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Nitrate uptake by *Karenia brevis*. I. Influences of prior environmental exposure and biochemical state on diel uptake of nitrate

Geoffrey A. Sinclair*, Daniel Kamykowski, Edward Milligan, Blake Schaeffer

Department of Marine, Earth, and Atmospheric Sciences, North Carolina State University, Campus Box 8208, Raleigh, North Carolina 27695-8208, USA

ABSTRACT: The ability of a *Karenia brevis* population to persist in an oligotrophic water column depends on how cell physiology and cell behavior contribute to the acquisition of light and nutrients that often are separated in space. We hypothesized that an aggregation of *K. brevis*, observed undergoing a diel vertical migration (DVM) in the bottom half of a 22 m water column on the West Florida Shelf, used the sediments as a nutrient source. We tested how the physiology of *K. brevis* contributed to the acquisition of nitrate by evaluating how nitrate uptake changed with prior environmental exposure. The experimental conditions simulated the extremes that cells might endure during DVM when migrating up into an oligotrophic water column versus cells that remained near the sediment-water interface. The first culture, representing cells that attained the maximum apex of their migration away from the sediments, was grown under relatively high light (350 µmol quanta m^{-2} s^{-1}) and reached nitrate-depleted conditions (<0.5 µM NO_3^-) prior to the experiment. The second culture, representing cells that remained near the sediment-water interface, was grown under relatively low light (60 µmol quanta m^{-2} s^{-1}) and nitrate-replete conditions (~20 µM NO_3^-) prior to the experiment. Cells exposed to nitrate-depleted environments for 12 h prior to the experiment enhanced nocturnal uptake compared to cells continuously exposed to nitrate-replete conditions. Changes in cell physiology may contribute to nitrate acquisition after descent from oligotrophic environments to areas with elevated nitrate concentrations.

KEY WORDS: *Karenia brevis* · Dinoflagellate · Physiology · Nocturnal uptake · Vertical migration

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INTRODUCTION

Dense aggregations in excess of 10^5 cells l^{-1} of *Karenia brevis*, a toxic dinoflagellate, are responsible for massive fish kills, neurolytic shell fish poisoning, human respiratory irritation, and millions of dollars of lost revenue for the state of Florida (Tester & Steidinger 1997). Initiation regions for these blooms are thought to be 18-74 km offshore, often in oligotrophic water columns (Dragovich et al. 1961, Steidinger 1975, Tester & Steidinger 1997). The ability of *K. brevis* to acquire nutrients in these oligotrophic conditions is critical to the growth and development of bloom events, but remains poorly understood.

Nitrogen (N) is often the limiting nutrient in marine systems (Hecky & Kilham 1988). Proposed sources of N that may initiate and sustain population growth in the oligotrophic offshore regions include upwelling events (Tester & Steidinger 1997) and *Trichodesmium* blooms (Walsh & Steidinger 2001, Walsh et al. 2003). The vertical distribution of cells both at the surface and near the bottom of a 22 m water column during an ECOHAB cruise suggested that another potential source of nitrogen for *K. brevis* was the sediment–water interface (see Fig. 1).

The majority (30 to 80%) of the phytoplanktonic nitrogen requirement in coastal environments 5 to 50 m in depth may originate from the sediments (Nixon 1981,

*Email: gasincla@ncsu.edu

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Blackburn & Henriksen 1983, Boynton & Kemp 1985). Increased nutrient availability near the sediments relative to that in an oligotrophic water column, combined with algal adaptation to low light, may also explain the distribution of benthic microalgae (Stevenson & Stoermer 1981, Sundback & Jonsson 1988). Considerable microphytobenthic primary productivity occurs at depths of 15 to 20 m in coastal temperate areas with sufficient light conditions (Bodin et al. 1985, Herndl et al. 1989). The presence of benthic microalgae, in many cases, may act as a filter to deprive overlying water of at least a portion of the nutrients that flux from the sediment (Tyler et al. 2003). This potential competition constrains the niche in which *Karenia brevis* may utilize a sediment derived nutrient source. The spatial limit on the distribution of the sediment-bound microalgae is determined by a bottom light intensity greater than compensation intensity (i.e. between 0.1 and 1 % of the photosynthetically active radiation (PAR) present at the surface; Cahoon 1999). One niche in which free swimming *K. brevis* populations may exploit a sediment-derived nutrient source is from sediment sources at water depths beyond the distribution of benthic microalgae. A second opportunity is indicated from comparisons of both day and night diffusive nutrient fluxes, which indicate that the uptake of nutrients by benthic microalgae involves a significant light-driven component (Sundback et al. 2004). If *K. brevis* can enhance nocturnal uptake, when uptake by benthic microalgae is depressed, it may provide another niche in which to acquire nutrients inshore of the light compensation depth for benthic microalgae.

