Vol. 186: 67–74, 1999 | MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser | Published September 17

Effect of ammonium on nitrate utilization by *Emiliania huxleyi*, a coccolithophore from the oceanic northeastern Pacific

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ABSTRACT: Explanations for the high nitrate (NO_3^-), low phytoplankton biomass of the subarctic NE Pacific have mainly centered on iron limitation and grazing pressure. Although ammonium (NH_4^+) has been proven to inhibit NO_3^- uptake by phytoplankton in numerous laboratory and field studies, this hypothesis has not been experimentally tested for the subarctic Pacific. The effect of increasing NH_4^+ concentrations on NO_3^- uptake by *Emiliania huxleyi*, a coccolithophore isolated from Ocean Station Papa in the subarctic NE Pacific, is examined. The effect of diel periodicity on NO_3^- uptake and other physiological parameters for *E. huxleyi* during a 14:10 h light:dark cycle are also reported. About 84% of the total daily NO_3^- was taken up during the light period. Cell division occurred only during the last part of the dark period. Chlorophyll *a*, nitrogen, and carbon quotas and cell volume increased during the light period and decreased during the dark. The presence of NH_4^+ inhibited NO_3^- uptake in *E. huxleyi*. Nitrate uptake rates were reduced to half the maximum value at 0.24 µM NH_4^+, and maximum realized inhibition was ~100% at 2.2 µM NH_4^+. If this laboratory result is extrapolated to field conditions, the inhibition of NO_3^- uptake rates for the small size class of phytoplankton would be predicted to be 38 to 70% for the range of ambient NH_4^+ concentrations found in the oceanic NE Pacific.

KEY WORDS: Nitrate uptake · Ammonium inhibition · Diel periodicity · *Emiliania huxleyi* · Ocean station Papa · northeastern Pacific · HNLC

INTRODUCTION

Numerous studies have shown that NH_4^+ exerts an effect on the NO_3^- metabolism of marine algae. Ammonium has been found to be the preferred nitrogen source for most species of marine phytoplankton as well as to inhibit the utilization of NO_3^- in a more direct manner (see review by Dortch 1990). Concentrations of NH_4^+ lower than 1 µM may readily inhibit NO_3^- uptake (e.g. McCarthy et al. 1975, Harrison et al. 1996). However, in some cases NH_4^+ had little or no effect on NO_3^- uptake (e.g. Kokkinakis & Wheeler 1987, Kristiansen & Lund 1989) and in other cases NH_4^+ enhanced NO_3^- uptake rates (e.g. Dortch et al. 1991). Therefore, although there is undoubtedly an interaction between NH_4^+ and NO_3^- uptake, it appears that the extent and threshold concentrations involved depend on the species under study, its physiological status, and the environmental conditions to which this particular species or the natural assemblage of phytoplankton has been exposed (e.g. Bates 1976, Dortch & Conway 1984, Dortch et al. 1991, Harrison et al. 1996, Lomas & Glibert 1999).

In the oceanic subarctic NE Pacific, concentrations of NO_3^- are high while NH_4^+ concentrations are low and fairly constant annually. Surface concentrations of NO_3^- are rarely below 5.0 µM and those of NH_4^+ range between 0.17 and 0.54 µM at Ocean Station Papa (OSP; 50°N, 145°W; Varela & Harrison 1999). Despite the high NO_3^- concentrations, phytoplankton biomass remains low even during times of the year when environmental conditions are favorable for rapid growth (Varela & Harrison 1999). A number of factors have been cited as responsible for the low rates of NO_3^- uptake, such as iron limitation and grazing pressure (e.g. Martin & Fitzwater 1988, Frost 1991, Miller et al. 1991, Boyd et al. 1996). Wheeler & Kokkinakis (1990)

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added a new candidate: NH_4^+ inhibition of NO_3^- uptake. They suggested that the constant recycling of NH_4^+ in subarctic waters can also limit the utilization of NO_3^- by phytoplankton, and thus prevent its depletion. However, their conclusions were only based on the field observation that NO_3^- uptake rates decreased with increasing ambient NH_4^+ concentrations. This observation deserves further testing both in the field and in the laboratory under conditions that simulate the natural environment and with unialgal cultures of ecologically relevant species isolated from subarctic waters.

