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Short-term interaction between nitrate and ammonium uptake in *Thalassiosira pseudonana*: effect of preconditioning nitrogen source and growth rate

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Abstract. The effect of preconditioning nitrogen source and growth rate on the interaction between nitrate and ammonium uptake was determined in *Thalassiosira pseudonana* (Clone 3H). A new method, using cells on a filter (Parslow et al. 1985), allowed continuous measurement of uptake from 0.5 to 9 min after the addition of nitrate, ammonium, or both, with no variation in concentration during the course of the experiment. For each preconditioning N source and growth rate, a series of uptake experiments was conducted, including controls with only nitrate or only ammonium, and others with different combinations of concentrations of nitrate and ammonium. For the first time, preference for ammonium was separated from inhibition of nitrate uptake by ammonium. Ammonium was the preferred N source, i.e. if nitrate and ammonium were presented separately, ammonium uptake rates exceeded nitrate uptake rates. Preference for ammonium varied with both preconditioning N source and growth rate. Inhibition of nitrate uptake by ammonium, determined by comparing nitrate uptake in the presence and absence of ammonium, was observed at ammonium concentrations $>1\ \mu M$, but was rarely complete. Inhibition of nitrate uptake by ammonium was less in the ammonium-limited culture than in the cultures growing on nitrate, but invariant with growth rate in the nitrate-grown cultures. Below $1\ \mu M$ ammonium, nitrate uptake was often stimulated and rates exceeded those in the controls without ammonium. Ammonium uptake was not inhibited by the presence of nitrate. *T. pseudonana* fits the classical view of the interaction between nitrate and ammonium uptake in some respects, such as preference for ammonium, and inhibition of nitrate uptake by ammonium concentrations $>1\ \mu M$. However, at ammonium concentrations typical of most marine environments, nitrate uptake occurs at rapid rates. In other respects, N uptake in *T. pseudonana* deviates from the classical view in the following ways: (1) stimulation of nitrate uptake by low concentrations of ammonium; (2) lack of inhibition of nitrate uptake by ammonium at low nitrate concentrations; and (3) variation in preference and inhibition with preconditioning, which is markedly different for other species. Because of the apparent enormous species variation in the interaction between nitrate and ammonium uptake and the lack of detailed information for a variety of species, it is difficult to generalize about the effect of ammonium on nitrate uptake, especially in the field, where prior N availability and species composition are not usually addressed.

Introduction

Nitrate uptake by phytoplankton is often inhibited in the presence of ammonium, but the degree of inhibition is highly variable and rarely as extreme as generally believed (Dortch 1990). Dortch proposed that the variability arises because inhibition is strongly influenced by environmental conditions, such as nitrogen availability and light level, and species composition. Some of the variability may also be due to the difficulty in separating the effect of ammonium on nitrate uptake from its effect on nitrate reduction and subsequent assimilation. With the renewed interest in the measurement of nitrate and ammonium uptake in natural populations as a means of determining new and regenerated production, an understanding of the interaction between nitrate and ammonium uptake is essential for designing experiments and interpreting results.

In laboratory studies, where the degree of N deficiency and the N source used for growth can be controlled, inhibition of nitrate uptake by ammonium is usually greatest in N-sufficient phytoplankton and decreases with increasing N deficiency (Caperon and Meyer 1972, Eppley and Renger 1974, Bienfang 1975, Conway 1977, Tischner 1981, Terry 1982). However, this pattern is not universal. Caperon and Meyer (1972) found no variation in inhibition of nitrate uptake with growth rate in *Dunaliella tertiolecta*, and Dortch and Conway (1984) observed that inhibition varied with N deficiency in *Skeletonema costatum*. In the latter study, it was also observed that inhibition of nitrate uptake by ammonium

is less in cultures preconditioned on nitrate alone than on ammonium alone, at any particular growth rate in *S. costatum*, but not in *Chaetoceros debilis*.

While most studies of inhibition focus on the effect of ammonium on nitrate uptake, nitrate has also been observed to inhibit ammonium uptake in four algal species (Caperon and Ziemann 1976, Ohmori et al. 1977, Dortch and Conway 1984), but not in a fifth species (Zevenboom and Mur 1981). Lack of data on this unexpected phenomenon makes it difficult to generalize, but the degree of inhibition is not as great as when ammonium inhibits nitrate uptake. The degree of inhibition may also be influenced by preconditioning (Dortch and Conway 1984).

Although external ammonium is thought to directly inhibit nitrate uptake (Collos 1989), the variation with preconditioning growth rate and N source implicates intracellular controls as well (Dortch and Conway 1984). The control could be exercised directly on nitrate uptake or indirectly by inhibiting a later assimilatory step, or by some combination of mechanisms (cf. Blasco and Conway 1982). A mixed inhibitory mechanism has two practical implications: (1) If the mechanisms are affected differently by environmental conditions, it will increase the difficulty of generalizing about, and explain some of the variability in, inhibition; (2) If control is exerted internally, intracellular conditions can change so rapidly after nitrate and ammonium additions that it may be difficult to determine the effect of preconditioning. This problem is exacerbated because most N uptake measurements are made over time periods which are long enough for assimilation to strongly affect uptake. While much less is known about the inhibition of ammonium uptake by nitrate, similar problems are likely.

Much of the early literature on interactions between nitrate and ammonium uptake assumed that nitrate uptake and assimilation were not separable. In fact, it has been proposed that the two processes are accomplished by the same enzyme (Butz and Jackson 1977, Jones and Morel 1988). Since nitrate reduction is strongly negatively influenced by ammonium, it was assumed that uptake was as well. However, in marine phytoplankton uptake is often completely uncoupled from assimilation, as demonstrated by the accumulation of large intracellular nitrate pools (e.g. Eppley and Coatsworth 1968, Collos and Slawyk 1976, Cresswell and Syrett 1979, 1981, DeManche et al. 1979, Dortch et al. 1979, 1984, Collos 1982, Dortch 1982) and high nitrate uptake rates in the absence of measurable nitrate reductase activity (Dortch et al. 1979, 1982, Collos 1982). Thus, before generalizations can be made about inhibition of uptake, it is necessary to separate uptake and assimilation as much as possible.

