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Interactions between NH_4^+ and NO_3^- uptake and assimilation: comparison of diatoms and dinoflagellates at several growth temperatures

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Abstract Ammonium concentrations of ~1 μ*M* are commonly cited as being the threshold for inhibition of NO_3^- uptake, but the applicability of this threshold to phytoplankton from different taxonomic classes has rarely been examined. Additionally, little is known about the influence of environmental variables (e.g. growth temperature) on the interaction between ambient NH_4^+ and NO_3^- uptake. Four species of estuarine phytoplankton, two diatom [*Chaetoceros* sp., and *Thalassiosira weissflogii* (Grunow) Fryxell et Hasle] and two dinoflagellate [*Prorocentrum minimum* (Pavillard) Schiller, and *Gyrodinium uncatenum* Hulburt], were grown on NO_3^- at several different temperatures (4, 10, 15, or 20 °C), and the impact of NH_4^+ additions on NO_3^- uptake/assimilation (non-TCA-extracted) and assimilation (TCA-extracted) was assessed. For all species at all temperatures, NO_3^- uptake/assimilation and assimilation rates decreased in a roughly exponential manner with increasing NH_4^+ concentrations but were not completely inhibited even at elevated NH_4^+ concentrations of 200 μ*M*. Estimated half-inhibition concentrations (K_i) were significantly greater in the diatom species (mean ± SE; 2.70 ± 0.67 μ*M*) than in the dinoflagellate species (1.26 ± 0.55 μ*M*). Half-inhibition constants were positively related to temperature-limited relative growth rate although not significantly. The observed inhibition of NO_3^- uptake and assimilation, as a percentage of NO_3^- uptake in the absence of NH_4^+, averaged about 80% and ranged from 49 to 100%. For all species, a significant ($P < 0.001$) positive correlation was found between percent inhibition of NO_3^- assimilation and temperature-limited relative growth rate. Two experiments on Chesapeake Bay phytoplankton during an April 1998 diatom bloom showed that in short-term (~1 h) temperature manipulation experiments, percent inhibition of NO_3^- uptake/assimilation was also positively related ($P = 0.05$) to experimental temperature. The observed relationships between temperature-limited relative growth rate and percent inhibition of NO_3^- assimilation rates for the species tested suggest that at the enzyme level, the inhibitory mechanism of NO_3^- assimilation is similar among species, but at the whole cell level may be regulated by species-specific differences in the accumulation of internal metabolites. These findings add not only to our understanding of species-specific variability and the role of growth temperature, but also provide additional data with which to evaluate current models of NH_4^+ and NO_3^- interactions.

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Introduction

Phytoplankton ecologists have generally assumed that NH_4^+ will be used preferentially over NO_3^- when both substrates are available (e.g. Dugdale 1976; McCarthy 1981; Syrett 1981). It is also believed that the uptake and assimilation of NH_4^+ inhibits the uptake/assimilation of NO_3^- with complete inhibition of NO_3^- uptake occurring at NH_4^+ concentrations >1 μ*M* in both culture (e.g. Eppley et al. 1969) and field experiments (e.g. McCarthy et al. 1975). However, Dortch (1990) noted that many studies have not included the appropriate controls to unequivocally assess the interaction between NH_4^+ and NO_3^- uptake, which led her to conclude that NH_4^+ inhibition of NO_3^- uptake was not as common as generally assumed.

Although a great deal is known about NH_4^+/NO_3^- interactions in selected species, relatively few studies (Eppley et al. 1969; Flores et al. 1980; Terry 1982; Dortch and Conway 1984; Maestrini et al. 1986) have compared multiple species, and only one of these studies (Eppley et al. 1969) has compared species from different taxonomic classes. Dortch et al. (1991) commented on "...the apparent enormous species variation in the interaction between nitrate and ammonium uptake...", but suggested that much of this variation might be due to differences

in methodology among studies. Additionally, the lack of a uniform (and clear) distinction between NH_4^+ inhibition of NO_3^- uptake (e.g. Cresswell and Syrett 1979; Florencio and Vega 1982) and NO_3^- assimilation (e.g. Syrett and Morris 1963) likely also contributes to this variability. The lack of generalizations about classes of phytoplankton as a whole greatly restricts the ability to predict and interpret NH_4^+/NO_3^- interactions in the field. Consequently, field studies where NO_3^- and NH_4^+ uptake rates were equal at NH_4^+ concentrations of ~10 μ*M* (e.g. Maestrini et al. 1982, 1986) and where NO_3^- uptake rates were negligible at NH_4^+ concentrations of >1 μ*M* (McCarthy et al. 1975) have remained largely unexplained with respect to each other.

The importance of nutrient preconditioning, growth rate (Dortch and Conway 1984; Dortch et al. 1991), and irradiance (Bates 1976) in regulating NH_4^+/NO_3^- interactions are known, but as yet growth temperature has not been considered as a regulatory variable. Two important conclusions have emerged from these studies: phytoplankton preconditioned to NH_4^+ are more susceptible to NH_4^+ inhibition of NO_3^- uptake, and NH_4^+ inhibition increases with decreasing growth rate (where nitrogen availability limits growth rate). In addition, high light conditions appear to lessen the NH_4^+ inhibition of NO_3^- uptake. Field studies that have observed high NO_3^- uptake rate at high NH_4^+ concentrations (e.g. Maestrini et al. 1982, 1986; Quéguiner et al. 1986) were conducted when water temperatures were < 20 °C and NO_3^- concentrations were high. These studies suggest the potential importance of growth temperature in NH_4^+/NO_3^- interactions.

The present study examined NH_4^+ inhibition of NO_3^- uptake in estuarine diatoms and dinoflagellates under identical growth conditions, and the importance of growth temperature to this interaction. We asked two specific questions. First, are there general differences in the half-inhibition concentration (K_i) and percent inhibition between diatoms and dinoflagellates? Second, is there a relationship between the degree of NO_3^- uptake inhibition and growth temperature? We hypothesize that although the exact mechanism by which NH_4^+ inhibits NO_3^- uptake/assimilation may be the same across taxa at the enzyme level, other species-specific characteristics modify the expression of the mechanism at the whole cell level. These data are discussed in terms of current models of NH_4^+ and NO_3^- uptake interactions.

Materials and methods

Culture conditions

Cultures of the diatoms *Chaetoceros* sp. and *Thalassiosira weissflogii* (Grunow) Fryxell et Hasle, and the dinoflagellates *Prorocentrum minimum* (Pavillard) Schiller and *Gyrodinium uncatenum* Hulburt were isolated from Chesapeake Bay by A. Lewitus and are currently maintained in the Horn Point Laboratory culture collection. Each species was grown on *f*/2 enriched river water medium (12.4 PSU, Guillard 1983) with nitrogen added as $NaNO_3^-$ at *f*/20 concentrations. All cultures were grown at 180 μE m^{-2} s^{-1} on a 14 h light:10 h dark cycle with the light period starting at 0600 hrs. *Chaetoceros* sp. and *P. minimum* were grown at 20, 10, and 4 °C, *T. weissflogii* was grown at 20 and 10 °C, and *G. uncatenum* was grown at 20 and 15 °C. The latter two species did not grow at 4 °C, and *G. uncatenum* did not grow at 10 °C. Consequently, there are a total of ten experiments presented in this study: five conducted on diatoms and five conducted on dinoflagellates. Each culture was grown through at least five generations to ensure acclimation to the growth temperature before being used in the experiments. Growth rates for each species at each temperature were determined by cell counts on a Coulter Multisizer II. Microscopic examination of each culture showed that the diatoms were cylindrical with the cell diameter and total valve height being similar. The dinoflagellates were roughly spherical. Therefore cell volumes were determined using the equations for a cylinder and sphere with diameters determined from the multisizer. For each experiment, live cell samples were taken for estimation of cell diameter and density during the course of the sample incubation.

