water column and in the sediments. Most of the relationships and parameters were obtained from previously reported data, and some parameters were estimated through model calibration. PNS was implemented in Turbo Pascal using a fixed time-step of one hour. An earthen pond stocked with *Colossoma macropomum* was monitored during a production cycle, nitrogen allocations were monitored, and the data collected were used to calibrate the model. Further, the model was validated using an independent set of data from ponds stocked with *Oreochromis niloticus*. All concentrations of the different N-species were well simulated with the exception of the nitrogen accumulated in the pond bottom. The model was also used to gain insight into the relative importance of transformation processes between the various N-compounds.

In order to improve the simulations of the PNS model, in Chapter 3 the dynamics of nitrogen in the pond bottom were investigated. The rate of mineralization of organic nitrogen in the sediments was calculated measuring both the increase of ammonium concentration and the decrease of organic matter in laboratory incubation experiments. The values for the mineralization rate constants were 5.2×10^{-4} d^{-1} and 3.83×10^{-5} d^{-1}, respectively. The flux of inorganic nitrogen forms was also determined to be in the range of 1.15 to 7.42 mg N m^{-2} d^{-1}, 0.02 to 0.46 mg N m^{-2} d^{-1} and 0.07 to 0.39 mg N m^{-2} d^{-1} for TAN, NO_2^- and NO_3^-, respectively. In another experiment, organic matter accumulation in fish ponds was quantified, and the data were used to construct, calibrate and validate a dynamic simulation model of organic matter deposition/decomposition in fish ponds. Through model calibration, the proportion of the principal accumulating materials in fish ponds (dead phytoplankton, fish faeces and uneaten feed) was determined. In the model, gross photosynthetic rate was estimated from an empirical relationship with feed input. After calibration, the model was validated using an independent data set. The model simulated well the concentrations of organic carbon and nitrogen in the sediments, with average relative errors of +0.10 for sediment organic carbon and +0.06 for sediment organic nitrogen.

Although not considered before, the sedimentation and suspension/ resuspension of nitrogen forms induced by fish may play an important role in the transfer of chemical components between the water column and the sediment. Thus, in Chapter 4 two methods to measure sedimentation and resuspension rates in fish ponds were compared. The influence of nutrient input, water parameters and fish size/number was followed

during a production cycle in tilapia ponds. Using a dilution analysis, sedimented particles were differentiated from resuspended ones, and the rates of sedimentation and resuspension were calculated. The rate of material collected in sediment traps increased from 88.5 g m^{-2} d^{-1} to 330 g m^{-2} d^{-1}. Despite the growth of the fish during the experiment, the relative resuspension did not change significantly and remained in the range of 42 to 47% of the total collected material. Comparing measured and expected organic carbon sedimentation rates, the measured sedimentation rate was on average 11 times higher. The difference represents an estimate of the proportion of trap contents that comes from sediment resuspension. When comparing measured and expected organic carbon resuspension rates, the measured resuspension rate was on average 8 times higher. Resuspended material was most likely organic matter, with lower density than inorganic soil. Total solids sedimentation and resuspension rates were both highly correlated ($P \leq 0.01$) to fish weight/biomass, chlorophyll-a, water suspended solids, total feed input, and Secchi depth.

With the information gathered in the previous chapters, in Chapter 5 the PNS model was expanded with the addition of seepage, sedimentation and resuspension rates. A logistic equation relating the rate of resuspension and the fish biomass was calculated and included in the model. The model was calibrated and validated using independent sets of data, and the model predictions improved 5-fold. The model was also used to evaluate the effect of different pond management strategies on the dynamics of nitrogen in fish ponds. It was demonstrated that feed protein level, pond aeration and water exchange have a strong effect on the concentration of different nitrogen forms in the system. In the final part of the thesis the convenience of modelling as a research and management tool is discussed, and the limitation of the model and further research needs are suggested.

SAMENVATTING

Stikstofstromen en -omzettingen in visvijvers:
een modelbenadering

Aquacultuur is één van de snelst groeiende sectoren van voedselproductie, met een gemiddelde jaarlijkse groei van bijna 10%. In 1998 was de totale productie van gekweekte dieren en planten bijna 40 miljoen ton, tegen 14 miljoen ton in 1990. Omdat de oceaanvisserijen niet noemenswaardig groeien zal de toekomstige aanvoer van viseiwitten uit de aquacultuur moeten komen.