Numerous physiological studies have focused their attention on the coccolithophore *Emiliania huxleyi* (Class Prymnesiophyceae) because of its great importance in global nutrient cycles (e.g. Keller et al. 1989, Sikes & Fabry 1994). *E. huxleyi* has been found to be the most abundant coccolithophore in surface waters of the oceanic subarctic NE Pacific (Honjo & Okada 1974) and forms part of the dominant <5 µm autotrophic size class (Taylor & Waters 1982, Booth et al. 1993, Varela 1997). The majority of studies on *E. huxleyi*, however, have been conducted on isolates obtained from the North Atlantic (e.g. Brand 1982), because until recently isolates from the oceanic NE Pacific or any other high nitrate, low chlorophyll (HNLC) region have not been available. In November of 1991, *E. huxleyi* was successfully isolated from water samples from OSP in the oceanic NE Pacific (see Muggli 1995). Since then, experiments on this isolate have focused mainly on metal nutrition (Muggli 1995) and sinking rates (Lecourt et al. 1996). Further studies are needed to understand other physiological aspects, such as the nitrogenous nutrition, of this coccolithophore from a HNLC region.

The main purpose of the present study was to investigate the effect of increasing NH_4^+ concentrations on NO_3^- uptake rate in *Emiliania huxleyi* grown under environmental conditions that simulated those of the oceanic NE Pacific. The diel periodicity of NO_3^- uptake rate and other physiological characteristics by *E. huxleyi* are also presented.

MATERIALS AND METHODS

Culture conditions and measurements. Recently isolated cells of *Emiliania huxleyi* had multiple layers of coccoliths, and, in order to preserve the original morphology, *E. huxleyi* has been maintained in nutrient-enriched microwave-sterilized OSP water since November 1991 (Muggli 1995). The maintenance medium was enriched with macronutrients (30 µM NO_3^- and 5 µM HPO_4^{2-}), metals (23 nM Mn, 8 nM Zn, 1 nM Cu, 2.5 nM Co, 100 nM Mo, 10 nM Se and 100 nM Fe), EDTA (10 µM) and vitamins (1×10^{-4} g l^{-1} thiamine and 5×10^{-7} g l^{-1} biotin) (Muggli 1995). Cultures were maintained at 16°C under low irradiance (ca 30 µmol photons m^{-2} s^{-1} provided by Vita-lite™ fluorescent tubes and measured with a Biophysical Instruments Inc. light meter, model QSL 100) on a 14:10 h light:dark (L:D) cycle.

Experimental cultures were established under environmental conditions that simulated the natural oceanic environment of *Emiliania huxleyi* for the summer period. Cells were grown in semi-continuous batch cultures with filter-sterilized (0.22 µm) nutrient-enriched artificial seawater medium (ESAW; Harrison et al. 1980) in 1 or 2 l acid-clean glass flasks. The original ESAW was modified by reducing the concentrations of Fe, Mn, Zn, Co and EDTA by a factor of 50, adding 1.2 nM Ni, 1.2 nM Mo, and 10 nM Se, decreasing the concentrations of NO_3^- to 30 µM and HPO_4^{2-} (as Na_2HPO_4) to 3 µM, and omitting Si. Cultures were exposed to saturating irradiance (ca 180 µmol photons m^{-2} s^{-1}; Muggli & Harrison 1996) with a 14:10 h L:D cycle while immersed in a temperature-regulated water bath maintained at 10.5 ± 0.5°C. Cultures were manually agitated once or twice daily, and were not exposed to bubbling in order to avoid contamination by NH_4^+ from the air. The concentration of total dissolved inorganic carbon (DIC) in the cultures was measured with an ADC type 225 Mk3 infra-red gas analyser during the logarithmic phase of growth. DIC values ranged between 1.92 and 2.05 mM, close to the typical DIC concentration in the oceans (i.e. around 2 mM). The pH of the medium was measured frequently and found to range between 7.9 and 8.1. Cells were acclimated to the described conditions for >10 generations.

Cell growth in the experimental cultures was monitored daily (9 h after the start of the light period) using cell counts and *in vivo* fluorescence. All culture transfers and sampling were conducted in mid-logarithmic growth phase. Cultures were transferred when cell densities approached 240×10^3 cells ml^{-1} (every 5 d on average) and were generally diluted 10-fold. Microscopic examination of the cultures was done at the time of the transfers to ensure that the morphology of the cells, mainly in terms of coccolith abundance, remained unchanged for the entire experiment. Cultures were discarded if the number of layers of coccoliths on the cells was decreasing in any of the flasks. Cultures were unialgal and sterile techniques were employed in order to avoid bacterial contamination.

