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Temperature regulation of nitrate uptake: A novel hypothesis about nitrate uptake and reduction in cool-water diatoms

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Abstract

Marine diatoms generally form large blooms during periods of cool temperature (<20°C), high NO_3^- fluxes (>25 μM-N), and turbulent mixing, but the adaptations that allow diatoms to bloom under these conditions are not well understood. We have conducted both NO_3^- uptake kinetics and direct short-term temperature manipulation studies on field diatom-dominated populations from Chesapeake and Delaware Bays during both spring and fall blooms. Absolute rates of NO_3^- uptake by a *Rhizoselenia*-dominated population did not appear to saturate even at concentrations as high as 180 μM-N. We observed contrasting patterns of NO_3^-, NH_4^+, and urea utilization as a function of experimental temperature (ambient ± 9°C). Over the temperature range of 7–25°C, absolute uptake rates of NO_3^- (ρ_{NO3-}) decreased an average of 46% with increasing temperature from 7 to 25°C (nine individual experiments), while ρ_{NH4+} and ρ_{UREA} increased with increasing temperature by an average of 179 and 86% (eight individual experiments), respectively. Based on these observations and the nature of the physical environment, we hypothesize that these diatom-dominated populations were taking up NO_3^- in excess of nutritional requirements, the reduction of which may serve as a sink for electrons during transient periods of imbalance between light energy harvesting and utilization. We suggest that the increase in non-nutritional NO_3^- uptake increases proportionately with the magnitude of the imbalance between light energy harvesting and imbalance. This hypothesis reconciles previous observations of low C : N uptake ratios, high release rates of dissolved organic nitrogen or NO_2^- by diatom-dominated assemblages, other observations of nonsaturating NO_3^- kinetics in field populations, and the apparent ''preference'' for NO_3^- by the netplankton size fraction. The two phenomena described here, nonsaturable kinetics and a negative relationship between NO_3^- uptake and short-term temperature shifts, have important ecological implications. The hypothesized ability of these diatom-dominated populations to better modulate the flow of photosynthetic electron energy, via NO_3^- reduction, in variable environments may provide a competitive advantage to diatoms and could potentially explain why diatoms frequently dominate in regions of cool temperature, high NO_3^- flux, and turbulent mixing. Also, models of new production may need to incorporate terms for temperature dependence of NO_3^- uptake. Finally, if a significant fraction of NO_3^- uptake is regulated by non-nutritional mechanisms in the cell, and if some fraction of nitrogen reduced by this mechanism is subsequently released in the form of NO_2^-, NH_4^+, or dissolved organic nitrogen (DON), then estimates of new production based solely on NO_3^- uptake could be seriously biased.

A large fraction of the annual autotrophic production in temperate estuaries is typically associated with spring phytoplankton blooms (Malone and Chervin 1979; Sharp et al. 1982; Pennock 1985; Cloern et al. 1989; Glibert et al. 1995; Smith and Kemp 1995). These blooms are commonly dominated by large diatoms (i.e., >20 μm), which appear to be favored by the high nutrient concentrations (Eppley et al. 1969*b*) and the pulsed nature of nutrient inputs (Turpin and Harrison 1979; Stolte et al. 1994; Stolte and Riegman 1996), as well as the turnover and rapid stabilization of the water column (Malone 1980). During these temperate spring blooms, as well as in coastal upwelling areas and polar regions, NO_3^- is the most abundant inorganic nitrogen source (e.g., Malone et al. 1988; Smith 1991; Glibert et al. 1995; Clarke and Leavey 1996) and supports most of the increase in phytoplankton biomass. Diatom dominance in these regions has been explained on a variety of ecological bases (e.g., Margalef 1978), such as high growth rates and greater competitive ability under pulsed nutrient conditions. However, to date, the physiological adaptations allowing diatoms to bloom under such conditions are not well understood.

It has been known for several decades that both phytoplankton growth (e.g., Droop 1968; Eppley and Thomas 1969) and nutrient uptake (e.g., Eppley et al. 1969*b;* McCarthy 1981; Harrison et al. 1996) exhibit Michaelis–Menten-type kinetics. These studies have guided our thinking in the modeling of nitrogen uptake in phytoplankton. Recently, however, several studies have been published that question the saturating nature of NO_3^- uptake in cultures of diatoms and chlorophytes under nutrient-replete conditions (Collos et al. 1992*b;* Watt et al. 1992; Collos et al. 1997). These studies have shown either linear or biphasic kinetics (up to concentrations of ~300 μM-N), with the first transition point occurring at ~60 μM-N. Furthermore, Collos et al. (1997) presented data for field populations showing similar linear or biphasic NO_3^- uptake kinetics, up to concentrations of 100 μM-N in populations dominated by diatoms and small (<5 μm) flagellates. There are a number of explanations for the varying reports of the saturation response in NO_3^- uptake kinetics: species-specific and/or cell size differences, diel

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variability in NO_3^- uptake, varying physiological state of the cultures, the use of a limited concentration range that is not fully representative of natural situations, as well as the inclusion of NO_3^- within internal pools during mass spectrometric analysis. The biphasic nature of an uptake system, such as that for NO_3^-, is commonly due to multiple steps in the overall transport (e.g., Neame and Richards 1972) pathway. Concentrations of NO_3^- >50 μM-N are common in coastal estuaries (Sharp 1983), and NO_3^- uptake kinetics should be investigated further at these high nutrient concentrations.

Diatom-dominated populations under spring bloom conditions are also subjected to an energetically turbulent environment, with the mixing layer frequently exceeding the euphotic layer. These populations therefore are exposed to irradiances ranging from darkness to full sunlight on time scales much shorter than their generation time. This transient light environment imparts transient energy stress within the cell due to imbalances between light energy harvesting and utilization, where cells acclimate to a ''mean'' irradiance (e.g., MacIntyre et al. 1996). Therefore, mechanisms by which cells under these conditions could balance the flow of electrons through light harvesting (e.g., Neale et al. 1993; Ting and Owens 1993) would impart a competitive advantage.

In this paper, we provide data showing nonsaturating NO_3^- uptake kinetics in a diatom-dominated field population. In addition, we describe the differential effects of short-term temperature shifts on NO_3^-, NH_4^+, and urea uptake. We observed a negative relationship between short-term temperature shifts and NO_3^- uptake but a positive relationship between short-term temperature shifts and NH_4^+ and urea uptake. We interpret these findings by presenting a novel hypothesis relating the balance of photosynthetic energy and material cycling within a cell to environmental forcing.

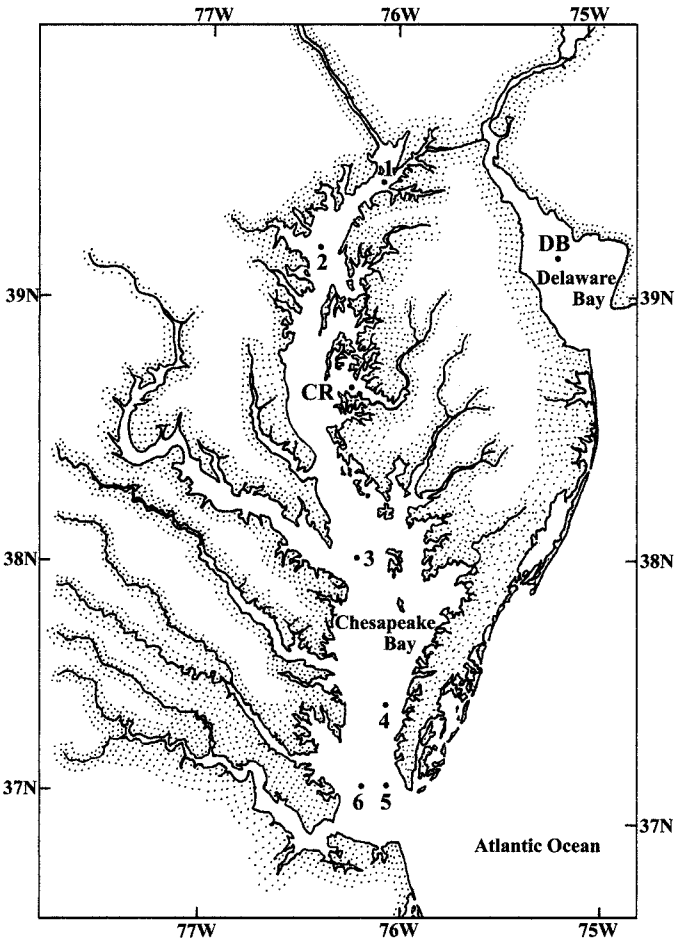

Fig. 1. Map of Chesapeake and Delaware Bays showing locations of the Delaware Bay station (DB), the Choptank River station (CR), and the Chesapeake Bay stations (1–6).

Methods

Study sites and environmental conditions—Field studies were conducted in both the Delaware (October 1995) and Chesapeake Bays (April 1996–May 1997), with several additional experiments conducted at a station on the Choptank River, a tributary of the Chesapeake Bay (March–April 1996; Sta. CR, Fig. 1). Two types of experiments were conducted during this study: NO_3^- uptake kinetics and NO_3^-, NH_4^+, and urea uptake as a function of short-term temperature shifts.