Aquacultuur in vijvers werd oorspronkelijk bedreven in gebieden waar water in ruime hoeveelheden beschikbaar was. In een aantal gebieden wordt de wateraanvoer nu beperkt door een toenemend gebruik in huishoudens, industrie en landbouw. Er is behoefte aan methoden om water efficiënter te gebruiken.

De intensificatie van aquacultuur moet leiden tot systemen met een hoge gebruiksefficiëntie van de hulpbronnen, die ook in sociaal-economisch opzicht acceptabel zijn. De effecten op het milieu moeten minimaal zijn om een continuë, duurzame productie in het systeem te garanderen en om negatieve effecten stroomafwaarts van het kweeksysteem te voorkomen. Voor de ontwikkeling van technieken voor verbeterd management van aquacultuursystemen met intensief gebruik van water maar geringe effecten op het milieu is een flinke onderzoeksinspanning nodig.

Eén voorwaarde om de efficiëntie van het watergebruik te verbeteren, de vervuiling te minimaliseren en de benutting van de hulpbronnen the optimaliseren is een beter begrip van de dynamiek van de nutriënten in het systeem. In dit proefschrift werd de dynamiek van stikstof in aarden visvijvers onderzocht met de bedoeling om het begrip van de processen van stikstofomzetting en stikstofstromen te verhogen en om de algehele stikstofbenutting te optimaliseren.

Stikstof is een essentiëel element in de aquacultuur omdat het een onderdeel is van de eiwitten die onmisbaar zijn voor groei. Daar staat tegenover dat stikstof ook een belangrijke vervuilende werking heeft, niet alleen voor het milieu rond het aquacultuursysteem maar ook omdat sommige stikstofverbindingen toxisch zijn voor de gekweekte dieren. Beheersstrategieën om de nadelige effecten van stikstofophoping in het systeem te beheersen en om lozing in het milieu te minimaliseren zijn voorgesteld,

maar de effectiviteit ervan is beperkt door de geringe kennis over de stikstofcyclus in visvijvers.

In Hoofdstuk 1 werden stikstofbalanzen opgesteld voor de complete groeicyclus van vier visvijvers met *Colossoma macropomum*. In tegenstelling tot eerder voor visvijvers opgestelde stikstofbalanzen, waar slechts de begin- en eindconcentraties van stikstof in de berekening werden opgenomen, kon hier met deze temporele benadering de allocatie van stikstof gedurende de hele groeicyclus gevolgd worden. De hoeveelheden stikstof in de waterkolom, in de vis en in de vijverbodem werden bepaald. De hoeveelheid stikstof die via het kwelwater verloren gaat werd geschat. De verzamelde gegevens werden ook gebruikt om de afbraaksnelheid van organische stikstof in de vijver te schatten. Gedurende de eerste twee perioden van 20 en 14 dagen werd ongeveer 65% van de toegediende stikstof teruggevonden, terwijl dat in de overige perioden 100% was. Een belangrijke gevolgtrekking daarvan is dat de volatilisatie van stikstof in de vorm van ammoniak of stikstofgas onbelangrijk is in deze systemen. Voeder werd onregelmatig toegediend, waarbij in het begin van het experiment teveel werd gegeven. Dit leidde tot ophoping van stikstof in de vijverbodem. Na drie to vier weken was een intensieve microbiële afbraak op gang gekomen, resulterend in het vrijkomen van anoganische stikstof. Ondanks het gebrek aan regelmaat in de voedering vertoonde de visgroei een regelmatig patroon, waaruit bleek dat de vis de detritus en het plankton benutte gedurende perioden met weinig kunstmatige voedering. De eerste-orde snelheidsconstante for afbraak ($0.237 \pm 0.019 d^{-1}$) was vergelijkbaar met waarden gevonden in andere studies en is representief voor de afbraaksnelheid van organische stikstof in de gehele vijver.