One way to separate uptake from the affects of assimilation is to measure initial uptake rates on the time scale of minutes. A new method developed for measuring rapid ammonium uptake over very short time-periods (Parslow et al. 1985) can be used for measuring nitrate uptake and nitrite excretion on this time scale. Phytoplankton cells are placed on a filter in line with an AutoAnalyzer® and uptake is measured from the decrease in concentration of a standard seawater solution as it passes across the filter. Ammonium uptake rates obtained by this method over longer time periods are comparable to other more standard methods (Parslow et al. 1985). An additional advantage to this method is that cells are exposed continuously to the same nitrogen concentration, whereas during even very short standard incubations, concentrations can decrease rapidly.

In this paper, the effect of preconditioning nitrogen source and growth rate on ammonium inhibition of nitrate uptake will be examined for one marine diatom, *Thalassiosira pseudonana*, using this method. The hypothesis that nitrate inhibits ammonium uptake will also be tested. Other papers in the series examine the effect of preconditioning N source and growth rate on nitrate-uptake kinetics (Dortch et al. 1991), the effect of preconditioning irradiance on inhibition (Yin 1988, Yin et al. unpublished data), nitrite excretion as a function of preconditioning N source, growth rate, and irradiance (Dortch et al. unpublished data), and the regulation of nitrate and ammonium uptake and their interaction by amino acids and amino acid analogs (Dortch et al. unpublished data).

Materials and methods

Culture methods

Thalassiosira pseudonana (Clone 3H) from the Northeast Pacific Culture Collection, Department of Oceanography, University of British Columbia, was grown on an artificial seawater medium (Harrison et al. 1980), according to Thompson et al. (1989). For nitrogen-deficient cultures, the medium contained 50 μM of either nitrate or ammonium. For the N-sufficient culture, 100 μM nitrate plus two times the normal $NaHCO_3$ was added.

All cultures were maintained at 18 °C under continuous light at a surface irradiance of 80 μE m^{-2} s^{-1}. Light was provided by Sylvania® VHO daylight fluorescent tubes filtered through 3 mm of blue Plexiglas®. Cultures were unialgal but not axenic. All cultures were stirred by magnetic bars at 60 rpm.

N-deficient chemostats were set up as described in Harrison et al. (1976), with medium delivered by constant-flow piston pumps (Fluid Metering Inc., New York). Dilution rates (see Table 1) were calculated from measurements of overflow volumes. A continuous N-sufficient culture growing at the maximum growth rate (μ_{max}) was obtained by starting a batch culture, measuring its initial growth rate, and then pumping high nitrate medium into the culture at the same rate as μ_{max} measured in the initial batch culture. The concentration of nitrate plus nitrite in the outflow of this N-sufficient culture was always high (Table 1). Care was taken during uptake experiments to remove subsamples of the culture at a rate which would not perturb steady state. Steady state was monitored daily by measuring in vivo fluorescence and cell counts (Table 1).

Uptake-rate measurements

The uptake method, apparatus, and uptake rate calculations are described in detail in Parslow et al. (1985) and an example of the raw data from one experiment is given in Fig. 1. Briefly, a standard solution of nitrate, ammonium or both, made up in nitrogen-depleted culture filtrate, was passed across a filter held between two Plexiglas® plates and fed into a three-channel Technicon AutoAnalyzer® measuring nitrate, nitrite, and ammonium (Friederich and Whitledge 1972) for 10 min ("std" in Fig. 1). After a brief rinse with N-depleted culture filtrate, a known volume of culture was removed from the chemostat and the cells contained in this volume were

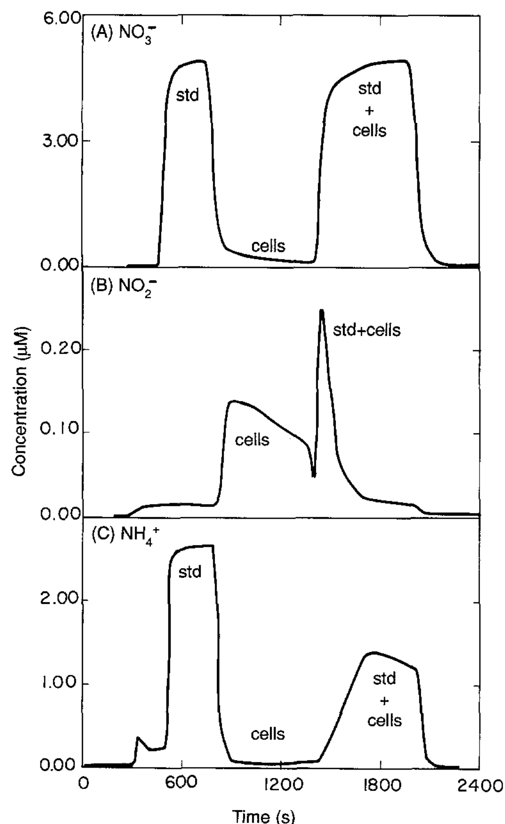


Fig. 1. *Thalassiosira pseudonana*; nitrate-limited, $\mu = 1.25\ d^{-1}$. Time course of nitrate, nitrite, and ammonium concentrations for uptake experiment in which cells were exposed to 5 μM NO_3^- and 2.74 μM NH_4^+. std: standard solution; sequence of events detailed in "Materials and methods – Uptake-rate measurements"

loaded on the filter ("cells" in Fig. 1). After another brief wash with culture filtrate, the initial standard was pumped across the cells on the filter for 10 min ("std + cells" in Fig. 1). Typically, phytoplankton on the filter removed some of the nitrate and ammonium and excreted nitrite as the solution passed. The exact amounts of nutrients removed or added were calculated by comparing nutrient concentrations when cells were present on the filter to the concentrations in the initial standard. To determine nitrite concentrations, a separate standard was run. A fresh filter was used for each uptake experiment. Light was provided by a slide projector. Since it was not possible to measure the irradiance on the filter, preliminary experiments were conducted to ensure that light levels were sufficient to saturate uptake. The filter holder which contained the cells was not temperature-controlled, but all solutions which flowed across the filter were kept at the growth temperatures, so the cells were not exposed to high temperatures.