For all experiments, surface samples were collected at first light (0600–0700-h local time) and passed through a 202-μm Nitex screen to remove large zooplankton. Samples were handled in subdued light prior to the experiment to minimize light stress, and all experiments were started within 1 h of sample collection. In addition to the standard experimental protocols described below, pigment samples (Harding unpubl. data) were collected to identify the major phytoplankton classes in 1996 and 1997 (Van Heukelem et al. 1994). The equations of Tester et al. (1995) were used to estimate the contribution of flagellates (both dinoflagellates and other flagellates; sum of eq. 1 and 3) and diatoms (eq. 2) to the total population. Samples were also collected for species identification (fixed in acid Lugol solution) during the NO_3^- kinetics experiments in May 1997.

Particulate nitrogen, chlorophyll a*, and nutrient concentrations*—Known volumes of the water were filtered (<100 mTorr) onto precombusted 25-mm GF/F filters (450°C, 1 h) after the initiation of the ^{15}N incubation. Both the filters and the filtrate were frozen immediately at −20°C for later analysis of particulate nitrogen (PN), chlorophyll *a* (Chl *a*), and ambient nutrient concentrations. Concentrations of PN (following combustion to N_2) were determined on a Control Equipment Elemental Analyzer (fall 1995 and spring 1996), using acetanilide as a standard, or by using a mass-pressure–calibrated inlet system for a nuclide mass spectrometer (starting in fall 1996) with NO_3^- as a standard. Known masses of NO_3^- (in solution) were spotted on GF/F filters, dried for 1.5 h, and then prepared for analysis as described below. The relationship between the known mass of NO_3^--N and the pressure within the inlet system for the mass spectrometer was used to determine the mass of nitrogen in each sample. For the range of these PN determinations, ~1–4 μM-N, relative standard deviations for the calibrated inlet

Table 1. Summary of experimental and environmental data during this study. The locations of stations are shown in Fig. 1. Experiment codes (Exp) are as follows: TM, temperature manipulation experiment; K, kinetic experiment. Ambient temperature readings (Temp) are in °C, nutrient and particulate nitrogen (PN) concentrations are all in μM-N and Chl *a* concentrations are in μg liter^{-1}. Using the equations of Tester et al. (1995), the percent of flagellate and diatoms was estimated for those dates where pigment data were available (*see* text for details). As a further characterization of the phytoplankton community, we provide taxonomic identification data from the Chesapeake Bay Living Resources Monitoring Program where available. nd, no data collected.

Date	Sta.	Exp.	Temp.	NO_3^-	NH_4^+	Urea	PN	Chl *a*	>20 μm Chl *a* (%)	% flagellates	% diatoms	Dominant species
16 Oct 95	DB	TM	17	12.37	1.88	0.45	12.81	9.75	nd	nd	nd	
18 Oct 95	DB	TM	16	22.44	1.62	0.40	10.83	9.80	nd	nd	nd	
20 Oct 95	DB	TM	20	27.23	1.67	0.71	8.89	11.83	nd	nd	nd	
13 Mar 96	CR	K	2.3	0.50*	0.48*	0.13*	14.81	10.08	nd	nd	nd	
8 Apr 96	CR	K	7.9	0.29*	0.44*	0.55*	27.00	8.88	nd	nd	nd	
24 Apr 96	CR	K	13.6	1.04*	0.95*	0.06*	20.56	10.28	nd	nd	nd	
5 May 96	2	TM	17	86.26	9.11	0.61	7.04	19.99	nd	2.3	25.5	
24 Jul 96	2	TM	26	11.80	5.13	1.98	23.26	18.36	nd	18.2	19.2	*Agmenellum, Microcystis, Chaetoceros subtilis*
23 Oct 96	2	TM	14.1	68.84	4.42	0.92	6.38	6.50	nd	0	27.1	*Skeletonema costatum, S. potamos, Aulacoseira*
24 Oct 96	1	TM	15.3	84.46	4.27	0.96	8.30	11.05	nd	0	28.1	
29 Oct 96	3	TM	17.6	0.48	2.33	1.12	6.28	11.41	40	2.2	46.9	
30 Oct 96	4	TM	17.7	1.39	3.98	0.89	8.20	10.57	50	7.5	35.2	
23 Apr 97	4	K	12.1	1.51	nd	nd	11.71	10.41	52	5.3	54.6	*Rhizoselenia*
2 May 97	5	TM	14.2	3.08	nd	nd	9.52	6.27	49	9.1	49.2	
2 May 97	6	K	14.2	4.86	nd	nd	9.94	6.77	42	nd	nd	*Rhizoselenia*

* Nutrient concentrations in the sample of resuspended cells.

system were <5% for duplicate standards. Low vacuum pressures were used in this study to minimize rupturing of cells; therefore, determination of PN by either of the methods used represents total nitrogen contained within cells, as proteinaceous material or soluble internal nitrogen pools. Samples for Chl *a* analysis were ground in 90% acetone on ice, and concentrations were determined fluorometrically (Parsons et al. 1984) on a Turner Designs model 10 fluorometer calibrated against a high-pressure liquid chromatography (HPLC)-measured Chl *a* standard (Fluka Chemical, model 25730). For several of the sample dates, Chl *a* in the >20-μm size fraction was determined as the difference in Chl *a* concentrations between whole-water samples and samples passing through the 20-μm Nitex screen.

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 an ammonium chloride solution (4.7%, pH 8.5) in 15-ml centrifuge tubes (Corning, model 25319-15). Samples were placed on a lateral shaking table for 90 min at 100 oscillations per minute. The cadmium was removed, and the color was developed with a combined sulfanilamide and N-(1-naphthyl)-ethylenediamine dihydrochloride reagent. The absorbance was read at 540 nm within 2 h. Concentrations of NH_4^+ and urea (following hydrolysis by urease; McCarthy 1970) were determined manually using the phenol-hypochlorite method of Parsons et al. (1984). All nutrient samples were analyzed within 3 weeks of collection. The limit of detection for all of these manual chemical analyses is 0.03 μM-N for triplicate samples of each N source.

NO_3^- uptake kinetics—At the time of the NO_3^- uptake kinetic experiments at Sta. CR, ambient NO_3^- concentrations were 40–80 μM-N; therefore, netplankton were gently concentrated by reverse filtration using a 20-μm Nitex screen. The concentrated sample was diluted in salinity-adjusted Sargasso Sea water (SSW) to lower the ambient NO_3^- concentration. This cell suspension was divided into 200-ml aliquots in 250-ml polycarbonate incubation flasks, and $^{15}NO_3^-$ (>98% enriched) was added in the following final $^{15}NO_3^-$ concentrations: 0.1, 0.25, 0.5, 1, 5, 10, and 25 μM-N. Samples were incubated under neutral density screening at 60% incident irradiance (range for experiments = 500–750 μmol m^{-2} s^{-1}) for 1 h. Although only NO_3^- was added back after dilution with SSW, it is unlikely that other elements became limiting in the 1-h incubation. Incubations were terminated by filtration onto precombusted 42.5-mm GF/F filters. Filters were rinsed thoroughly with 0.2-μm filtered estuarine water, dried overnight at 50°C, and stored in a desiccator for later isotopic analysis (described below).

Ambient NO_3^- concentrations at Chesapeake Bay Sta. 4 and 6 were low (<5 μM-N); therefore, samples were only prescreened through the 202-μm Nitex screen to remove the larger zooplankton. Incubation volumes were the same as for experiments conducted at Sta. CR, with $^{15}NO_3^-$ added to the 200-ml sample to yield the following final $^{15}NO_3^-$ concentrations: 0.5, 1, 5, 10, 20, 50, 95, and 182 μM-N. Samples were incubated under neutral density screening at 60% incident irradiance (range = 200–450 μmol m^{-2} s^{-1}) for 1 h. At the end of the incubation period, the sample was divided into two equal 100-ml aliquots and filtered onto pre-

