

## Ammonium uptake and growth limitation in marine phytoplankton

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### Abstract

We used continuous culture techniques and a sensitive fluorescence method to quantify relationships among ammonium ( $\text{NH}_4^+$ ) concentration, cellular  $\text{NH}_4^+$  uptake rates, and growth rate limitation for five marine algal species ranging in diameter from 3 to 11  $\mu\text{m}$ . The growth rate of two high-nutrient-requiring coastal diatoms, *Thalassiosira weissflogii* and *Thalassiosira pseudonana*, were limited at  $\text{NH}_4^+$  concentrations  $<90\text{--}100\text{ nmol L}^{-1}$ , while those of low-nutrient-adapted species, the oceanic coccolithophore *Emiliana huxleyi*, the coastal pelagophyte *Aureoumbra lagunensis*, and the coastal trebouxiophyte *Nannochloris atomus*, were limited at concentrations  $<30\text{--}40\text{ nmol L}^{-1}$ . In the nitrogen-limited cyclostats, the residence times of dissolved ammonium were extremely short (4–12 min at chlorophyll *a* levels of 20–30  $\mu\text{g L}^{-1}$ ), and these short times had to be taken into account to obtain accurate  $\text{NH}_4^+$  measurements. Ammonium and nitrate concentrations in stratified surface ocean waters (3–15  $\text{nmol L}^{-1}$ ) fall within the range that substantially limited algal growth rates in our experiments, providing evidence for nitrogen limitation in these waters. Ammonium uptake rates and associated specific growth rates were much lower for *T. weissflogii* (10–11  $\mu\text{m}$  in diameter) than for the other smaller (3.1–4.5  $\mu\text{m}$  in diameter) species under ammonium limitation owing to the limits imposed by  $\text{NH}_4^+$  diffusion to the cell surface. Diffusion per unit of cell volume varies with the inverse square of the cell diameter and, thus, greatly restricts growth of large-celled species under  $\text{NH}_4^+$  limitation. The resultant selection of small-celled algal species in ammonium-limited ocean waters should promote rapid grazing and nutrient cycling and minimum nutrient loss via settling of intact cells and zooplankton fecal material.

Competition for limiting nutrients is a fundamental force shaping the composition and structure of phytoplankton communities and planktonic food webs (Sommer 1989; Thingstad and Sakshaug 1990). This competition is based in large measure on differences in relationships among dissolved nutrient concentrations, cellular nutrient uptake rates, and specific growth rates among species. It has been argued that nitrogen (N) is the most important limiting nutrient in coastal waters and the open ocean based on N:phosphorus (P) ratios of inorganic nutrient pools and results of nutrient addition experiments (Dugdale 1967; Ryther and Dunstan 1971; Falkowski and Raven 1997). In low-nitrogen waters, ammonium ( $\text{NH}_4^+$ ) is often the dominant nitrogen source utilized by phytoplankton, while nitrate, which is energetically more difficult to assimilate, is only a minor source (Harrison et al. 1996; Mulholland and Lomas in press). However, despite the likely importance of  $\text{NH}_4^+$  in influencing marine carbon cycles and food web dynamics, the ammonium concentrations that limit algal growth rate are uncertain because of difficulties in measuring ammonium at the low concentrations that limit algal growth rate (Goldman and McCarthy 1978).

Although a continuum undoubtedly exists, phytoplankton (and indeed all plants) can be roughly divided into two broad categories: r-selected and K-selected species (Kilham and Hecky 1988; Sommer 1989). r-Selected species, typified by diatoms, have high maximum growth rates and should require high concentrations of nutrients to sustain those rates (Kilham and Kilham 1980). Diatoms dominate algal communities in nutrient-rich environments such as coastal

upwelling systems and annual spring blooms (Kilham and Kilham 1980). K-selected species typically have lower maximum growth rates and are well adapted for growth in low-nutrient environments (Kilham and Hecky 1988), where recycled ammonium is usually the principal inorganic nitrogen source (Platt and Harrison 1985; Harrison et al. 1996).

We used continuous culture techniques and a sensitive fluorescence ammonium method to quantify relationships among  $\text{NH}_4^+$  concentration, cellular nitrogen uptake rates, cellular nitrogen content, and N-limited growth rate in five species of marine phytoplankton. In our ammonium-limited cyclostat cultures, nutrient-enriched seawater medium was continuously introduced at a rate that equaled the removal rate of algal culture. A steady state developed under nutrient-limiting conditions in which the rate of ammonium input (via addition of fresh medium) equaled the rate of algal uptake and the daily specific growth rate of the algae equaled the culture dilution rate (the rate of addition of new medium [ $\text{L d}^{-1}$ ] divided by the culture volume) (Thingstad and Sakshaug 1990). Almost all of the available nitrogen was taken up by the algae, and the residual ammonium adjusted to a concentration that just supported the specific growth rate. By varying the dilution rate (and thus the specific growth rate) and by measuring the  $\text{NH}_4^+$  concentration in the culture, we were able to quantify relationships among N-limited growth rate, cellular ammonium uptake rate, and the  $\text{NH}_4^+$  concentration in the medium. In previous continuous culture studies, ammonium levels were always below the detection limit of standard analytical methods (Goldman and McCarthy 1978). We overcame this difficulty by using a new sensitive fluorometric method that has a detection limit of  $\sim 3\text{ nmol L}^{-1}$  (Holmes et al. 1999).

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Our five experimental algal species represented different taxonomic groups, cell sizes, marine habitats, and r- and K-selected reproductive strategies. The species included small and large coastal diatoms (*Thalassiosira pseudonana*, ~4.5- $\mu\text{m}$  diameter, and *Thalassiosira weissflogii*, 10–11- $\mu\text{m}$  diameter), the small coastal pelagophyte (*Aureoumbra lagunensis*, ~4.0- $\mu\text{m}$  diameter), the small coastal trebouxioiophyte (*Nannochloris atomus*, 3.0- $\mu\text{m}$  diameter), and the oceanic coccolithophore (*Emiliana huxleyi*, 4.1- $\mu\text{m}$  diameter). The diatoms have high maximum growth rates and are putative r-selected species, while the remaining three species have low maximum growth rates and are likely K-selected species (Sunda et al. 2006). *A. lagunensis* has caused ecologically damaging “brown tide” blooms in Laguna Madre, Texas, over the past 16 yr (Buskey et al. 2001), while *N. atomus* caused similar harmful “green tide” blooms in shallow bays in Long Island, New York, in the 1950s (Ryther 1989). Thus, these latter two algae are harmful algal bloom (HAB) species.

In addition to r- and K-selected differences, cells of differing sizes were selected to investigate the relationship between nutrient limitation and cell size. Theoretically, small size should permit higher rates of nutrient uptake and growth under nutrient-limiting conditions as a result of higher surface-to-volume ratios and nutrient diffusion to the cell surface per unit of cell volume (Pasciak and Gavis 1974; Kiorboe 1993). Although it has been suggested that the uptake of nutrients by larger cells may be limited by the rate of nutrient diffusion to the cell surface (Pasciak and Gavis 1974), this has never been shown experimentally for ammonium.

## Methods

Axenic cultures of *T. pseudonana* (clone CCMP 1335), *T. weissflogii* (CCMP 1336), *A. lagunensis* (CCMP 1509), *N. atomus* (CCMP 509), and *E. huxleyi* (CCMP 373) were obtained from the Center for the Culture of Marine Phytoplankton, Booth Bay Harbor, Maine. Experiments were conducted in continuous cyclostat cultures using procedures similar to those previously described (Liu et al. 2001). Cultures were grown at 20°C or 25°C in polycarbonate culture bottles with internal Teflon stirrers (Nalgene) containing 1 liter of culture medium. Two species (*A. lagunensis* and *N. atomus*) were cultured at both temperatures, while the three other species were tested only at a single temperature (*T. weissflogii* and *T. pseudonana* at 20°C and *E. huxleyi* at 25°C). The choice of temperatures was based on the thermal tolerances of the species.