Experiment protocol

After each culture was acclimated to the growth conditions, the volume of culture was scaled up to 2.5 liters by dilution with fresh medium over several transfers. This "ramping up" of the culture volume allowed cells to remain in exponential growth phase. On mornings on which experiments were conducted, 50 μ*M* NO_3^- was added. Measured concentrations of NO_3^- after this addition were between 90 and 110 μ*M*. One hour before the lights came on, 200-ml subsamples were partitioned, and NH_4^+ was added at the following concentrations: 0, 2, 4, 8, 10, 20, 25, 50, 100, and 200 μ*M*. The subsamples were returned to the growth incubator. After 2 h, $^{15}NO_3^-$ (as $NaNO_3^-$ and 99% enriched) was added at ~10% of initial NO_3^- concentrations and incubated for an additional hour. From each 200 ml subsample, three 50 ml aliquots were filtered onto 42 mm precombusted (450 °C, 1 h) Poretics glass fiber filters (GF-75) and washed copiously with isotonic NaCl solution. At this point, one filter was frozen, one filter was treated with 10% ice-cold trichloroacetic acid (TCA), and the last filter was extracted with boiling distilled water with the extract being kept for determination of internal nitrogen pools (Thoresen et al. 1982). The first filter represents both the uptake and assimilation of NO_3^-, and the TCA-extracted filter represents only the $^{15}NO_3^-$ assimilated into proteinaceous material. Only one species/growth temperature combination was conducted each day so all experiments were conducted at the same time of day, unconfounded by diel patterns of metabolic processes.

Field experiment

Between 16 and 17 April 1998, two experiments were conducted during a spring diatom bloom in Chesapeake Bay. Between 0700 and 1000 hrs each morning, water samples were collected from mid-Chesapeake Bay (38°00′N; 076°12′W) and dispensed among tubes in a temperature gradient block ± 8 °C of ambient temperature (Lomas and Glibert 1999). After 10 to 15 min of acclimation to the experimental temperature, $^{15}NO_3^-$ was added to triplicate samples at 100 μ*M* with no added NH_4^+ and one sample with 50 μ*M* added NH_4^+. Samples were incubated for 1 h and then processed as described in the culture experiment section. Ambient nutrient samples were also collected and analyzed as below.

Sample analysis

Known volumes of culture (or field sample) were filtered onto 25 mm precombusted (450 °C, 1 h) Poretics glass fiber filters for chlorophyll *a* (chl *a*) and particulate carbon/nitrogen (PC/PN) analyses immediately after the initiation of the ^{15}N incubation. Samples for chl *a* were frozen at −20 °C until analysis, and samples for PC/PN were dried overnight at 50 °C. The filtrates were frozen immediately at −20 °C for later analysis of ambient

NO_3^- concentrations. Concentrations of PC/PN were determined on a Control Equipment Elemental Analyzer using acetanilide as a standard. Particulate N values were also determined on the filtered ^{15}N sample without TCA extraction using a mass-pressure calibrated inlet system for a Nuclide mass spectrometer with NO_3^- as the standard. For the range of these PN determinations, ~1 to 4 μmol N, relative standard deviations for the calibrated inlet system were <5% for duplicate standards. Comparison of PN values determined by these two methods showed no significant difference (slope of regression line 1.075 ± 0.089, $R^2 = 0.96$, $n = 10$, $P > 0.33$).

Samples for chl *a* analysis were ground in 90% acetone on ice, and concentrations determined fluorometrically (Parsons et al. 1984) on a Turner Designs Model 10 fluorometer calibrated against an HPLC (high-performance liquid chromatography) measured chl *a* standard. Ambient NH_4^+ concentrations in the field experiments were determined using the phenol hypochlorite method of Parsons et al. (1984). Concentrations of NO_3^- were determined using the "spongy" cadmium method of Jones (1984). Briefly, cadmium metal (0.2 g) was added directly to 5-ml samples with 1 ml of a neutralized ammonium chloride solution (4.7%) in 15-ml centrifuge tubes (Corning No. 25319–15). Samples were placed on a lateral shaking table for 60 min at 100 oscillations min^{-1}. The cadmium was removed, and the color was developed with a combined sulfanilimide and *N*-(1-napthyl)-ethylenediamine dihydrochloride reagent. The absorbance was read at 540 nm within 2 h. All nutrient samples were analyzed within 3 wk of collection. The limit of detection for this NO_3^- analysis method is 0.03 μ*M* for triplicate samples.

Samples for isotopic composition were prepared for analysis using the general procedures of Fiedler and Proksch (1975). Samples were ground with copper oxide (Baker No.1820–05, prepared for use by combusting at 600 °C for 3 h), placed into Pyrex glass ampoules (precombusted at 450 °C for 1 h) with copper metal accelerator (Alpha Resources Inc.), evacuated and sealed. Samples were combusted at 550 °C for 2.5 h and then analyzed on a Nuclide mass spectrometer (Glibert et al. 1991). Precision of triplicate standard samples was ± 0.001 at.%, with a 99.7% recovery of calculated standard additions. Absolute uptake rates were calculated according to the formulas of Dugdale and Goering (1967), and were not corrected for isotope dilution as little dilution would be expected at the relatively high concentrations used (Glibert et al. 1982).

Calculation of K_i and percent inhibition

Values for K_i and percent inhibition of NO_3^- uptake were determined by using a variation of the Michaelis–Menten equation (Eq. 1, present study; Harrison et al. 1996) following normalization of the NO_3^- uptake rate data to the NO_3^- uptake rate measured in the absence of NH_4^+:

$$\rho_{NO3}(\mathrm{rel}) = \left[1 - \left(\frac{\%I \times [NH_4^+]}{K_t + [NH_4^+]}\right)\right], \quad (1)$$

where $[NH_4^+]$ is the added NH_4^+, ρ_{NO3}(rel) is the relative NO_3^- uptake rate where values range from 1 to 0, $\%I$ is the maximum percent inhibition of NO_3^- uptake by NH_4^+, and K_i is the half-inhibition concentration of NH_4^+. In this context, $\%I$ is comparable to $\%I$ of Dortch and Conway (1984, their Eq. 1) although calculated differently.

