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Nitrogen transformations and factors leading to nitrite accumulation in a hypertrophic marine fish culture system

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ABSTRACT: Nitrogen transformations in a highly eutrophic model ecosystem were studied. The heterotrophic (fish and sedimentation) and autotrophic (seaweed) components of the ecosystem were separated into 3 units. The seaweed purified fish effluents from organic matter and ammonia, and enriched them with dissolved oxygen (DO). Particles were sedimented out and the treated water was recirculated to the fish unit. Both assimilation of ammonia and production of oxidized nitrogen (ToxN) occurred mainly in the seaweed unit. ToxN production potential was highest in organic films on the walls (0.16 mmol N l^{-1} d^{-1}), and less in the water body (0.055 mmol N l^{-1} d^{-1}) and on the seaweed fronds (0.036 mmol N l^{-1} d^{-1}). The overall rate of ToxN production potential in the whole seaweed unit reached 0.73 mol d^{-1}. The specific rate there was 0.74 g N m^{-2} d^{-1} (expressed per m^2 of tank wall), about 3 times the highest published rate for marine nitrification. In the other compartments, processes of production and consumption prevented net ToxN accumulation. Nitrite in the seaweed tanks accumulated in a diurnal fashion, at a rate that averaged 50% of accumulation rate of ToxN. Laboratory incubations of film samples collected from the seaweed unit revealed that, within the ranges of conditions examined (16 to 28°C and pH 7 to 9), ToxN accumulated fastest at pH 8 and at higher temperatures. Nitrite accumulation was enhanced as temperatures and pH values were elevated. Both nitrification and denitrification might have contributed to the observed nitrite accumulation. It was estimated that denitrification in the sedimentation unit consumed up to 19% of the total daily nitrogen input to the system.

KEY WORDS: Hypertrophic marine ecosystems · Nitrogen · Nitrite · Nitrification · Denitrification · Mariculture · Seaweed · Fish · Model

INTRODUCTION

With the increase in eutrophication of coastal waters and enclosed seawater bodies, the factors that control nitrogen transformations in marine hypertrophic ecosystems become more relevant. The roles in these processes of waterborne bacteria and algae, of sediments and animals and of season are of particular interest. Often, but not always, processes in the sediment dominate nitrate production and consumption in coastal waters, and often the rate of nitrate reduction depends on the rate of ammonification and nitrate production (e.g. Blackburn et al. 1988, Rysgaard et al. 1996).

In open and coastal marine waters, N_2 gas and nitrate are the most abundant inorganic nitrogen forms. This situation is in contrast to hypertrophic marine ecosystems where, in addition to these forms, ammonia and nitrite are often present at appreciable concentrations (reviewed by Paasche 1988). Nitrite is extremely toxic to aquatic life and, as such, its accumulation needs to be understood.

As an intermediate product of both nitrification and denitrification, nitrite accumulates when factors prohibit complete oxidation of ammonia to nitrate (nitrification) or complete reduction of nitrate to nitrogenous gases (denitrification).

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Information on nitrite production by marine denitrifiers is scarce, especially for natural hypertrophic seawater (reviewed by Paasche 1988). It may be assumed, however, that the biogeochemical factors controlling marine denitrifiers are similar to those found for freshwater denitrifying isolates. In the latter organisms, nitrite accumulation is influenced by carbon limitation and composition (Almeida et al. 1995, van Rijn et al. 1996), kinetic differences in reductases involved in reduction of nitrate to nitrogen gas (Betlach & Tiedje 1981), availability of dissolved oxygen (DO) (Hochstein et al. 1984) and light (Barak et al. 1998). Relatively high nitrite concentrations (up to 23 µM) in nutrient-rich coastal seawater have been associated with unstable denitrification rates (Codispoti et al. 1986). Nitrite peaks in lower oceanic layers, known as secondary nitrite maxima, were thought to result from incomplete denitrification at low DO concentrations (Tiedje 1988).

Nitrite accumulation by ammonia oxidizers occurs near the base of the euphotic zone, particularly in nutrient-rich estuarine water (reviewed by Paasche 1988), where it has been associated with a seasonally unstable water column. Rates of oxidation of ammonia and nitrite are believed to be regulated by DO concentration and light intensity (Olson 1981, Helder & de Vries 1983), and by innate kinetic differences between rates of ammonia and nitrite oxidation (Forster 1974, Poxton et al. 1981, Kaplan 1983, Nijhof & Bovendeur 1990). The last publication has also documented the extremely slow growth rate of marine nitrifiers, even compared with such bacteria in freshwater.

The physical proximity of the organisms responsible for nitrogen transformations and the instability of physicochemical conditions in natural hypertrophic ecosystems hinder their investigation. In this study, results are presented on nitrogen transformations and on factors leading to persistent nitrite accumulation simultaneously with nitrate, in a simplified marine hypertrophic ecosystem. The processes were investigated in a well-defined, compartmentalized model of an intensive mariculture system for fish and seaweed. The system was characterized by steady input rates of water and nutrients and by the daily discharge of the anaerobic sediments (Neori et al. 1993, 1996). Relatively high and persistent production rates of nitrite and nitrate had been found in this system (Krom et al. 1995, Neori et al. 1996). The physical separation between the autotrophic and heterotrophic compartments and the lack of significant sediment buildup have allowed the detailed characterization of these transformations.

MATERIALS AND METHODS

Experimental system. The culture system has been described in detail elsewhere (Neori et al. 1996). Briefly, the system has 3 main compartments (Fig. 1): a fish tank (1800 l), shaded to prevent phytoplankton growth; 3 identical parallel seaweed recirculating biofilters (each 2130 l, 3 m^2) through which the fish tank water is recirculated at an overall rate of about 900 l h^{-1}; and a sedimentation tank (400 l), situated between the fish and the seaweed tanks. The system receives daily 2000 l of clean seawater.