The culture medium consisted of filtered Gulf Stream seawater (salinity, 36) containing 12  $\mu\text{mol L}^{-1}$   $\text{NH}_4\text{Cl}$  (6–24  $\mu\text{mol L}^{-1}$  for *T. weissflogii*), 2  $\mu\text{mol L}^{-1}$   $\text{Na}_2\text{HPO}_4$ , 40  $\mu\text{mol L}^{-1}$   $\text{Na}_2\text{SiO}_3$ , 10  $\text{nmol L}^{-1}$   $\text{Na}_2\text{SeO}_3$ , vitamins (0.074  $\text{nmol L}^{-1}$  vitamin  $\text{B}_{12}$ , 0.4  $\text{nmol L}^{-1}$  biotin, and 60  $\text{nmol L}^{-1}$  thiamin), and an ethylenediaminetetraacetic acid (EDTA)–trace metal buffer (100  $\mu\text{mol L}^{-1}$  EDTA, 1  $\mu\text{mol L}^{-1}$  iron, 50  $\text{nmol L}^{-1}$  manganese, 100  $\text{nmol L}^{-1}$  zinc, 40  $\text{nmol L}^{-1}$  copper, and 40  $\text{nmol L}^{-1}$  cobalt). Light was provided at an intensity of 100  $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$

from fluorescent bulbs (Vita-Lite) on a 14:10 light:dark cycle. Sterile media were pumped continuously into the culture vessel, and an equal volume of culture was removed by peristaltic pumps. The culture dilution rate was quantified by measuring the volume of culture removed over a given time period. Prior to experiments the culture vessels and attached Teflon tubing were sterilized by autoclaving. The input culture medium was sterilized separately by microwave treatment (Keller et al. 1988).

Culture samples were removed for daily measurements of total cell volume, cell numbers, and average volume per cell using a Beckman–Coulter Multisizer 3 electronic particle counter. Samples were also removed from acclimated, “steady-state” cultures for measurements of dissolved ammonium (Holmes et al. 1999) and occasional analyses of chlorophyll *a* (Chl *a*). Samples were removed on separate days for measurements of total particulate (cellular) nitrogen with a Costech carbon (C):N analyzer. Prior to this analysis the cells were filtered onto combusted GF/F glass-fiber filters and fumed with HCl to remove inorganic carbon (Liu et al. 2001). Samples were taken during the middle of the light period and were analyzed in triplicate, except for particulate N and C, which were analyzed in duplicate samples. For three of the cultures, samples were also sampled over the entire diel period for comparison with the midday sample results.

Ammonium concentrations were measured by the recently described fluorometric method of Holmes et al. (1999). Measurements were made in 8-mL aliquots of unfiltered culture samples and, in some cases, in filtered samples for comparative purposes. Most commonly used filters could not be used for our analyses because they either absorb ammonium ions (e.g., GF/F glass-fiber filters) or because they could not be adequately cleaned to prevent contamination of the samples. Also, to avoid ammonium contamination from the atmosphere, samples could only be briefly exposed to laboratory air. For filtration, samples were passed under gentle pressure through acid-washed 0.4- $\mu\text{m}$ -pore IC-MILLEX-LH hydrophilic Teflon cartridge filters (Millipore).

The degree of nitrogen limitation of growth rate was determined by comparing the specific growth rate in the cyclostat cultures with the maximum growth rate under nutrient sufficiency. Under steady-state conditions in our cyclostat cultures, the average daily specific growth rate equaled the culture dilution rate. To measure the maximum specific growth rate, cells were grown in semicontinuous batch cultures in the same culture media as in the cyclostat cultures, but with a higher  $\text{NH}_4\text{Cl}$  concentration (32  $\mu\text{mol L}^{-1}$ ). The cultures were serially transferred to fresh media well before they reached their maximum cell volumes, and, thus, they never experienced nutrient limitation. Specific growth rates were determined by linear regression of curves of the natural log of total cell volume versus time after correcting for serial dilution of the cultures.

Cellular nitrogen uptake rates in the cyclostat cultures were computed by multiplying the moles of cellular nitrogen per liter of cell volume or per cell at a given growth rate by the specific growth rate of the algae. The

uptake rate per cell was compared with the computed maximum diffusion rate of  $\text{NH}_4^+$  to the cell surface based on an assumed spherical geometry:

$$\rho_{\max} = 4\pi r[\text{NH}_4^+]D \quad (1)$$

where  $\rho_{\max}$  is the rate that would exist if all of the ammonium diffusing to the cell surface were taken up and the surface concentration were reduced to zero (Pasciak and Gavis 1974). The diffusion coefficients for  $\text{NH}_4^+$  ( $D = 17.6 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$  at  $20^\circ\text{C}$  and  $19.8 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$  at  $25^\circ\text{C}$ ) equaled infinite dilution values corrected downward by 5% to take into account the lower viscosity of seawater relative to that of pure water and the slightly lower ionic hydration (Li and Gregory 1974). The correction value of 5% was assumed to equal that measured for  $\text{K}^+$ , an ion with the same charge and ionic radius as ammonium and the same value of  $D$  at infinite dilution for a given temperature (Li and Gregory 1974). The radius of the cell ( $r$ ) in Eq. 1 was computed from the mean volume per cell and the relation between the volume and radius of a sphere ( $V = 4\pi r^3/3$ ). *N. atomus*, *A. lagunensis*, and *E. huxleyi* have spherical shapes, and thus the above equations should apply exactly. Cells of the two centric diatoms are equidimensional rounded cylinders, and the above equations do not apply exactly. However, their use should result in only minor errors, since the aspect ratios for these cells, the ratios of length to diameter, are near unity (Pasciak and Gavis 1974).

Statistical tests (linear regressions and  $t$ -tests) were conducted with the Microsoft Excel computer program.

## Results and discussion

*Short residence times of dissolved ammonium*—We observed rapid algal uptake of ammonium ions and low steady-state ammonium concentrations in the cyclostat cultures. As a consequence, ammonium residence times were short, and concentrations decreased rapidly once a sample was removed from the cyclostat culture. In time-course experiments with *T. weissflogii* and *T. pseudonana*, the measured  $\text{NH}_4^+$  concentration,  $[\text{NH}_4^+]$ , fit the equation

$$\ln[\text{NH}_4^+]_t - \ln[\text{NH}_4^+]_{t=0} = -kt \quad (2)$$

(Fig. 1; Table 1), derived from integration of a first-order rate equation for  $\text{NH}_4^+$  loss via algal uptake:

$$-d[\text{NH}_4^+]/dt = k[\text{NH}_4^+] \quad (3)$$

where  $k$  is the specific  $\text{NH}_4^+$  loss rate ( $\text{min}^{-1}$ ) and  $t$  is the time elapsed between the removal of the sample from the culture and the addition of the analytical reagent mixture. This mixture contained  $4.2 \text{ mmol L}^{-1}$  sodium borate,  $12.6 \text{ } \mu\text{mol L}^{-1}$  sodium sulfite,  $3 \text{ mmol L}^{-1}$  orthophthalaldehyde (OPA), and  $171 \text{ mmol L}^{-1}$  ethanol, in terms of final concentrations in the culture sample. It contained one or more biological inhibitors (e.g., ethanol, sulfite, and OPA) that appeared to stop cellular ammonium uptake. This is evidenced by the rapid decrease in measured ammonium (at specific rates of  $0.07$ – $0.24 \text{ min}^{-1}$ ) with the

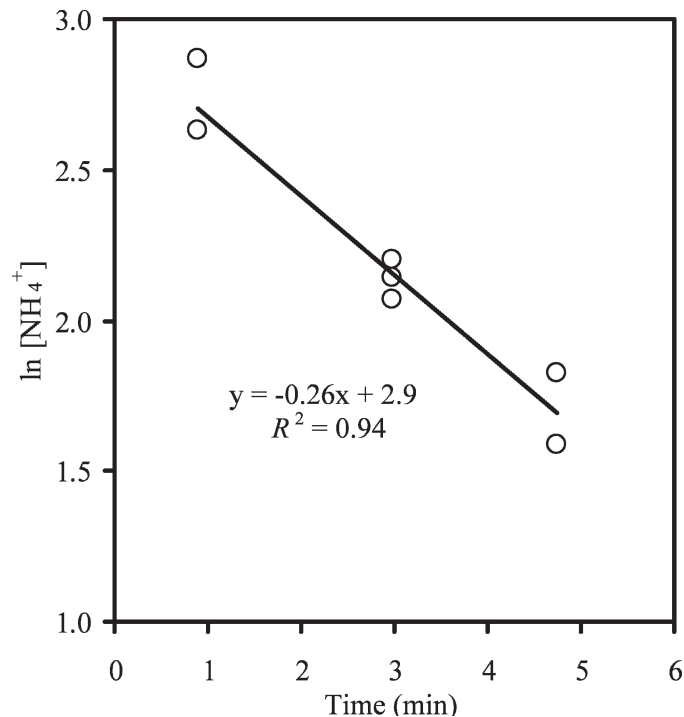


Fig. 1. Natural log  $[\text{NH}_4^+]$  versus time elapsed between sample removal from a *T. pseudonana* cyclostat culture (total nitrogen =  $12 \text{ } \mu\text{mol L}^{-1}$ ) and the addition of ammonium reagents to aliquots of an unfiltered culture sample.