For each preconditioning growth rate and N source (nitrate or ammonium) nitrate uptake was measured with 0.5 μM nitrate and 0.1, 0.25, 0.5, 0.75, 1.0, 2.5, or 5.0 μM ammonium. Controls with 5 μM nitrate only or 5 μM ammonium only were also run. Nitrite excretion was measured and will be reported elsewhere (Dortch et al. unpublished data). Net nitrate uptake rates were corrected for nitrite excretion; all data are gross nitrate uptake rates. Experiments could be run at the rate of one per hour; thus, between 6 and 15 experiments could be run in a day. Because nitrate uptake kinetics and other special experiments were conducted at the same time, it took several days to run all the experiments on a particular chemostat. Controls and some other experiments (5 μM ammonium plus 5 μM nitrate) were repeated daily or more often. The mean coefficient of variation (CV) for ammonium uptake was 13%, regardless of nitrate concentration. For nitrate uptake, the CV was

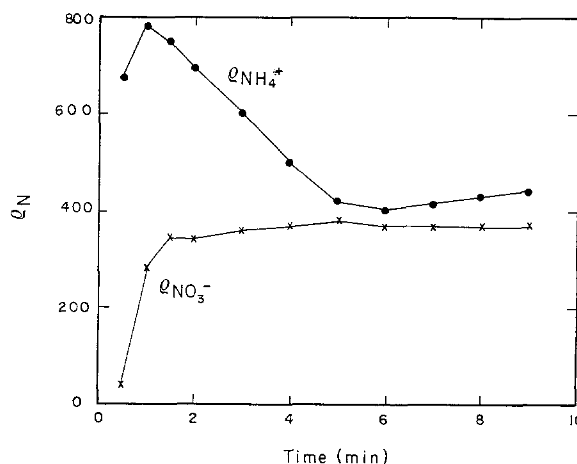


Fig. 2. *Thalassiosira pseudonana*; nitrate-limited, $\mu = 1.25\ d^{-1}$. Time course of nitrate and ammonium uptake rates [ϱ_N; mmol (liter cell vol)$^{-1}$ h^{-1}] when each was added alone at 5 μM

21% when only nitrate (5 μM) was present, but this increased to 58% in the presence of 5 μM ammonium because rates became low and quite variable.

Analytical methods

On a daily basis, in vivo fluorescence was measured with a Turner Model 10 fluorometer and cell volumes and counts on a Coulter Counter Model TAII (Table 1). When all uptake experiments had been completed for a culture, the entire culture was used for measuring many steady-state characteristics of the cells (Harrison et al. unpublished data); some data for the particular day that characteristics were measured are given in Table 1. Chlorophylls *a* and *c* were measured spectrophotometrically after acetone extraction (Parsons et al. 1984). Samples for particulate nitrogen and carbon were filtered onto precombusted GF/C filters, stored frozen over desiccant, and analyzed on a Carlo Erba CHN Analyzer. Steady-state nutrient concentrations were measured on samples filtered through glass-fiber filters.

Results

Time course of uptake

The time courses of nitrate and ammonium uptake in *Thalassiosira pseudonana*, when each nitrogen source was added alone at a concentration of 5 μM to a nitrate-limited culture, were quite different (Fig. 2). Initial nitrate uptake rates were low and did not reach a maximum until 1.5 to 2 min after nitrate additions. In some experiments there was an initial efflux of nitrate at 0.5 min (noted more frequently by Yin 1988). It is unclear whether this possible efflux was real or an experimental artifact due to smearing in the cadmium column on the leading edge of the nitrate peak. Consequently, nitrate uptake rates before 1 min have been excluded from calculations. The variation between replicates and the response to experimental treatments was most variable from 1 to 3 min. Thus, nitrate uptake rates were averaged over 1 to 3 min and 4 to 9 min. Although most experiments were only

Table 1. *Thalassiosira pseudonana.* Steady-state characteristics. For each growth rate (μ), top row represents mean $\pm$ 1 SE and (number of measurements) made on same days as nitrate and ammonium inhibition experiments were conducted, second row gives data for day all chemical analyses were made at conclusion of all uptake experiments. For the nitrate-limited chemostat at $\mu = 1.25\ d^{-1}$*, first row is for period in which regular inhibition experiments were conducted, second row refers to experiments to determine mechanism of inhibition, and third row gives chemical analyses at conclusion of all uptake experiments. av vol: $\mu m^3\ cell^{-1}$; no. cells: cells $l^{-1} \times 10^8$; total vol: liter cell vol (liter culture vol)$^{-1} \times 10^{-5}$