The fish tank was stocked with gilthead seabream *Sparus aurata* L. at a density of 17 kg m^{-3}. The fish were fed daily (08:30 h) with 600 g of a 40% protein pelleted diet as described by Krom & Neori (1989). The tanks were aerated and agitated via holes in the tank base. The biofilter tanks were stocked with the green seaweed *Ulva lactuca* at an initial stocking density of 1 kg m^{-2} (the optimum, based on Neori et al. 1991). The seaweed was harvested weekly and immediately restocked at the original density.

Measurements of inorganic nitrogen transformations in the culture system. Nitrification rate was defined for practical considerations as the net accumulation rate of total oxidized nitrogen (ToxN = NO_2^- + NO_3^-). Rates of ToxN production in the tanks were calculated from inflow and outflow concentrations of the nitrogenous species, multiplied by the water exchange rates, as in Krom et al. (1995). Potential rates of ToxN production in the tanks were measured by stopping the water exchange, topping up the water with ammonium salt to concentrations between 100 and 150 µM,

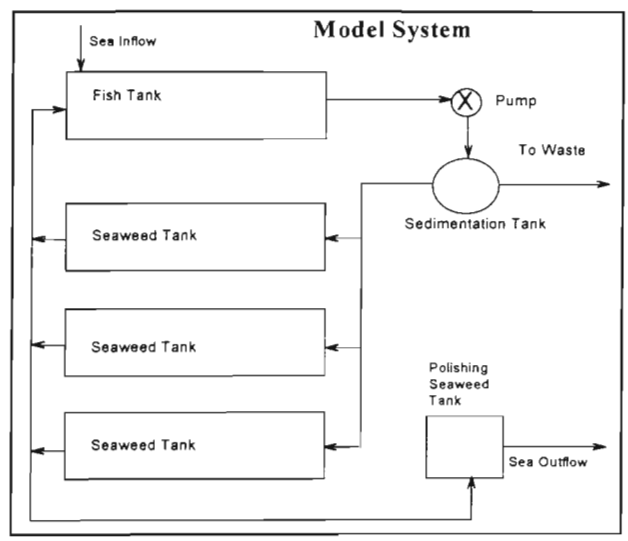

Fig. 1. A schematic diagram of the fishpond-seaweed biofilter system. Arrows indicate the direction of water flow

sampling the water every 1 to 4 h and drawing time-courses of ToxN accumulation. Linear regressions provided the desired rates. Replicates with non-significant regression lines were discarded.

ToxN production rates were measured in the 3 main compartments comprising the model mariculture system: the seaweed tanks, the fish tank and the sedimentation tank. Prior to the measurements the seaweed tanks were harvested and restocked with 3 kg of shaded seaweed. Fish were removed from the fish tank to eliminate possible effects of fish on inorganic nitrogen concentrations in the system. Water in the sedimentation tank was internally circulated to preserve conditions as close as possible to those prevailing during normal operation. In order to assess possible ToxN production on the fish skins, inorganic nitrogen transformations were examined in a separate, aerated, clean tank containing fish.

Within the seaweed tanks, 3 locations were further examined for activity of nitrifying bacteria: the seaweed, the organic film on the tanks' walls and the tank water. To isolate these 3 possible nitrification sites, each of the 3 seaweed tanks was prepared and operated differently. Tank A, a shaded tank from which the seaweed was removed and in which the walls were scrubbed to remove the organic film, was examined for ToxN production potential in the water. Tank B, as Tank A but stocked with 3 kg of seaweed and not shaded, was examined for ToxN production potential on the seaweed fronds and in the water. In this tank, ammonia concentration was maintained at 100 to 150 µM by a continuous dripping of an ammonium solution, to compensate for the ammonia assimilation by the seaweed. Tank C, a shaded tank in which the walls were not cleaned and without seaweed, was examined for ToxN production potential in the organic films on the walls and in the water. This organic film developed during several months under the operation regime described above. Neori et al. (1996) have shown that at such age, both forms of ToxN were produced in the system at significant rates.

Laboratory experiments. All the laboratory experiments below were carried out in the following manner: organic film, removed from the walls of a seaweed tank of the model mariculture system, was incubated (24 ± 1°C, except for the temperature experiments) in 2 l flasks filled with 1.5 l of 0.5 mm filtered seawater from the same seaweed tanks. The organic film material from a tank added to each 1.5 l flask was in proportion to the ratio between the area from which the film was collected and the water volume in the tank. That is, from a 2000 l tank, with a wet wall area of 5 m^2, the film from 37.5 cm^2 was scrubbed into the 1.5 l flask.

Effect of DO concentration on ToxN production potential: Three triplicated treatments (9 flasks in total) were bubbled by 3 gas mixtures, containing 3 different oxygen levels: (1) a mixture of 40% oxygen in nitrogen; (2) a mixture of 10% oxygen in nitrogen; (3) no oxygen (pure nitrogen gas). Filtered seawater from a seaweed tank served as a control for each treatment. Triplicate water samples, taken at 0, 5 and 15 h from the onset of the experiment, were analyzed for total ammonia nitrogen (TAN), nitrite and nitrate. DO concentrations and pH levels were measured at the beginning and end of each incubation.