In Hoofdstuk 2 werd kennis over de omzettingsprocessen van stikstof in visvijvers gebruikt voor de constructie, calibratie en validatie van een dynamisch simulatiemodel for stikstofomzettingen en stikstofstromen in aarden visvijvers. Deze Pond Nitrogen Simulator (PNS) berekent de concentraties van de verschillende vormen van stikstof in de waterkolom, de vis en de vijverbodem. Het model bestaat uit drie modulen: vis, fytoplankton en bodem-water. De vismodule is gebaseerd op bioenergetische en fysiologische principes, en wordt gebruikt om te berekenen enerzijds hoeveel stikstof in de vorm van ammonium en faeces door de vis wordt uitgescheiden, en anderzijds hoeveel stikstof wordt vastgelegd in visbiomassa. De hoeveelheid voer die

niet wordt opgegeten wordt ook in overweging genomen. De fytoplanktonmodule is gebaseerd op de fysisch-chemische principes van algengroei en wordt gebruikt om de benutting van anorganische stikstofvormen door het fytoplankton en de afzetting van dood fytoplankton op de vijverbodem te berekenen. De water-bodemmodule berekent de passieve stikstofflux tussen de waterkolom en de vijverbodem. De meeste vergelijkingen en parameterwaarden werden verkregen uit eerder gepubliceerd onderzoek. Sommige waarden werden geschat tijdens de calibratie van het model. PNS werd uitgevoerd in Turbo Pascal met een vaste tijdstap van 1 uur. Een vijver werd bezet met *Colossoma macropomum* en gedurende een volledige productiecyclus werd de allocatie van stikstof gevolgd. De gegevens werden gebruikt voor calibratie van het model. Daarna werd het model gevalideerd met een onafhankelijke set gegevens van vijvers met *Oreochromis niloticus*. Alle concentraties van de verschillende stikstofvormen werden goed gesimuleerd, behalve de stikstofophoping in de vijverbodem. Het model werd ook gebruikt om inzicht te verkrijgen in het onderlinge belang van de omzettingsprocessen van de verschillende stikstofvormen.

Om de simulaties met het PNS-model te verbeteren, werd in Hoofdstuk 3 de dynamiek van stikstof in de vijverbodem nader onderzocht. De mineralisatiesnelheid van organische stikstof in de bodem werd berekend op basis van de gemeten toename van de ammoniumconcentratie en de afname van het organische stofgehalte in de bodem in een incubatie-experiment in het laboratorium. De waarden voor de snelheidsconstante voor mineralisatie waren respectievelijk 5.2×10^{-4} d^{-1} and 3.83×10^{-5} d^{-1}. De flux van anorganische stikstofvormen werd bepaald op 1.15 to 7.42 mg N m^{-2} d^{-1}, 0.02 to 0.46 mg N m^{-2} d^{-1} and 0.07 to 0.39 mg N m^{-2} d^{-1} voor respectievelijk totaal ammoniumstikstof, nitrietstikstof en nitraatstikstof. In een volgend experiment werd de ophoping van organische stof in vijvers gemeten, en de resultaten werden gebruikt voor de constructie, calibratie en validatie van een dynamisch model voor de afzetting en afbraak van organische stof in visvijvers. Door middel van calibratie van het model werd de bijdrage van de verschillende soorten organisch materiaal (dood fytoplankton, faeces van de vis en niet-opgegeten voer) bepaald. De bruto fotosynthesesnelheid werd in het model geschat uit een empirische relatie met de voederhoeveelheid. Na de calibratie werd het model gevalideerd met een onafhankelijke set gegevens. Het model simuleerde de concentraties van organische koolstof en stikstof

in de bodem goed, met gemiddelde "relative errors" van +0.10 for organische koolstof en +0.06 voor organische stikstof in de bodem.