N source, μ, (d^{-1})	$\mu:\mu_{max}$	In vivo fluor.	Cells			Chlorophyll ($\mu g\ l^{-1}$)		Particulate (μM)		External N (μM)		
			av vol.	no. cells	total vol.	*a*	*c*	N	C	NO_3^-	NO_2^-	NH_4^+
NO_3^-												
0.430 ±0.0244 (8)	0.23	4.02 ±0.152 (9)	32 ±3.8 (7)	9.3 ±1.45 (7)	2.62 ±0.149 (7)							
0.45	0.25	3.9	44	5.6	2.49	79.63 (1)	11.04 (1)	49.04 (2)	473.8 (2)	0.064 ±0.0174 (3)	0.078 ±0.0229 (3)	0.10 ±0.051 (3)
0.940 ±0.0110 (4)	0.50	4.80 ±0.230 (4)	53.1 ±2.08 (4)	3.8 ±0.50 (4)	2.05 ±0.166 (4)							
0.95	0.53	4.10	54.3	3.8	2.07	83.80 (1)	11.96 (1)	52.02 (2)	465.3 (2)	0.238 ±0.0115 (3)	0.112 ±0.0133 (3)	0.095 ±0.0240 (3)
1.25* ±0.0132 (3)	0.66	3.68 ±0.209 (3)	40.31 ±0.213 (3)	6.21 ±0.058 (3)	2.510 ±0.0184 (3)							
1.25* ±0.032 (5)	0.66	3.44 ±0.246 (6)	40.04 ±0.144 (3)	6.11 ±0.048 (3)	2.433 ±0.0233 (3)							
1.22	0.65	4.98	35.58	6.83	2.43	110.47 (1)	14.54 (1)	53.87 (2)	385.9 (2)	0.469 ±0.0127 (3)	0.294 ±0.0035 (3)	0.062 ±0.0111 (3)
1.89 ±0.057 (4)	1.00	7.7 ±0.38 (3)	41.9 ±2.04 (3)	6.8 ±0.73 (3)	2.9 ±0.44 (3)							
1.77	0.97	9.0	36.4	9.0	3.3	203.5 (1)	32.79 (1)	74.67 (2)	496.5 (2)	4.41 ±0.066 (3)	4.17 ±0.088 (3)	0.23 ±0.035 (3)
NH_4^+												
0.86 ±0.051 (3)	0.46	3.46 ±0.060 (3)	35.7 ±0.72 (3)	8.3 ±0.51 (3)	2.95 ±0.139 (3)							
0.76	0.42	3.50	31.3	9.3	2.91	72.671 (1)	9.38 (1)	58.86 (2)	462.4 (2)	0.0174 ±0.00069 (3)	0.051 ±0.0239	0.08 ±0.059 (3)

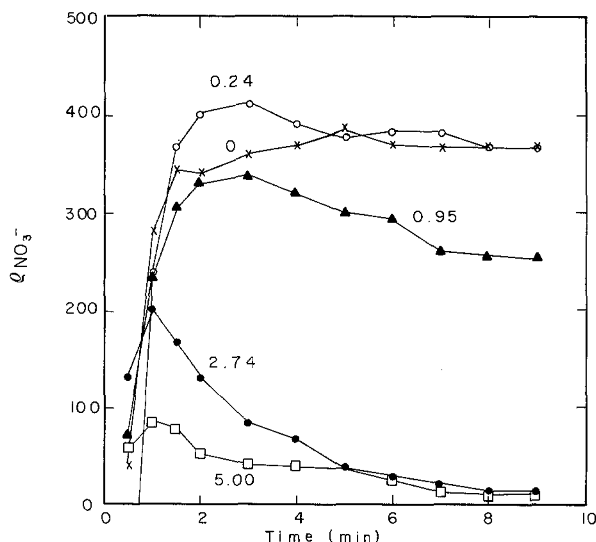


Fig. 3. *Thalassiosira pseudonana*; nitrate-limited, $\mu = 1.25\ d^{-1}$. Time course of nitrate uptake [$\varrho_{NO_3^-}$; mmol (liter cell vol)$^{-1}$ h^{-1}] when 5 μM was added in presence of different ammonium concentrations (0 to 5 μM), indicated by value adjacent to each curve. Some experiments were omitted from figure for clarity

carried out for 10 min, nitrate uptake rates remained constant or decreased slightly for up to 30 min.

In contrast, ammonium uptake rates were initially high and decreased to a relatively constant value between 4 and 6 min (Fig. 2). As with nitrate uptake, ammonium uptake was most variable in the first 0.5 to 2.0 min and, therefore, that period was separated from the subsequent 3 to 9 min.

The effect of ammonium on the time course of nitrate uptake depended on the ammonium concentration (Fig. 3). At low ammonium concentrations, nitrate uptake was either equal to or greater than that in the control, indicating stimulation. However, at intermediate ammonium concentrations ($\sim 1\ \mu M$) the initial (0.5 to 2 min) increase in nitrate uptake rate could not be maintained and the subsequent rates decreased. When the ammonium concentration was increased further, the initial increase in nitrate uptake became less pronounced and the subsequent decreases were more rapid. Finally, at 5 μM ammonium, nitrate uptake was reduced to a low, generally declining rate. This pattern was observed regardless of preconditioning growth rate or N source, although the magnitudes of the rates were dependent on preconditioning.

Preference for ammonium

Preference for nitrate or ammonium can be assessed by comparing uptake rates of each N source alone at 5 μM (Table 2). In all cases, ammonium uptake rates greatly exceeded nitrate uptake rates. The greatest preference was observed in the ammonium-limited culture, especially during the period of rapid ammonium uptake, and in the nitrate-sufficient culture. Among the nitrate-limited cultures, preference for ammonium decreased with increasing growth rate for both time periods, but the differences were not significant.

Table 2. *Thalassiosira pseudonana*. Comparison of nitrate and ammonium uptake rates [mmol (liter cell vol)$^{-1}$ h^{-1}] when each nitrogen source was added alone at 5 μM. Values are means ± 1 SD (number of replicate experiments/number of data points per experiment), 1 to 3, 4 to 9, 0.5 to 2, and 3 to 9 min after nitrate or ammonium addition. Maximum nitrate uptake [ϱ_{max} NO_3^- from Dortch et al. (1991); detailed in "Discussion – Preference for ammonium"]