Effects of ammonia and pH on ToxN production potential: The ToxN production potential in the film as a function unionized ammonia (NH_3) (in the range of 0 to 0.5 mM NH_3) at 3 pH levels (7 ± 0.2, 8 ± 0.2 and 9 ± 0.2) was examined twice. The desired NH_3 concentrations for each pH were attained by adding various amounts of ammonium salt to the water according to the equilibrium constant of ammonia, temperature and salinity of the water. Using the tables of Bower & Bidwell (1978), the percentages of NH_3 in TAN in seawater at 24°C were calculated to be 0.7, 2 and 17.3% for pH 7, 8 and 9, respectively. Tris (hydroxymethyl) aminomethane-maleic acid, Tris-HCl and Tris (hydroxymethyl) aminomethane-HCl were used to maintain pH values of 7, 8 and 9, respectively. A pH meter (Radiometer PHM 84) with a glass electrode was used for pH measurements. Nitrite was added to the flasks at an initial concentration of 0.2 mM. Samples were taken at 0, 2, 6 and 24 h after onset of the experiment for analyses of ammonia, nitrite and nitrate. At the same times, pH was measured. Solutions of HCl and NaOH were added to correct the pH when necessary.

Effect of temperature on ToxN production potential: Three temperature experiments were carried out. In each of them, 2 temperatures were compared for their effects on nitrogen transformations in the organic film from the seaweed tanks of the model mariculture system. In the second and third experiments, the interaction between the effects of pH and temperature on nitrogen transformations was also examined.

Temperature Expt 1 (January 1996): Treatments maintained at 16°C (L) and 21°C (H) were compared in quadruplicates. This temperature difference was the same experienced in the model mariculture system at that time (January). Ammonia and nitrite (50 µM final concentration of each) were added at the onset of the experiment to flasks which had been inoculated with organic film from a seaweed tank, as described above, at the 2 designated temperatures 24 h earlier. Two of the replicates from each treatment (LL and HH) were then kept at the initial temperature for 48 h afterwards. The other 2 replicates from each treatment (LH and HL) were

transferred 24 h after the onset of the experiment from 16 to 21°C and vice versa. These duplicates served as controls. Water samples were drawn from each flask for the analysis of ammonia, nitrite and nitrate at the onset of the experiment and then about every 8 h for 2 d.

Two more experiments were carried out, to clarify the temperature effect and its interaction with pH (Focht & Verstraete 1977).

Temperature Expt 2 (February 1996): Two different pH levels, 8 and 9, and 2 temperatures, 17.5 (L) and 28°C (H), were examined in triplicates for their effects on the nitrogen transformations. The pH was adjusted in the water in each flask, 6 flasks (3 L and 3 H) at pH 8 and, 6 flasks (3 L and 3 H) at pH 9. The water in each flask was then aerated for several hours, to equilibrate the DO level. From then on, the experiment proceeded as in Expt 1 (24 h of equilibration with the organic film at the designated temperatures and then the addition of ammonia and nitrite at the onset of the experiment). Water samples were drawn from each flask for the analysis of ammonia, nitrite and nitrate at the onset of the experiment and then every about 10 h for 2 d. Each sampling was accompanied by the measurement of pH and its adjustment, as described above.

Temperature Expt 3 (April 1996): A third temperature experiment was conducted, similar to the second one, except that the L temperature ranged from 18 to 19°C, due to technical limitations of our culture room.

Chemical and physical analysis. Water samples were filtered through acid-washed, glass-fiber filters (Whatman GF/C) within 15 min of collection. The filtered samples were then refrigerated for up to 2 d before analysis by an Autoanalyzer (Technicon AAII). This storage had been shown in our laboratory to be absolutely safe (Krom et al. 1985). Nitrite and nitrate were analyzed as in Glibert & Loder (1977), TAN as in Krom et al. (1985). Precision of each method in our laboratory is given in Krom et al. (1995). DO was measured with a YSI electrode (Yellow Springs Instrument Co.) after calibration against the Winkler method.

Statistical analysis. A multivariate analysis of the ToxN production potential rates was made by SPSS software. Groups were first tested for normal variance. In the ammonia-pH study, the experiment date was taken as a third non-parametric independent variable.

RESULTS

Location and characterization of ToxN production potential in the model fish-seaweed mariculture system

Nitrogen transformations in each of the compartments comprising the mariculture system were measured under conditions in which the flow of water through the compartments was entirely stopped for 24 h.

Fish tank. DO concentrations in the fish tank (with the fish removed) during the 24 h period remained above 5.3 mg l^{-1}, temperature ranged between 25 and 26.3°C and the pH between 7.92 and 8.12 (data not shown). Ammonia increased at an overall rate of 51 µmol N l^{-1} (92 mmol $tank^{-1}$) d^{-1}, nitrite concentrations increased at a rate of 28 µmol N l^{-1} (50 mmol $tank^{-1}$) d^{-1}, while nitrate concentrations varied inconsistently and decreased overall at a rate of 49 µmol N l^{-1} (88 mmol $tank^{-1}$) d^{-1} (Fig. 2A).

Fish skin was not a site for nitrogen transformations as no ToxN accumulated in an ammonia-supplemented, clean tank stocked with fish (data not shown).

Sedimentation tank. DO concentration dropped to undetectable levels within 6 h after water flow was ceased while the temperature increased from 28 to 34°C after 12 h (data not shown). An increase in the nitrite concentration was measured in the first 2 h (Fig. 2B). Thereafter, for the next 8 h, nitrite concentrations decreased to undetectable levels at an average rate of 4.9 µmol l^{-1} h^{-1} (118 µmol N l^{-1} [47 mmol $tank^{-1}$] d^{-1}). Nitrate concentrations decreased rapidly to undetectable levels at an average rate of 24 µmol l^{-1} h^{-1} (575 mmol N l^{-1} [230 mmol $tank^{-1}$] d^{-1}). Ammonia accumulated at an overall rate of 26.9 µmol l^{-1} h^{-1} (0.65 mmol N l^{-1} [258 mmol $tank^{-1}$] d^{-1}), with noteworthy leaps during the first 2 h and between 8 and 10 h after onset of measurements. The latter coincided with the disappearance of ToxN (nitrite and nitrate).