Hoewel de sedimentatie en resuspensie onder invloed van vis tot op dit punt nog niet in overweging werden genomen, spelen deze processen een belangrijke rol bij de overdracht van stikstofvormen tussen de waterkolom en de vijverbodem. In Hoofdstuk 4 werden daarom twee methoden voor het meten van de sedimentatie- en resuspensiesnelheden in visvijvers vergeleken. De effecten van nutriëntentoevoer, waterkwaliteit en visgrootte en -aantallen werden geobserveerd gedurende een productiecyclus in tilapiavijvers. Met behulp van een verdunningsmethode werden gesedimenteerde deeltjes onderscheiden van geresuspendeerde deeltjes, en werden de snelheden van sedimentatie en resuspensie berekend. De snelheid waarmee materiaal zich verzamelde in sedimentcollectors ging omhoog van 88.5 g m^{-2} d^{-1} naar 330 g m^{-2} d^{-1}. Ondanks de groei van de vis gedurende het experiment veranderde de relatieve resuspensie niet significant en bleef tussen 42 en 47 % van de totale hoeveelheid verzameld materiaal. Vergelijking van de gemeten en berekende sedimentatiesnelheden van organische koolstof liet zien dat de gemeten sedimentatiesnelheid gemiddeld 11 maal zo hoog was. Dit verschil geeft aan hoeveel van de inhoud van de sedimentcollectoren afkomstig was van geresuspendeerd materiaal. Vergelijking van de gemeten en berekende resuspensie van organische koolstof liet zien dat de gemeten resuspensiesnelheid ongeveer 8 maal zo hoog was. Het geresuspendeerde materiaal was waarschijnlijk organische stof, met een lagere dichtheid dan anorganische bodemdeeltjes. De totale sedimentatie- en resuspensiesnelheden van de vaste deeltjes waren sterk gecorrelleerd (P ≤ 0.01) met visgewicht en -biomassa, chlorofyl-a, gesuspendeerde deeltjes in het water, totale voederhoeveelheid en Secchi-diepte.

Met de in de vorige twee hoofdstukken verzamelde informatie werd het PNS-model in Hoofdstuk 5 uitgebreid met modules voor de effecten van kwelverliezen, sedimentatie en resuspensie. Een logistische vergelijking die de resuspensiesnelheid berekent op grond van de visbiomassa werd geschat en opgenomen in het model. Het model werd gecalibreerd en gevalideerd met een onafhankelijke set gegevens, en de voorspellingen van het model verbeterden met een factor vijf. Het model werd ook gebruikt om het effect van verschillende strategieën voor vijverbeheer op de stikstofdynamiek in visvijvers te voorspellen. Het eiwitgehalte van het voer, de mate van aëratie en de

hoeveelheid waterververversing hadden een sterk effect op de concentratie van verschillende stikstofvormen in het systeem. In het laatste gedeelte van het proefschrift worden de toepasbaarheid van modellen als hulpmiddel bij onderzoek en beheer en de beperkingen van het model besproken en worden suggesties gedaan voor vervolgonderzoek.

RESUMEN

Flujos e intercambios de nitrógeno en estanques de peces:
un modelo matemático.

La acuicultura es uno de los sectores productores de alimento de mayor crecimiento, con un promedio anual de casi 10%. Mientras que en 1990 la producción de animales y plantas cultivados fue de 14 millones de toneladas métricas, en 1996 la producción total fue de 34 millones de toneladas. Debido a que las pesquerías oceánicas no están aumentando sustancialmente sus desembarques, en el futuro, la fuente de proteínas de origen acuático tendrá que provenir de la acuicultura.

La acuicultura en estanques se estableció originalmente en regiones en donde los recursos acuáticos eran abundantes. Sin embargo, en la actualidad este recurso empieza a ser limitante debido a la creciente demanda para uso doméstico, industrial y agrícola. Se deben por lo tanto desarrollar métodos que permitan utilizar el agua de manera más eficiente..

La intensificación de la acuicultura debe llevar a sistemas eficientes y que sean además factibles socioeconómicamente. El impacto ambiental debe ser bajo, de tal modo que permita una producción sostenible y continua. Para mejorar el manejo de sistemas de producción acuáticos se requiere de considerable investigación para desarrollar técnicas de uso intensivo de agua con bajo impacto.

Una de las maneras de mejorar la eficiencia del uso del agua, disminuir/minimizar la contaminación potencial, y optimizar la utilización de recursos es entendiendo mejor la dinámica de los nutrientes dentro de la unidad productiva. En esta tesis se investigaron los intercambios y flujos del nitrógeno en los estanques de acuicultura con el fin de optimizar su utilización.