Results

Phytoplankton growth rate, chemical composition, and internal N pools

With the exception of *Gyrodinium uncatenum*, growth rates for *Chaetoceros* sp., *Thalassiosira weissflogii*, and *Prorocentrum minimum* were exponential with respect to growth temperature, and exhibited Q_{10} values for growth between 1.95 and 2.46 (Fig. 1). Growth of *G. uncatenum* was very slow at 15 °C which prohibited calculation of a Q_{10} value. Generally, each species increased in mean cell diameter with decreasing temperature, except for *Chaetoceros* sp. which decreased slightly in diameter at 4 °C (Table 1). The increase in size was most dramatic in *P. minimum* with an increase in cell diameter from 12.8 to 17.2 μm as growth temperature

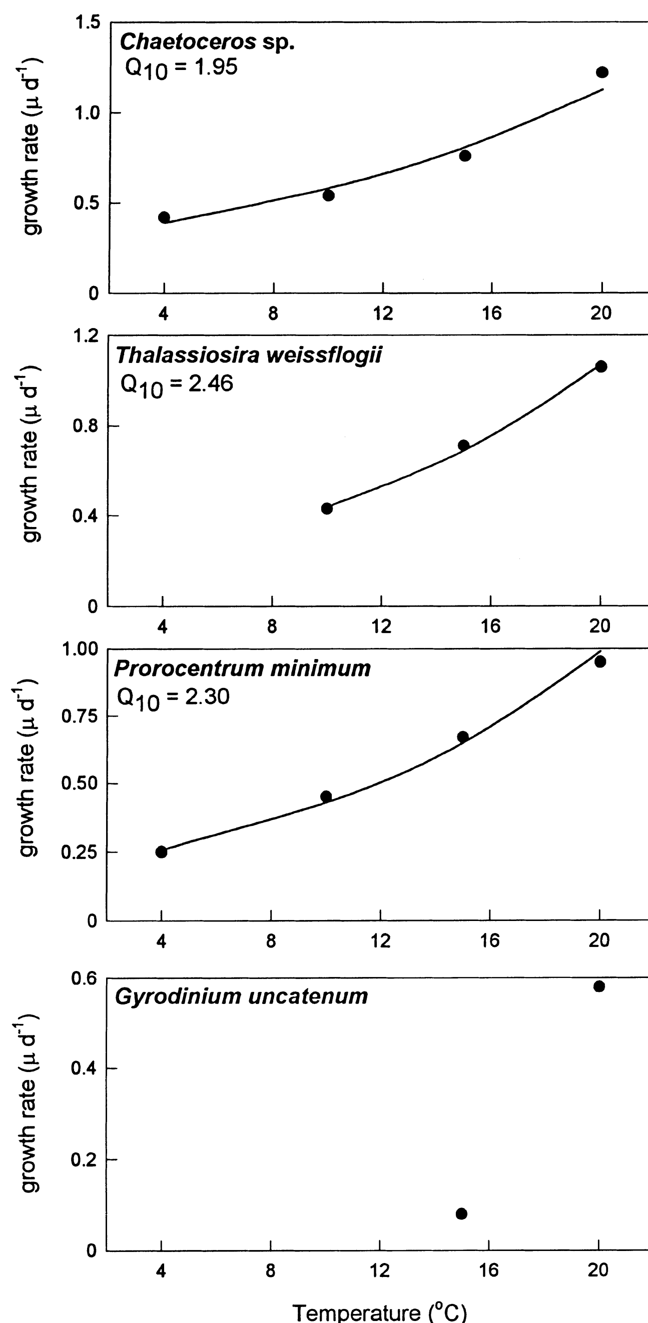

Fig. 1 Growth rates of the species examined in this study as a function of growth temperature. Solid line represents the model $\mu = \alpha \cdot \exp(\beta \cdot T)$

Table 1 Summary of growth and chemical composition data for two species of diatoms (*Chaetoceros* sp., *Thalassiosira weissflogii*) and two dinoflagellates (*Prorocentrum minimum*, *Gyrodinium uncatenum*) for each growth temperature. Mean values for each parameter is followed by SE in parentheses. Cellular C:N ratios were determined using cell carbon and total cell nitrogen values

Parameter	*Chaetoceros* sp.			*Thalassiosira weissflogii*		*Prorocentrum minimum*			*Gyrodinium uncatenum*	
	4 °C	10 °C	20 °C	10 °C	20 °C	4 °C	10 °C	20 °C	15 °C	20 °C
Growth rate (d^{-1})	0.42	0.54	1.22	0.43	1.06	0.25	0.45	0.95	0.08	0.58
Cell diameter (μm)	4.1	4.4	4.5	12.9	12.1	17.2	14.5	12.8	32.5	30.2
	(0.01)	(0.11)	(0.03)	(0.11)	(0.05)	(0.18)	(0.78)	(0.09)	(0.48)	(0.11)
Cell volume (μm^3)	52	66	71	1680	1385	2685	1642	1109	17942	14470
	(0.2)	(4.9)	(1.4)	(43.3)	(15.5)	(89)	(271)	(22)	(805)	(155)
Cell carbon	11.68	10.70	13.42	501.83	102.09	656.36	601.54	495.40	9712.5	9335.42
(pg $cell^{-1}$)	(0.35)	(1.15)	(0.33)	(2.13)	(5.69)	(54.24)	(19.55)	(33.53)	(426.79)	(900.09)
Total cell nitrogen	1.53	1.88	1.74	154.11	16.75	107.5	91.69	99.08	906.7	1510.6
(pg $cell^{-1}$)	(0.01)	(0.03)	(0.04)	(7.47)	(0.26)	(2.29)	(1.03)	(1.07)	(9.15)	(43.09)
TCA-insoluble cell	1.32	1.58	1.51	99.9	15.40	96.34	83.00	79.7	813.7	1281.1
nitrogen (pg $cell^{-1}$)	(0.026)	(0.01)	(0.01)	(6.31)	(0.27)	(0.77)	(0.69)	(1.05)	(9.63)	(15.16)
C:N ratio	7.63	5.69	7.713	3.26	6.09	6.11	6.56	5.00	10.71	6.18
Cell chl *a*	0.42	0.44	0.62	2.52	4.75	13.30	6.49	60.44	69.7	286.6
(pg $cell^{-1}$)	(0.01)	(0.01)	(0.01)	(0.19)	(0.16)	(1.38)	(0.36)	(1.02)	(4.32)	(21.11)
C:chl ratio	27.8	24.3	21.6	199.1	21.5	49.4	92.7	8.2	139.3	32.6

was reduced from 20 to 4 °C. Cellular carbon quotas increased with decreasing temperature (i.e. increasing size) as expected (Table 1). Total cellular nitrogen quotas did not fit a general pattern. For all species, there was a difference between total cellular nitrogen quota calculated using the PN values from non-TCA-extracted and TCA-insoluble nitrogen quotas calculated using the PN values from TCA-extracted filters due to the inclusion of internal nitrogen pools in the non TCA-extracted samples (Table 1). For the dinoflagellates, this internal nitrogen pool constituted 10 to 15% of the cellular nitrogen. The diatoms exhibited different patterns, with the internal pool constituting 15 to 25% of the cellular nitrogen in *Chaetoceros* sp., and 10 to 60% in *T. weissflogii*, the higher percentage being at 10 °C. The C:N ratio (using total cell nitrogen) was relatively conservative as a function of growth temperature for *Chaetoceros* sp. and *P. minimum*, and was consistent with the Redfield ratio of 6.6 (Redfield et al. 1963). The other two species, however, exhibited dramatic changes in the C:N ratio between the two experimental temperatures. *G. uncatenum* exhibited a 75% increase in the C:N ratio at 15 °C, whereas *T. weissflogii* exhibited a 50% decrease in C:N ratio to a value of 3.26. As the light regime was the same at each growth temperature, cellular chl *a* levels decreased with decreasing growth temperature, resulting in a general increase in the cellular C:chl ratios with decreasing temperature.