Seaweed tank. In the shaded seaweed tank, temperature increased from 27.5 to 31.5°C, pH level ranged between 8.0 to 7.9 and DO concentration decreased from 5.5 to 5.0 mg l^{-1}. While ammonia concentration first dropped and then stabilized (not including the addition of ammonium salt after 3 h), concentrations of both nitrite and nitrate increased steadily during the entire 24 h of the experiment (Fig. 2C). Overall accumulation rates of these oxidized N-forms were 2.0 and 2.8 µmol l^{-1} h^{-1}, respectively (0.048 and 0.067 mmol N l^{-1} d^{-1}, and 102 and 143 mmol $tank^{-1}$ d^{-1}, respectively), totaling 0.73 mol d^{-1} in the 3 seaweed tanks. Overall, 58% of the ToxN accumulation was in the form of nitrate (complete nitrification). Absolute and relative rates of ammonia and nitrite oxidation varied over the experimental period. During the first morning, nitrite and nitrate accumulated at rates of 1 and 2.2 µmol l^{-1} h^{-1}, respectively. Of the total ToxN accumulated, 69% was recovered as nitrate. That afternoon, the respective rates of the 2 processes increased to 2 and 2.6 µmol l^{-1} h^{-1}, respectively, with 56.5% of the accumulated ToxN recovered as nitrate. During the early evening, nitrite and nitrate accumulated still faster, at rates of 2.8 and 3.1 µmol l^{-1} h^{-1}, respectively (52.5% nitrate recovery), and during the

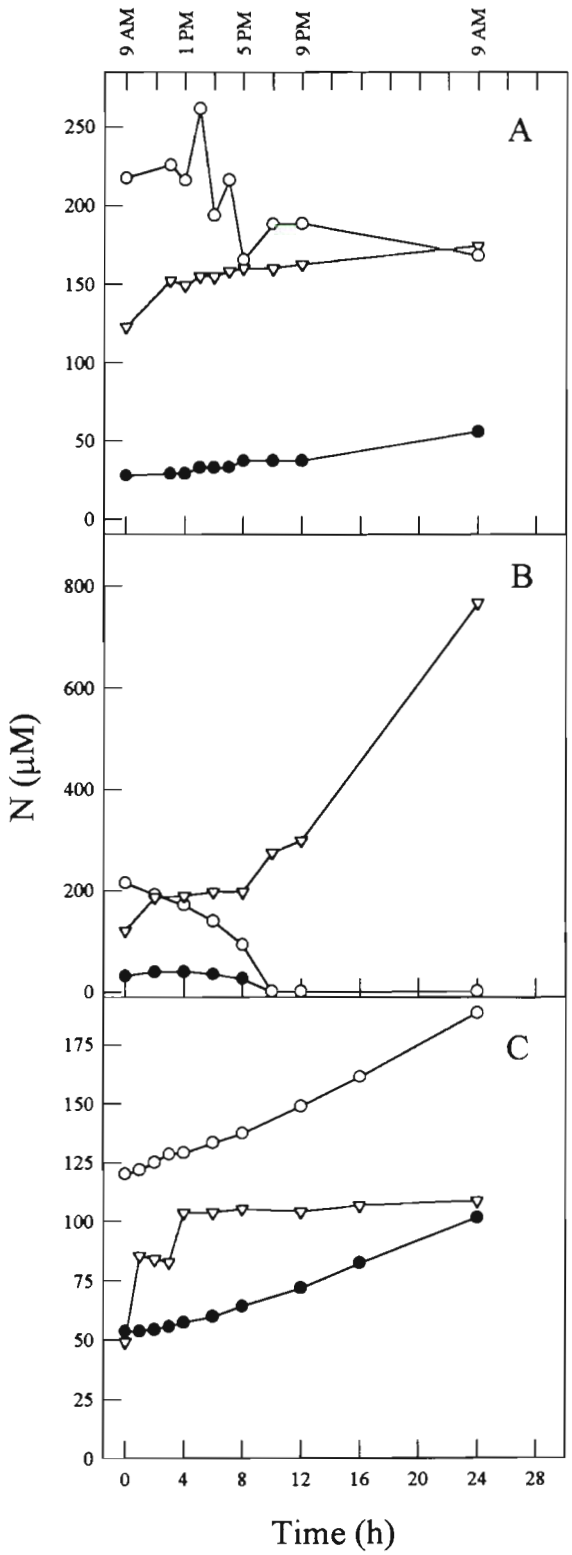

Fig. 2. Concentrations of (▽) TAN, (●) nitrite and (○) nitrate in (A) the fish tank, (B) the sedimentation tank and (C) the seaweed tank. Tanks were operated without water exchange over a 24 h period

night and early morning, the rates were 2.4 and 3.4 µmol l^{-1} h^{-1}, respectively (58% nitrate recovery).

Location of the ToxN production within the seaweed tanks

ToxN production potential in the water (Tank A, Table 1). DO concentration ranged between 6.3 and 5.7 mg l^{-1}. The pH was rather constant at 8.45 to 8.55 and temperature ranged between 27.1 and 31.1°C (data not shown). A stable net ToxN production rate of 0.12 mol N d^{-1} was measured throughout the entire 48 h of the experiment. Only 50% of the accumulated ToxN was nitrified completely to nitrate (Table 1).