El nitrógeno es un elemento esencial en acuicultura ya que forma parte de las proteínas de los organismos cultivados. Sin embargo, también es una importante forma de contaminación, no solo para el ambiente circundante sino que también porque algunas formas son tóxicas para los animales en cultivo. Se han propuesto estrategias de manejo para controlar los efectos perjudiciales de la acumulación de nitrógeno en los sistemas, y para minimizar su descarga hacia el ambiente circundante. Sin embargo, su efectividad está limitada por el escaso conocimiento que se tiene hasta el momento sobre el ciclo de nitrógeno en estanques acuícolas.

En el capítulo 1 se elaboraron balances de nitrógeno en cuatro estanques de tierra sembrados con *Colossoma macropomum* durante un ciclo de crecimiento completo. En contraste con previos balances de nitrógeno elaborados en estanques de peces y en los que se consideraron solo las concentraciones iniciales y finales de nitrógeno, el enfoque temporal seguido en ese trabajo permitió determinar la ubicación de nitrógeno durante todo el ciclo de cultivo. Se cuantificó el nitrógeno en la columna de agua, en los peces y en los sedimentos y se estimó su pérdida a través de la percolación en el fondo del estanque. Con esta información se pudo estimar además la tasa de descomposición de nitrógeno orgánico en los estanques. La recuperación de nitrógeno durante los dos primeros períodos de 20 y 14 días respectivamente fue cercana al 65%, mientras que posteriormente la recuperación de nitrógeno fue cercana al 100%. Una importante conclusión fue que en esos sistemas en particular, la importancia de la volatilización de nitrógeno en forma de amoniaco o N_2 era limitada. Como se aplicó el alimento de forma irregular, con un exceso de alimentación al inicio del experimento, el nitrógeno orgánico se acumuló en los sedimentos. Tres a cuatro semanas después se desarrolló una intensa degradación microbial que liberó nitrógeno inorgánico. A pesar de la irregularidad en la alimentación, los peces crecieron de forma continua, lo que indica que utilizaron alimento planctónico o detrital durante los períodos de baja alimentación. La constante de primer orden de la tasa de descomposición ($0.237 \pm 0.019/d$) es comparable a las de otros estudios y representa la tasa de descomposición de nitrógeno orgánico en todo el sistema.

En el capítulo 2, se utilizaron los datos sobre los intercambios del nitrógeno en estanques de peces para construir, calibrar y validar un modelo matemático de simulación dinámica. El modelo (Simulador de Nitrógeno en Estanques SNE o PNS) calcula las concentraciones de los diferentes compuestos nitrogenados en la columna de agua, en los peces y en los sedimentos. El modelo incluye tres módulos: peces, fitoplancton, y sedimentos - agua. El módulo de peces está basado en principios bio - energéticos y fisiológicos, y se utiliza para calcular el nitrógeno excretado por los peces en forma de amoníaco y heces, así como el nitrógeno incorporado a la biomasa de los organismos. Se considera también la fracción de alimento no consumida por los peces. El módulo de fitoplancton está basado en principios físico-químicos del crecimiento algal. Se utiliza también para estimar la utilización de las diversas formas de nitrógeno inorgánico por parte del fitoplancton, así como la deposición de fitoplancton muerto en el fondo del estanque. El módulo sedimento - agua toma en consideración el flujo pasivo de nitrógeno entre la columna de agua y los sedimentos, así

como las transformaciones microbiológicas de las diferentes formas orgánicas e inorgánicas tanto en la columna de agua como en los sedimentos. La mayoría de las relaciones y parámetros fueron obtenidos de datos previamente reportados en la literatura, y algunos parámetros fueron estimados a través de la calibración del modelo. SNE fue implementado en Turbo Pascal utilizando un tiempo de integración fijo de una hora. Para calibrar el modelo se utilizó un estanque de tierra sembrado con *Colossoma macropomum* durante un ciclo de producción, determinando y cuantificando las ubicaciones del nitrógeno. Además, el modelo fue validado usando un conjunto independiente de datos colectados en estanques de tierra sembrados con *Oreochromis niloticus*. Todas las concentraciones de las diferentes formas de nitrógeno fueron bien simuladas, con la única excepción del nitrógeno acumulado en el fondo del estanque. El modelo también fue utilizado para entender mejor la importancia relativa de los procesos de transformación entre los diferentes compuestos de nitrógeno.