Unlike benthic microalgae that are constrained to the sediments, *Karenia brevis* can migrate vertically up in the water column at a rate of approximately 1 m h^{-1} in the absence of water motion (Liu et al. 2001a). Traditionally, dinoflagellates undergoing diel vertical migration (DVM) ascend during the day, in support of photosynthetically driven carbon fixation, and descend at night to access sub-surface nitrogen, phosphorus and assorted trace elements in support of biochemical synthesis (Cullen 1985, Kamykowski et al. 1998). *K. brevis*, described as shade-adapted (Shanley & Vargo 1993, Walsh et al. 2003), is compensated for growth at about 0.3 % and can saturate growth with as little as 2.25 % surface irradiance (assuming a 2000 µmol quanta m^{-2} s^{-1} noon maximum; Shanley 1985, Shanley & Vargo 1993). If *K. brevis* ascends from the sediment to light intensities from 1 to 10 % surface PAR, it may extend the region within which *K. brevis* may access the sediment and still obtain enough light to support cell growth. During the day, upward migration of *K. brevis* increases light exposure but also decreases exposure to nutrients. Downward migration at night could increase nutrient exposure and enable uptake in the dark. Dark uptake is generally depressed in most phytoplankton species (Cochlan et al. 1991, Clark & Flynn 2002). In order to maximize the benefits of a near-bottom nutrient source, while fitting into the temporal niche permitted by depressed nocturnal uptake by benthic microalgae, *K. brevis* would need to couple migration behavior with the physiological capacity to take up adequate nutrients in the dark.

This study examined the ability of *Karenia brevis* to take up nitrate in light and in dark under nutrient regimes similar to those of the near-bottom *K. brevis* population observed on the 2000 ECOHAB cruise (Fig. 1). The population aggregated near the sediment–water interface at night, dispersed into water column during the day, and aggregated again near the bottom in the evening. Boundary conditions were defined by the sediment and by the distance a cell could swim upward during a 12 h period. One culture represented cells at the apex of migration exposed to higher light intensity but a nitrate-depleted water column. The second culture represented near-bottom cells exposed to lower light and higher nutrients. The objective of the batch culture approach used here was to isolate nitrate uptake responses to environmental variables from the confounding effects of migration behavior. In a companion paper (Sinclair et al. 2006, this volume), we investigated the importance of migration behavior in influencing environmental exposures and as a nutrient acquisition mechanism, by exploring the migration of *K. brevis* between the nutrient regimes examined in this study.

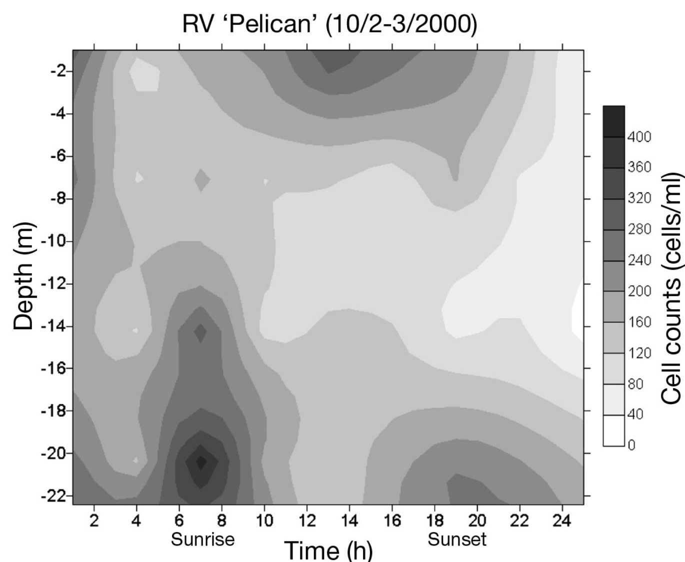

Fig. 1. *Karenia brevis*. Near-bottom cell aggregations and subsequent day-time dispersal up the water column. Samples taken during the ECOHAB 2000 cruise (D. Kamykowski pers. comm.), beginning at 00:00 h and every 2 h thereafter for 24 h (2–3 Oct), at 5 m depth intervals

MATERIALS AND METHODS

Two non-axenic 6 l batch cultures were started from the same *Karenia brevis* parent culture (strain Apalachicola) and grown in modified L20 media (Guillard & Hargraves 1993) which contained no copper, and had nitrate as the only nitrogen source. Autoclaved and filtered (0.2 µm) seawater (salinity 35) taken from the Gulf Stream was used as the base. The 2 batch cultures were grown under a 12:12 light:dark cycle with lights on at 06:30 h EST and off at 18:30 h EST.

One culture (referred to herein as 'Deplete') was depleted of nitrate in the media (<0.5 µM NO_3^-) 12 h prior to the experiment andgrown under light delivering approximately 350 µmol quanta m^{-2} s^{-1} to the surface plane of the culture and at 25°C. The Deplete culture represented cells that have undergone a 12 h upward migration from near bottom into an oligotrophic water column, where they would experience higher light and temperature levels and lower nitrate concentrations (Fig. 1). A second culture (referred to herein as 'Replete') was grown under light delivering <60 µmol quanta m^{-2} s^{-1} to the surface plane of the culture at 22°C. Nitrate was not limiting (~20 µM NO_3^-) prior to the experiment. The Replete culture represented cells remaining near the sediment surface, exposed to lower light and temperature levels and higher nitrate concentrations.