Preconditioning N source, μ (d^{-1})	$\varrho_{NO_3^-}$ at 5 μM		$\varrho_{NH_4^+}$ at 5 μM		ϱ_{max} NO_3^-	
	1–3	4–9	0.5–2	3–9	1–3	4–9
NO_3^-						
0.43	408 ±56.9 (6/3)	430.1 ±13.87 (6/3)	964 ±62.5 (4/3)	651 ±122.2 (3/7)	738	751
0.94	400 ±49.1 (1/4)	409.2 ±5.60 (1/5)	902 ±78.0 (2/4)	575 ±99.4 (2/7)	864	764
1.25	333 ±34.8 (2/4)	372.2 ±6.39 (2/6)	728 ±50.2 (1/4)	461 ±70.2 (1/7)	1050	730
1.83	124 ±22.3 (4/4)	137.6 ±9.91 (4/6)	420 ±96 (2/4)	272 ±23.7 (2/7)	141	154
NH_4^+						
0.86	68.8 ±7.63 (3/4)	75.2 ±3.20 (3/6)	782 ±57.4 (1/4)	504 ±71.6 (1/7)	83	86

Inhibition of nitrate uptake by ammonium

Inhibition can best be expressed by comparing nitrate uptake rates in the presence of ammonium with those in its absence (Dortch 1990) (relative $\varrho_{NO_3^-}$; Fig. 4). At ammonium concentrations between 0.1 and 0.5 μM there was often, but not always, significant stimulation of nitrate uptake. Significant inhibition did not occur until ammonium concentrations $>1\ \mu M$, but even at 5 μM ammonium, nitrate uptake was not zero. Finally, the negative effects of ammonium were more apparent after 3 min than before 3 min.

In general, for nitrate-grown cultures the variation in degree of inhibition with growth rate was not pronounced. Stimulation of nitrate uptake by low concentrations of ammonium may be greater at high than at low growth rates. At the highest ammonium concentrations there was little variation in inhibition. In contrast, while growth on ammonium did not result in any greater stimulation in nitrate uptake at low ammonium concentrations, inhibition by high ammonium concentrations was not as severe.

Although inhibition (relative $\varrho_{NO_3^-}$) did not vary with μ in nitrate-grown cells and was less in ammonium-limited cells, absolute uptake rates showed a different pattern (Fig. 5), because preconditioning also affected nitrate up-

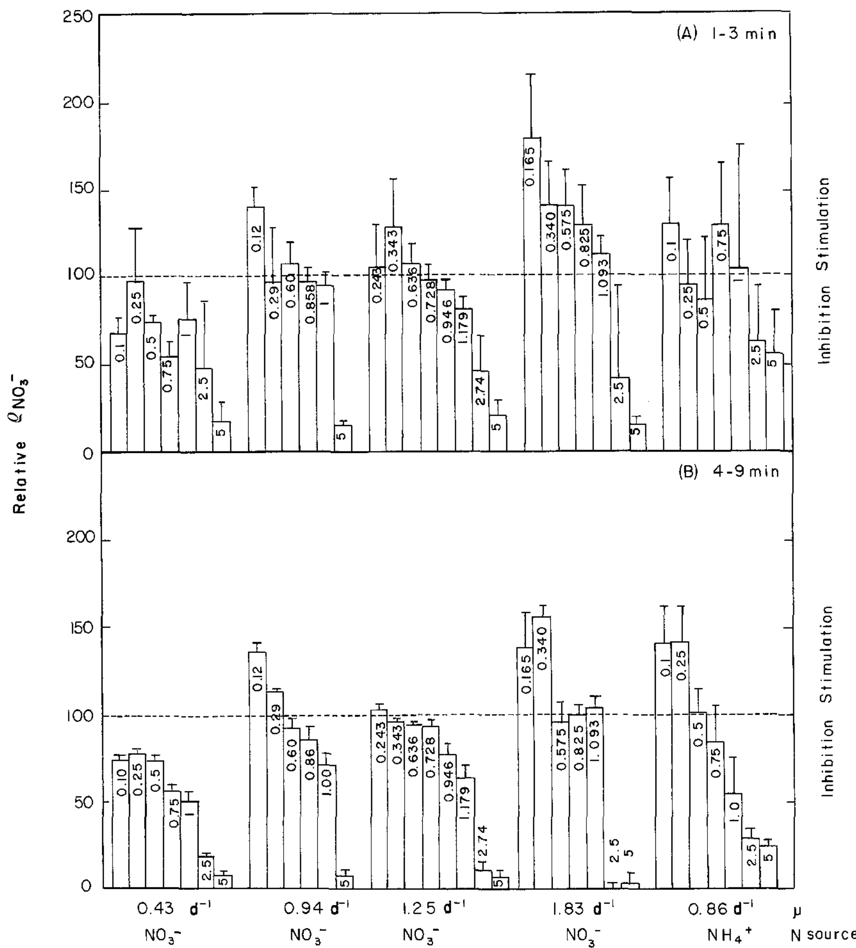
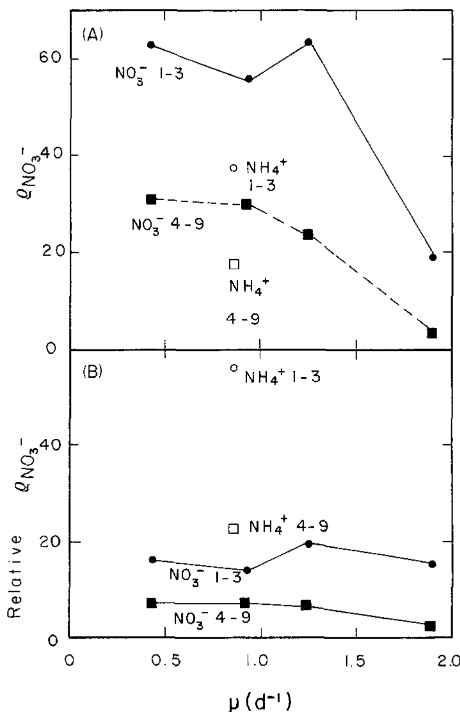


Fig. 4. *Thalassiosira pseudonana.* Effect of ammonium concentration (in μM on each histogram) on relative $\varrho_{NO_3^-}$ (%; nitrate uptake in presence of ammonium ÷ nitrate uptake in absence of ammonium × 100) over 1 to 3 min (A) and 4 to 9 min (B), as a function of preconditioning N source for growth and growth rate (μ; d^{-1}). Nitrate concentration was 5 μM for all experiments. Error bars indicate ±1 standard deviation



take rates in the controls without ammonium (Dortch et al. 1991). Thus, in the presence of 5 μM ammonium, absolute nitrate uptake rates were lower in ammonium-grown cells than in nitrate-grown cells at a similar μ (Fig. 5A), despite less inhibition (Fig. 5B). In addition, absolute uptake rates in the presence of ammonium decreased with increasing μ (Fig. 5A), even though the degree of inhibition was constant (Fig. 5B).