Internal NO_3^- and NH_4^+ pools ranged from non-detectable to a maximum of ~100 m*M* for *Chaetoceros* sp., *Thalassiosira weissflogii*, and *Prorocentrum minimum* as NH_4^+ concentration increased (Fig. 2). Neither internal NO_3^- nor internal NH_4^+ were detected in *Gyrodinium uncatenum* and therefore this species is omitted from Fig. 2. Internal NO_3^- in *Chaetoceros* sp. was undetectable at 20 °C, averaged ~5 m*M* at 10 and 4 °C, and was relatively independent of external NH_4^+ concentrations. Internal NH_4^+ pools were greater than internal NO_3^- pools in this species and tended to increase with external NH_4^+ concentration. *T. weissflogii*, on the other hand, had greater internal NO_3^- pools than internal NH_4^+ pools, in most cases. Internal NO_3^- pools at 20 °C were 22 m*M* and decreased slowly with increasing external NH_4^+ concentration to about 15 m*M*. At 10 °C, internal NO_3^- pools were ~5 m*M*, whereas internal NH_4^+ pools were undetectable. Internal NO_3^- pools for *P. minimum* were undetectable at 10 and 4 °C, while at 20 °C the internal NO_3^- pool started at 20 m*M* and quickly decreased to 10 m*M* as NH_4^+ concentration increased.

NH_4^+ inhibition of NO_3^- uptake: comparison between diatoms and dinoflagellates

Diatoms exhibited a different response from the dinoflagellates with respect to short-term NH_4^+ inhibition of NO_3^- uptake. Nitrate uptake rates, as a function of added NH_4^+, decreased in a near exponential manner for each culture at all growth temperatures. The patterns of decrease in NO_3^- uptake rates at 20 °C are representative of patterns at other growth temperatures (Fig. 3). Values of K_i of NH_4^+ for each culture for both uptake/assimilation (non-TCA-extracted) and assimilation only (TCA-extracted) ranged from 0.24 to 4.64 μ*M* (Table 2). Pooling all the experiments for each taxonomic group showed that diatoms had significantly higher K_i concentrations than dinoflagellates for both uptake/assimilation (P = 0.033) and assimilation (P = 0.028) (Fig. 4A). Percent inhibition of NO_3^- uptake, however, was not significant for either uptake/assimilation or assimilation (Fig. 4B). More importantly, even at NH_4^+ concentrations of 200 μ*M*, NO_3^- uptake rates were only inhibited by an average 80%. Separating the experi-

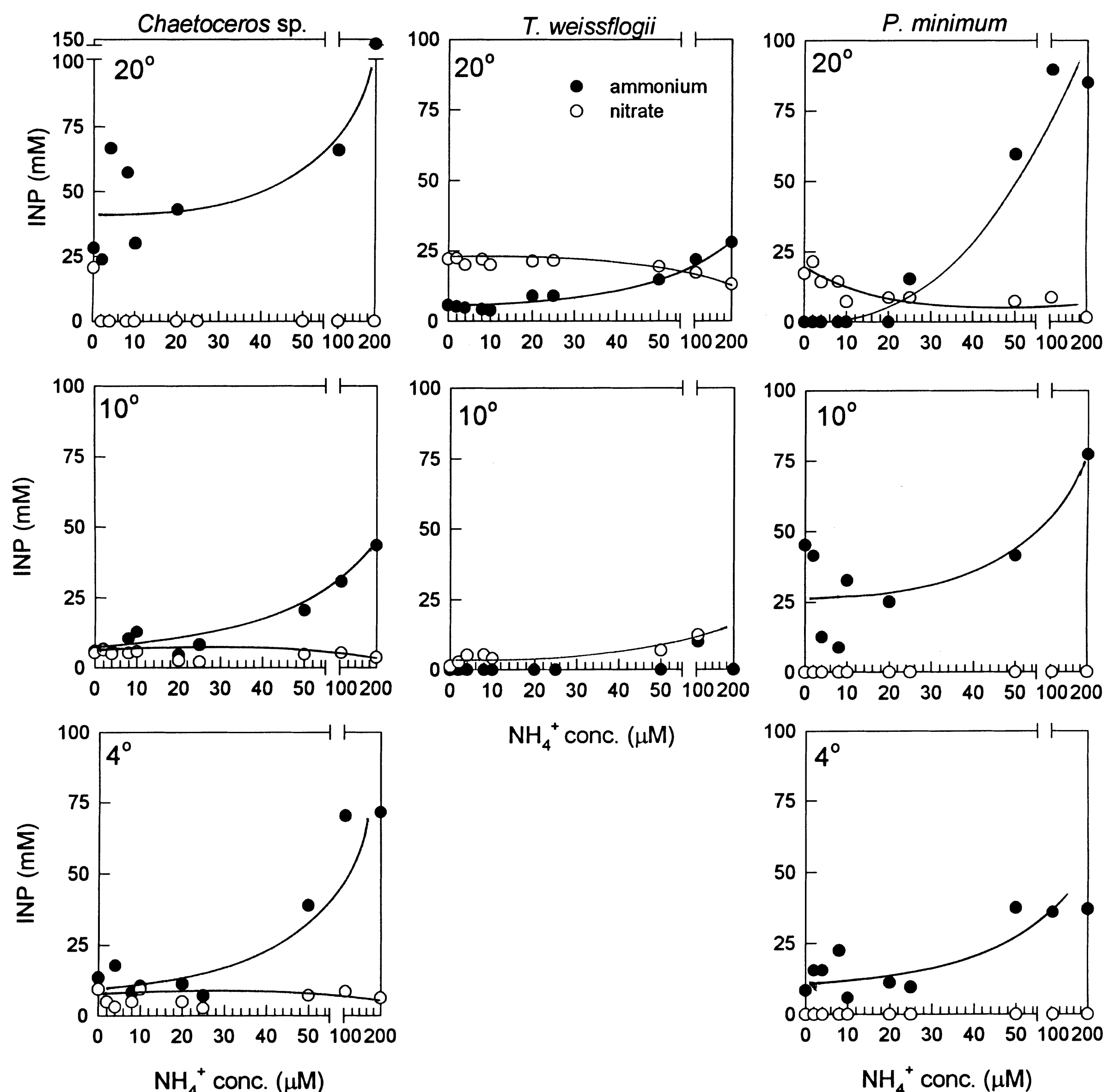

Fig. 2 Internal NO_3^- (○) and NH_4^+ (●) pools (*INP*) for *Chaetoceros* sp., *Thalassiosira weissflogii* and *Prorocentrum minimum* grown at the temperatures indicated in the panels. Lines fitted by eye only to show trends in internal pool sizes and do not represent any mathematical curve fitting

ments into the four individual species failed to show significant differences (likely due to small number of degrees of freedom; Table 2).