Two separate experiments were performed:

(1) Diel periodicity of NO_3^- uptake rate and other physiological characteristics in a 14:10 h L:D cycle in the absence of NH_4^+. During a 24 h period, samples were drawn every 3 h from triplicate cultures for the determination of cell number, cell volume, *in vivo* fluorescence, chlorophyll *a* (chl *a*), particulate nitrogen

(PN), particulate carbon (PC), and dissolved NO_3^- concentrations. Nitrate uptake rates (fmol NO_3^- cell^{-1} h^{-1}) were calculated for each 3 h period from the disappearance of NO_3^- from the medium and then normalized to the average cell number over the same period. Ammonium concentration was also measured, even if no additions of NH_4^+ were made.

(2) NO_3^- uptake rate with NH_4^+ additions. These experiments were carried out by adding NH_4^+ to the NO_3^--grown cultures of *Emiliania huxleyi* and monitoring the concentrations of NO_3^- and NH_4^+ in the medium. Ammonium additions to multiple cultures ranged from 0 (control) to ca 3 µM. Immediately after the NH_4^+ addition, samples were drawn for the determination of cell number, cell volume, *in vivo* fluorescence, chl *a*, PN, PC, and dissolved NO_3^- and NH_4^+ concentrations. Cultures were re-sampled for the same measurements every 24 h for 5 to 7 d until 24 h after NH_4^+ became undetectable (<0.05 µM). Nitrate uptake rates (fmol NO_3^- cell^{-1} d^{-1}) were estimated from the disappearance of NO_3^- from the medium over 24 h normalized to the average cell number over that period. The concentration of NH_4^+ for the same 24 h period was calculated as the average between the beginning and the final concentrations. Replicate cultures were run simultaneously as well as staggered in time.

Analytical methods. Growth rate was calculated as the slope of the natural log of cell number versus time, and of the natural log of *in vivo* fluorescence versus time. Cell numbers and average cell volumes were determined with a Coulter Counter® Model TA II particle counter equipped with a population accessory using a 70 µm aperture. Before making measurements with the Coulter Counter®, the pH of the sample was decreased to ca 5 with 5% HCl to dissolve the coccoliths, which interfere with cell counts in some of the channels (Muggli & Harrison 1996). *In vivo* fluorescence was measured with a Turner Designs™ Model 10-AU fluorometer.

Samples for chl *a*, PN and PC (including coccoliths) were filtered through pre-combusted Whatman® GF/F filters (ca 0.7 µm nominal porosity) using a vacuum pressure differential of <125 mm Hg, and stored at –20°C in a desiccator until analysis. Chl *a* was measured using *in vitro* fluorometry with a Turner Designs™ Model 10-AU fluorometer (Parsons et al. 1984). After drying at 60°C, PN and PC samples were analysed with a Carlo Erba Model NA-1500 Elemental Analyzer (Verardo et al. 1990).

Samples for dissolved NH_4^+ and NO_3^- were gently filtered through pre-combusted Whatman® GF/F filters into acid-clean polypropylene bottles and processed immediately or kept at 4°C until analysis within a few hours. Ammonium and NO_3^- concentrations were measured colorimetrically with a Technicon Autoanalyzer® II following the procedures of Slawyk & MacIsaac (1972) and Wood et al. (1967), respectively.

Nitrate uptake rates were estimated from the disappearance of NO_3^- from the medium, as explained above. Due to the length of the period over which the rates were calculated (3 h in Expt 1 and 24 h in Expt 2), the term 'uptake' includes transport of nitrogen through the membrane and also assimilation into amino acids and proteins (i.e. growth). Efforts were made to carry out short-term measurements in Expt 2, but, due to the relatively low biomass of the cultures, nutrient disappearance was more precisely calculated over a 24 h period.

Models. The NO_3^- uptake rate versus NH_4^+ concentration data were modeled using 2 non-linear least-squares fits:

(1) a modified Michaelis-Menten equation of the form:

$$V\mathrm{NO_3^-} = V_{max} - \left(\frac{V_{max} \cdot I_{max} \cdot \mathrm{NH_4^+}}{K_i + \mathrm{NH_4^+}}\right) \quad (1)$$

where $V\mathrm{NO_3^-}$ is the NO_3^- uptake rate (fmol NO_3^- cell^{-1} d^{-1}); V_{max} is the maximum rate of NO_3^- uptake at undetectable NH_4^+ concentrations (fmol NO_3^- cell^{-1} d^{-1}); I_{max} is the maximum realized inhibition (values from 0 to 1); K_i is the NH_4^+ concentration at which V_{max} is reduced by half (µM); and NH_4^+ is the NH_4^+ concentration (µM).