A series of experiments was conducted with the nitrate-limited chemostat at $\mu = 1.25\ d^{-1}$ to determine the mechanism of inhibition and the inhibition constant, K_I, for ammonium, which was suspected to be in the region of 1 μM. Unlike the other experiments, nitrate concentrations were varied (between 0.25 and 5 μM), as well as ammonium concentrations (Fig. 6). The intent was to

Fig. 5. *Thalassiosira pseudonana.* Nitrate uptake rates [$\varrho_{NO_3^-}$; mmol (liter cell vol)$^{-1}$ h^{-1}] (A) and relative nitrate uptake rates (see Fig. 4) (B) in the simultaneous presence of 5 μM nitrate and 5 μM ammonium, as a function of preconditioning N source (NO_3^-: ●, ■; NH_4^+: ○, □) and growth rate (μ; d^{-1}) and the time interval (1 to 3 min: ●, ○; 4 to 9 min: ■, □)

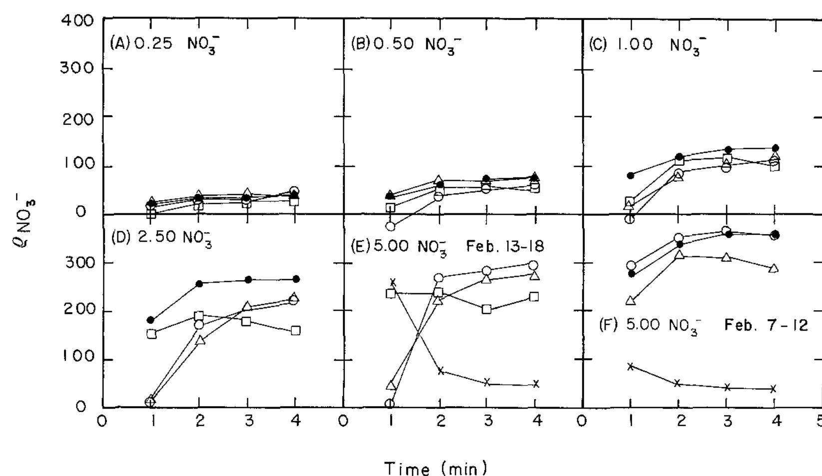


Fig. 6. *Thalassiosira pseudonana*; nitrate-limited, $\mu = 1.25\ d^{-1}$. Time course of $\varrho_{NO_3^-}$ [mmol (liter cell vol)$^{-1}$ h^{-1}] as a function of nitrate (μM) and ammonium [•: controls (0 μM); o: 0.5 μM; △: 1.0 μM; □: 1.5 μM; ×: 5 μM] concentrations (A)–(D). All experiments with ammonium run from 13–18 February; controls run from 7–12 February

then perform standard Michaelis-Menten and Lineweaver-Burke plots and determine the mechanism.

Neither plot was readily interpretable for four reasons that are obvious in retrospect: (1) The experiments were only run for 4 min and inhibition could be quite variable during that time. (2) Nitrate uptake was not saturated at 5 μM at this preconditioning growth rate (Dortch et al. 1991) and, therefore, higher nitrate concentration should have been included. (3) Stimulation and lack of inhibition were often observed at ammonium concentrations $<1\ \mu M$ and in this culture, possibly at 1.5 μM as well, especially if nitrate concentrations were low. (4) There was a shift in uptake rates which affected the controls. The controls (nitrate uptake in the absence of ammonium) were a regular series of experiments to determine nitrate uptake kinetics (Dortch et al. 1991), which were conducted several days before (7–12 February) these experiments to determine K_I and the inhibitory mechanism (13–18 February). Although there were no controls run during the latter period, there were three treatments common to both periods, shown in Fig. 6 E and F. In general the rates were lower from 13–18 February. For one treatment (5 μM nitrate and 0.5 μM ammonium), comparison of replicates for both time periods (9 replicates, 7–12 February; 3 replicates, 13–18 February) showed that the differences were significant. This is the only time such a shift in a culture was observed, but there was nothing in the steady-state data (Table 1) or the culture log-book to explain it. These experiments (13–18 February) were not included in the calculations of replication included in the "Materials and methods". The major consequence of the shift is that the controls may in fact be too high, which would decrease apparent inhibition or make stimulation more evident and further complicate the determination of inhibition kinetics.

Despite these problems with the experiments to determine the inhibitory mechanism, they showed that inhibition did not occur at low nitrate concentrations (Fig. 6A and B). The precise variation with nitrate concentration cannot be determined. It is clear, however, that at low nitrate and ammonium concentrations, no inhibition of nitrate uptake by ammonium occurred and, in fact, stimulation may have occurred.