time elapsed between sampling and the addition of the reagent mixture to unfiltered samples (Fig. 1; Table 1), even though the half-time for ammonium reaction with the analytical reagent OPA is  $\sim 40 \text{ min}$  (Holmes et al. 1999). In addition, the reagent mixture totally stopped flagellar motility within 10–15 s (the minimum time needed for microscopic observation) in three of the algal species we examined (the dinoflagellate *Karenia brevis*, the cryptophyte *Rhodomonas salina*, and the raphidophyte *Heterosigma akashiwo*). In a subsequent experiment, the addition of the OPA and ethanol mixture (the OPA was dissolved in the ethanol) caused the same complete stoppage of flagellar motility in *K. brevis*, while the ethanol added alone slowed but did not stop flagellar movement. Thus, the OPA and ethanol appear to be the main inhibitory components of the analytical reagent mixture.

Despite its inhibitory effects on cells, the addition of the reagent mixture to *A. lagunensis* culture samples caused no measurable ( $<1 \text{ nmol L}^{-1}$ ) increase in extracellular dimethylsulfoniopropionate (DMSP), despite the high intracellular concentration of this osmolyte ( $\sim 100 \text{ mmol L}_{\text{cell}}^{-1}$  and  $100 \text{ nmol per liter of culture}$ ; data not shown). This result indicates that the reagent mixture does not cause general leakage of intracellular solutes.

In the time-course experiments with unfiltered cyclostat culture samples, the values of  $k$  determined from regression analyses agreed with the theoretical values computed by multiplying the  $[\text{NH}_4^+]$  in the input medium by the culture dilution rate and dividing by the extrapolated initial  $[\text{NH}_4^+]$  (Table 1). This agreement again supports the validity of

Table 1. Negative regression slopes ( $k$ ) for plots of the natural log ( $\ln$ )  $[\text{NH}_4^+]$  versus time (min) elapsed between the addition of analytical reagents to unfiltered culture samples and removal of samples from the cyclostat cultures.

Species	Input $[\text{NH}_4^+]$ ( $\mu\text{mol L}^{-1}$ )	Dilution rate ( $\text{d}^{-1}$ )	Time interval (min)	$k$ (regression) ( $\text{min}^{-1}$ )	$k^*$ (theoretical) ( $\text{min}^{-1}$ )	Initial $[\text{NH}_4^+]$ ( $\text{mmol L}^{-1}$ )	$R^2$	$n$	$p$ (regression slope)
<i>Thalassiosira weissflogii</i>	12	0.357	1.0–5.0	0.103 $\pm$ 0.025	0.106 $\pm$ 0.097	28.1 $\pm$ 2.5	0.70	9	0.005
	12	0.357	1.0–5.0	0.070 $\pm$ 0.014	0.080 $\pm$ 0.005	37.1 $\pm$ 1.8	0.78	9	0.0015
	12	0.468	1.0–7.3	0.097 $\pm$ 0.011	0.095 $\pm$ 0.005	41.0 $\pm$ 2.0	0.89	11	0.01
	12	0.467	1.2–7.2	0.123 $\pm$ 0.009	0.078 $\pm$ 0.003	49.8 $\pm$ 1.6	0.88	9	<0.001
<i>Thalassiosira pseudonana</i>	24	0.373	1.2–6.5	0.109 $\pm$ 0.012	0.146 $\pm$ 0.009	42.5 $\pm$ 2.7	0.88	12	<0.001
	24	0.373	1.0–7.0	0.133 $\pm$ 0.005	0.131 $\pm$ 0.003	47.4 $\pm$ 1.1	0.98	12	<0.001
	24	0.484	1.2–5.0	0.170 $\pm$ 0.015	0.173 $\pm$ 0.009	46.5 $\pm$ 2.4	0.95	9	<0.001
	12	0.552	0.9–4.8	0.26 $\pm$ 0.03	0.24 $\pm$ 0.03	18.9 $\pm$ 1.7	0.94	8	<0.001

\* Theoretical  $k$  values equal the  $\text{NH}_4^+$  input rate (input  $[\text{NH}_4^+]$  times the dilution rate in minutes) divided by the  $[\text{NH}_4^+]$  at the time of sampling. The latter was computed from the Y-intercept of the linear regression, which equals the  $\ln$   $[\text{NH}_4^+]_{t=0}$ . Error bars here and in adjacent columns give  $\pm$  standard error (SE).

our measured ammonium values. Time courses were not run for most samples, and in these cases we made measurements as quickly as possible (after 1 min for unfiltered samples and after 3 min for filtered samples) and extrapolated back to the time zero  $[\text{NH}_4^+]$  using the theoretically calculated specific loss rate.

At the usual total nitrogen concentration of 12  $\mu\text{mol L}^{-1}$  culture (20–30  $\mu\text{g L}^{-1}$  of Chl *a*, depending on the growth rate), average specific  $\text{NH}_4^+$  loss rates were 0.13–0.23  $\text{min}^{-1}$  for the four smaller species, which yielded extremely short  $\text{NH}_4^+$  residence times of 4–8 min (Table 2). Estimated residence times were longer (10–12 min) for *T. weissflogii* (Table 2), which had a lower specific rate of ammonium uptake because of its large size.

Based on theory and our experimental observations, ammonium residence times are inversely related to total cell N and associated algal biomass (Table 1). Short residence times, similar to those in our cultures, should also occur at similar algal biomass levels and cell sizes (3–11  $\mu\text{m}$ ) in N-limited recycled environments, where ammonium input and cellular uptake rates are often roughly in balance, as occurs in the cyclostats (Glibert 1993; Mulholland and Lomas in press). In such high-biomass recycled systems, short residence times will result in rapid adjustment in ammonium concentrations toward new steady-state values with changes in rates of ammonium inputs from external or internal sources (e.g., from nutrient cycling) or in rates of loss from algal uptake. Such changes invariably occur with variations in wind-driven or tidal mixing, solar heating of surface waters, vertical migration of zooplankton or algal cells, or diel variations in algal growth. And as observed in our cyclostat cultures, such susceptibility to rapid change can present a substantial challenge to accurate measurement of in situ ammonium concentrations.

#### Effect of varying ammonium levels in the input medium—

It is often assumed in nutrient-limitation models of aquatic ecosystems that below some critical ratio of the concentration of limiting nutrient to that of other potentially limiting nutrients (the N:P ratio in the inflowing medium in present experiments), the relationship between the growth rate of an algal species and the concentration of limiting nutrient is independent of the source nutrient ratios (Tilman 1982; Roelke et al. 2003). Although there are few actual data to support this hypothesis (and none in the case of ammonium), this extension of Liebig's Law of the Minimum is nonetheless widely used because it greatly simplifies the modeling of algal growth dynamics in nutrient-limited aquatic ecosystems. We tested the validity of this hypothesis by conducting cyclostat experiments with *T. weissflogii* at three different ammonium concentrations in the inflowing medium (6, 12, and 24  $\mu\text{mol L}^{-1}$ ) and a single phosphate concentration (2  $\mu\text{mol L}^{-1}$ ). The associated molar N:P ratios (3, 6, and 12) were in all cases below the Redfield ratio (16), the approximate molar ratio in deep ocean water and that needed to achieve maximum growth rate in many marine phytoplankton (Redfield et al. 1963; Goldman et al. 1979). At a constant dilution rate (0.61  $\pm$  0.04  $\text{d}^{-1}$ ) in these experiments, the total cell volume per liter of culture (5.6  $\pm$  1.2, 11.1  $\pm$  0.0, and 21.4  $\pm$

Table 2.  $\text{NH}_4^+$  concentrations and residence times and nitrogen uptake rates as a percentage of the maximum rate of  $\text{NH}_4^+$  diffusion to the cell surface for cyclostat cultures with an  $[\text{NH}_4^+]$  of  $12 \mu\text{mol L}^{-1}$  in the input medium. Errors provide value  $\pm$  standard deviation (SD) for  $n$  samples, where each sample was measured in triplicate.