(2) a simple exponential model of the form:

$$V\mathrm{NO_3^-} = A + B \cdot e^{-C \cdot \mathrm{NH_4^+}} \quad (2)$$

where $A + B$ is V_{max}; C is the exponential decay constant; and $V\mathrm{NO_3^-}$ and NH_4^+ are as above.

RESULTS

Growth rate of *Emiliania huxleyi*

Under the experimental conditions, the growth rate of *Emiliania huxleyi* was 0.75 ± 0.02 divisions d^{-1} (mean ± 1 SE, n = 52) when calculated from cell numbers and was not significantly different from the growth rate (0.70 ± 0.01 d^{-1}; mean ± 1 SE, n = 55) calculated from *in vivo* fluorescence (*t*-test, $p > 0.01$).

Diel periodicity of NO_3^- uptake rate and other physiological parameters in *Emiliania huxleyi*

Nitrate concentrations in the culture medium showed a more rapid decrease during the 14 h light period than during the 10 h dark period (Fig. 1). The slopes of NO_3^- disappearance for the periods 0–3 and

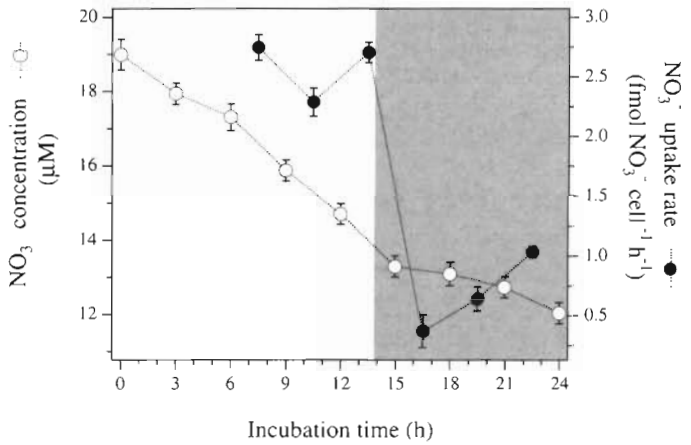

Fig. 1. Changes in nitrate concentration and nitrate uptake rate (calculated for each 3 h period, starting at 6 h; see text for details) for *Emiliania huxleyi* cultures during a 14:10 h L:D cycle. The gray area (14 to 24 h) indicates the dark period. Each symbol represents the mean of triplicate cultures ± 1 SE. If no error bars are visible, they are smaller than the symbol

3–6 h were lower than the slopes from 6–15 h (Fig. 1), likely due to the presence of NH_4^+ (see next section) as well as lower culture biomass (see Fig. 2B). Ammonium concentrations of 0.1 µM were measured during the first 6 h of the experiment. However, for the rest of the incubation period, NH_4^+ was undetectable (<0.05 µM). Because the objective of this experiment was to determine the diel variability of NO_3^- uptake in the absence of NH_4^+, only NO_3^- uptake rates from 6 to 24 h are shown in Fig. 1. Cell-specific NO_3^- uptake rates were significantly higher during the light period (2.53 ± 0.04 fmol NO_3^- $cell^{-1}$ h^{-1}; mean ± 1 SE) than during the dark period (0.69 ± 0.02 fmol NO_3^- $cell^{-1}$ h^{-1}, mean ± 1 SE) (*t*-test, $p \ll 0.001$). Thus, in a 14:10 h L:D cycle, 84% of the total daily NO_3^- was taken up during the light period, and the remaining 16% during the dark period.

During the L:D cycle, other physiological parameters were also measured (Fig. 2). Cell numbers showed a rapid increase during the last part of the dark period, but remained constant during most of the light period and the first 4 h of darkness (Fig. 2A). In contrast, *in vivo* fluorescence increased during the light period but changed little during the dark (Fig. 2B). As a result of these diel patterns of cell numbers and *in vivo* fluorescence, fluorescence per cell increased during the light period, but decreased during the dark (Fig. 2C).

Chl *a* increased constantly in the cultures, while the rate of accumulation of PN and PC was higher during the day than during the night (data not shown). When these parameters were expressed as cell quotas (Fig. 2D to F), they all showed a similar pattern of increase during the light period and decrease during the dark. Cell volume also increased during the light hours, but decreased at night (Fig. 2G).