Table 3. *Thalassiosira pseudonana*. Mean relative ammonium uptake in the presence and absence of nitrate (relative $\varrho_{NH_4^+}$; %) ± 1 SD. Number of experiments (# exp) with 5 μM NO_3^- and 5 μM NH_4^+/number of experiments with 5 μM NH_4^+ only are shown. Uptake measured from 0.5 to 2 min (4 measurements per experiment) and 3 to 9 min (7 measurements per experiment) after nitrogen addition. * Relative $\varrho_{NH_4^+}$ was significantly <100; one-tailed t-test, $P \leq 0.05$

Preconditioning N source, μ (d^{-1})	Relative $\varrho_{NH_4^+}$		# exp
	0.5–2	3–9	
NO_3^-			
0.43	97±1.6	96±1.8*	2/2
0.94	94±4.9	95±6.5	5/2
1.25	107±9.7	102±4.0	2/1
1.83	98±0.8*	104±4.2	3/2
NH_4^+			
0.86	106±4.2	113±8.2	3/1

Inhibition of ammonium uptake by nitrate

Inhibition of ammonium uptake by nitrate was determined by calculating relative ammonium uptake in the presence and absence of 5 μM nitrate (Table 3). Although in several cases ammonium uptake in the presence of nitrate was significantly less than that in its absence, the differences were very slight. Thus, it appears that in *Thalassiosira pseudonana*, nitrate has little effect on ammonium uptake, regardless of preconditioning μ or N source.

Discussion

Time course of uptake

The time courses of nitrate uptake in *Thalassiosira pseudonana* show that uptake rates were not constant and suggest that several processes occur simultaneously when nitrate and ammonium are both added. Initially nitrate uptake rates increased. A similar increase was also seen in controls without ammonium. It would be expected that

at the time the nitrate was first added to the cells, the uptake rate would be similar to that in the chemostat, but extrapolation of the initial nitrate uptake rates back to 0 min, even in the controls, gave negative uptake rates (nitrate excretion). The mechanism cannot be determined, but the time scale of the increase (0 to 2 min) is more indicative of derepression of nitrate uptake than induction. It is interesting that even though nitrate uptake rates in the ammonium-limited culture were quite low, the time courses of nitrate uptake with or without added ammonium were similar to those in the nitrate-grown cultures.

At the same time that derepression of nitrate uptake occurred, ammonium either stimulated (at low concentrations) or inhibited (at high concentrations) nitrate uptake. At the highest ammonium concentrations, inhibition of nitrate uptake was so great that the initial derepression was hardly observed. The fact that at each ammonium concentration the degree of inhibition of nitrate uptake increased with time suggests that it is the build-up of an intracellular nitrogen compound which inhibits nitrate uptake. Since external concentrations were constant for the duration of each uptake experiment, any change in rate must be controlled intracellularly.

The cells on the filter method for measuring uptake was used to separate the effect of ammonium on nitrate uptake from the effect on assimilation. The need to separate uptake and assimilation has been addressed by a variety of innovative methods: growing phytoplankton on nitrite so they contain no nitrate reductase (Eppley and Coatsworth 1968), isolation of cell-membrane fractions (Falkowski 1975), production of cells with non-functional nitrate reductase which contains tungsten substituted for the usual molybdenum (Serra et al. 1978), measurement of nitrogen disappearance from the medium and appearance in various forms in the cell (Cresswell and Syrett 1979, Dortch et al. 1979, Collos 1982, Dortch 1982), and use of $^{36}ClO_3^-$ as a radioactive and unassimilable analog of nitrate (Balch 1987). The problems with most of these approaches are that they often significantly alter the cell physiology and biochemistry or are difficult to relate to what happens in the real world, although they also give insight into some processes. By using the cells on the filter method, it was assumed that if uptake were measured over short enough time periods, the effect on assimilation would not yet have occurred. This assumption cannot be tested with the available data. Certainly, it is evident that the intracellular environment changed rapidly, especially in response to ammonium. Whether the changes in nitrate uptake were due to intracellular ammonium, a product of ammonium assimilation, or a change in nitrate assimilation cannot be evaluated. But it would be extremely difficult to measure nitrate uptake on any shorter time scales than are possible with this method. It has the added advantage of maintaining constant external N concentrations for the duration of the experiment.

Dortch and Conway (1984) hypothesized that total N uptake remained constant, but that the relative uptake of nitrate and ammonium varied depending on environmental conditions. The method they used for measuring uptake introduced such high variability in total uptake data that it was not possible to test this hypothesis. Using total N uptake rates at high and low ammonium concentrations from the experiments shown in Fig. 3 as an example, it can be seen that total N uptake was not constant (Table 4). For both time intervals, the total N uptake in the presence of high concentrations of ammonium was significantly greater than at low concentrations. This occurred because ammonium uptake rates were so much greater than nitrate uptake rates in this species even when each was added alone, i.e., there was a strong preference for ammonium (Dortch 1990).

Table 4. *Thalassiosira pseudonana*; nitrate-limited, $\mu = 1.25\ d^{-1}$. Mean total nitrogen (nitrate plus ammonium) uptake rate [mmol N (liter cell vol)$^{-1}$ h^{-1}] ± 1 SD (number of data points) in presence of 5 μM nitrate and either high (5.0 μM) or low (0.34 μM) ammonium

Time interval (min)	Total N uptake	
	5.0 μM NH_4^+	0.34 μM NH_4^+
0.5–2	840 ± 67 (4)	470 (2)
3–9	494 ± 67.0 (7)	388 ± 12.2 (7)

Preference for ammonium

The preference for nitrate or ammonium should be assessed by measuring the ϱ_{max} (or V_{max}) for uptake of each separately or, as an approximation, by measuring uptake of each separately at saturating concentrations (Dortch 1990). When these experiments were conducted it was known that ammonium uptake was saturated at 5 μM (Parslow et al. 1985) and assumed that nitrate uptake was saturated at this concentration also. However, subsequent analysis of the nitrate-uptake kinetics data showed that higher nitrate concentrations were required, particularly in the nitrate-limited cultures (Dortch et al. 1991). Thus, preference for nitrate or ammonium could only be assessed by comparing uptake rates of each alone at the same concentrations, 5 μM. In all cases ammonium uptake rates exceeded nitrate uptake rates.