Con el fin de mejorar las simulaciones del modelo SNE, en el capítulo 3 se investigó la dinámica de nitrógeno en el fondo del estanque. Se determinó la tasa de mineralización de nitrógeno orgánico en el sedimento en incubaciones realizadas en el laboratorio, midiendo tanto el incremento en la concentración de amonio/amoníaco, como la disminución de la materia orgánica. Los valores para la constante de la tasa de mineralización fueron 5.2×10^{-4} d^{-1} y 3.83×10^{-5} d^{-1}, respectivamente. El flujo de las diferentes formas de nitrógeno inorgánico osciló entre valores de 1.15 y 7.42 mg N m^{-2} d^{-1} para amonio/amoníaco (TAN), 0.02 y 0.46 mg N m^{-2} d^{-1} para nitritos (NO_2^-), y entre 0.07 y 0.39 mg N m^{-2} d^{-1} para nitratos (NO_3^-). En otro experimento, se cuantificó la acumulación de materia orgánica en estanques de peces y se usó esta información para elaborar, calibrar y validar un modelo de simulación dinámica para la deposición/descomposición de materia orgánica en estanques de peces. A través de la calibración del modelo, se determinó la proporción de los principales materiales que se acumulan en los estanques (fitoplancton muerto, heces de peces y alimento no consumido). En el modelo, se estimó la tasa bruta de fotosíntesis a partir de una relación empírica con la cantidad de alimento ofrecido, validándolo después de la calibración, con un conjunto de datos independiente. El modelo simuló bien las concentraciones de carbón orgánico y nitrógeno en los sedimentos con errores relativos de +0.10 para el carbón orgánico y +0.06 para el nitrógeno orgánico.

Aunque no había sido considerado antes, la sedimentación y suspensión/resuspensión de formas de nitrógeno, inducida por los peces, puede jugar un papel importante en la

transferencia de componentes químicos entre la columna de agua y los sedimentos. De este modo, en el capítulo 4 se compararon dos métodos para la medición de las tasas de sedimentación y resuspensión en estanques de peces. Durante un ciclo de producción en estanques con Tilapia se registraron las variables entrada de nutrientes en forma de alimento, los parámetros del agua, y el número/tamaño de los peces. Utilizando un análisis de dilución, las partículas sedimentadas fueron diferenciadas de las resuspendidas, y se calcularon las tasas de sedimentación y resuspensión. La tasa del material colectado en las trampas para sedimentos aumentó de 88.5 $g\,m^{-2}\,d^{-1}$ hasta 330 $g\,m^{-2}\,d^{-1}$. Pese al crecimiento de los peces durante el experimento, la resuspensión relativa no cambió apreciablemente y permaneció en el rango de 42 a 47% de todo el material colectado. La tasa de sedimentación de carbono orgánico medida fue en promedio 11 veces mayor que la esperada, posible si se considera que la diferencia proviene de la resuspensión de sedimentos. Cuando se comparan las tasas de resuspensión de carbono orgánico esperado y medido, la tasa de resuspensión medida fue en promedio 8 veces mayor. El material resuspendido era fundamentalmente materia orgánica, con una menor densidad que el suelo inorgánico. La sedimentación y resuspensión de los sólidos totales estuvieron altamente correlacionados ($P \leq 0.01$) con el peso/biomasa de los peces, con la concentración de clorofila-a en el agua, con los sólidos suspendidos en el agua, con la cantidad de alimento ofrecida, y con la profundidad Secchi.