NH_4^+ inhibition of NO_3^- uptake: temperature effects

Inhibition by NH_4^+ of NO_3^- uptake/assimilation and assimilation, as a percentage of the rate determined in the absence of NH_4^+, decreased with temperature for all species, with the exception of uptake/assimilation by *Gyrodinium uncatenum* which increased slightly (Table 2). Values for K_i generally increased with decreasing growth temperature, although substantial scatter was present in the data. Species-specific differences in the formation of internal NO_3^- pools might complicate interactions between NH_4^+ and NO_3^- uptake/assimilation and growth temperature, and therefore only NO_3^- assimilation rates (determined from TCA-extracted samples) were considered as a function of growth temperature. Percent inhibition of NO_3^- assimilation rates for all species decreased as a function of growth temperature. As all species exhibited a similar relationship between growth temperature and percent inhibition of NO_3^- assimilation, all data were pooled and found to be significantly related to temperature-limited relative growth rate (i.e. relative to growth at 20 °C; Fig. 5).

Nitrate concentrations in the field study ranged from 25 to 30 μ*M*, and with the addition of 100 μ*M* $^{15}NO_3^-$ were similar in final concentration to those used in the culture component of this study. The high addition of NO_3^- was used to facilitate comparisons between the

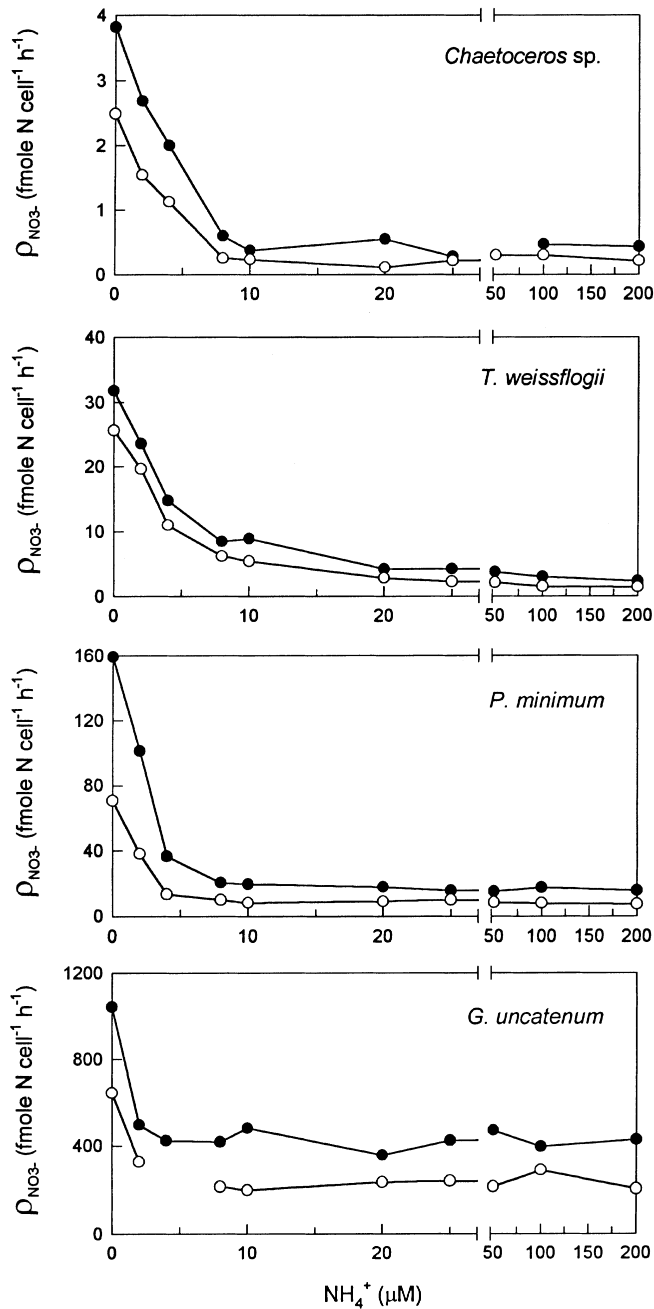

Fig. 3 Representative patterns of NO_3^- uptake as a function of added NH_4^+ for each species at 20 °C. Both non-TCA-extracted NO_3^- uptake rates (●) and TCA-extracted NO_3^- uptake rates (○) are shown

field and culture components. Ambient NH_4^+ concentrations during the field study ranged from 2.16 to 3.02 μM, and consequently NO_3^- uptake rates were almost assuredly inhibited to some degree prior to the addition of the NH_4^+ pulse. The addition of 50 μM NH_4^+ only resulted in a maximum of a 25% further reduction in NO_3^- uptake rates. However, the reduction in NO_3^- uptake rates was greater at the higher experimental temperatures (Fig. 6A). This resulted in a significant (P = 0.05) negative relationship between percent inhibition and temperature (Fig. 6B), very similar to that observed for the culture data.

Discussion

This study was designed to compare several diatoms and dinoflagellates with respect to interactions between NH_4^+ and NO_3^- uptake and assimilation as well as to examine the importance of growth temperature in influencing this interaction. Different phytoplankton species and growth temperatures were used in an attempt to provide a better understanding of potential patterns in NH_4^+/NO_3^- interactions and to reconcile some of the disparate reports in the literature. Additionally, a more detailed assessment of the impact of NH_4^+ on NO_3^- uptake rates was conducted to generate data to compare with various models of NH_4^+ inhibition of NO_3^- uptake.

Phytoplankton growth rate, chemical composition, and internal N pools

Temperature-dependent growth rates for *Chaetoceros* sp., *Thalassiosira weissflogii*, and *Prorocentrum minimum* increased in an exponential manner as originally shown by Eppley (1972), and fell within the range of Q_{10} (given change in a rate process for a given 10 °C change in temperature) values observed for a variety of phytoplankton (e.g. Thompson et al. 1992). *Gyrodinium uncatenum*, on the other hand, grew as expected at 20 °C, but extremely slowly at 15 °C (Fig. 1). This slow growth rate could be due to poor "physiological health" or a lack of ability to grow at low temperatures. As an indicator of physiological health, the variable fluorescence (F_v/F_m) was measured on all cultures before use in each experiment. Variable fluorescence values of ~0.65 are associated with physiologically healthy phytoplankton (Kolber and Falkowski 1993). A value of 0.64 was measured for *G. uncatenum* at 15 °C; by this measure the culture was healthy but growing very slowly. Another possibility for the low growth rate is a genotypic acclimation to growth at warm (i.e. >20 °C) temperatures. This explanation is consistent with the data of Nielsen and Tønseth (1991) where a congener, *Gyrodinium aureolum*, grew slowest at low temperature and salinity.