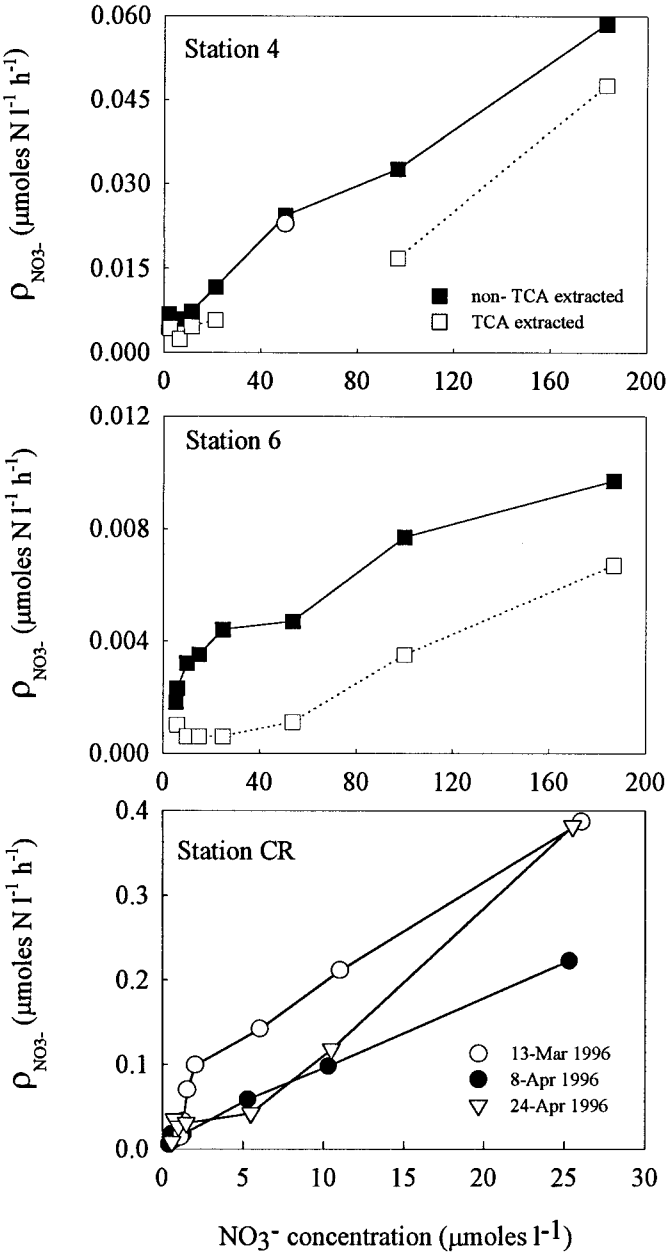

Fig. 2. Nitrate uptake kinetic curves for samples collected at Sta. 4, 6, and CR. Locations and dates of sampling are given in Table 1. For Sta. 4 and 6, the solid squares and lines represent the specific uptake rates for samples not extracted with TCA, and the open squares and dashed lines represent the specific uptake rates for samples that have been extracted with TCA. Sta. 4 has one TCA-extracted rate that appears to have been poorly extracted and is represented by an open circle. At Sta. CR, data for three experimental dates are provided: 13 March 1996, 8 April 1996, and 24 April 1996. Note the difference in x-axis scales between panels.

combusted 42.5-mm GF/F filters. Following a rinse with 0.2-μm filtered estuarine water, one filter was extracted with ice-cold 10% trichloroacetic acid (TCA) for 5 min to remove any TCA-soluble internal pools in order to separate uptake and assimilation of $^{15}NO_3^-$. Following TCA extraction, the filter was rinsed again with 0.2-μm filtered water. The replicate filter was simply rinsed with 0.2-μm filtered water. Both filters were frozen at −20°C until being returned to the lab, where they were dried at 50°C overnight and stored until being processed for isotopic analysis.

Temperature gradient block and temperature manipulation experiments—For the temperature manipulation experiments, a self-contained temperature gradient block and light source were used. An aluminum block (15 in. wide × 24 in. long × 5 in. deep) was bored through with 25-mm holes in an eight-column by 11-row array. This array allows for a maximum of eight replicates at the same experimental temperature and up to 11 possible experimental temperatures, although in these studies, only six experimental temperatures were used. At either end of the temperature block (in the direction of the 11 rows), six holes were drilled through the width of the block so that water could circulate through the block in a closed-loop fashion. By setting the water baths at each end of the block to different temperatures, a uniform temperature gradient was formed along the length of the block. Typically, a range of 18°C (±9°C of ambient temperature) was achieved in these manipulations. All surfaces of the block were painted white to increase the reflectance within each sample well. A series of 6–20-W fluorescent bulbs were maintained 6 in. above the surface of the temperature block, providing an average irradiance of ~250 μmol m^{-2} s^{-1} within each sample well. This entire system (temperature gradient block and light source) was contained in a wooden housing to minimize the influence of outside temperature and irradiance levels.

Several hours before the initiation of an experiment, the ambient temperature was measured and set as the midpoint of the temperature range that covered ±9°C of ambient. For each experiment, samples of 30-ml volume were dispensed into 25-mm test tubes. This volume was contained entirely within the depth of the temperature block and therefore, was subject only to the experimental temperature. In those situations where biomass was low and a 30-ml sample provided insufficient mass for mass spectrometric analysis, additional 30-ml samples were used and were pooled on the same filter at the end of the incubation. Samples were placed in the temperature block and allowed to acclimate to the experimental temperature for ~5 min before the addition of ^{15}N substrates (all >98% enriched). After the acclimation period, ^{15}N substrates were added to a final concentration of 10 μM-N. Incubations were for 1 h and were terminated by filtration as above.

^{15}N isotopic analysis—Samples were prepared for analysis using the general procedures of Fiedler and Proksch (1975). Samples were ground with copper oxide (Baker, model 1820-05, prepared for use by combusting at 600°C for 3 h), placed into Pyrex glass ampoules (precombusted at 500°C for 1 h) with copper metal accelerator (Alpha Resources), 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 atom%, with a 99.7% recovery of calculated standard additions. Absolute uptake rates were calculated according to the formulas of Dugdale and Goering (1967) and

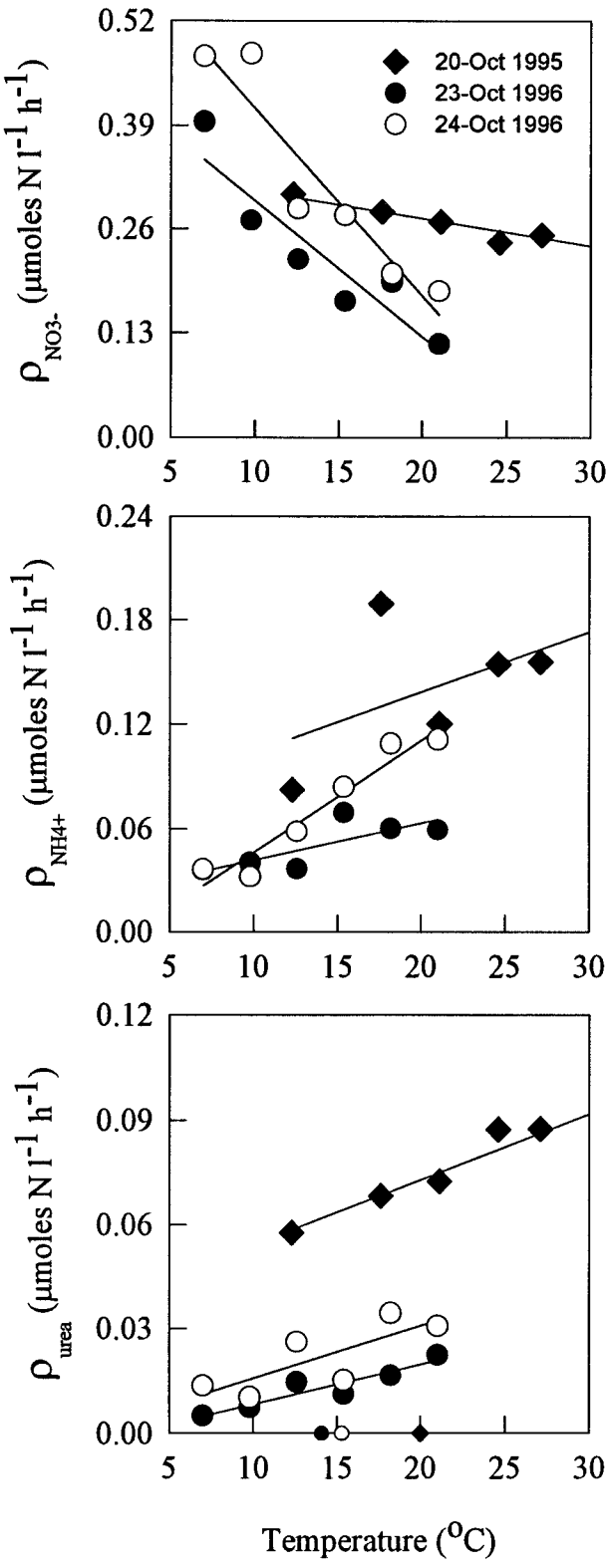

Fig. 3. Representative absolute uptake rates for NO_3^-, NH_4^+, and urea for samples from 20 October 1995, 23 October 1996, and 24 October 1996, and incubated over a range of temperatures ±9°C of ambient. The solid lines represent the best fit regression to each date. The smaller symbols on the x-axis denote the ambient temperature for the respective day.

were not corrected for isotope dilution (Glibert et al. 1982*b*), as little dilution would be expected at the relatively high concentrations used.

Results

Nutrient concentrations, particulate nitrogen, and Chl a—Over the temporal and spatial scales of data collection, large ranges in all environmental variables were observed (Table 1). Ambient NO_3^- concentrations ranged from 0.3 to >80 μM-N, while NH_4^+ and urea concentrations were much lower, ranging from 0.44 to 9.11 μM-N and 0.06 to 1.98 μM-N, respectively. The highest concentrations of NO_3^- and NH_4^+ were observed in the northern Chesapeake Bay due to the inflow of Susquehanna River water. The range in Chl *a* and PN was fourfold. Generally, the highest PN and Chl *a* concentrations were observed during the spring. In those cases where Chl *a* concentrations were divided into total and >20-μm fractions, the >20-μm fraction (netplankton) averaged 46% of the total. During spring and fall 1996, HPLC pigment analysis and estimation of flagellate and diatom contributions to total population showed that diatoms comprised 20–50% of the total population and always contributed substantially more to the total population than flagellates. During the 24 July 1996 sampling, flagellates and diatoms contributed equally to the total population (Table 1). For two of the stations occupied in 1996, we were able to compare our sampling times and locations with similar sampling times and locations of the Chesapeake Bay Programs Phytoplankton taxonomic collection. The taxonomic data support the dominance by diatoms during these sampling periods. During the 1997 spring bloom, the dominant species identified was the diatom *Rhizoselenia* spp., although a number of other large diatom species were observed.