Species	Growth rate ( $\text{d}^{-1}$ )	Diameter ( $\mu\text{m}$ )	$[\text{NH}_4^+]$ ( $\text{nmol L}^{-1}$ )	Turnover time (min)	Uptake rate ( $\text{mol L}^{-1} \text{d}^{-1}$ )	Percent diffusion rate	$n$
<i>Thalassiosira weissflogii</i> (20°C)	$0.87 \pm 0.02^*$	$12.6 \pm 0.1$	9,000–26,000				3
	$0.75 \pm 0.04$	$10.1 \pm 0.0$	$75.0 \pm 7.8$	$12.1 \pm 1.7$	$1.04 \pm 0.05$	$82.6 \pm 11.7$	3
	$0.63 \pm 0.03$	$10.3 \pm 0.0$	$55.5 \pm 4.5$	$10.7 \pm 1.4$	$0.71 \pm 0.01$	$80.4 \pm 12.4$	8
	$0.55 \pm 0.00$	$11.0 \pm 0.0$	$46.9 \pm 9.7$	$10.7 \pm 2.5$	$0.53 \pm 0.00$	$81.5 \pm 14.8$	4
	$0.45 \pm 0.02$	$11.1 \pm 0.1$	$41.9 \pm 3.5$	$10.5 \pm 1.0$	$0.39 \pm 0.02$	$66.4 \pm 5.7$	8
	$0.36 \pm 0.01$	$11.2 \pm 0.0$	$31.8 \pm 1.8$	$10.5 \pm 0.6$	$0.31 \pm 0.02$	$70.0 \pm 3.8$	5
	$0.32 \pm 0.01$	$10.1 \pm 0.1$	$28.7 \pm 0.8$	$10.6 \pm 0.4$	$0.34 \pm 0.02$	$70.6 \pm 6.3$	4
	$0.25 \pm 0.01$	$10.1 \pm 0.0$	$21.9 \pm 2.4$	$10.5 \pm 1.2$	$0.18 \pm 0.01$	$47.9 \pm 5.9$	4
<i>Thalassiosira pseudonana</i> (20°C)	$1.45^*$	$4.8 \pm 0.1$	19,000–28,000				4
	$1.29 \pm 0.00$	$4.7 \pm 0.0$	$81.3 \pm 14.7$	$7.6 \pm 1.4$	$2.63 \pm 0.02$	$38.8 \pm 2.5$	2
	$0.90 \pm 0.02$	$4.6 \pm 0.0$	$44.2 \pm 7.7$	$5.9 \pm 1.0$	$1.93 \pm 0.10$	$48.3 \pm 2.5$	3
	$0.56 \pm 0.00$	$4.4 \pm 0.1$	$18.1 \pm 3.4$	$3.9 \pm 0.7$	$0.73 \pm 0.06$	$36.8 \pm 2.8$	4
	$0.33 \pm 0.01$	$4.3 \pm 0.1$	$12.2 \pm 2.5$	$4.4 \pm 0.9$	$0.55 \pm 0.07$	$40.5 \pm 7.9$	6
<i>Aureoumbra lagunensis</i> (20°C)	$0.45 \pm 0.00^*$	$5.7 \pm 0.1$	~20,000–30,000				3
	$0.32 \pm 0.00$	$4.2 \pm 0.1$	$18.1 \pm 2.7$	$7.1 \pm 1.4$	$0.38 \pm 0.01$	$22.4 \pm 4.5$	7
	$0.25 \pm 0.00$	$4.1 \pm 0.1$	$14.6 \pm 2.8$	$7.1 \pm 1.3$	$0.25 \pm 0.01$	$17.3 \pm 3.8$	4
(25°C)	$0.73 \pm 0.00^*$	$5.8 \pm 0.3$	~20,000–30,000				
	$0.64 \pm 0.01$	$4.4 \pm 0.0$	$35.2 \pm 3.0$	$6.6 \pm 0.6$	$0.60 \pm 0.00$	$16.7 \pm 1.7$	3
	$0.32 \pm 0.00$	$4.2 \pm 0.1$	$14.3 \pm 2.4$	$5.3 \pm 0.9$	$0.22 \pm 0.00$	$14.1 \pm 2.2$	3
	$0.23 \pm 0.01$	$3.8 \pm 0.1$	$10.0 \pm 0.5$	$5.1 \pm 0.6$	$0.16 \pm 0.01$	$11.9 \pm 1.7$	3
<i>Nannochloris atomus</i> (20°C)	$0.71 \pm 0.01^*$	$3.5 \pm 0.1$	~20,000–30,000				
	$0.65 \pm 0.03$	$3.1 \pm 0.1$	$29.0 \pm 6.4$	$5.4 \pm 1.0$	$1.10 \pm 0.11$	$21.0 \pm 2.7$	6
(25°C)	$0.94 \pm 0.03^*$	$3.1 \pm 0.2$	~20,000–30,000				
	$0.63 \pm 0.01$	$3.3 \pm 0.1$	$24.8 \pm 2.1$	$4.7 \pm 0.4$	$0.93 \pm 0.01$	$19.1 \pm 1.6$	3
	$0.32 \pm 0.01$	$3.3 \pm 0.0$	$13.7 \pm 1.6$	$5.1 \pm 1.0$	$0.37 \pm 0.01$	$14.5 \pm 2.7$	7
<i>Emiliania huxleyi</i> (25°C)	$0.54 \pm 0.01^*$	$5.0 \pm 0.3$	~20,000–30,000				
	$0.33 \pm 0.00$	$4.1 \pm 0.0$	$21.4 \pm 2.9$	$7.7 \pm 1.1$	$0.38 \pm 0.01$	$14.9 \pm 1.9$	2

\* Maximum growth rates measured in nutrient-sufficient batch cultures. Errors provide value  $\pm$  standard deviation (SD) for  $n$  samples, where each sample was measured in triplicate.

$1.2 \mu\text{mol L}^{-1}$ , respectively) was proportional to the  $[\text{NH}_4^+]$  in the input medium, which verified that algal growth was limited by nitrogen. The relationship between specific growth rate and  $[\text{NH}_4^+]$  in the cultures was independent of the  $\text{NH}_4^+$  concentration in the input medium (Fig. 2), thus verifying the Liebig hypothesis for N:P ratios of  $\leq 12$  (75% of the Redfield value). In all subsequent cyclostat experiments with other species, we used an N:P ratio of 6 in the inflowing medium (37% of the Redfield value), based on ammonium and phosphate concentrations of 12 and  $2 \mu\text{mol L}^{-1}$ , respectively.

*Ammonium measurement in unfiltered cultures and culture filtrates*—Dissolved ammonium concentrations were measured in unfiltered and filtered culture samples for four of the five experimental species (Table 3; Fig. 2). There were no statistically significant differences between the two sets of ammonium measurements for *E. huxleyi* and *T. weissflogii*; however, measured values were higher in the filtered than in the unfiltered samples for the other two species (Table 3). For *A. lagunensis* and *N. atomus*, the filtered values were, respectively, 29% and 75–89% higher, on average, than the unfiltered values.