ToxN production potential on the seaweed fronds (Tank B, Table 1, Fig. 3). The highest peak of DO concentration, 8.7 mg l^{-1}, was measured in the morning. DO concentration declined to the lowest level of 5.1 mg l^{-1} at night. A pH of 9.92 was measured at noon and it declined to 8.59 at night. Temperature varied from 25.5 at night to 31.8°C in the afternoon. In this tank ToxN accumulation showed a diurnal pattern. Overall, the rate of ToxN accumulation on the seaweed in the whole tank averaged 0.08 mol N d^{-1}. During the day, most of the accumulated ToxN (1.56 µmol l^{-1} h^{-1}, 2.8 mmol N $tank^{-1}$ h^{-1}) was nitrate. In contrast, at night, nitrite was the predominant oxidized nitrogen form that accumulated (Table 1, Fig. 3). On average, 90% and 30% of the accumulated ToxN was nitrate during the day and nighttime, respectively. The highest pH and temperatures coincided with the switch from nitrate to nitrite accumulation. The periods in which nitrite was the dominant ToxN accumulation product coincided with highest levels of pH, temperature and NH_3 and with lower levels of DO (Fig. 3).

ToxN production potential in biofilm on the tanks' walls (Tank C, Table 1). DO concentration varied between 5.7 and 6.3 mg l^{-1} and was negatively correlated with water temperature (data not shown). Temperature varied between 26.7 and 31.3°C while pH levels ranged between 8.31 and 8.56. Relative to other sites within the seaweed tank, the highest net ToxN production potential was measured here (0.345 mol d^{-1}; 53 mmol N m^{-2} d^{-1}; 0.74 g N m^{-2} d^{-1}) (Table 1). Over half of the accumulated ToxN was recovered as nitrate in this treatment.

Factors affecting ToxN production in the organic film on the seaweed tanks—laboratory experiments

Effect of DO concentration on ToxN production potential. The rate of ToxN accumulation was similar at 9.3 and 4.5 mg O_2 l^{-1} (Table 2). At undetectable

Table 1. Nitrite and nitrate potential production rates (µmol l^{-1} h^{-1} ± SE) over 48 h in (A) seaweed tank without seaweed and without wall growth, (B) seaweed tank with seaweed and without wall growth, and (C) seaweed tank without seaweed and with wall growth

Tank	Period examined	Ammonia	Nitrate	ToxN	Nitrate/ToxN
A	Entire 48 h	−2.38 ± 0.2	1.15 ± 0.02	2.34 ± 0.03	0.5
B	Day 1		1.56 ± 0.04	1.52 ± 0.14	1.0
	Night 1		0.26 ± 0.13	1.2 ± 0.09	0.2
	Day 2		1.94 ± 0.28	2.31 ± 0.21	0.8
	Night 2		0.28 ± 0.10	1.09 ± 0.09	0.26
C	Entire 48 h	−4.50 ± 0.55	3.7 ± 0.08	6.75 ± 0.07	0.55

levels of DO, this rate dropped by 76%. The oxidation rate of nitrite to nitrate dropped insignificantly between 9.3 to 4.5 mg O_2 l^{-1} but nitrate was consumed at undetectable DO levels. Nitrite, however, did accumulate even at undetectable levels of DO (Table 2).

Interaction between the effects of ammonia concentration and pH level on ToxN production potential in the organic film from the wall of a seaweed tank. No significant differences in the rates of the 2 nitrification steps were found between the 2 replicate experiments (1-way ANOVA). Therefore, the data from both experiments were combined in the following statistical analyses.

In a 2-way ANOVA to evaluate the combined effects of NH_3 and pH on nitrification rate, the only informative effects were those of pH on ToxN accumulation (Table 3a; $p = 0.088$, 1-tail) and the negative combined effects of pH and ammonia on nitrate accumulation (Table 3b; $p = 0.07$, 1-tail). In a 1-way ANOVA for effect of pH on ToxN accumulation, it was absolutely significant ($F_{[2,18]} = 3.71$; $0.01 < p < 0.05$). The pH level significantly ($p < 0.05$) influenced the rate of accumulation of ToxN, so that at pH 7 the rate of ToxN production was less than half that at either pH 8 or 9 (Table 4). However, the pH level did not significantly influence the absolute rate of nitrate accumulation. There was only an indication of a lower nitrate accumulation rate at pH 9 (Table 4). At pH 7, three-quarters of the accumulated ToxN (i.e. of total nitrification) was nitrate and at higher pH levels this ratio dropped to a quarter or less (Table 4). In the range of concentrations tested (up to 0.5 mM), unionized ammonia did not significantly affect ToxN production potential.

Interaction between the effects of temperature and pH on ToxN production potential. The rise in temperature by 5 to 10°C significantly increased the rates of total ToxN production potential and reduced the ratio between the accumulation of nitrate and ToxN (Table 5). On the contrary, as was found in the ammonia-pH experiment described above, a pH change between 8 and 9 did not affect either stages of ToxN production potential at any temperature (Table 5). The trends but not the extents of the temperature effect between the 3 experiments should be compared, as each experiment took place at a different time, and therefore the environmental conditions and the precise characteristics of the organic film differed.

In temperature Expt 1, a temperature increase of 5°C caused a significant ($p < 0.05$) near doubling of the ToxN production potential already after 24 h (data not shown). Switching between 2 replicates of the treatments after 24 h, that is, the transfer of 2 flasks incubated at the low temperature to the high temperature (LH) and vice versa (HL), also switched the relationship between the rates, confirming the results of the temperature effects in the first 24 h.