NO_3^- uptake kinetics—Absolute NO_3^- uptake rates did not saturate for samples collected at Sta. 4 and 6 (Fig. 2), even up to concentrations of 180 μM-N. The highest concentration used in samples taken from Sta. CR was only 25 μM-N, but for all three samples, NO_3^- uptake rates did not saturate. The pattern of ^{15}N incorporation in TCA-extracted samples at Sta. 4 and 6 was similar to that of non–TCA-extracted samples; these rates also did not appear to saturate. Absolute uptake rates of NO_3^- for TCA-extracted samples at Sta. 4 were relatively constant at concentrations <15 μM-N, but they increased in a nonsaturating manner at higher concentrations. Absolute NO_3^- uptake rates of TCA-extracted samples averaged about 80% of the absolute NO_3^- uptake rates of non–TCA-extracted samples. Absolute NO_3^- uptake rates of TCA-extracted samples at Sta. 6 were extremely low and constant (within error of the method at these low rates) between <1 and 50 μM-N. At higher concentrations, as at Sta. 4, uptake rates of TCA-extracted samples increased linearly and averaged 60% of non–TCA-extracted samples.

Temperature manipulation experiments—All temperature manipulation experiments conducted with diatom-dominated assemblages exhibited the same trends: absolute uptake rates of NO_3^- (ρ_{NO3-}) decreased with increasing temperature, while absolute uptake rates of NH_4^+ and urea (ρ_{NH4+} and ρ_{UREA})

Table 2. Summary of NO_3, NH_4, and urea uptake data for all temperature manipulation experiments. Columns are for the date of sampling, number of experimental temperatures used to generate the linear regression, slope of the absolute uptake velocity vs. temperature linear regressions, coefficient of determination, total % change in the uptake velocity over the experimental temperature range, and % change $°C^{-1}$. The total percent changes are relative to the rate observed at the coldest temperature for each experiment. Therefore, the percent change for NO_3^- is negative, while it is positive for NH_4^+ and urea. Also included are the experimental temperature range examined and the ambient surface temperature at the time of sample collection.

Date	N	Slope (μM-N $(h\ °C)^{-1}$)†	Coefficient of determination	Total % change	% change $°C^{-1}$	Exp. temp. range (°C)	Ambient temp. (°C)
Nitrate							
16 Oct to 20 Oct 95	15	−0.0053**	0.36	−11 to −35	−1.1 to −2.2	12–30	16–20
5 May 96	6	−0.0230**	0.88	−15	−1.0	9–24	17
23 Oct to 24 Oct 96	12	−0.0200**	0.72	−62 to −71	−4.4 to −5.1	7–21	14–15
29 Oct to 30 Oct 96	12	−0.0023**	0.84	−69 to −70	−4.3	9–25	18
2 May 97	4	−0.0036*	0.83	−58	−3.6	7–23	14
Ammonium							
16 Oct to 20 Oct 95	11	0.0059*	0.31	85 to 108	5.3 to 6	12–30	17–20
5 May 96	6	0.0058	0.28	60	4.0	9–24	20
23 Oct to 24 Oct 96	12	0.0043**	0.60	166 to 307	12 to 22	7–21	14–15
29 Oct to 30 Oct 96	12	0.0040**	0.74	235 to 290	14.7 to 18.1	9–25	18
Urea							
16 Oct to 20 Oct 95	15	0.0018	0.02	0 to 54	5.1	12–30	16–20
5 May 96	6	0.0	0.02	0	6.1	9–24	15
23 Oct to 24 Oct 96	11	0.0013**	0.51	126 to 350	9 to 25	7–21	14–15
29 Oct to 30 Oct 96	12	0.0003	0.08	36 to 38	2.3 to 2.4	9–25	18

* $P<0.05$; ** $P<0.01$ using the Student's *t*-test.
† Significance level of the slope.

increased with increasing temperature. The slope of this trend differed, however, between sampling dates (Fig. 3; Table 2). Comparison of the overall response to experimentally manipulated temperature in each experiment was achieved by normalizing rates within a given experiment for a single substrate to the rate at the lowest experimental temperature (Fig. 4). Overall, ρ_{NO3-} decreased an average of 46% with increasing temperature within each experiment, while ρ_{NH4+} and ρ_{UREA} increased with increasing temperature by an average of 179 and 86%, respectively. Because of the variability of responses, the results of these uptake experiments were separated into two response groups, with the experiments from fall 1995 and spring 1996 exhibiting a weaker response to temperature than the experiments from fall 1996 and spring 1997 (Fig. 4A,C,E vs. B,D,F). Although the responses differed between experiments, most of the responses were highly significant (Table 2). Over the experimental temperature range, on average, ρ_{NO3-} decreased 1.4 ± 0.7% $°C^{-1}$ and 4.1% ± 0.8% $°C^{-1}$ for fall 1995/spring 1996 and fall 1996/spring 1997, respectively. Over the same experimental temperature range, ρ_{NH4+} increased 5.1 ± 1.0% $°C^{-1}$ and 16.7 ± 4.3% $°C^{-1}$, while ρ_{urea} increased 5.6 ± 0.7% $°C^{-1}$ and 9.7 ± 10.5% $°C^{-1}$ for these same respective periods.

The negative relationship between NO_3^- uptake and short-term shifts in experimental temperature in the range of 7–20°C was only observed during fall and spring periods when diatoms were a large fraction of the total chlorophyll biomass. During a cruise in July 1996, when the ambient temperature was 26°C and flagellates and diatoms contributed equally to the assemblage, ρ_{NO3-} increased with temperature over the range of 7–20°C (Fig. 5). At 25°C, ρ_{NO3-} for this sampling date decreased 40% over the rate observed at 20°C.

Discussion

A major goal of this study was to conduct physiological experiments to examine the mechanisms underlying the dominance of diatoms in cool, NO_3^--rich environments. We found that NO_3^- uptake kinetics were nonsaturating and that short-term temperature stress (operationally equivalent to an increase in generation of biochemical energy relative to its consumption) resulted in a negative relationship between NO_3^- uptake and temperature. Although these data address two relatively distinct aspects of nitrogen physiology, we feel that they can both be explained in terms of a single underlying physiological pathway. Here, we discuss each of these observations in detail and present a unifying hypothesis whereby the reduction of NO_3^- may be used as a means to buffer short-term imbalances between biochemical energy production and consumption.

NO_3^- uptake kinetics—Generally, it is believed that NO_3^- uptake kinetics curves follow a Michaelis–Menten formulation and saturate at NO_3^- concentrations <20–30 μM-N (e.g., Eppley et al. 1969*b;* McCarthy 1981), but several recent studies have observed nonsaturable NO_3^- uptake kinetics in both chlorophytes (Watt et al. 1992) and diatoms (Collos et al. 1992*b*, 1997). The data we have presented clearly show that diatom-dominated field assemblages exhibit nonsaturating NO_3^- uptake kinetics at concentrations up to at

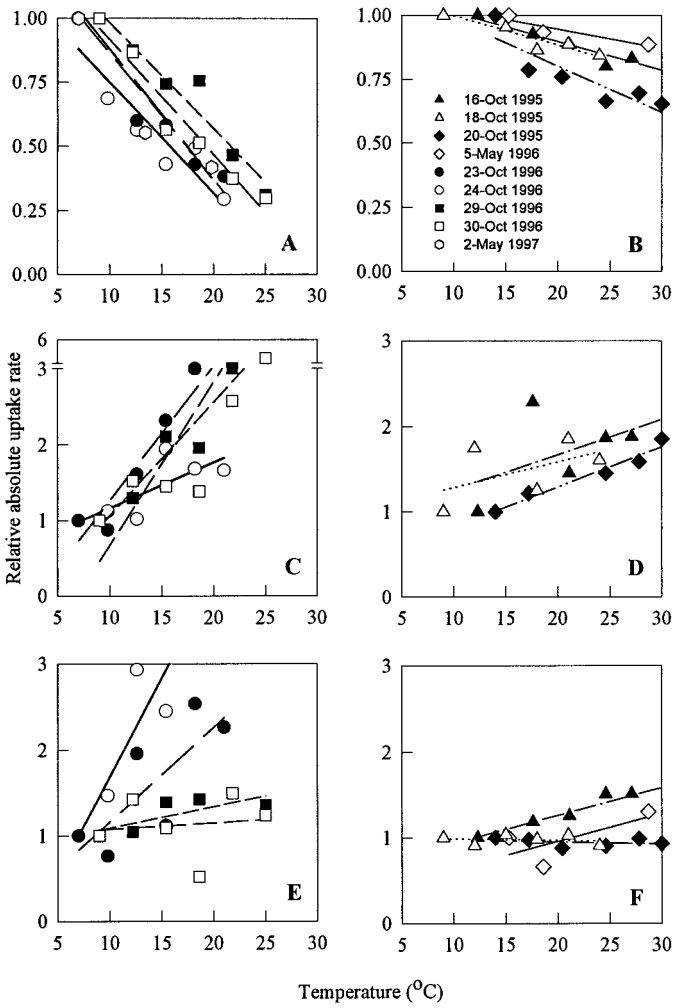

Fig. 4. Relative absolute uptake rates for NO_3^- (A and B), NH_4^+ (C and D), and urea (E and F). For each experimental date, the absolute uptake rates are normalized to the absolute uptake rate measured at the lowest experimental temperature to facilitate comparison between experiments. The absolute uptake rate data for each date are represented by the symbols defined in the figure and line descriptors: (dash-dot-dot line) 16 October 1995, (thin solid line) 18 October 1995, (dash-dot line) 20 October 1995, (dotted line) 5 May 1996, (thick solid line) 23 October 1996, (long-dash line) 24 October 1996, (medium-dash line) 30 October 1996, (short-dash line) 29 October 1996, and (thick medium-dash line) 2 May 1997. For each substrate, data are separated into periods representing fall 1996 and spring 1997 (A, C, and E) and fall 1996 and spring 1997 (B, D, and F) to more clearly demonstrate the variability in the slope of this response.