The higher ammonium concentrations in culture filtrates may reflect physical damage to cells during filtration and subsequent leakage of intracellular ammonium into the culture medium. Although intracellular ammonium pools represent only a small percentage of cellular nitrogen (0–2%; Dortch et al. 1984), the leakage of intracellular ammonium into the medium could significantly increase dissolved ammonium concentrations in at least some situations. For example, if the intracellular  $\text{NH}_4^+$  was only 0.2% of cellular nitrogen, its complete leakage from damaged cells at a total cellular nitrogen concentration of  $12 \mu\text{mol}$  per liter of culture would increase extracellular ammonium by  $24 \text{ nmol L}^{-1}$ , an amount sufficient to affect our results. Physical damage to algal cells can occur even under mild filtration conditions and has been shown to result in significant leakage of intracellular solutes. Recently it was shown that the leakage of the intracellular osmolyte DMSP out of phytoplankton cells during standard filtration procedures results in substantial increases in dissolved DMSP in seawater samples (Kiene and Slezak 2006). The amount of leakage of intracellular solutes such as DMSP and ammonium ions depends on the intracellular concentration and the extent of cell damage

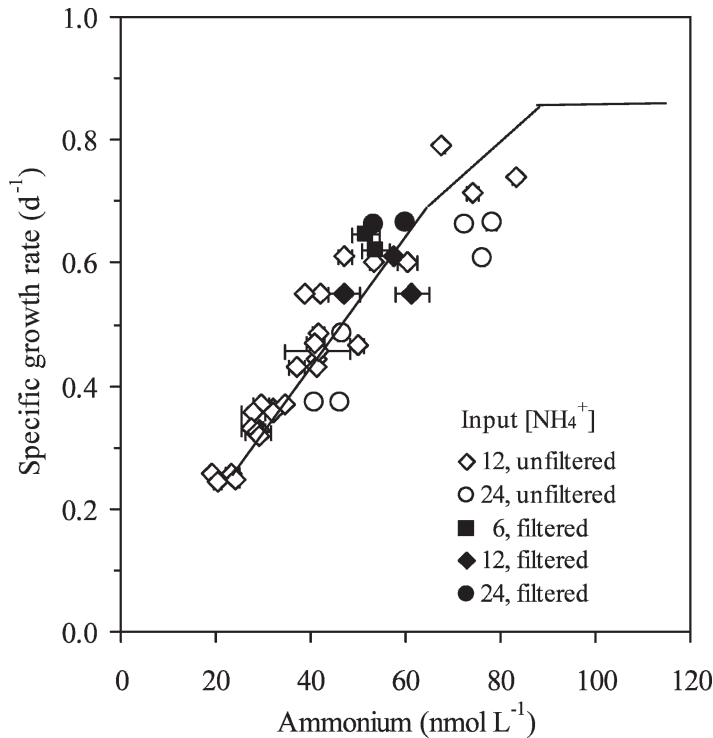


Fig. 2. Relationship between specific growth rate of *T. weissflogii* and dissolved ammonium concentration measured in unfiltered culture samples and in samples filtered through 0.4- $\mu\text{m}$ -pore Teflon filters. Depending on the experiment,  $[\text{NH}_4^+]$  in the input medium was 6, 12, or 24  $\mu\text{mol L}^{-1}$ . Error bars provide value  $\pm$  standard error (SE). The diagonal lines were fitted by eye to the data. The maximum growth rate (0.85  $\text{d}^{-1}$ ) in nutrient-sufficient batch cultures is indicated by the horizontal line.

during filtration. As a result of our experimental tests and existing literature data, ammonium concentrations were usually measured only in unfiltered culture samples.

**Diel variations**—The cyclostat cultures were grown under a light:dark cycle to simulate the natural diel cycle and to yield maximum growth rates similar to those in the natural environment at the same temperatures and photoperiod. However, because the light:dark cycle imposes a diel cycle on photosynthesis and growth, and often on cell division, our cultures, like the environment,

cannot attain a true steady state on an hour-to-hour basis (Sunda and Huntsman 2004). However, they do attain a steady state in terms of average daily values (e.g., average daily specific growth rate). They also should attain steady-state values for ammonium concentrations because of the short residence times (4–6 min) relative to the length of the day–night cycle (1,440 min).

We investigated diel variations in ammonium concentration, total biovolume, and average volume per cell in cyclostat cultures of *A. lagunensis*, *E. huxleyi*, and *N. atomus*. The *A. lagunensis* culture exhibited the largest diel differences, a result that was expected since its specific growth rate (0.66  $\text{d}^{-1}$ ) was almost twice that (0.34  $\text{d}^{-1}$ ) for cultures of the other two species. The *A. lagunensis* culture had a 36% higher total cell volume per liter of culture and a 29% lower ammonium concentration at the end of the light period than at the beginning (Fig. 3). The lower ammonium concentration apparently reflects an increase in uptake rate resulting from the higher total volume of algal cells, which occurs because cells often grow at higher rates during the day, as a direct consequence of photosynthetic C-fixation (Sunda and Huntsman 2004). The roughly inverse relationship between steady-state ammonium concentrations and cell biovolume indicates that specific ammonium uptake rates (normalized to cell volume and dissolved ammonium) are similar during the day and night, and, thus, there appears to be no direct light requirement for ammonium uptake. This conclusion also applies to the slower growing *E. huxleyi* and *N. atomus* cultures, in which ammonium concentration and total cell volume were similar throughout the diel cycle (Fig. 3A,B).

For *A. lagunensis* and *E. huxleyi*, the average volume per cell decreased by 36% and 24%, respectively, during the dark period (Fig. 3C), indicating that these species primarily divide at night, as occurs for many algal species. For *N. atomus*, however, average volume per cell and total cell volume were the same regardless of time of day, which indicates that this species grows and divides at similar specific rates during the day and night.

Based on the results of the present and previous diel studies (Sunda and Huntsman 2004), we chose to sample our cultures in the middle of the light period for the majority of samples in which only a single daily sample was collected.

Table 3. Dissolved  $\text{NH}_4^+$  concentrations measured in unfiltered and filtered cyclostat culture samples.

Species	Temp. ( $^{\circ}\text{C}$ )	Growth rate ( $\text{d}^{-1}$ )	Unfiltered $[\text{NH}_4^+]$ ( $\text{nmol L}^{-1}$ )*	<i>n</i>	Filtered $[\text{NH}_4^+]$ ( $\text{nmol L}^{-1}$ )*	<i>n</i>	<i>t</i> -Test <i>p</i> value
<i>Thalassiosira weissflogii</i>	20	0.58 $\pm$ 0.03	48.4 $\pm$ 8.7	15	55.4 $\pm$ 7.7	9	0.15
<i>Nannochloris atomus</i>	20	0.64 $\pm$ 0.04	25.5 $\pm$ 5.4	11	44.5 $\pm$ 7.1	11	<0.001
<i>N. atomus</i>	25	0.33 $\pm$ 0.01	13.7 $\pm$ 2.9	12	25.9 $\pm$ 6.3	12	<0.001
<i>Aureoumbra lagunensis</i>	25	0.32 $\pm$ 0.00	14.3 $\pm$ 2.2	9	18.4 $\pm$ 2.3	7	<0.001
<i>Emiliania huxleyi</i>	25	0.33 $\pm$ 0.00	24.6 $\pm$ 3.5	14	26.7 $\pm$ 10.0	15	0.34

\* Error bars provide value  $\pm$  standard deviation (SD) for *n* individual ammonium measurements. Usually there were three replicate measurements per day.