Effect of increasing NH_4^+ concentrations on NO_3^- uptake rate in *Emiliania huxleyi*

Increasing NH_4^+ concentrations resulted in a decrease in NO_3^- uptake rates (Fig. 3). When NH_4^+

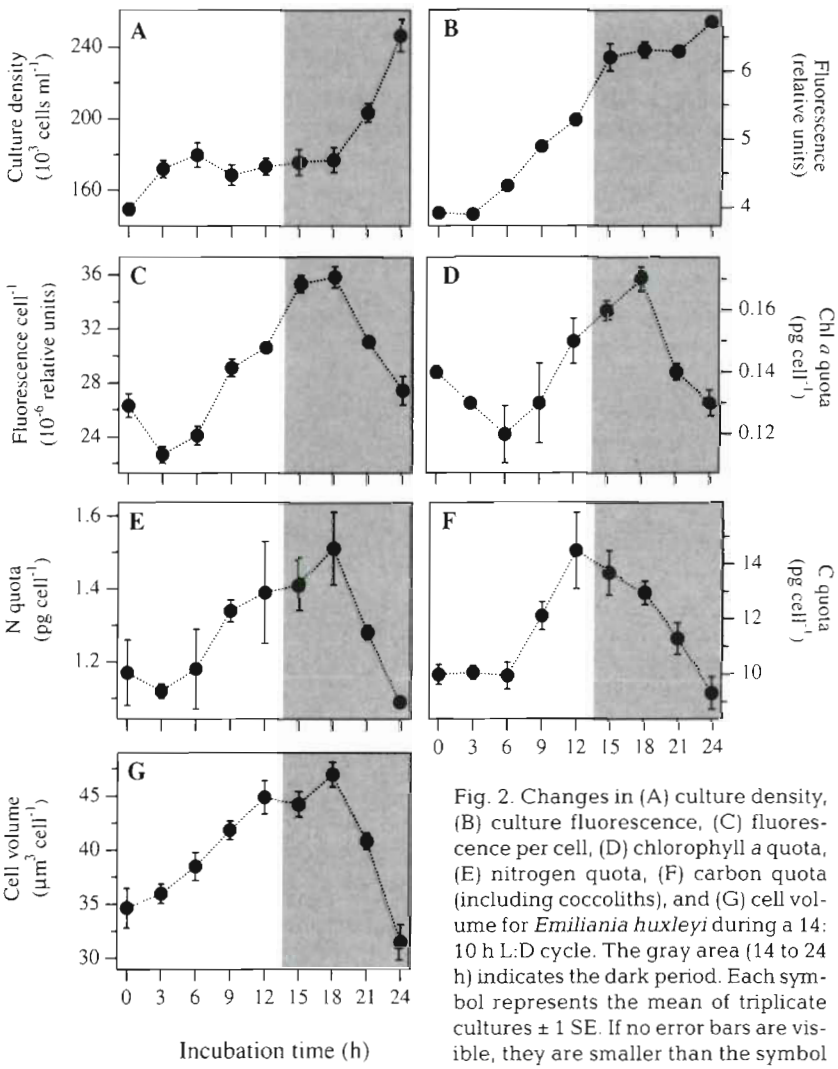

Fig. 2. Changes in (A) culture density, (B) culture fluorescence, (C) fluorescence per cell, (D) chlorophyll *a* quota, (E) nitrogen quota, (F) carbon quota (including coccoliths), and (G) cell volume for *Emiliania huxleyi* during a 14:10 h L:D cycle. The gray area (14 to 24 h) indicates the dark period. Each symbol represents the mean of triplicate cultures ± 1 SE. If no error bars are visible, they are smaller than the symbol

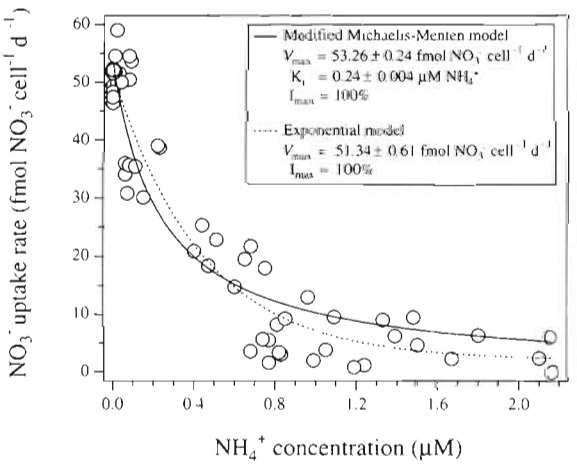

Fig. 3. Nitrate uptake rate at increasing ammonium concentrations for cultures of *Emiliania huxleyi* grown in a 14:10 h L:D cycle. Each symbol represents a determination from a single culture (n = 56). The models applied to the data as well as the fitted parameters (± 1 SE) are also included

concentrations were undetectable in the culture medium, NO_3^- was taken up at maximal rates; however rates decreased sharply when NH_4^+ was present even at low concentrations (<0.5 µM), and was almost completely inhibited at 2.2 µM NH_4^+.