Cellular carbon, nitrogen, and chl *a* concentrations followed the temperature-dependent trends expected based on results for other phytoplankton species (Goldman and Mann 1980; Geider 1987; Nielsen and Tønseth 1991; Thompson et al. 1992; Nielsen 1996). Cellular C:N ratios were generally constant as a function of growth temperature with the exception of *Thalassiosira weissflogii* grown at 10 °C. The decrease in the C:N ratio was due to a greater relative change in cellular nitrogen than carbon. Furthermore, the fact that particulate cellular nitrogen did not increase at 10 °C to the same extent that total cellular nitrogen increased suggests that much of the nitrogen leading to the low

Table 2 Summary of NH_4^+ half-inhibition concentrations (K_i) and percent inhibition for both non-TCA-extracted samples and TCA-extracted samples. Values for K_i and maximum percent inhibition were determined by curve fitting the data to an inverse Michaelis–Menten function as described by Eq. 1 in the text

Species, μ_T (°C)	K_i (μmol N l^{-1})		% Inhibition	
	not TCA-extracted	TCA-extracted	not TCA-extracted	TCA-extracted
Chaetoceros sp.				
20	3.46	3.42	97	97
10	4.64	4.15	99	100
4	1.60	1.56	77	65
Thalassiosira weissflogii				
20	3.46	3.42	97	97
10	1.45	0.31	75	59
Prorocentrum minimum				
20	0.24	1.22	96	93
10	0.42	0.42	60	73
4	1.99	0.66	80	58
Gyrodinium uncatenum				
20	2.82	0.52	60	66
15	0.83	0.91	62	49

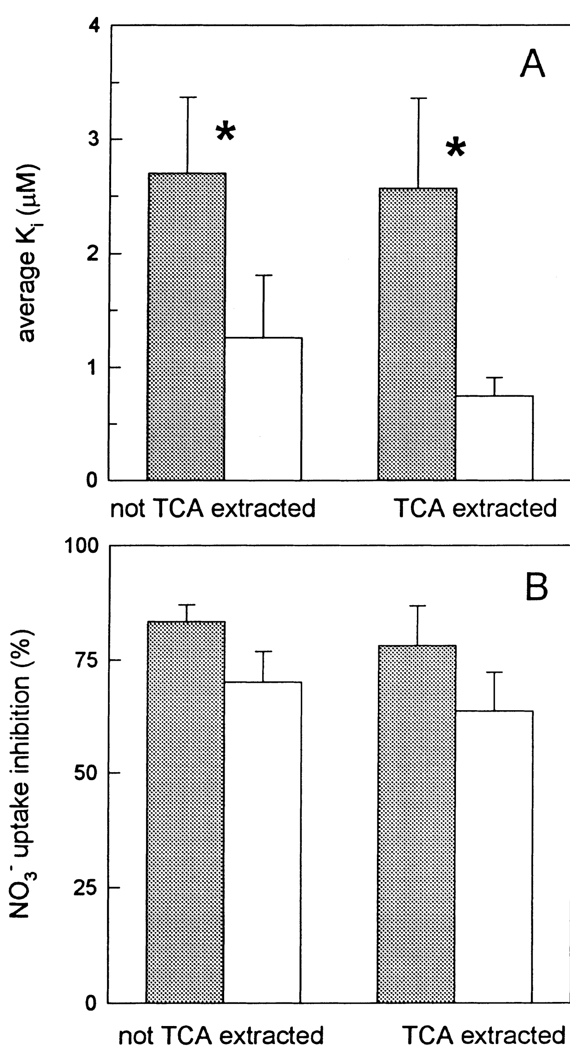

Fig. 4 Comparison of inhibition parameters, K_i and percent inhibition, for diatoms (*shaded bars*) and dinoflagellates (*open bars*). Parameters are calculated for both non-TCA-extracted samples and TCA-extracted samples. Averages (±SE) are for all species–temperature combinations (n = 5). Asterisks (*) denote a significant difference between values for diatoms and dinoflagellates

C:N ratio was maintained as an internal nitrogen pool. Although this pool may be NO_3^-, the lower overall internal concentration of NO_3^- might suggest the presence of another large internal pool, such as amino acids or proteins (Fig. 2).

Concentrations of internal NO_3^- and NH_4^+ measured in this study, as well as the relative patterns of internal pool size, were similar to those observed by Dortch et al. (1984) for a number of phytoplankton species. The congeneric species, *Chaetoceros gracilis*, grown on NO_3^- has a much larger NH_4^+ pool than NO_3^-, and *Thalassiosira pseudonana* and *T. nordenskioldii* grown

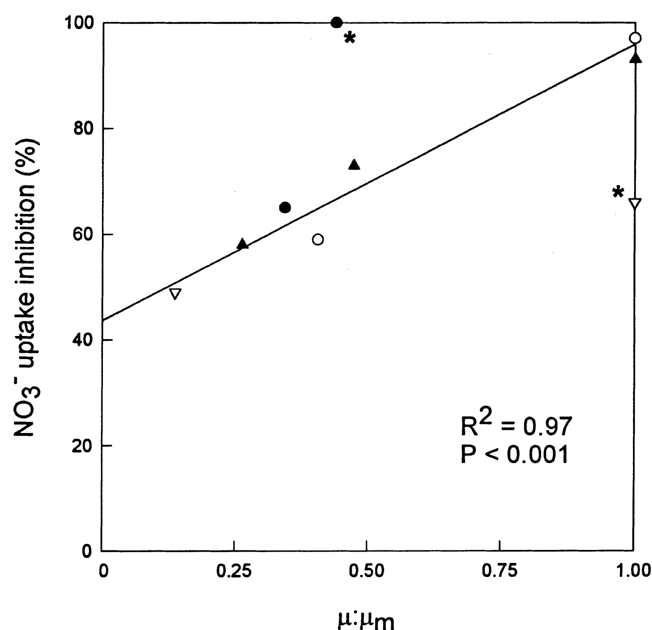

Fig. 5 Nitrate uptake inhibition, as a percent, plotted as function of temperature-limited relative growth rate. Species represented by the following symbols: *Chaetoceros* sp. (●), *Thalassiosira weissflogii* (○), *Prorocentrum minimum* (▼), *Gyrodinium uncatenum* (▽). Parameters reported are for TCA-extracted samples. The two values with an asterisk are excluded from the regression equation

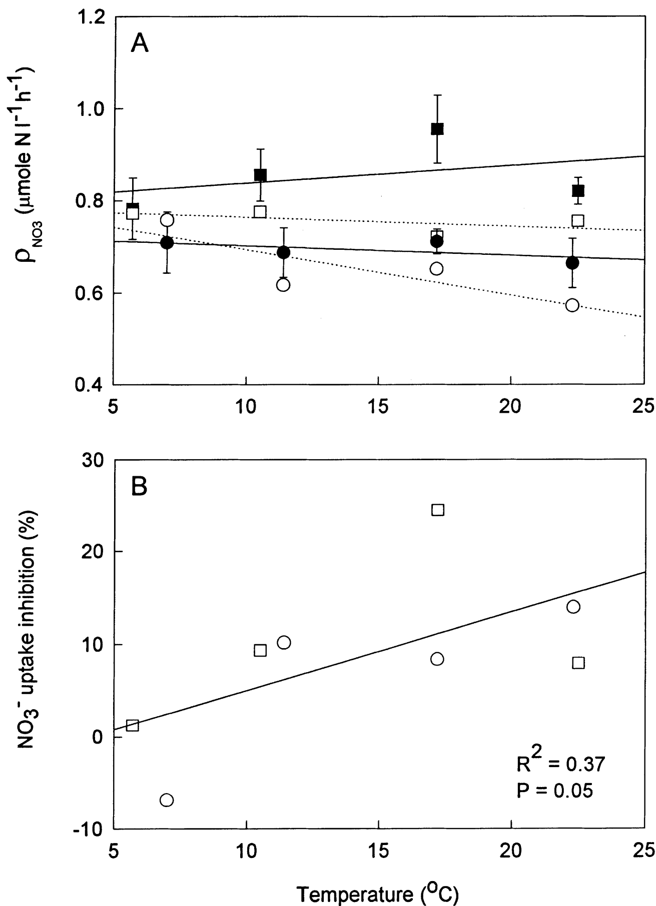

Fig. 6 A Nitrate uptake rates for Chesapeake Bay phytoplankton determined on 16 April 1998 (*squares*) and 17 April 1998 (*circles*). Rates were measured without (*solid symbols*) and with (*open symbols*) the addition of 50 µ*M* NH_4^+. Least squares regression lines are given on each date for NO_3^- uptake rates without (*solid*) and with (*dotted*) added NH_4^+. **B** Inhibition of NO_3^- uptake rates, as a percentage, for the data presented in Panel A. Inhibition of NO_3^- uptake rates was calculated according to Eq. 1 in the text, even though NH_4^+ was present at ~3 µ*M* initially

on NO_3^- exhibited a much larger NO_3^- pool than NH_4^+. These differences are likely due to differences in vacuole size and the mechanisms related to the transport of NO_3^- and NH_4^+ into the cell, and may have an impact on NH_4^+/NO_3^- interactions (e.g. Syrett 1981).