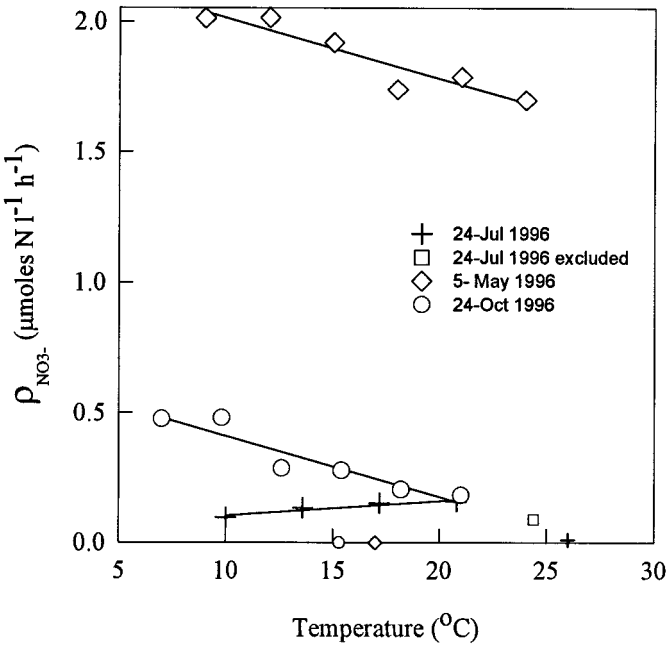

Fig. 5. Absolute NO_3^- uptake rates from temperature manipulation experiments conducted during a flagellate-dominated population (24 July 1996). The solid lines represent the best fit regression through the data at temperatures <20°C. The data at 25°C (represented by the open square) were excluded from calculating the best fit regression line. For comparison of trends, data from 5 May 1996 and 24 October 1996 taken at the same station are included. The smaller symbols on the x-axis denote the ambient temperature for the respective day.

least 180 μM-N. Although the nitrogen uptake kinetics literature is quite extensive, with the exception of Collos et al. (1992*b*, 1997) and Watt et al. (1992), we are unaware of other studies in which concentrations above ~30 μM-N were used in the experimental design. Furthermore, little appreciation has been given to kinetic curves that show other than a typical saturation response (but *see* Dortch et al. 1991). For example, in one of the most commonly cited papers on NO_3^- uptake kinetics in cultured phytoplankton, Eppley et al. (1969*b*) showed that for the diatom *Rhizoselenia robusta*, NO_3^- uptake did not saturate up to concentrations of 20 μM-N but that a trend toward possible saturation was apparent. This trend, in fact, was almost identical to our Chesapeake Bay Sta. 6 (dominated by *Rhizoselenia* spp.; Fig. 2b), where up to ~20 μM-N NO_3^- uptake rates appeared to be saturating but then increased linearly up to ~180 μM-N. Clearly, it cannot be assumed that uptake kinetics will saturate by measuring only the uptake responses to concentrations <30 μM-N.

Although on time scales of cell division it is unlikely that

Table 3. Summary of Michaelis–Menten half-saturation constant (K_m) values for nitrate reductase in marine diatoms. All values are given as millimoles of substrate per liter.

Organism	K_m (mM-N)	Reference
Ditylum brightwellii	0.110	Eppley et al. 1969*a*
Thalassiosira pseudonana	0.062	Amy and Garrett 1974
Skeletonema costatum	0.24	Serra et al. 1978
T. pseudonana	0.063–0.083	Packard 1979
T. pseudonana	0.04	Smarelli and Campbell 1980
S. costatum	0.295	Gao et al. 1993
T. pseudonana	0.047 ± 0.006	Berges and Harrison 1995
S. costatum	0.146 ± 0.022	Berges and Harrison 1995

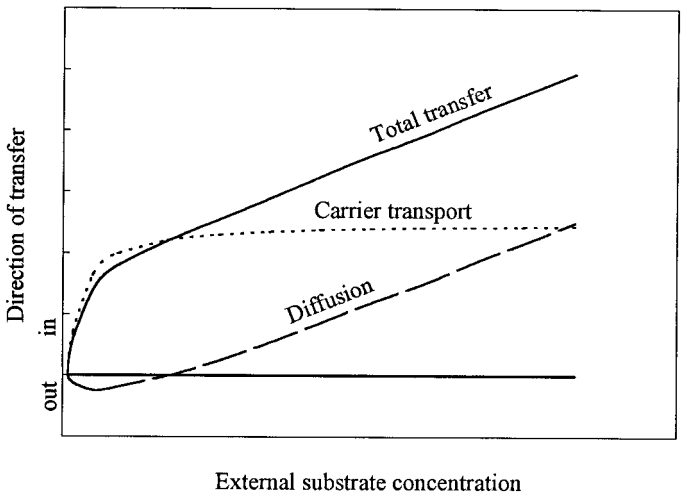

Fig. 6. Components of a concentrative transfer mechanism. The dotted line represents the carrier-mediated uptake of a substrate as a function of the external concentration of that substrate. The dashed line represents flux of substrate due to diffusional transport alone. At low external concentrations, the concentration gradient generated by the carrier transport system is sufficient to result in a diffusion of the substrate out of the cell. As the external substrate concentration increases, the diffusional flux is into the cell and becomes additive with the carrier-mediated transport to yield a nonsaturating transfer of material into the cell (solid line). Modified from Neame and Richards (1972).

these nonsaturating rates will be maintained, there are several possible explanations why they would be observed in incubation periods of ~1 h. These kinetics may reflect an uncoupling of uptake and growth. Early studies by Eppley and Renger (1974) and Conway and Harrison (1977) found that NH_4^+-limited cultures exhibited NH_4^+ uptake rates in excess of immediate growth rate requirements for nitrogen. McCarthy and Goldman (1979) examined the uncoupling of uptake and growth in steady-state NH_4^+-limited cultures at several growth rates and observed that as the experimental growth rate approached the maximal growth rate, short-term NH_4^+ uptake and growth rates became more closely coupled. Since these early studies, a number of authors have observed this rapid uptake in a variety of cultures and field studies (e.g., Glibert and Goldman 1981; Horrigan and McCarthy 1981; Goldman and Glibert 1982; Horrigan and McCarthy 1982; Lomas et al. 1996), but these studies have largely focused on NH_4^+. Raimbault and Gentilhomme (1990) and Garside and Glover (1991), using nanomolar additions of NO_3^-, have demonstrated that diatoms rapidly take up short-term pulses of NO_3^-, resulting in the formation of significant internal NO_3^- pools (Raimbault and Mingazzini 1987). However, studies to date demonstrating that rapid uptake does occur have been undertaken solely with nitrogen-limited cells. Given the typically high ambient NO_3^- concentration we measured in the field, it is not likely that the populations we studied were nitrogen limited to a significant degree.

Nonsaturable kinetics may also result from the retention of ^{15}N within internal pools and the inclusion of this ^{15}N with the particulate fraction during isotopic analysis (Collos et al. 1997). The ability of diatoms to form these internal pools is consistent with the work of Dortch et al. (1984), who observed that diatoms in the presence of high NO_3^- concentrations have the ability to form internal pools with concentrations on the order of 10s of millimolars. We sought to address this issue by using TCA to extract these internal pools from samples before the analysis of ^{15}N excess in the particulate pool. Although our data clearly show that some of the ''particulate ^{15}N'' is due to the inclusion of nitrogen that has yet to be assimilated, the NO_3^- uptake kinetic curve for TCA-extracted samples did not saturate either. The nonsaturating nature of the TCA-extracted samples could be due to a lack of saturation of nitrate reductase (NR). A sufficient number of studies have been conducted to provide an estimate of the half-saturation constant (K_m) values of NR in marine diatoms (Table 3), which range from 40 to 300 μM-N. Clearly, NR appears geared for rapidly assimilating NO_3^- that is present in the cells at high concentrations.

Lastly, nonsaturable kinetics may be due to simple diffusion into the cells (Fig. 6). In fact, the means by which cells are able to form and maintain an internal pool of a constituent that is greater than the external concentration of that constituent requires a diffusion-controlled transfer into the cell (Neame and Richards 1972). Collos et al. (1997), however, concluded that nonsaturable kinetics were not an artifact of diffusion by citing the upper concentration achieved by cells as being several millimolars, which would result in a diffusive gradient out of the cell; however, neither Collos et al. (1997) nor we actually measured the internal NO_3^- concentrations in these populations under ambient con-

Table 4. Summary of published inverse temperature relationships for cultures and field populations utilizing NO_3^-. The percent change in the measured parameter $°C^{-1}$ for each study was calculated as described in Table 1. For those studies where raw data were not published, data were extrapolated from the published parameter/temperature plots.