With the modified Michaelis-Menten model, the best fit to the data (n = 56) yielded a V_{max} (± SE) of 52.00 ± 0.22 fmol NO_3^- cell^{-1} d^{-1}, a K_i (± 1 SE) of 0.42 ± 0.01 µM NH_4^+, and an I_{max} (± SE) of 1.2 ± 0.01. Since experimental values showed maximum inhibition of up to 100% and the theoretical range for I_{max} is from 0 to 1 (0 to 100% inhibition), the model was run again constraining I_{max} to 1. When I_{max} = 1, the estimates of V_{max} and K_i were 53.26 ± 0.24 fmol NO_3^- cell^{-1} d^{-1} and 0.24 ± 0.004 µM NH_4^+, respectively.

The exponential model estimated A = 2.10 ± 0.30, B = 49.24 ± 0.32 and C = 2.28 ± 0.34 (mean ± SE, n = 56). Thus, V_{max} ($A+B$) was 51.34 ± 0.61 fmol NO_3^- cell^{-1} d^{-1} and I_{max} was approximately 100% ($A/[A+B]$). The estimates from the exponential fit were in good agreement with those from the modified Michaelis-Menten model.

DISCUSSION

Over the 5 yr period since *Emiliania huxleyi* was isolated, much care has been taken to maintain this isolate under conditions typical of the oceanic NE Pacific in order to avoid adaptation to unrealistic culture conditions. Physiological parameters of *E. huxleyi* measured during the present study were in close agreement with the measurements made soon after its isolation (Muggli & Harrison 1996).

Diel periodicity of NO_3^- uptake rate and other physiological parameters in *Emiliania huxleyi*

All the physiological characteristics measured in *Emiliania huxleyi* showed diel changes during a 14:10 h L:D cycle. The stimulation of NO_3^- utilization by light is thought to be primarily at the assimilation rather than at the transport level (see Vincent 1992). The higher rate of NO_3^- uptake observed here for *E. huxleyi* during the light period is likely the result of the dependency of the NO_3^--assimilating enzymes on photosynthetic energy. Data from Eppley et al. (1971) confirm this suggestion; they found that the activity of NO_3^- (NR) and NO_2^- reductases measured in cell-free extracts of *E. huxleyi* was higher during day than night. These results have also been observed for other algal groups (see Syrett 1981). Berges et al. (1995) reported that diel patterns of NR activity closely followed changes in nitrogen uptake rate under high light in the diatom *Thalassiosira pseudonana*. The diel pattern of NO_3^- uptake rate observed by Berges et al. (1995) closely resembles the pattern shown in this study for *E. huxleyi*.

The diel periodicity of NO_3^- uptake rate and NR activity observed in laboratory studies is supported by field experiments (e.g. Eppley et al. 1970, Cochlan et al. 1991a,b, Berges et al. 1995). In the oceanic subarctic NE Pacific, phytoplankton NO_3^- uptake rates showed a clear diel pattern, with daytime rates about twice nighttime values (Cochlan et al. 1991a). A similar pattern was also reported by Wheeler et al. (1989) and Wheeler & Kokkinakis (1990). Although NR activity was not measured in the subarctic NE Pacific, diel variations would probably show patterns similar to those found by Cochlan et al. (1991a) for NO_3^- uptake rates.

The nocturnal synchronization of cell division observed during the present study has been reported previously for other isolates of *Emiliania huxleyi* (e.g. Paasche 1967, Nelson & Brand 1979, Van Bleijswijk et al. 1994) as well as for other algal species (e.g. Nelson & Brand 1979, Berdalet et al. 1992). Of all the algal groups, the most complex and varied patterns of diel periodicity in cell number have been exhibited by diatoms. Diatom division rates have been shown to increase in the light (Nelson & Brand 1979, Chisholm & Costello 1980), in the dark (Eppley & Renger 1974), in both light and dark periods (Chisholm & Costello 1980), or to remain constant over the L:D cycle (Nelson & Brand 1979, Berges et al. 1995).

The diel pattern of *in vivo* fluorescence, fluorescence per cell and cell volume observed by Van Bleijswijk et al. (1994) and of chl *a* per cell reported by Paasche (1967) for other isolates of *Emiliania huxleyi* coincided with the findings of this study. When comparing the diel periodicity of cell volume of *E. huxleyi* with those

of diatom species, more complex patterns are observed for diatoms, with 2 peaks found during the 24 h diel period (e.g. Berges et al. 1995) as opposed to 1 for *E. huxleyi*.