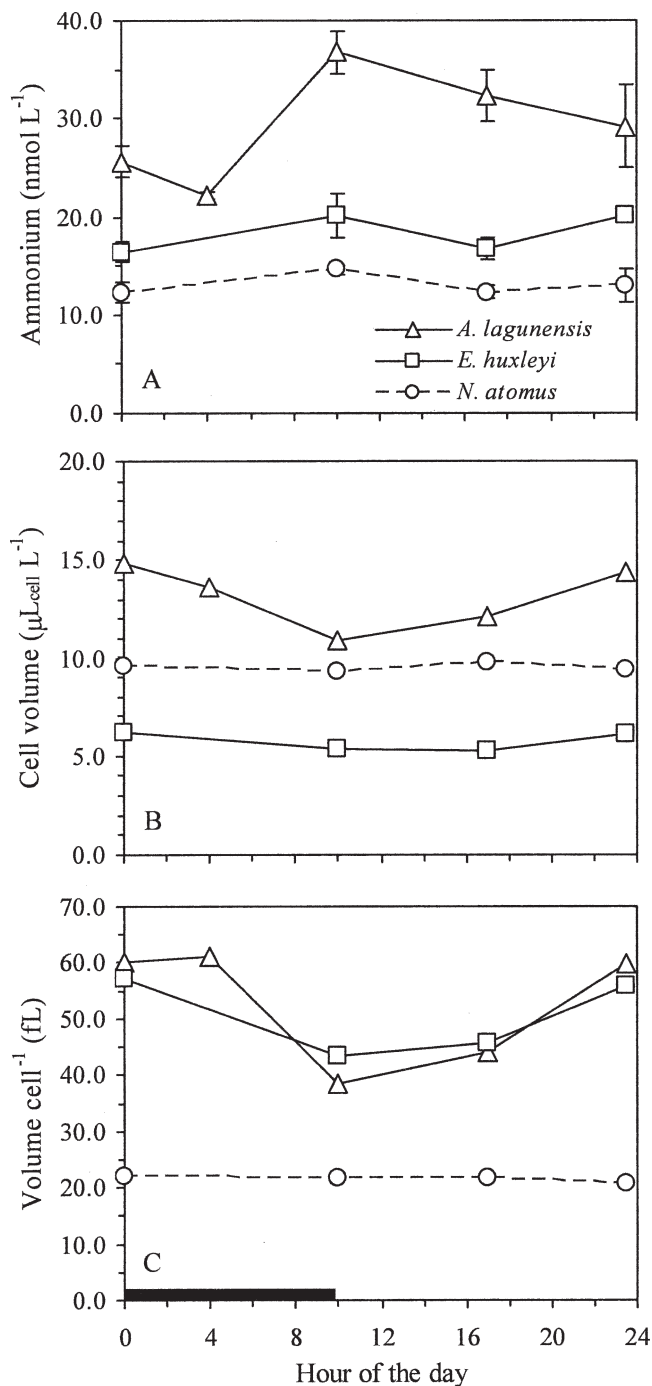


Fig. 3. Diel variations in (A) dissolved ammonium concentration, (B) total volume of cells per liter of culture, and (C) average volume per cell in cyclostat cultures at 25°C. Data are given for *A. lagunensis* at a dilution rate of 0.66 d<sup>-1</sup> and for *E. huxleyi* and *N. atomus* cultures at a dilution rate of 0.34 d<sup>-1</sup>. Error bars (panel A) provide value  $\pm$ SD ( $n = 3$ ).

*Relationships between specific growth rate and ammonium concentration*—In our diel experiments at 25°C, *N. atomus* and *E. huxleyi* grew at an average specific growth rate of 0.34 d<sup>-1</sup> at daily average ammonium concentrations ( $\pm$ standard deviation [SD]) of  $13.0 \pm 1.0$  and  $18.3 \pm$

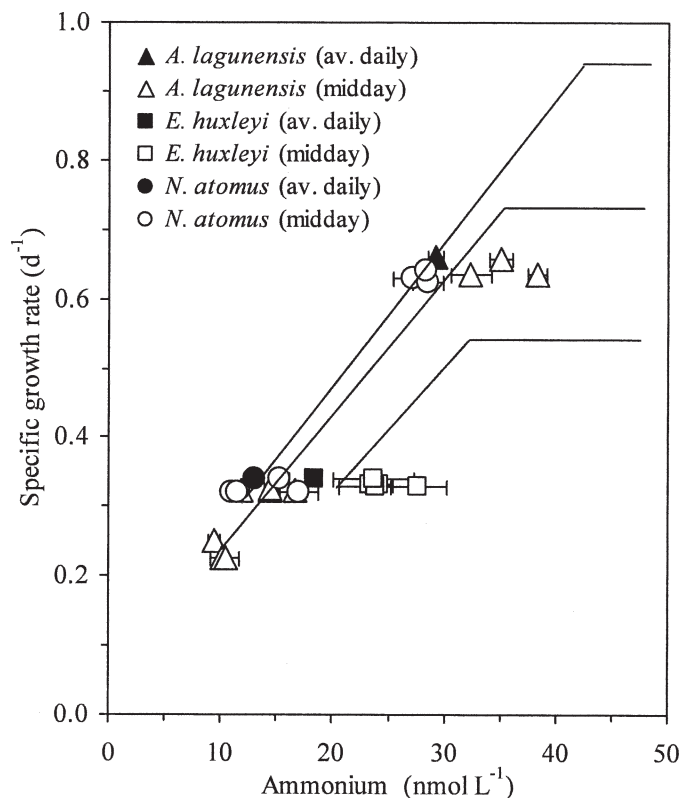


Fig. 4. Relationships between specific growth rate and ammonium concentration in cultures of *A. lagunensis*, *E. huxleyi*, and *N. atomus* at 25°C. Data are given for average daily values ( $\pm$ SE) taken from the diel data plotted in Fig. 3 and mean ( $\pm$ SE) for triplicate analyses made at midday on separate days in unfiltered culture samples.  $[\text{NH}_4^+]$  in the input growth medium was  $12 \mu\text{mol L}^{-1}$ . The diagonal lines for *A. lagunensis* and *N. atomus* were fitted by eye to the data and are weighted toward the average daily values. The slope for the growth rate versus  $[\text{NH}_4^+]$  relationship for *E. huxleyi* is an estimate based on the slopes for the other two species. Horizontal lines indicate the maximum growth rates of the species measured in nutrient-sufficient batch cultures.

$1.2 \text{ nmol L}^{-1}$ , respectively. *A. lagunensis* grew at a specific rate of  $0.66 \text{ d}^{-1}$  at an average daily ammonium concentration of  $29.2 \pm 2.1 \text{ nmol L}^{-1}$ . These values agreed reasonably well with values measured at midday for cultures growing at the same specific growth rate, given the day-to-day variation in ammonium measurements (Fig. 4). Based on our N-limited cyclostat data and measured maximum growth rates in batch cultures, the three species achieved specific growth rates of  $\sim 60\%$  of their maximum values at  $[\text{NH}_4^+]$  concentrations of 20–24  $\text{nmol L}^{-1}$  (Fig. 4).

Relationships between specific growth rate and midday ammonium concentrations were measured at 20°C for the two diatoms (*T. pseudonana* and *T. weissflogii*) as well as for *A. lagunensis* and *N. atomus*. The specific growth rate of the diatoms was limited at  $[\text{NH}_4^+]$  below 90–100  $\text{nmol L}^{-1}$ , while *N. atomus* was limited at a concentration below  $\sim 30 \text{ nmol L}^{-1}$  (Fig. 5; Table 2). Likewise, *A. lagunensis* grew at 71% of its maximum rate at an  $[\text{NH}_4^+]$  of

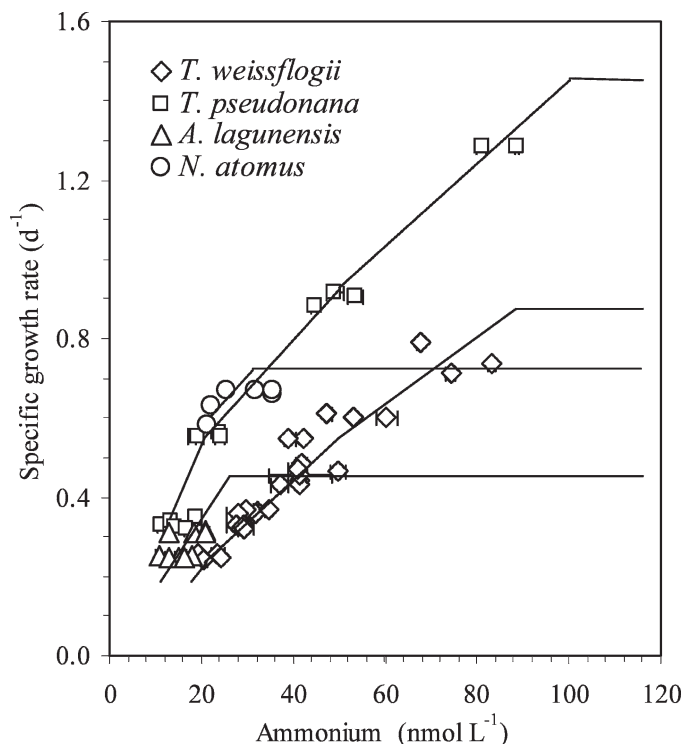


Fig. 5. Specific growth rate versus dissolved  $[\text{NH}_4^+]$  for *T. weissflogii*, *T. pseudonana*, *N. atomus*, and *A. lagunensis* at 20°C.  $[\text{NH}_4^+]$  was measured in unfiltered samples.  $[\text{NH}_4^+]$  in the input growth medium was 12  $\mu\text{mol L}^{-1}$ . Error bars give  $\pm\text{SE}$ . Maximum growth rates measured in nutrient-sufficient batch cultures are indicated by horizontal lines.