Con la información obtenida en los capítulos anteriores, en el Capítulo 5 se amplió el modelo SNE con la adición de las tasas de percolación de agua en el fondo del estanque, de sedimentación y de resuspensión. Se elaboró una ecuación logística relacionando la tasa de resuspensión y la biomasa de peces y se incluyó también en el modelo. El modelo fue calibrado y validado utilizando conjuntos independientes de datos, lográndose que sus predicciones mejoraran 5 veces. El modelo también fue utilizado para evaluar el efecto de diferentes estrategias de manejo de estanques sobre la dinámica del nitrógeno en estanques de peces. Se demostró que el nivel de proteína en el alimento, la aireación artificial, y el recambio de agua pueden afectar de forma significativa las diferentes formas de nitrógeno en el sistema. En la parte final de la tesis se discute la conveniencia del modelaje como una herramienta de investigación y manejo, así como las limitaciones del modelo desarrollado, y se sugieren investigaciones adicionales.

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CURRICULUM VITAE

Ricardo Antonio Jiménez Montealegre was born on January 12th, 1962 in the city of San José, Costa Rica; son of Rodrigo Jiménez-Zeledón and Beatriz Montealegre-Moreno. His elementary studies were completed at the Escuela Buenaventura Corrales (1974). In 1979, he graduated from the Colegio Seminario (secondary school) in San José, Costa Rica, after which he enrolled in the Universidad Nacional (UNA). He obtained a B.Sc.-degree in Marine Biology in 1985 and the Lic.-degree in Marine Biology with emphasis in Aquaculture in 1989, graduating with honors (*Summa cum laude*). In 1990 he was awarded a scholarship by NUFFIC (The Netherlands) under an International Cooperation Project between the Escuela de Ciencias Biológicas (UNA) and the Fish Culture and Fisheries Group of Wageningen Agricultural University. In 1992 he obtained his M.Sc.-degree in Aquaculture with *Distinctions*. From 1992 to date, he has been a teacher/researcher in the UNA. He applied for a PhD position in the WU. He started working on this thesis in the middle of 1994.

Abstract

Jiménez-Montealegre, R. 2001. Nitrogen transformations and fluxes in fish ponds: a modelling approach.

Nitrogen is a key element in aquatic environments, and in Aquaculture it is an important pond management variable. In current aquaculture research two important goals are to maintain the water quality within the system, and to improve the retention of nutrients applied to the system in order to minimize the discharge. The principal objectives of this study were to integrate the information available of nitrogen processes in fish ponds into a predictive model, and to investigate further the nitrogen dynamics between the water, the sediments and the biota present in this systems. First, a nitrogen balance in fish ponds was followed along a growing cycle; by combining estimates of the deposition rates of uneaten feed, faeces and dead phytoplankton with measurements of nitrogen accumulation in the sediment, the rate of decomposition of organic matter in the sediment was evaluated. The cumulative recovery at the end of the experiment was almost 100%, meaning that the nitrogen budget in the system studied can be fully explained without any consideration of nitrogen volatilisation, due to either denitrification or ammonia volatilisation. The interactions between various N-species are complex and difficult to integrate. A model that calculates the amounts of various N-compounds in the water column and in the sediment was constructed, and used to gain insight into the relative importance of transformation processes between the various N-compounds. The model was divided into three modules: fish, phytoplankton and sediment-water. All concentrations of the various N-species present were simulated well except the N retained in organic matter in the sediment. To improve our understanding of the bottom organic matter dynamics, and make the model a more comprehensive predictive tool, an estimation of the principal sources of organic matter that accumulate in fish pond bottoms was assessed. Organic matter accumulation in fish ponds was quantified, and the data was used to construct, to calibrate and to validate a dynamic simulation model of organic matter deposition/decomposition in fish ponds. Besides, the rates of sedimentation and resuspension were measured along a growing cycle, following the influence of nutrient input, water parameters, fish biomass and fish size on these processes. Using a dilution analysis method to differentiate between sedimented and resuspended particles, sedimentation and resuspension rates were calculated. The rate of material collected in sediment traps increased from 88.5 to 330 g/m^2 per day along the growing cycle, but the relative resuspension did not change significantly, being always in the range of 42 to 47% of the total collected material. The processes of sedimentation of organic matter and resuspension were included in the original model. The proportion of three principal sources of organic matter that accumulate in the pond bottom were also included as parameters of the sedimentation process. A logistic equation relating the rate of resuspension and the fish biomass was calculated; and seepage, as a potential loss of nitrogen from the system, was also considered. The additions to the model represented a substantial improvement to model simulations.

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