In temperature Expts 2 and 3, a temperature difference of about 10°C for 48 h caused significant increases of the ToxN production potential by up to 3-fold at both pH values and slightly decreased the ratio between the accumulation of nitrate and ToxN (Table 5). Hence, at higher temperatures more nitrite accumulated.

DISCUSSION

In the hypertrophic model ecosystem studied, the production and consumption of ToxN was most probably mediated by nitrifying and denitrifying bacteria although, based on our results, the influence of other nitrogen transformation processes cannot be excluded. In addition to autotrophic nitrifiers, heterotrophic nitrifiers might have been responsible for ammonia oxidation to nitrite and nitrate. This latter process usually takes place in organic-rich, low-pH environments (Killham 1986) and as such it might be assumed that its contribution to ammonia oxidation in our experimental system was minor. Dissimilatory nitrate reduction to ammonia is an additional process in which nitrite forms an intermediate product. Usually this process takes place under electron-acceptor limiting conditions, i.e. in environments with high C/N ratios (Tiedje 1988). The process can be differentiated from denitrification by measurement of nitrogen gases produced during nitrate reduction. As in the present study no such measurements were conducted, the possibility that dissimilatory nitrate reduction to ammonia also affected the nitrate reduction and nitrite accumulation rates in the system cannot be discarded. In this study, the N-deficit of the system cor-

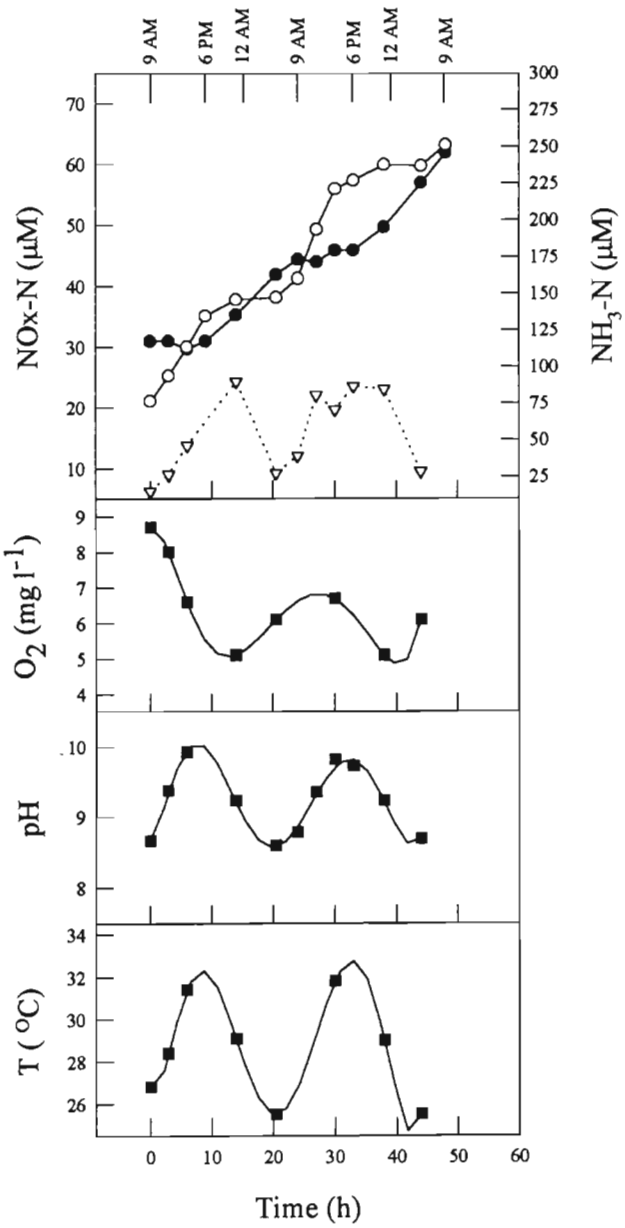

Fig. 3. (Top frame) concentrations of (▽) unionized ammonia, (●) nitrite and (○) nitrate, and (lower frames) dissolved oxygen, pH and temperature, in a seaweed tank during 48 h. Concentrations of unionized ammonia were calculated from TAN concentrations and pH, according to Bower & Bidwell (1978)

responded to the measured nitrate reduction. It seems, therefore, that denitrification and not dissimilatory nitrate reduction to ammonia was responsible for most of the nitrate reduction in this system.

Based on the results obtained in this study it is possible to locate the important processes in the studied experimental hypertrophic ecosystem and identify the factors that underlie them.

Table 2. Effect of oxygen levels on nitrite and nitrate potential production rates (µmol l^{-1} h^{-1} ± SE) in organic film scraped from a seaweed tank; laboratory incubations. Values with the same superscript are not significantly different ($p < 0.05$), using Student-Newman Keuls post-hoc tests

Oxygen (mg l^{-1})	ToxN	Nitrate	Nitrate/ToxN
9.3	1.88 ± 0.14^{a}	0.48 ± 0.19^{a}	0.25 ± 0.04
4.5	1.87 ± 0.20^{a}	0.35 ± 0.05^{a}	0.19 ± 0.03
~0	0.45 ± 0.09^{b}	-0.35 ± 0.33^{b}	*

*Nitrate reduction

Table 3. Two-way ANOVA of potential production rate of (a) ToxN and (b) nitrate in organic film scraped from a seaweed tank, for pH and NH3 effects; laboratory incubations. The experimental design was factorial, with 3 levels of NH_3 concentration ranges (<50 µM, 60 to 200 µM, and 210 to 500 µM) and 3 levels of pH (7, 8, and 9)