The strong diel periodicity in NO_3^- uptake rates and other physiological characteristics for *Emiliania huxleyi* required a 24 h sampling interval during the NO_3^--NH_4^+ uptake interaction experiment.

Effect of NH_4^+ on NO_3^- uptake rate

Results from this study suggest an inhibitory effect of NH_4^+ on NO_3^- uptake in the subarctic isolate of *Emiliania huxleyi*. Maximal NO_3^- uptake rates at undetectable concentrations of NH_4^+ were rapidly reduced when NH_4^+ was present. Although inhibition of NO_3^- uptake by NH_4^+ has been observed in many laboratory studies on unialgal cultures of phytoplankton (see Dortch 1990), this is the first study to investigate this phenomenon for any phytoplankton species isolated from a HNLC region.

The culture conditions adopted in the present study for *Emiliania huxleyi* were very similar to those found in the surface waters of the oceanic subarctic NE Pacific during the summer period, in terms of temperature, photoperiod and composition of the medium, and thus, extrapolation to a field scenario is reasonable. Cochlan & Harrison (1991) also simulated natural conditions as close as possible when studying nitrogen uptake interactions in the picoplanktonic alga *Micromonas pusilla*, and found that NO_3^- uptake rate was completely inhibited at 1 µM NH_4^+. Maximal uptake rate of NO_3^- was only obtained when NH_4^+ was exhausted from the medium. Similar findings were reported for more commonly used species such as *Chlorella vulgaris* (e.g. Syrett & Morris 1963), *Skeletonema costatum* (e.g. Bates 1976, Dortch & Conway 1984), and *Thalassiosira pseudonana* (e.g. Dortch et al. 1991, Berges et al. 1995, Yin et al. 1998).

In all of the above studies, significant or complete inhibition of NO_3^- uptake was observed between 0.5 and 1 µM NH_4^+. In the isolate of *Emiliania huxleyi* studied here inhibition at 0.5 and 1 µM NH_4^+ was ca 68 and 81% (derived from the Michaelis-Menten model), respectively, and complete inhibition was not achieved below 2.2 µM. Differences between laboratory studies are not uncommon because inhibition is species specific and is also affected by the preconditioning of the cultures (e.g. Dortch & Conway 1984, Dortch et al. 1991), light levels (e.g. Bates 1976, Yin et al. 1998) and other environmental factors, such as temperature (Lomas & Glibert 1999). Thus, multiple responses can be expected from different laboratory studies and, principally, from the field.

The extrapolation of the response by unialgal cultures of phytoplankton in the laboratory to that by a natural assemblage of phytoplankton in the field is problematic. However, laboratory experimentation is still a powerful approach for studying phytoplankton physiology, which would be difficult to assess in the field, where the physiological responses of phytoplankton assemblages are a product of the compounding effects of environmental parameters and the response by individual species. In the subarctic NE Pacific, Varela & Harrison (1999) showed that at ambient NH_4^+ concentration <0.5 µM the N-specific uptake rate of NO_3^- by the natural assemblages of phytoplankton varied widely, indicating that factors other than the absolute concentration of NH_4^+, i.e. irradiance and/or iron levels (La Roche et al. 1996), are affecting these rates. At $NH_4^+ > 0.5$ µM, NO_3^- uptake rates were lower, however those rates corresponded to low light levels (Varela & Harrison 1999). Although limiting iron and irradiance levels may have accounted for the low rates in the Varela & Harrison (1999) study, compounding inhibitory effects of NH_4^+ on NO_3^- uptake cannot be disregarded.

Field studies on natural phytoplankton assemblages have suggested that NH_4^+ inhibition of NO_3^- uptake commonly occurs in the oceans (e.g. McCarthy et al. 1975, Blasco & Conway 1982, Cochlan 1986, Wheeler & Kokkinakis 1990, Muggli & Smith 1993, Harrison et al. 1996). Wheeler & Kokkinakis (1990) presented the first evidence of a NH_4^+-NO_3^- interaction in the oceanic NE Pacific. They indicated that NO_3^- assimilation was completely inhibited at 0.1 to 0.3 µM NH_4^+, and the shape of the relationship was linear. The results obtained for *Emiliania huxleyi* in this laboratory experiment agree in that a negative relationship exists. However, inhibition of NO_3^- uptake was never complete for *E. huxleyi* at concentrations as low as 0.1 to 0.3 µM NH_4^+ and the relationship was non-linear. It was observed, however, that at 0.24 µM NH_4^+, NO_3^- uptake rate by *E. huxleyi* was reduced to 50% of its maximal rate and that the rate only reached undetectable values at 2.2 µM NH_4^+. Although the results of the present study and those of Wheeler & Kokkinakis (1990) do not entirely agree, both studies demonstrate that a suppression of NO_3^- uptake occurs both in subarctic waters and in cultures of a phytoplankton species isolated from this region.