Parameter	Species	Study type	T°C range	% change $°C^{-1}$	Reference
Cellular protein conc.	*Skeletonema costatum*	Culture	7 and 20°C	−3.5	Jørgensen 1968
Cellular nitrogen conc.	*S. costatum, M. lutheri*	Culture	10–30°C	−3	Goldman 1977
Cellular nitrogen conc.	*Asterionella, Scenedesmus*	Culture	10–20°C	−8.2	Rhee and Gotham 1981
NR activity	*Skeletonema, Thalassiosira*	Culture	5–25°C	−4	Kristiansen 1983
^{15}N uptake velocity	Spring diatom bloom	Field	0–18°C	−13	Glibert et al. 1995
NR activity	Chlorophyte	Culture	5–55°C	−2.2	Loppes et al. 1996
^{15}N uptake velocity	Diatom blooms	Field	7–25°C	−2.9	This study

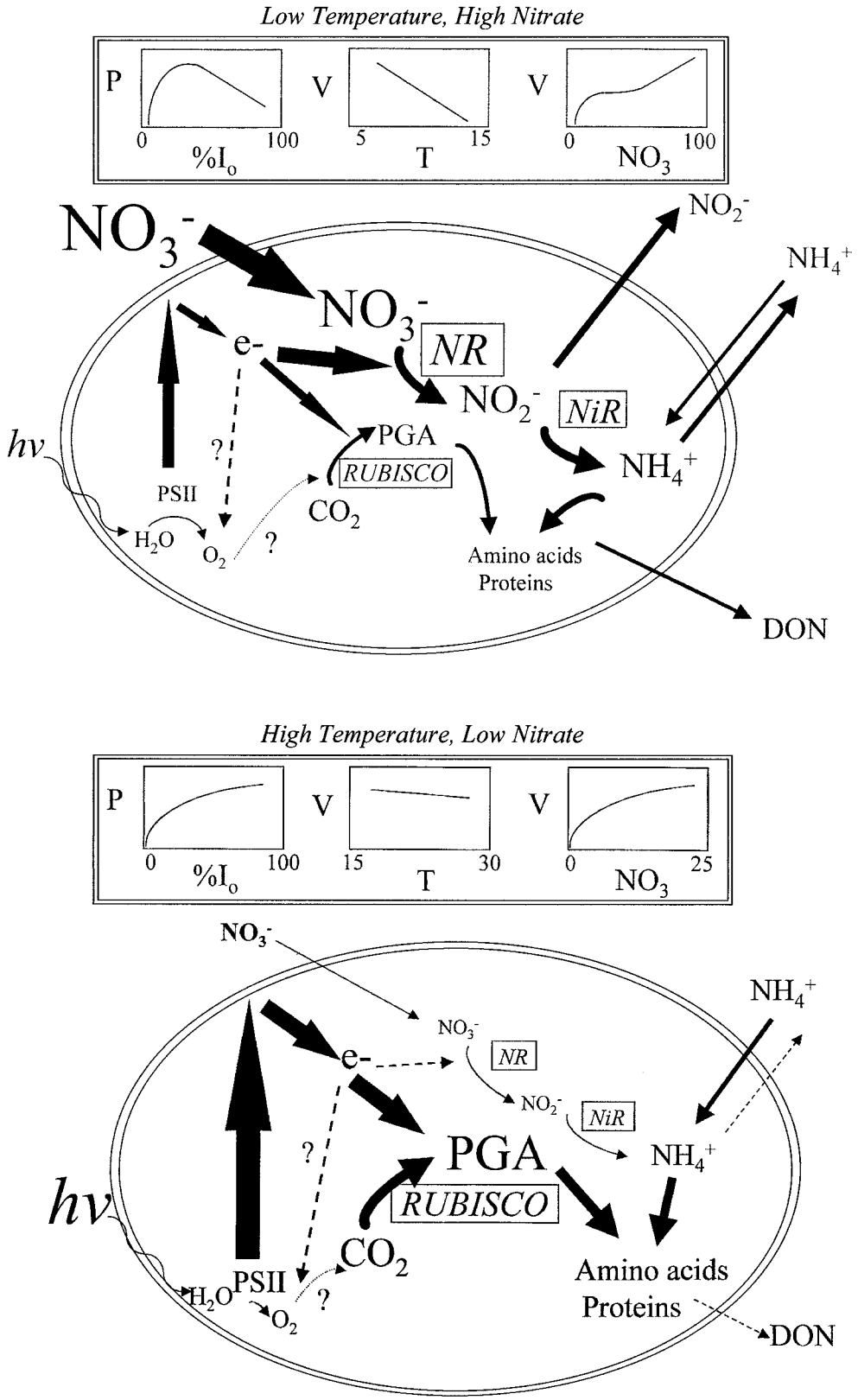

Fig. 7. Summary relationships of photosynthesis vs. irradiance and NO_3^- uptake (as ρ, μM-N h^{-1}) as a function of temperature and substrate concentration (upper panels) and hypothesized electron flow patterns during periods of low temperature, high NO_3^- (panel A) and high temperature, low NO_3^- (panel B). Under conditions of low temperature and high NO_3^-, phytoplankton assemblages are very photosynthetically efficient, but the overall photosynthetic capacity is lower. These cells exhibit enhanced NO_3^- uptake, as observed by the negative relationship of NO_3^- uptake with temperature and nonsaturable NO_3^- kinetics (over concentration ranges from 0 to >100 μM-N).

centrations. Falkowski (1975) has shown for several diatoms that the cell membrane-associated active carrier transporter for NO_3^- is very efficient ($K_m < 1$ μM-N) but does saturate (Fig. 6), and therefore diffusion may well be an important process contributing to nonsaturating NO_3^- uptake kinetics. Furthermore, Dortch (1982) has shown that a pulse of NO_3^- at 100 μM-N is required to form internal nitrogen pools of several millimolar concentrations. The mechanisms leading to nonsaturable uptake kinetics clearly need to be further examined under more highly controlled conditions.

Nitrogen uptake as a function of temperature—Positive physiological relationships between temperature and photosynthesis and growth have been well characterized (*see* reviews by Eppley 1972; Geider 1987; Raven and Geider 1988), but the relationship between nitrogen uptake and temperature has not been as well characterized to date. Although the relationship is assumed to be similar to that of photosynthesis, several studies have provided data suggestive of a negative relationship between NO_3^- utilization and temperature (Table 4). Furthermore, this is the first study to examine multiple nitrogen substrates and show contrasting patterns for temperature dependence of nitrogen uptake.

A substantial body of literature (Nalewajko and Garside 1983; Probyn 1985; Probyn and Painting 1985; Koike et al. 1986; Probyn et al. 1990; Dauchez et al. 1996) has developed showing that in cool, nutrient-rich (generally NO_3^-) environments, the >20-μm biomass (mainly diatoms) takes up a disproportionate fraction of total NO_3^- uptake. Several other studies have observed that diatoms take up substantial amounts of NO_3^- even when NH_4^+ is present in excess of 10 μM-N at cool temperatures (Maestrini et al. 1982, 1986). Although these studies do not provide a physiological basis for the observation of enhanced or preferred uptake of NO_3^-, they do suggest that NO_3^- is being taken up preferentially at low temperatures by cells >20 μm. Moreover, using the Relative Preference Index (RPI, in which the uptake of one nitrogen form relative to total nitrogen uptake is compared to the contribution that nitrogen form makes to the total nitrogen pool measured), originally developed by McCarthy et al. (1977), it has been found that exceptions to the observation of universal preference for NH_4^+ occur in cool, NO_3^--rich, diatom-dominated systems (Glibert et al. 1982*a;* Boyer et al. 1994; Glibert et al. 1995).

Studies conducted to date on the relationship between nitrogen uptake and temperature can be separated into two general categories: (1) steady-state culture experiments, and (2) field studies focusing primarily on seasonal relationships. Due to the inherent differences in temporal scales between these types of studies, they are measuring different aspects of phytoplankton physiology; therefore, there is no a priori reason why these studies all should yield the same result.

Steady-state culture studies, in which the influence of growth temperature has been examined, have typically reported these results as changes in cellular chemical composition (e.g., N/cell, C/cell, protein/cell, etc.) as a function of temperature-limited growth, as opposed to directly measuring nitrogen uptake rates. These studies have observed that for many phytoplankton species, N/cell increases at lower temperatures due to the increased synthesis of enzyme systems (Jørgensen 1968; Goldman 1977; Goldman and Mann 1980; Rhee and Gotham 1981; Thompson et al. 1992). Furthermore, it appears that N/cell is more sensitive to growth temperature than C/cell, leading to C : N ratios that decrease with decreasing temperature, which can be below the Redfield ratio (e.g., Thompson et al. 1992). This also can be explained by the increased synthesis of enzymes that are typically more rich in N than other cellular constituents. These steady-state observations imply that a number of species, in particular diatoms, growing at 10°C would incorporate relatively more N than the same species growing at 25°C. Consequently, observed uptake rates might be expected to exhibit a negative relationship with temperature (Table 4).

To the contrary, there are field studies in which nitrogen uptake rates were found to be positively related to temperature. For example, Harrison et al. (1996) observed that NO_3^- and NH_4^+ uptake rates collected in the North Atlantic were positively related to temperature over large temporal and spatial scales. During this study, not only were there gradients in temperature, but also nutrient concentrations, total biomass, and likely species composition (*see also* Slawyk 1979). This makes interpretation of the nitrogen uptake rates solely as a function of temperature difficult (e.g., Kanda

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Under conditions of high temperature and low NO_3^-, however, phytoplankton assemblages are less photosynthetically efficient but exhibit a greater photosynthetic capacity, NO_3^- uptake is not negatively related to temperature to the same degree, and NO_3^- uptake saturates following Michaelis–Menten kinetics (over concentration ranges from 0 to ~25 μM-N).