Ammonium uptake rates at 5 μM can be assumed to represent ϱ_{max} for ammonium for a particular time period. If they are compared with the ϱ_{max} for nitrate (Table 2 from Dortch et al. 1991), a strong preference for ammonium is still apparent for the nitrate-sufficient and ammonium-limited cultures, but not the nitrate-limited cultures. Thus, it can be concluded that in *Thalassiosira pseudonana* there is a preference for ammonium, with the caveat that the preference is dependent on nitrate concentrations and preconditioning.

Comparison with other species is difficult because of the lack of data. Dortch (1990) hypothesized that preference for ammonium would be greater in N-deficient than N-sufficient phytoplankton. While such a pattern is observed in four species (Dortch et al. 1982, Dortch and Conway 1984, Syrett et al. 1986), it is not true of *Thalassiosira pseudonana* (Eppley and Renger 1974, and present study). There are even fewer studies in which preference

as a function of preconditioning N source was determined. In N-limited *Skeletonema costatum*, the preconditioning N source does not affect preference (Dortch 1980), whereas in N-limited *T. pseudonana* (present study), preference for ammonium is greater when ammonium is the preconditioning N source.

Stimulation

The lack of inhibition of nitrate uptake at ammonium concentrations $<1\ \mu M$ was expected, but the occurrence of possible stimulation was a surprise. A search of the literature turned up several other instances of stimulation of nitrate uptake at low ammonium concentrations (Conover 1975, Caperon and Ziemann 1976, Glibert et al. 1982, Yin 1988). In all cases the ammonium concentrations which result in stimulation are approximately the same as the often-quoted threshold value for the inhibition of nitrate uptake by ammonium (summarized in Dortch 1990).

Inhibition

At ammonium concentrations $>1\ \mu M$, nitrate uptake was inhibited in comparison with controls without ammonium. The degree of inhibition did not vary as a function of growth rate for cultures grown on nitrate, but there was less inhibition in the ammonium-limited culture. Both results differ from studies with other species. Usually inhibition varies inversely with the degree of N deficiency, although the reverse also occurs (Dortch 1990). The effect of the preconditioning N source has only been examined in two species (Dortch and Conway 1984). In *Skeletonema costatum* inhibition was less in cultures growing on nitrate than on ammonium, but in *Chaetoceros debilis* there was no difference. However, for *Thalassiosira pseudonana* inhibition was less for ammonium-grown cells. These varying results serve to point out the significant species differences in inhibition which cannot at present be explained or predicted.

Nitrate inhibition of ammonium uptake has been observed, usually incidentally to other studies. (Caperon and Ziemann 1976, Ohmori et al. 1977, Terry 1982, Dortch and Conway 1984). In other deliberate attempts to study it, it has not occurred (Kuenzler et al. 1979, Zevenboom and Mur 1981, Lund 1987). Similarly, ammonium uptake in *Thalassiosira pseudonana* was unaffected by nitrate.

Biochemical mechanisms

The greater preference for ammonium probably results from the higher V_{max} and lower K_S for ammonium uptake (Parslow et al. 1984a) than for nitrate uptake (Dortch et al. 1991), which allows more rapid ammonium uptake at any concentration. Variations in preference as a function of preconditioning arise from its effect on the kinetics of uptake. Another factor determining preference, especially in short-term uptake experiments, is that ammonium is taken up at maximal, in fact very high rates, immediately. In contrast, nitrate uptake begins slowly and reaches its maximum rate several minutes later, suggesting derepression. In the case of severe N starvation, nitrate uptake may not occur for a number of hours in some species, implying that nitrate uptake capacity must be induced (Dortch et al. 1982, Parslow et al. 1984b). Thus, preference is determined by specific biochemical mechanisms.

These studies provide considerable information about inhibitory mechanisms and the question of external vs intracellular control (Dortch and Conway 1984, Collos 1989). In one series of experiments an attempt was made to determine whether external ammonium was a competitive or non-competitive inhibitor of nitrate uptake and the K_I, but the stimulation of nitrate uptake at low concentrations of ammonium in particular made the results uninterpretable in terms of inhibition kinetics. An alternative approach, using a linear model based on classical competitive enzyme inhibition kinetics developed by Collos (1989), was also taken. Good linear fits to the model were obtained ($r^2>0.75$), which indicated that much of the variation in nitrate uptake rates could be explained by variations in the relative concentrations of external nitrate and ammonium and phytoplankton biomass. However, the slopes for experiments at each preconditioning growth rate and N source were very different, suggesting that intracellular control was a major factor. Furthermore, the intercepts indicated nitrate excretion when nitrate made up approximately less than 50% of the external N. This last result is difficult to interpret, but indicates that the model may not be as appropriate for *Thalassiosira pseudonana* as it is for other species (Collos 1989). Two other lines of evidence implicate intracellular control: (1) When nitrate and ammonium are added simultaneously, several minutes are required for the full inhibitory effect, as if the ammonium must be taken up and some inhibitor produced. (2) Inhibition varies with preconditioning N source, which could only occur if there is intracellular control. Thus, while external ammonium certainly influences nitrate uptake, intracellular control also occurs. Studies with possible regulatory compounds will be reported elsewhere (Dortch et al. unpublished data).

Ecological significance

Nitrate and ammonium uptake in *Thalassiosira pseudonana* are very complex processes. The relative and absolute rates of nitrate and ammonium uptake are highly dependent on both present and past environmental conditions and would be extremely difficult to predict. This species shows a nearly classical interaction between nitrate and ammonium uptake, i.e., preference for ammonium and inhibition of nitrate uptake by ammonium but not of ammonium uptake by nitrate. Yet under most environmental conditions likely to be encountered in the field, including the entire range of normal ambient ammonium concentrations, nitrate uptake will occur at rea-

sonable rates, which will sometimes exceed ammonium uptake rates.

The species differences in the interaction between nitrate and ammonium uptake described in Dortch (1990) make it impossible to extrapolate from the detailed study of this single diatom to the response of other phytoplankton, even small diatoms. In order to generalize about how ammonium availability and nitrogen supply will affect nitrate uptake and new production in the field, similar detailed studies must be made on other phytoplankton.

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