NH_4^+ inhibition of NO_3^- uptake: comparison between diatoms and dinoflagellates

Overall the K_i concentrations determined in this study for all species are higher than those previously reported as inhibiting NO_3^- uptake in both culture (e.g. Eppley et al. 1969; Dortch et al. 1991) and field studies (e.g. Olson 1980; Wheeler and Kokkinakis 1990). As all of the species examined in this study were isolated from the Chesapeake Bay, the elevated K_i values may be the result of an adaptation to an environment where nutrient levels are continually high, as proposed by Maestrini et al. (1986). Alternatively, these values for K_i might be overestimated due to the depletion of added NH_4^+ during the course of the experiment, although culture biomass was purposely kept low to minimize depletion of NH_4^+. We estimate that the maximum consumption of NH_4^+ would be ~0.5 µ*M* at 20 °C and would decrease substantially at lower growth temperatures. Concentrations of NH_4^+ in sample filtrates after the completion of several of the experiments confirmed that even less NH_4^+ was consumed than predicted. Furthermore, there was no difference in NH_4^+ consumption between the diatoms and dinoflagellates.

Most studies have examined a single NH_4^+ concentration, which precludes not only the estimation of K_i concentration, but also the extent to which NO_3^- uptake is inhibited. Moreover, differences in conditioning of cultures and the separation of inhibitory effects on either NO_3^- uptake or assimilation make comparisons qualitative. In general, however, previous culture studies support the patterns of higher K_i concentrations and lower percent inhibition at a given NH_4^+ concentration for diatoms, which we also observed. Dortch and Conway (1984) found that N-sufficient cultures of *Skeletonema costatum* were only inhibited 6% by 5 to 20 µ*M* NH_4^+ and that even at NH_4^+ concentrations of ~13 µ*M* NO_3^- uptake rates were approximately one-third those of the NH_4^+ uptake rates. Bates (1976) and Lund (1987) further showed that *S. costatum* was only inhibited ~60% by 13 and 10 µ*M* NH_4^+ additions, respectively. *Thalassiosira pseudonana*, on the other hand, has been shown to exhibit similar NO_3^- uptake rates with and without daily pulses of 2 µ*M* NH_4^+ (Berges et al. 1995) as well as being completely inhibited by NH_4^+ concentrations of ~2 µ*M* (Dortch et al. 1991). Eppley et al. (1969) showed that NO_3^- uptake started at ~5 µ*M* in the diatom *Ditylum brightwellii* and at ~1 µ*M* in the dinoflagellate *Gonyaulax polyedra*, and values for K_i were estimated to be ~2 µ*M* and < 1 µ*M*, respectively (interpolated from their Figs. 6 and 8). Nakamura (1985) has shown that the dinoflagellate *Chattonella antiqua* exhibited a K_i concentration of 2 µ*M* NH_4^+ for NO_3^- uptake. In general, it appears that diatoms tend to be less susceptible to NH_4^+ inhibition as determined by estimated K_i concentrations, but additional studies in which exact K_i concentrations are determined are needed. Studies in which nitrate reductase activity in cultured phytoplankton have been examined may also support the observations presented here (e.g. Berges et al. 1995), although in some instances culture conditions might confound these results (e.g. Harrison 1976). It appears that when differences in culturing methods are eliminated, generalizations may be made about species composition and NH_4^+ inhibition of NO_3^- uptake. If further studies provide similar results, these generalizations may greatly enhance the ability to accurately model NO_3^- uptake in the presence of NH_4^+.

NH_4^+ inhibition of NO_3^- uptake: temperature effects

The decrease in NO_3^- inhibition as growth temperature decreases is, as far as we know, a previously unreported observation and in direct contrast to the relationship observed by Dortch (Dortch and Conway 1984; Dortch et al. 1991) for N-limited phytoplankton at a single growth temperature. In those studies increasing N-limitation resulted in greater inhibition of NO_3^- uptake by NH_4^+. All four species examined in the present study followed the same trend between percent inhibition of NO_3^- uptake and temperature limited growth rate (Fig. 5) which is consistent with a non-competitive feedback mechanism of the short-term NH_4^+ inhibition of NO_3 uptake (e.g. Zevenboom and Mur 1981). One possible explanation for the observed decrease in percent inhibition of NO_3 uptake at low growth temperatures may be differences in the temperature optima of the enzymes associated with NO_3^- reduction and NH_4^+ assimilation. For example, nitrate reductase activity in the marine diatom *Skeletonema costatum* has been shown to have a temperature optimum of 10 to 15 °C (Kristiansen 1983; Gao et al. 1993), whereas the temperature optimum for glutamate synthase (GOGAT) in this species is 25 °C (Clayton and Ahmed 1986). Growth at low temperature may limit the capacity of GOGAT to assimilate the experimentally added NH_4^+ above and beyond the NH_4^+ that is derived from NO_3^-, and therefore the feedback mechanism might not be as strongly observed at the lower growth temperature. The similar relationship between percent inhibition of NO_3^- uptake rates and temperature observed in the field population (Fig. 6B) lends considerable support to the idea that temperature may influence the interaction between NH_4^+ and NO_3^- uptake by acting at the level of enzyme activity.

This trend for decreasing inhibition of NO_3^- uptake by NH_4^+ at low temperatures is generally consistent with the observations of Lomas and Glibert (1999) where diatom-dominated field populations under short-term excess energy stress (imposed by a rapid drop in temperature at a light intensity greater than E_k for photosynthesis) take up proportionately more NO_3^- than NH_4^+ compared to the same populations at ambient temperature. The experiments presented in this study can be thought of as the fully acclimated analogue to the temperature shift experiments conducted in the field portion of this study, and by Lomas and Glibert (1999), where light was held constant and temperature was changed. We speculate that there is some connection between high cellular energy levels and a preferential utilization of NO_3^- by marine phytoplankton.

Comparison with NH_4^+/NO_3^- interaction models

Developing realistic models of NO_3^- uptake is important not only for understanding phytoplankton physiology, but also for modeling new production (sensu Dugdale and Goering 1967). Several models attempt to describe the interactions between NH_4^+ and NO_3^- uptake, and these can be separated into two basic forms: external NH_4^+ concentration regulation (competitive interaction, Collos 1989; Parker 1993) and biochemical regulation (non-competitive or feedback interaction, DeManche et al. 1979; Flynn and Fasham 1997; Flynn et al. 1997). The external regulation models attempt to model the interaction on either the relative ratio of available NO_3^- and NH_4^+ (Collos 1989) or the ambient NH_4^+ concentration relative to the Michaelis–Menten constant for NH_4^+ uptake (Parker 1993). The biochemical models are based upon the notion that internal NH_4^+, or some product of its assimilation, is a feedback regulator of NO_3^- uptake and/or assimilation.