The lower schematic diagrams summarize our hypothesis of electron flow within a phytoplankton cell, which leads to the experimental observations mentioned above. In both panels, the size of symbols and arrows roughly depicts the size or importance of the pool or pathway. The flow of electron energy from photosystem II (PSII) may be divided into flow to carbon, flow to nitrogen, or flow to other dissipative processes such as O_2. Under low temperature, high NO_3^- conditions, carbon fixation (i.e., RUBISCO activity) and subsequent synthesis of phosphoglyceric acid (PGA) is temperature restricted and consequently is an inadequate electron sink. Under these low temperature, high NO_3^- conditions, we hypothesize that the high NO_3^- concentrations are used as an additional electron sink to prevent photoinhibitory damage to PSII. The excess electron energy (the fraction of total electron energy not being used by RUBISCO activity) is used to reduce NO_3^- to NH_4^+ via NR and nitrite reductase (NiR). As NO_3^- is reduced, it may either be released as NO_2^-, NH_4^+, or DON (e.g., amino acids and proteins). As temperatures warm and NO_3^- concentrations decline (i.e., as a spring bloom progresses), RUBISCO activity is no longer limited by temperature and able to utilize the higher daily light doses for higher photosynthetic productivity. The lower NO_3^- concentrations limit a cell's ability to form large internal pools, and therefore, the importance of NO_3^- as an electron sink is greatly reduced. Furthermore, nitrogen loss rates are likely to be lower, as the nitrogen that is taken up will be used to support higher amino acid biosynthesis rates.

et al. 1985). Separating the effects of changing biomass and temperature on NO_3^- uptake can be partially accounted for by normalizing to chlorophyll; however, over seasonal scales, there can also be substantial changes in the taxonomic composition of the biomass (e.g., Karentz and Smayda 1984; Glibert et al. 1995). Le Bouteiller (1986) observed a positive relationship between temperature and NO_3^- uptake rates. However, the temperature range was 18–30°C, and data from all depths were plotted together. Therefore, the relationship between temperature and NO_3^- uptake may have been confounded by the light dependence of NO_3^- uptake (e.g., MacIsaac and Dugdale 1972).

To examine the response of nitrogen uptake in field populations without the interference of changes in biomass, species composition, and nutrient history, we conducted the temperature shift experiments we present here. The positive relationship between NH_4^+ and urea uptake and temperature fits well with previous studies and the expected pattern for temperature-controlled enzyme mechanisms; however, the wide variance in response to these temperature shifts suggests that other factors are also important in regulating the actual response to temperature manipulations. The uptake of NO_3^- was negatively related to temperature in all experiments conducted during the winter or spring. Within the literature, only one other study was found for comparison, where the relationship between experimental temperature and nitrogen uptake was measured on short time scales. Smith and Harrison (1991) found that for Antarctic phytoplankton, both NO_3^- and NH_4^+ uptake responded positively to temperature based on a 4–8-h incubation period. At first, this study may appear contrary to ours, but upon closer examination, this may not be the case. The temperature range examined by Smith and Harrison was from −2 to 8°C, while we examined 7–25°C. Therefore, these two data sets cover different temperature ranges.

Energy balance hypothesis—The data we have presented show clearly that field populations with strong diatom components exhibit not only a high capacity for NO_3^- uptake but that this capacity increases under transient conditions of low temperature stress and excess light energy stress. More importantly, this capacity is maintained by cells under nitrogen-replete conditions. We suggest that these physiological characteristics of NO_3^- uptake may be regulated by the energy balance within the cell, as the nutritional requirements are clearly being met within these cells under the high nutrient conditions.

Pathways of carbon and nitrogen assimilation are linked via their requirements for energy (ATP) and reductant (NADPH) that are most efficiently generated through the light reactions of photosynthesis and transported between the chloroplast and the cytoplasm via the malate/oxaloacetate shuttle (Heber 1974; Lillo 1994). Typically, phytoplankton from cold waters, including polar systems and temperate systems in winter, display low E_k values and fairly low rates of photosynthesis at light saturation (e.g., Bunt and Lee 1970; Li and Platt 1982; Tilzer et al. 1986; Davison 1991). At cold temperatures, photoinhibition of photosynthesis has also been shown to be severe even for samples collected from near-surface waters for short-term incubations (Gallegos and Platt 1981; Glibert et al. 1985). Photoinhibition under low temperatures has been hypothesized to be due to temperature limitation of the enzymes in the dark reactions of photosynthesis (Kirk 1983). Therefore, if the light and dark reactions of photosynthesis are regulated differently by temperature, electrons generated in the light reactions must be dissipated, or damage to the photosynthetic apparatus may result.

Electrons generated through the light reactions of photosynthesis can be dissipated by a variety of biosynthetic and nonbiosynthetic pathways (*see* Maxwell et al. 1994, 1995; Geider et al. 1996). The nonbiosynthetic pathways include fluorescence and heat production from light-harvesting antennae, as well as a variety of downstream ''futile cycles,'' such as the oxygen-consuming Mehler reaction, although diatoms do not appear to have Mehler activity (Scheppe 1997). Xanthophyll cycling has also been shown to be an important pathway for energy dissipation in marine phytoplankton (Willemoes and Monas 1991; Olaizola et al. 1994; Olaizola and Yamamoto 1994). However, the fact that photoinhibition has been observed in field populations from cooler waters suggests that the sum of these pathways may be insufficient at times to balance the flow of electrons within these cells. Consequently, additional pathways are likely to be invoked to dissipate these high energy electrons from light harvesting (e.g., Wood et al. 1992; Neale et al. 1993; Ting and Owens 1993). We hypothesize that when water temperatures are low and NO_3^- concentrations are high, diatoms take up and store NO_3^- at high intracellular concentrations and use this NO_3^- as an oxidant to dissipate the periodic overflow of electron energy through the activity of NR. At low temperatures, while carbon uptake and metabolism may be limited by temperature and unable to buffer sudden changes in the flow of electrons from the light reactions (particularly on sudden changes in light and/or temperature), NO_3^- uptake and reduction should remain high, allowing for dissipation of these electrons. Therefore, NO_3^- reduction may be characterized as an alternative ''futile cycle'' for these diatom assemblages (Fig. 7A). The adaptive advantages for possessing mechanisms to dissipate excess electron energy flow in unstable water columns are numerous (e.g., Cullen and MacIntyre 1998).

We suggest, therefore, that in cool water, NO_3^- reduction would be a better mechanism to buffer imbalances in electron flow than ribulose bisphosphate carboxylase (RUBISCO). For this hypothesis to hold, one important requirement is that the temperature optima for enzymes of carbon fixation (RUBISCO) and NO_3^- uptake (NR) must be different, with NR being favored at low temperatures (<10°C) and RUBISCO being favored at higher temperatures (>10°C; Fig. 7A,B). The observation that NR activity in *Skeletonema costatum* is maximal around 10–15°C (Packard et al. 1971; Kristiansen 1983; Dohler 1991) and inactivated above 20°C (Gao et al. 1993, 1997) and the observation that RUBISCO activity is maximal at >30°C (Li et al. 1984; Smith and Platt 1985; Descolas-Gros and de Billy 1987) in both temperate and Antarctic diatoms are consistent with this requirement.

Additionally, if NO_3^- uptake and reduction are regulated by the generation of reductant and energy from photosynthetic processes under conditions of high NO_3^- concentration and low temperature, excess nitrogen to carbon uptake