18  $\text{nmol L}^{-1}$ , while *T. pseudonana* needed a much higher concentration ( $\sim 60 \text{ nmol L}^{-1}$ ) to achieve 71% of its maximum rate (Fig. 5). Thus, the diatoms need much higher ammonium concentrations to grow at or near their maximal rates than do the two HAB species.

Generally, higher ammonium concentrations were required at 20°C than at 25°C to achieve the same N-limited specific growth rate, although the differences were not always statistically significant (Table 2). For example, for *A. lagunensis* the ammonium concentration required to yield a specific growth rate of 0.32  $\text{d}^{-1}$  at 20°C ( $18.1 \pm 2.7 \text{ nmol L}^{-1}$ ) was 27% higher than that ( $14.3 \pm 2.4 \text{ nmol L}^{-1}$ ) required to support the same specific growth rate at 25°C, although the difference was of marginal significance ( $p = 0.060$ ,  $t$ -test of mean daily values). Likewise, the ammonium level needed to support a specific growth rate of 0.248  $\text{d}^{-1}$  in *A. lagunensis* at 20°C ( $14.6 \pm 2.8 \text{ nmol L}^{-1}$ ) was 46% higher than that ( $10.0 \pm 0.5 \text{ nmol L}^{-1}$ ) needed to support a similar specific growth rate (0.234  $\text{d}^{-1}$ ) at 25°C ( $p = 0.033$ ,  $t$ -test of mean daily values).

The high ammonium concentrations needed to support the maximum growth rate of *T. pseudonana* and *T. weissflogii* are consistent with the putative r-selected status of diatoms (Kilham and Kilham 1980). Diatoms dominate algal communities in environments with high concentrations of available macronutrients, such as coastal upwelling

systems, an observation that is entirely consistent with the present data. The ability of *E. huxleyi* to grow at 61% of its maximum rate at an  $[\text{NH}_4^+]$  of 18–24  $\text{nmol L}^{-1}$  (Figs. 3, 4) clearly identifies it as a K-selected species. This is expected for *E. huxleyi*, because this clone was isolated from the Sargasso Sea, where surface ammonium concentrations have been measured to be only 3–15  $\text{nmol L}^{-1}$  (Brzezinski 1988), well within the range for ammonium limitation in the present experiments.

The low-ammonium adaptation of *A. lagunensis* and *N. atomus* is surprising, however, as both are bloom-forming species isolated from eutrophic coastal environments. *A. lagunensis* has formed dense “brown tide” blooms of up to 60  $\mu\text{g L}^{-1}$  Chl *a* in nutrient-enriched coastal lagoons in Texas (Buskey et al. 2001; Sunda et al. 2006), and *N. atomus* has formed similar dense “green tide” blooms in eutrophic bays in Long Island, New York (Ryther 1989). Mechanisms that can allow such low-nutrient-adapted species to proliferate in eutrophic coastal waters are discussed in a recent article (Sunda et al. 2006). Both species possess robust grazing-deterrent mechanisms (Gobler and Sunda 2006; Sunda et al. 2006), a common attribute of K-selected phytoplankton (Sommer 1989); these mechanisms allow them to proliferate at the expense of more easily grazed competing species. The lower grazing rates also decrease grazing-mediated recycling of nutrients and thus lower available nutrient concentrations. This further benefits the brown and green tide bloom species, which are well adapted to low-nutrient conditions. This inherent positive feedback has been proposed to contribute substantially to the bloom development of these HAB species (Sunda et al. 2006).

*Cell size effects on growth and ammonium uptake*—Under ammonium limitation at 20°C, the large diatom, *T. weissflogii*, grew more slowly than did the three other smaller species at equivalent  $[\text{NH}_4^+]$  (Fig. 5). This difference was not caused by variations in cellular nitrogen, because all four species had similar cell nitrogen concentrations for a given growth rate (Fig. 6). Rather, the difference was related to variations in uptake rates. Uptake rates (normalized to cell volume) were four times higher for *T. pseudonana* (4.3–4.7- $\mu\text{m}$  diameter) and *N. atomus* (3.1- $\mu\text{m}$  diameter) than they were for *T. weissflogii* (10–11- $\mu\text{m}$  diameter) at equivalent  $\text{NH}_4^+$  levels (Fig. 7).

The size-related differences in uptake rates appear to be linked to physical limits imposed by ammonium diffusion and intracellular transport. Cellular uptake of ammonium and other nutrients is a two-step process involving diffusion of nutrient molecules from the bulk medium to the cell surface (through the cell’s aqueous surface boundary layer) and subsequent transport into the cell by transport proteins associated with the outer cell membrane. Either process can limit uptake depending on the relative magnitude of rates, but ultimately the cell cannot transport nutrients faster than their maximum rate of diffusion to the cell surface (the hypothetical limiting rate for which all of the nutrient diffusing to the cell surface is taken up, and the surface concentration is reduced to zero). Diffusion rate per cell varies in direct proportion to the cell diameter (see Eq. 1),

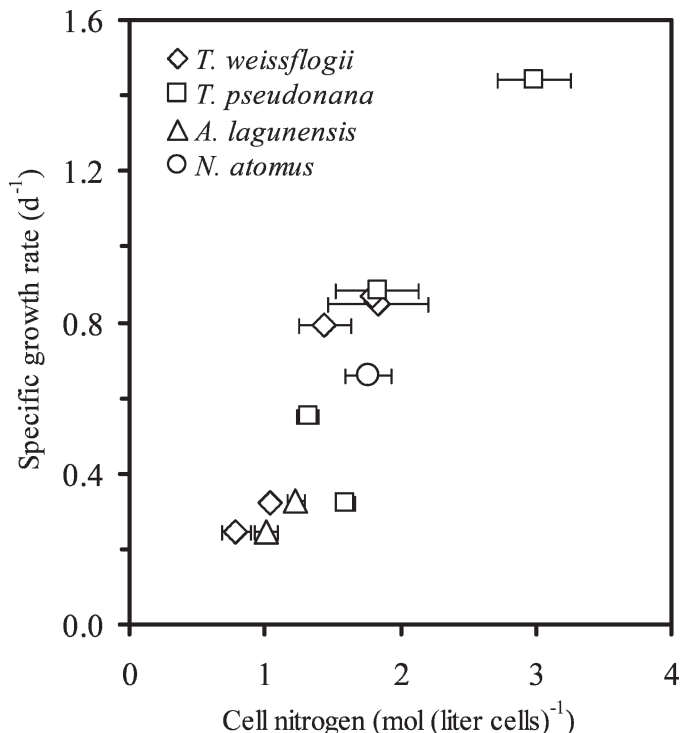


Fig. 6. Relationships between specific growth rate and cell nitrogen concentration (mol [liter of cell volume]<sup>-1</sup>) for *T. weissflogii*, *T. pseudonana*, *N. atomus*, and *A. lagunensis* at 20°C. Error bars provide value  $\pm$  SD for  $n = 4-8$ , where  $n$  is the total number of individual cell nitrogen measurements.

while the cell volume increases with the cube of the diameter. Consequently, maximum diffusion rates per unit of cell volume vary with the inverse square of the cell diameter ( $1/d^2$ ) (Pasciak and Gavis 1974). By contrast, transport across the outer membrane (normalized to cell volume) is ultimately restricted by the cell's surface-to-volume ratio, which is inversely proportional to the cell diameter ( $1/d$ ). Thus, both processes favor uptake and growth in smaller cells under nutrient-limiting conditions; but because of its higher-order (squared) dependence on cell diameter, diffusion limitation becomes a much more important limiting process with increasing cell size. Our present observations confirm these theoretical predictions. The uptake rate for the large diatom (*T. weissflogii*, 10–11- $\mu$ m diameter) at 20°C was  $73\% \pm 14\%$  ( $\pm$ SD,  $n = 40$ ) of the diffusion rate to the cell surface (Table 2, column 7), close to the theoretical maximum (Hudson and Morel 1993). For the smaller diatom *T. pseudonana* (4.5- $\mu$ m diameter) at the same temperature, the uptake rate was  $41\% \pm 7\%$  ( $\pm$ SD,  $n = 15$ ) of the maximum diffusion rate, while for the two slightly smaller nondiatom species, *A. lagunensis* (4.1- $\mu$ m diameter) and *N. atomus* (3.1- $\mu$ m diameter), the uptake rates were  $20\% \pm 5\%$  ( $n = 9$ ) and  $21\% \pm 3\%$  ( $n = 6$ ), respectively, of the maximum diffusion rate (Table 2).