Source	df	MS	*F*
(a) ToxN			
pH	2	11.41	2.65
NH_3	2	4.60	1.07
pH × NH_3	3	0.74	0.14
(b) Nitrate			
pH	2	0.65	1.40
NH_3	2	0.17	0.36
pH × NH_3	3	0.81	2.40

Table 4. Potential production rates (µmol l^{-1} h^{-1} ± SE) of ToxN and nitrate as a function of pH in organic film scraped from a seaweed tank; laboratory incubations. Values with the same superscript are not significantly different ($p < 0.05$), using Student-Newman Keuls post-hoc tests. NH_3 data were not tabulated because of insignificant effect (see in text)

pH	ToxN	Nitrate	Nitrate/ToxN
7	0.20 ± 0.13^{b}	0.13 ± 0.06	0.77 ± 0.26^{a}
8	0.50 ± 0.25^{a}	0.13 ± 0.06	0.23 ± 0.08^{b}
9	0.44 ± 0.23^{ab}	0.07 ± 0.07	0.13 ± 0.13^{b}

Site of ToxN production potential

Most of the ToxN production and the highest nitrification rate (ToxN production potential) within the system were found in the seaweed tanks. Most of the nitrifying activity was found within the organic film on the submerged walls of these tanks, and only a little in the water or on the seaweed. The rates of nitrification per surface of the wall (up to 0.74 g N m^{-2} d^{-1}) were nearly 3 times higher than the rates reported by Nijhof & Bovendeur (1990) in a seawater nitrification biofilter (up to 0.28 g N m^{-2} d^{-1}) and also higher than the maxi-

Table 5. Potential production rates of ToxN and nitrate (μmol l^{-1} h^{-1} ± SE) at different temperatures and pH levels in organic film obtained from a seaweed tank; laboratory incubations. Expt 1. Incubation for 48 h at 16 ± 1°C (LL) or at 21 ± 1°C (HH); for 24 h at 16 ± 1°C followed by 24 h at 21 ± 1°C (LH); for 24 h at 21 ± 1°C followed by 24 h at 16 ± 1°C (HL). Expt 2: Incubation for 48 h at 17.5 ± 1°C (L) or 28 ± 1°C (H). Expt 3: Incubation for 48 h at 18.5 ± 1°C (L) or 28 ± 1°C (H). Values with the same superscript are not significantly different ($p < 0.05$), using Student-Newman Keuls post-hoc tests

Expt	pH	Temperature	ToxN	Nitrate	Nitrate/ToxN
1	8.2	LL	1.97 ± 0.38^{c}	0.33 ± 0.01^{b}	0.17 ± 0.05
1	8.2	LH	2.77 ± 0.11^{b}	0.45 ± 0.01^{a}	0.16 ± 0.01
1	8.2	HH	3.61 ± 0.09^{a}	0.38 ± 0.03^{ab}	0.1 ± 0.006
1	8.2	HL	1.88 ± 0.22^{c}	0.30 ± 0.03^{ab}	0.16 ± 0.01
2	8	L	0.19 ± 0.08^{b}	0.08 ± 0.01	0.49 ± 0.10^{a}
2	8	H	0.1*	0.05 ± 0.10	0.32*
2	9	L	0.25 ± 0.03^{ab}	0.09 ± 0.03	0.37 ± 0.10^{a}
2	9	H	0.37 ± 0.06^{a}	0.04 ± 0.01	0.10 ± 0.02^{b}
3	8	L	0.17 ± 0.03^{b}	0.06 ± 0.02^{b}	0.34 ± 0.07
3	8	H	0.52 ± 0.08^{a}	0.13 ± 0.05^{a}	0.25 ± 0.06
3	9	L	0.20 ± 0.07^{b}	**	**
3	9	H	0.52 ± 0.07^{a}	0.12 ± 0.01^{a}	0.23 ± 0.03

*Only 1 replicate survived
**Nitrate reduction

mum rates recorded in freshwater nitrification biofilters (van Rijn 1996). The stable nature of the system, the abundant surface area provided by the organic matter, the constant flow of DO-rich water along the walls, the high ambient water temperatures and the ample supply of ammonia, released by degradation and ammonification of organic matter (as found in laboratory experiments), probably explain this high nitrification rate. Similar nitrification rates were reported by Caffrey et al. (1993) from a semi-artificial, marine sediment microcosm enriched by labile organic matter.

The overall ToxN production (nitrification) potential for the 3 seaweed-biofilter tanks of 0.73 mol N d^{-1} equals about 33% of the total daily N input (fish excretions). This potential, measured with the addition of ample ammonia, is about 3 times higher then the actual values determined during normal mariculture operation by Krom et al. (1995) for this model ecosystem. Therefore, the overall normal ammonia oxidation process was substrate-limited, most probably as a consequence of competition by the seaweed.

Competition for ammonia, based on the differences in ammonia affinity between seaweed and nitrifying bacteria that settle on them (see in Lobban et al. 1985), might explain why nitrification on the seaweed was very low. It may also be possible that the fast seaweed growth and concomitant harvest could not be matched by the slow-growing (Nijhof & Bovendeur 1990) seawater nitrifiers. The high pH values associated with the surface of the photosynthesizing seaweed might be an additional factor preventing the development of nitrifiers on it.