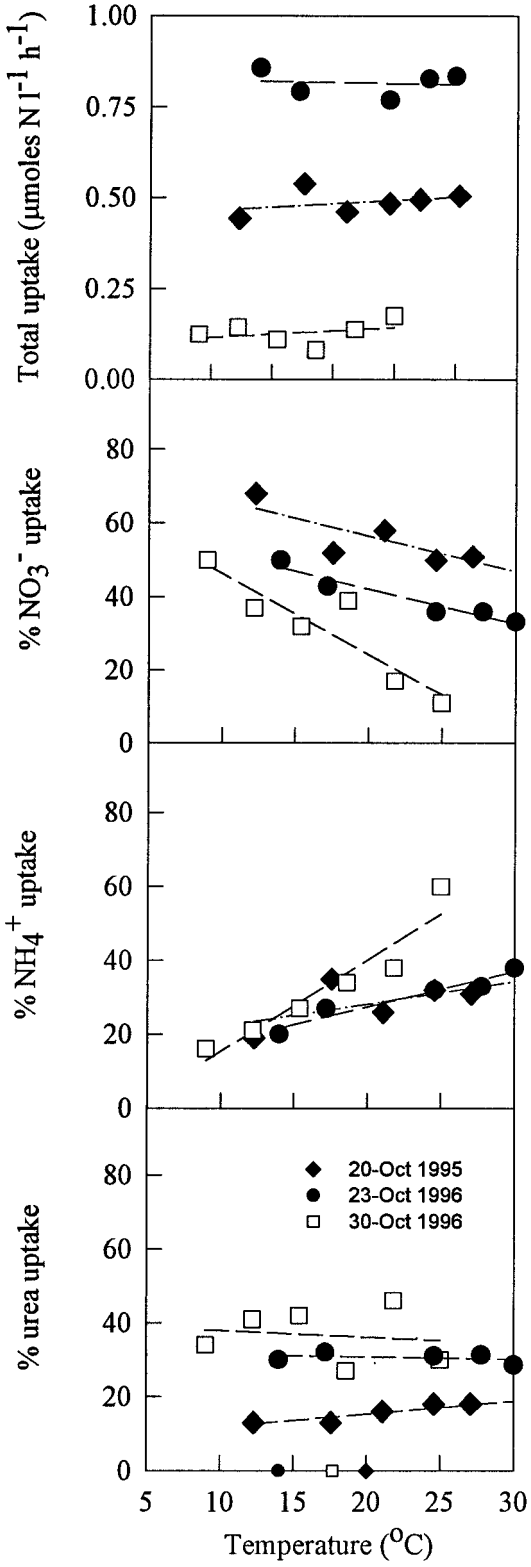

Fig. 8. Total nitrogen uptake (sum of NO_3^-, NH_4^+, and urea) and the percent contribution to the total of each substrate as a function of temperature. Only three experimental dates are presented for illustration: 20 October 1995, 23 October 1996, and 30 October 1996. The 20 October 1995 and 30 October 1996 dates show that there is no significant change in total nitrogen uptake as a function of temperature, while the $\%NO_3^-$ uptake increased sharply with a concomitant decrease in $\%NH_4^+$ uptake. On both dates, NO_3^- uptake represented >50% of total nitrogen uptake at the lowest temperature. The experiment on 23 October 1996 exhibited a large increase in total nitrogen uptake as temperature decreased, due to the overwhelming importance of NO_3^- uptake to total nitrogen uptake. These representative experiments clearly show that an increase in preference for NO_3^- uptake occurs as temperature decreases. The smaller symbols on the x-axis denote the ambient temperature for the respective day.

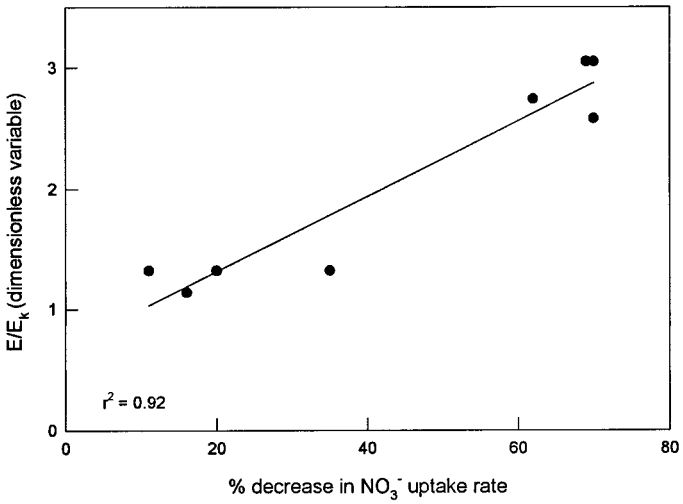

Fig. 9. Relationship between the degree of energy imbalance (experimental irradiance/photosynthetic half-saturation constant, E/E_k; MacIntyre and Harding unpubl. data) and the total percent decrease in NO_3^- uptake for each experiment. Data for spring 1997 are not yet available and therefore are not included. The linear regression is significant at the $P < 0.001$ level.

should be observed. The first recorded observation of ''apparent'' excess NO_3^- uptake was observed by Yentsch and Vaccaro (1958), who demonstrated for diatoms a ''poor balance between NO_3^- lost from solution and nitrogen found in growing algae.'' Several other early studies observed low carbon to nitrogen (C : N) assimilation ratios for spring diatom blooms growing on NO_3^- (McAllister et al. 1961; Antia et al. 1963), and several recent studies in four different waterbodies have observed C : N uptake ratios in the range of 2–4, considerably lower than predicted by Redfield stoichiometry (McCarthy and Nevins 1986; Dugdale et al. 1992; Glibert et al. 1995; McCarthy et al. 1996; Metzler et al. 1997). In the latter studies, heterotrophic uptake of carbon was invoked to reconcile these low ratios, but excess NO_3^- uptake may also be a possibility.

Our data provide a test of this hypothesis in two ways. First, NO_3^- is taken up disproportionately relative to NH_4^+ at low temperatures (Fig. 8). For the three representative experiments chosen, total nitrogen uptake remained roughly constant across the temperature ranges tested, while the uptake of NO_3^- decreased and the uptake of NH_4^+ increased as temperatures were increased from 10 to 30°C. In these three experiments, urea uptake was largely independent of exper-

Table 5. Comparison of environmental data for the high and low response groups of NO_3^- uptake as a function of temperature. Mixed layer depths are based on CTD profiles conducted when samples were collected. Euphotic depth and photosynthetic half-saturation irradiance were kindly provided by H. MacIntyre (Delaware Bay stations) and L. Harding, Jr. (Chesapeake Bay stations). PI curves were conducted at the same station and time as the temperature manipulation experiments.

Parameter	High response group	Low response group
Decrease in NO_3^- uptake (%)	63	20
T_{amb} (°C)	14–18	16–20
NO_3^- (μM-N)	12.4–86.3	0.5–84.5
NH_4^+ (μM-N)	1.6–9.1	2.3–4.4
Urea (μM-N)	0.5–0.7	0.9–1.1
PN (μM-N)	7.0–12.8	6.4–11.7
Chl *a* (μg liter^{-1})	9.8–20.0	6.3–11.4
Mixed layer depth (m)	7–18	10
Euphotic depth (m)	1–8	3
Photosynthetic half-saturating irradiance (μmol m^{-2} s^{-1})	79–97	180–290

imental temperature (Fig. 4), resulting in a relatively constant percent urea uptake. If these nitrogen substrates were being taken up only to meet nutritional demands, total nitrogen uptake should decrease with decreasing temperature, especially on this very short nonacclimated time scale. Clearly, this is not the case and is due to the increase in NO_3^- uptake as temperature decreased. Interestingly, as a percentage of total nitrogen uptake, urea did not change substantially over the temperature range even though the absolute rate increased as temperature increased.

The second test of this hypothesis is provided by comparison of the rates of NO_3^- uptake with photosynthetic parameters measured independently but simultaneously on the same bulk water samples (MacIntyre and Harding unpubl. data). An estimate of the imbalance between light harvesting and utilization can be expressed as the experimental irradiance divided by the half-saturation irradiance calculated from the photosynthesis vs. irradiance curve (E/E_k). The greater this ratio, the greater the imbalance between light harvesting and the utilization of CO_2 as a sink for the light-harvested energy. Plotting the ratio of E/E_k against the total decrease in NO_3^- uptake within each experiment (Fig. 9) shows a very strong ($r^2 = 0.92$) and significant ($P < 0.001$) positive relationship. Moreover, the experiments fall into the same two response groups (high and low) as separated in Fig. 4A,B. Table 5 compares parameters for both the high and low response groups, and the only major differences are in the ambient temperatures of populations at the time of sample collection and the calculated E_k values for the populations.

Finally, if NO_3^- does serve as an electron acceptor and its uptake is regulated in response to the need for energy dissipation in the cell, and if excess nitrogen is taken up relative to carbon, the question then becomes, What is the fate of this nitrogen? Although there are a number of possible fates, two are consistent with our hypothesis of NO_3^- as an electron acceptor and have support from the literature. First, release of DON has been observed in both culture and field populations utilizing primarily NO_3^- (e.g., Hellebust 1965; Strickland et al. 1969; Newell et al. 1972; Schell 1974; Conover 1975; Nalewajko 1977; Chan and Campbell 1978; Sharp 1984; Price et al. 1985; Collos et al. 1992*a;* Eppley and Renger 1992; Bronk and Glibert 1994; Bronk et al. 1994; Pujo-Pay et al. 1997). From these studies, the range of DON release rates has been found to be 6–79% of the NO_3^- taken up during the incubation period. Furthermore, field studies of Butler et al. (1979) and Collos et al. (1996) have shown significant linear relationships between the disappearance of NO_3^- and the appearance of DON. Second, the release of NO_2^- has also been commonly observed during the uptake of NO_3^- (e.g., Serra et al. 1978; Olson et al. 1980; Anderson and Roels 1981; Collos 1982; Ward et al. 1984). Clearly, phytoplankton can and do release nitrogen once reduced from NO_3^-, but the amount and form of this released nitrogen depends on a wide range of factors, from nutritional to environmental (Bronk and Glibert 1994).

Summary

In summary, we have presented data on NO_3^- uptake kinetics and the relationship between short-term temperature shifts and nitrogen uptake in diatom-dominated field populations. We observed nonsaturating NO_3^- uptake rates as well as a negative relationship between NO_3^- uptake and temperature. We interpreted these results in terms of a hypothesis whereby phytoplankton, which are exposed to conditions of transient energy stress, reduce NO_3^- to maintain the energy balance within the cell. While this hypothesis clearly requires further scrutiny, it is the first physiological explanation for the observed ''preference'' for NO_3^- by diatoms and nonsaturable kinetics even under nutrient-replete conditions. This hypothesis further reconciles observations of low C : N uptake ratios and high release rates of DON or NO_2^- by diatom-dominated assemblages. Inasmuch as the uptake of NO_3^- is central to models of new production, understanding both the direct and interactive effects of temperature, species composition, and kinetics of NO_3^- uptake will improve the predictive nature of these types of models. Furthermore, if indeed a significant fraction of NO_3^- uptake is regulated by non-nutritional mechanisms in the cell, and if some fraction of nitrogen reduced by this mechanism is subsequently released in the form of NO_2^-, NH_4^+, or DON, then estimates of new production based solely on NO_3^- uptake could be seriously biased (Collos et al. 1992*a;* Bronk and Glibert 1994).

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