Ammonium uptake rates that are near the maximum cell surface diffusion rate come with an energetic cost because they reduce the ammonium concentration at the cell surface ( $[\text{NH}_4^+]_s$ ) (Hudson and Morel 1993). The surface concen-

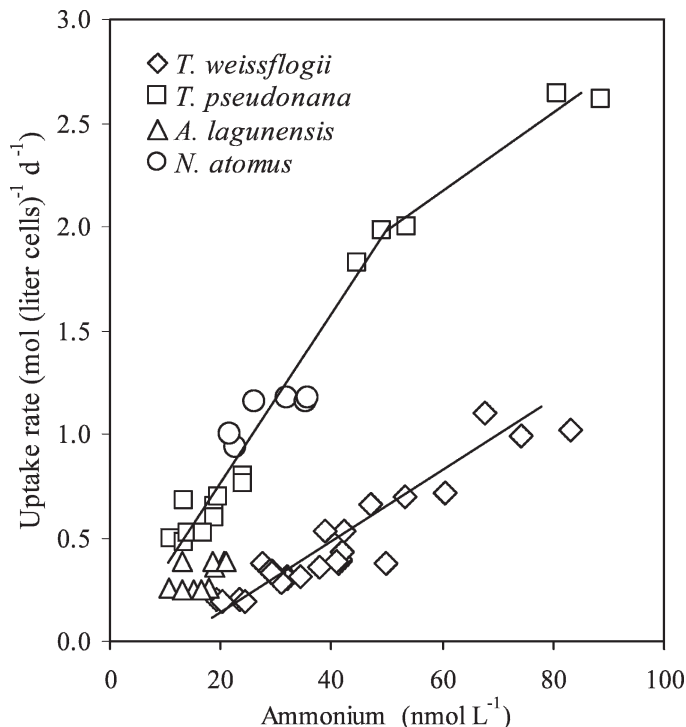


Fig. 7. Cellular ammonium uptake rate (mol [liter of cell volume]<sup>-1</sup> d<sup>-1</sup>) versus  $[\text{NH}_4^+]$  for *T. weissflogii*, *T. pseudonana*, *N. atomus*, and *A. lagunensis* at 20°C. Data are for experiments shown in Figs. 5 and 6. All points plotted are based on mean daily measurements.  $[\text{NH}_4^+]$  error bars are not shown.

tration is given by the equation:

$$[\text{NH}_4^+]_s = [\text{NH}_4^+]_M(1 - V/\rho) \quad (4)$$

where  $[\text{NH}_4^+]_M$  is the ammonium concentration in the bulk medium and  $V$  and  $\rho$  are the cell-specific ammonium uptake rate and maximum diffusion rate, respectively. Thus, for an uptake rate that is 73% of the diffusion rate, as occurs, on average, for *T. weissflogii*, the surface ammonium concentration is reduced to 27% of that in the bulk medium. As a consequence, the cell has to work that much harder to pump ammonium ions across the outer cell membrane against a much steeper concentration gradient than would exist if there were no diffusion limitation. In addition, the lower surface concentration lowers the rate of encounter of ammonium ions with cellular membrane transport sites by an amount proportional to the decrease in concentration. This lower encounter rate proportionately lowers the rate of binding of ammonium ions by cellular membrane uptake sites and, thus, proportionately lowers the rate of intracellular uptake. Thus, although cells can take up nutrients such as ammonium at rates close to the rate of diffusion to the cell surface, this uptake comes with a substantial cost in terms of energy and number of surface membrane transport sites needed to meet the cell's growth demand. This cost may be prohibitive in highly nutrient-limited environments, such as the open ocean, where a species' ability to outcompete its rivals ultimately depends on its specific growth rate. In such environments,

algal species can greatly minimize this cost through a decrease in cell size. However, such a size decrease comes with the cost of increased susceptibility to rapid grazing by microzooplankton (Thingstad and Sakshaug 1990; Kiorboe 1993).

Diffusion limitation of  $\text{NH}_4^+$  uptake appears to be more severe than that observed for many other nutrients. Diffusion limitation of iron uptake by marine phytoplankton was computed to occur only for cells of  $>40 \mu\text{m}$  in diameter at  $20^\circ\text{C}$  (Sunda 2001), while uptake for smaller cells was inversely related to cell diameter and was limited by passage of iron across the outer cell membrane (Sunda and Huntsman 1997). And in model calculations (Pasciak and Gavis 1974), diffusion limitation of nitrate uptake was predicted only for cells of  $>50 \mu\text{m}$  in diameter, while we observed diffusion limitation of  $\text{NH}_4^+$  uptake in cells as small as  $4.5 \mu\text{m}$  in diameter.

Given the benefit of small size in enhancing uptake rates per unit of cell volume, it is perhaps not surprising that four of the five algal species (all but *N. atomus*) decreased their average size in response to limiting  $\text{NH}_4^+$  concentrations (Table 2). The largest change was observed in *A. lagunensis* at  $25^\circ\text{C}$ , where the mean cell diameter decreased from  $5.8 \pm 0.3$  to  $3.8 \pm 0.1 \mu\text{m}$  with increasing N-limitation of growth rate. The 34% decrease in cell diameter in this species increased the rate of diffusion per unit of cell volume by 2.3-fold, providing a substantial benefit under N-limiting conditions. Decreases in cell size have also been observed previously for N-limitation and iron-limitation of algal growth rate (Sunda and Huntsman 1995; Keller et al. 1999) and may be a general cellular response to nutrient limitation.

Because diffusion-limited uptake is so sensitive to cell size, the extension of diffusion-limited  $\text{NH}_4^+$  uptake down to small cell sizes means that ammonium-limited algal communities will be under severe competitive selection pressure for small-celled algal species. Such small-species selection indeed is observed in surface layers of much of the tropical and subtropical ocean (and in temperate waters during warmer months), where thermal stratification from solar heating severely restricts the input of nutrients from deeper waters via advection and mixing processes. Ammonium and nitrate concentrations in these surface ocean waters are extremely low ( $3\text{--}15 \text{ nmol L}^{-1}$  in the North Atlantic central gyre; Brzezinski 1988; Harrison et al. 1996), well within the concentration range that limited algal growth in the present study. In such low-nitrogen surface waters in the Atlantic and Pacific,  $\geq 70\text{--}80\%$  of the Chl *a* occurs in algal cells that are  $< 2\text{--}3 \mu\text{m}$  in diameter (Dickson and Wheeler 1993; Pérez et al. 2005), indicating a strong selective pressure for small algal cells. Such small cells are rapidly grazed by protozoans and other microzooplankton, resulting in efficient recycling of nitrogen and rapid regeneration of ammonium (Thingstad and Sakshaug 1990). The small size of algal cells and zooplankton grazers minimizes loss of nutrients from particulate settling, increasing the retention of nitrogen and other nutrients within the euphotic zone and minimizing further depletion of limiting nutrients (Thingstad and Sakshaug 1990). Thus, the selection of small-celled phytoplankton in N-limited

marine waters has a profound influence, not only on species composition of algal communities but also on the related structure and function of marine food webs and cycling of nutrients and carbon. These effects may become more pronounced with future greenhouse warming of the earth's atmosphere, which should increase surface ocean temperatures and cause greater thermal stratification and consequent nutrient limitation in ocean waters.

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