No clear evidence was obtained on the contribution of nitrification to the nitrogen transformations occurring in the fish tank, but the overall rates were low there. Nitrite in this tank was produced not only by nitrification but probably also by denitrification as nitrate concentrations at times increased (nitrification) and at other times decreased (denitrification). The latter process might occur in the less aerated areas within this tank. Evidence, however, that the observed nitrite buildup resulted also from nitrification and not only from denitrification was provided in a previous study of this experimental system (Krom et al. 1995). There, both nitrite and nitrate accumulated in the fish tank. This nitrification, however, occurred in the recirculated water while no nitrification occurred on the walls of the fish tank. The organic film on the walls of this tank was much thinner in appearance than that in the seaweed tanks, probably as a result of grazing and scraping by the fish.

In the sedimentation tank, ammonification and denitrification were the dominant nitrogen transformation processes as attested by the fast rate of TAN accumulation and the depletion of ToxN. It might be assumed, however, that within the oxygenated water flowing through this tank some nitrification took place. This was supported by the finding that nitrite and nitrate were produced upon aerobic incubation in the laboratory of organic film obtained from this tank (not shown). In the field test, during the initial aerobic period, nitrification and denitrification in the sedimentation tank occurred simultaneously and ammonia was apparently produced and consumed at approximately even rates. After several hours, once DO and ToxN were depleted, ammonia accumulated rapidly. Therefore, the value of denitrification rate, as determined in this study by the disappearance of ToxN, was an underestimate of the actual denitrification rate in the sedimentation basin under the normal, partially aerated operation conditions. Considering the ammonia oxidation and subsequent reduction of oxidation products, the actual denitrification rate rises from an apparent value of 29 μmol l^{-1} h^{-1} to a more realistic value of 49 μmol l^{-1} h^{-1}, or 0.47 mol $tank^{-1}$ d^{-1}, about 19% of the entire N-input. It is instructive to notice that this value is about the same as the N-deficit described for the N-budget of this system in Krom et al. (1995).

Environmental factors affecting ToxN production

As observed in other nitrifying systems (Belser 1979, Kaplan 1983), pH, DO and temperature were shown to affect the rates of oxidation of ammonia and nitrite by the nitrifying bacteria present in this marine model ecosystem. TAN levels recorded in the model system did not influence ToxN production potential at levels that have been reported to inhibit nitrification in freshwater systems (Belser 1979). At the levels of unionized ammonia examined in this study, oxidation rates of ammonia and nitrite were not affected. Marginally better nitrification rates were found at ambient ammonia concentrations of 100 µM. At these optimal concentrations, nitrite accumulated for reasons explained below.

The pH optimum for nitrification was pH 8 as found in freshwater systems (Focht & Verstraete 1977). The interactions of the effects of pH with ammonia and temperature were weak.

At DO concentrations of 4.5 and 9.3 mg l^{-1}, DO did not limit nitrifying activity. This finding is in accordance with work on freshwater nitrifying bacteria, in which K_m for oxygen of *Nitrosomonas* and *Nitrobacter* ranged from 0.3 to 1.0 mg O_2 l^{-1} (Focht & Verstraete 1977). Ammonia-oxidizing nitrifiers are considered less sensitive to changing or extreme environmental factors than nitrite-oxidizing nitrifiers (Focht & Verstraete 1977, Belser 1979, Kaplan 1983, Ward 1986). With respect to oxygen this was also evident in our experiments as it was found that in flasks flushed with nitrogen gas (undetectable DO), ammonia oxidation took place together with nitrate reduction. The higher sensitivity to environmental conditions of nitrite oxidizers relative to ammonia oxidizers was also found at high ambient temperatures in high pH waters. Under these conditions, relative to nitrite oxidation, ammonia oxidation increased and nitrite accumulated. Similar observations were made on nitrite-oxidizing bacteria in sewage (Wong-Chong & Loehr 1978) and in other freshwater environments (Fdz-Polanco et al. 1996). Catalan-Sakairi et al. (1996) suggested that at high pH values, nitrite-oxidizing nitrifiers are limited by available inorganic carbon since at high pH values most of the inorganic carbon is either present in the bicarbonate or carbonate forms.

It is believed that nitrite oxidizers are more photosensitive than ammonia oxidizers (Olson 1981). Strong light on a nitrifying bacterial population can therefore lead to nitrite accumulation. The only non-shaded compartment of the experimental system studied here was the seaweed's. There, ToxN was produced mainly on the walls, which were shaded by the seaweed themselves. Incomplete nitrification occurred mainly at night. Therefore, light does not seem to exert any direct effect on nitrification in this model system.

CONCLUSIONS

Inorganic nitrogen transformations in hypertrophic ecosystems are complex and, rather than understanding all of these processes, the aim of this study was to locate and identify the process(es) which caused the observed nitrite accumulation in this model hypertrophic marine system. It is shown that incomplete nitrification taking place within the organic films in the seaweed tanks contributed to most of the nitrite in the system. Elevated temperatures and pH values during the day led to higher overall nitrification rates due to increased ammonia oxidation rates. As a result, from the early afternoon nitrite accumulated. Ammonia oxidation rates dropped during the night due to a decrease in temperature and pH values, so that by early morning nitrate again became the dominant product of nitrification. Without photosynthetic activity (as in the shaded seaweed tanks), pH values did not increase. Temperature variation alone was apparently not sufficient to change the relative rates of the 2 nitrification processes.

It is concluded that total nitrification in this oxic hypertrophic system was correlated, in addition to ammonia and DO, to high ambient temperatures and the abundant immobilization surfaces provided by the walls of the seaweed tanks. Nitrite accumulation in the system was enhanced by the effect of high temperatures and high pH values on nitrification in the seaweed tanks and, to a lesser extent, by the effect of low DO concentrations on the balance between nitrification and denitrification in the fish and sedimentation tanks.

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