We compared the outputs of these models to the data generated in this study for insight into the mechanism of interaction in these species. In developing his model of competitive interaction, Collos (1989) specified four criteria (only two N sources available, no chemical transformations such as nitrification, batch mode perturbation, and data on uptake rates and initial N concentrations) for choosing data sets, all of which were available in the present study. However, plotting NO_3^- uptake rates as a function of external NO_3^- availability did not result in the positive linear increase as predicted by the model for some species (Collos 1989). In none of our experiments was a linear relationship observed as would be suggested by a competitive interaction of external NO_3^- and NH_4^+ for transport sites on the cell surface (Fig. 7). All experiments did exhibit some degree of correlation between uptake and availability, but only four of the ten experiments were significant ($P < 0.05$). These findings do not invalidate the model but simply suggest that it may not be applicable to all species, or that other factors are more important than external N concentrations. In fact, relating NO_3^- uptake rates as a function of relative internal NO_3^- availability increased the significance of the correlation for *Chaetoceros* sp., although it dropped the significance of the correlation for *Thalassiosira weissflogii* (Table 3). Parker's model (1993; Eq. 2), although not strictly competitive, relates either the relative NO_3^- uptake rate or the relative nitrate reductase (NR) synthesis rate to the ambient NH_4^+ concentration by the following formula:

$$NO_3^- \text{ uptake rate or NR synthesis rate} = \frac{1}{1 + \left(\frac{[NH_4^+]}{K_s}\right)}, \quad (2)$$

where $[NH_4^+]$ is the ambient NH_4^+ concentration and K_s is the half-saturation concentration for NH_4^+ uptake by a given phytoplankton species. Mathematically this model will yield an exponential decrease in NO_3^- uptake rate as NH_4^+ increases without a specific feedback regulator, even though there is significant evidence that this is the case (discussed by Flynn et al. 1997).

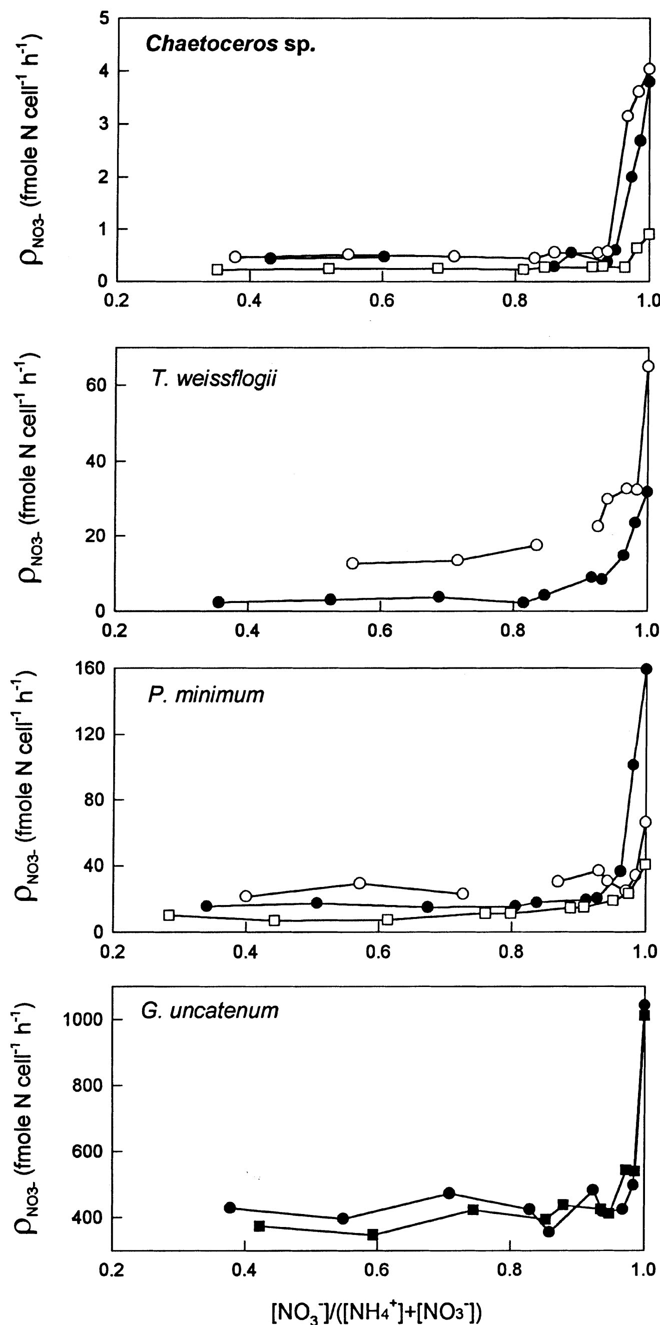

Fig. 7 Cellular NO_3^- uptake rates as a function of the fraction of external NO_3^- for each species and temperature. Growth temperatures for each species indicated by the following symbols: 20 °C (●), 15 °C (■), 10 °C (○) and 4 °C (□). All rates are for non-TCA-extracted samples

The models of DeManche et al. (1979) and Flynn (Flynn and Fasham 1997; Flynn et al. 1997) allow the direct feedback of internal N-metabolites on the uptake and assimilation rates of NO_3^-. Consequently, these models involve numerous variables for which we had no data and so could not test whether they fit our data directly. However, these models both predict an exponential decline in NO_3^- uptake rates as ambient NH_4^+ increases. Qualitatively, our data do fit these model predictions suggesting that in all four of these species a feedback mechanism may be regulating the interaction of NO_3^- and NH_4^+ to the overall N metabolism.

Table 3 Correlations of NO_3^- uptake rate by *Chaetoceros* sp. and *Thalassiosira weissflogii*, with NO_3^- as a fraction of external NO_3^- and NH_4^+ concentrations (R_{ext}) and internal (R_{int}) NO_3^- and NH_4^+ concentrations. Only the species-growth temperature (μ_T) combinations where both internal NO_3^- and NH_4^+ concentrations were detected are presented. *, $P < 0.05$; **, $P < 0.01$

Species	μ_T	R_{ext}	R_{int}
Chaetoceros sp.	10	0.578*	0.802**
Chaetoceros sp.	4	0.516	0.579*
T. weissfloggi	10	0.706*	0.566

Conclusions

This study allows two important generalizations: the two diatom species studied exhibited higher K_i concentrations than the two dinoflagellates, and uptake rates of NO_3^- by all species were less inhibited by NH_4^+ at lower growth temperatures than at higher growth temperatures. Furthermore, field phytoplankton assemblages were also less inhibited by NH_4^+ at lower experimental temperatures. Although only four species (two diatom and two dinoflagellate) were studied, these data are encouraging in that generalizations about classes of phytoplankton may be applicable following examination of additional species at multiple growth temperatures. The general agreement between this study and others suggests that the mechanistic relationships determined in the laboratory can be used to interpret and potentially model similar interactions in the field.

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