Ecological implications

On the basis of the findings of this laboratory study on *Emiliania huxleyi*, inhibition of NO_3^- uptake by NH_4^+ may be one of the factors contributing to the low rates of NO_3^- uptake in surface waters of the oceanic

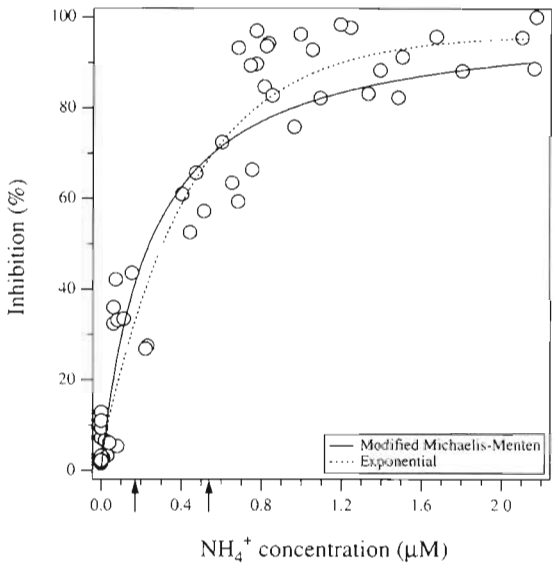

Fig. 4. Percent inhibition of nitrate uptake rate at increasing ammonium concentrations for cultures of *Emiliania huxleyi* grown in a 14:10 h L:D cycle. Same data as used in Fig. 3 but transformed to percentages. Each symbol represents a determination from a single culture (n = 56). The models used to fit the data are also included. Arrows point to minimum and maximum ammonium concentrations measured at OSP in the NE Pacific during 1992 to 1994 by Varela & Harrison (1999)

subarctic NE Pacific during those times of the year when temperature and irradiance are saturating for growth.

Although it has been shown that NO_3^- uptake rates by phytoplankton in the oceanic NE Pacific may be impaired by iron limitation (e.g. Martin & Fitzwater 1988, Boyd et al. 1996), this was only the case for cells >5 μm (Boyd et al. 1996). Iron enrichment did not affect the N-specific uptake rates of NO_3^- by phytoplankton cells <5 μm (Boyd et al. 1996). In addition, laboratory results on NO_3^--grown *Emiliania huxleyi* demonstrated that under the low iron concentrations typical of OSP growth rate was not limited; *E. huxleyi* reduced its requirements for photosynthate and iron by decreasing its cell volume (Muggli & Harrison 1996). This field and laboratory evidence suggests that during the summer, NO_3^- uptake in the <5 μm size-fraction is not impaired by low iron levels. However, the specific uptake rate of NO_3^- by the small cells at OSP is still low (Varela 1997), implying that some limitation is still imposed on NO_3^- uptake in the small cells. This laboratory experiment suggests that the constant availability of NH_4^+ in oceanic waters of the NE Pacific may be partly responsible for these depressed NO_3^- uptake rates.

The impact of this NH_4^+-NO_3^- uptake interaction in *Emiliania huxleyi* on the subarctic ecosystem cannot be neglected since this species forms part of the dominant size class (<5 μm) of this region. The degree to which the utilization of NO_3^- is prevented in the small phytoplankton size class will depend on the NH_4^+ concentration present in the water. This laboratory experiment suggests inhibition of NO_3^- uptake rate by NH_4^+ of between 38 and 70% (Fig. 4) at the range of NH_4^+ levels (0.17 to 0.54 μM, respectively) measured at OSP (Varela & Harrison 1999). This may partly contribute to the low *f*-ratios (0.27 on average) found at OSP throughout the water column at various times of the year (Varela & Harrison 1999).

Acknowledgements. We extend our appreciation to D. Crawford, T. Larson, R. Guy, F. Whitney, T Parsons and S. Calvert for comments on the manuscript and to M. Soon for PN/PC values. We also thank 3 anonymous reviewers for their valuable comments. D.E.V. was supported by UBC University Graduate and Chevron Fellowships.

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Editorial responsibility: Yves Collos (Contributing Editor), Montpellier, France

Submitted: May 10, 1998; Accepted: April 19, 1999
Proofs received from author(s): August 20, 1999