Nitrate uptake was evaluated both during the day (12:30 h) and at night (23:00 h). The 12:30 h time was chosen to provide adequate exposure to light prior to the sampling, and 23:00 h was chosen to complete the incubation well before cell division, which generally occurs between 03:00 and 04:00 h (Van Dolah & Leighfield 1999). NO_3^- uptake in the Deplete culture was tested with additions of 0.5 and 11 µM NO_3^- from a stock solution of 10% [^{15}N]NO_3^-. 11 µM NO_3^- was added to the Replete culture from stock solutions that contained 30% [^{15}N]NO_3^- so that the final percentage of [^{15}N]NO_3^- was 10.6% of the final concentration (31 µM [^{15}N]NO_3^-) of nitrate in the incubation flask (Lomas & Glibert 1999). The flasks were incubated in a radial photosynthetron (Babin et al. 1994) adapted according to Schaeffer et al. (2004) in order to test the effects of light and temperature on nitrate uptake. The sub-samples from both the Deplete culture and Replete culture used for uptake incubations were exposed to different light levels (350 and <60 µmol quanta m^{-2} s^{-1}) and different temperatures (25 and 22°C) for 2 h.

Each culture was monitored during the 24 h of the experiment for intracellular nitrate pools, free amino acids and total intracellular N. Measurements were taken at 06:00, 12:30, 18:00 and 23:00 h to compare diel variation in intracellular N reserves between the two cultures.

Cell counts for all samples were made using a Coulter Multisizer II Particle Analyzer Counter (Beckman Coulter) with threshold settings of 13.3 and 30 µm for cell diameter. Approximate cell volumes were calculated based on the equivalent spherical diameter applied to volumetric formula for a sphere. Filtrations for intracellular nitrate pool, intracellular free amino acids, and total cellular N used precombusted Whatman GF/C 25 mm filters. Whatman GF/C 12 mm filters were used for ^{15}N filtrations. After filtration, cells were washed with artificial seawater and frozen at –20°C prior to analysis. Nitrate in the media prior to the experiments was tracked using both manual techniques (Parsons et al. 1984) as well as with a Lachat QuikChem 8000 Continuum Series Autoanalyzer.

Sample volume for intracellular nitrate and intracellular free amino acids was equivalent to 2×10^4 cells. Intracellular nitrate pools were evaluated following the protocol of Thoresen et al. (1982), involving extraction by passing 10 ml boiling deionized water through the filters. Filtrate was analyzed with the Lachat QuikChem 8000 Continuum Series Autoanalyzer. Both intracellular nitrate and intracellular free amino acids were calculated on a per cell basis.

Free amino acid assays followed the protein and free amino acid extraction protocol for phytoplankton reviewed by Clayton et al. (1988). Fluorescence was measured at 390 nm excitation and 475 nm emission with a Turner fluorometer. Amino acid concentration was then determined with a standard curve using glutamate as the amino acid standard.

Total intracellular N samples were processed on a Carlo Erba nutrient analyzer. The volume filtered corresponded to approximately 21 µg N $filter^{-1}$ as determined by prior sensitivity analysis. Nitrate uptake was evaluated using a mass spectrometer in line with the Carlo Erba nutrient analyzer. According to clean [^{15}N-tracer techniques, all incubation vials and storage vials were acid washed with 10% HCl. The nitrate uptake rate was calculated by the formula:

$$V_{nitrate} = \frac{(^{15}\text{N atom \% excess})}{(^{15}\text{N atom \% enrichment}) \times \text{time}}$$

(Dugdale & Goering 1967) and corrected for cell concentration to yield uptake units of pmol N $cell^{-1}$ h^{-1}. Since the 2 cultures started with different internal concentrations of total cellular N, uptake rates were normalized to total cellular N for purposes of comparison (V h^{-1}).

All samples for intracellular nitrate, intracellular free amino acids, total cellular N, and uptake responses were taken in triplicate to allow statistical comparisons. Statistics were performed with SAS Institute software using a standard 1-way ANOVA to make basic sample comparisons, and a 2-way ANOVA to test for interaction effects between light and temperature.

RESULTS

Deplete culture

The Deplete culture, acclimated to 350 µmol quanta m^{-2} s^{-1} and 25 °C, exhibited growth rates of 0.5 doublings d^{-1} prior to the experiment when nitrate was present in the media. The 24 h experiment started when the nitrate level in the media became undetectable (<0.5 µM NO_3^- at 06:00 h the morning of the experiment). This correlated with the cessation of culture growth (Fig. 2). The culture had a mean cell diameter of 20.85 (±0.05) µm over the 24 h of the experiment. Both internal nitrate pools and free amino acids fluctuated slightly throughout the day. There was a significant decrease in the intracellular nitrate pool from 0.07 (±0.002) pg $cell^{-1}$ at 12:30 h to 0.06 (±0.0002) pg $cell^{-1}$ at 18:00 h ($n = 6$, $F = 39$, $p < 0.01$). Fluctuations in intracellular free amino acid levels tended to follow the same diel pattern as intracellular nitrate but were not significantly different from mean levels of 0.42 (±0.09) pg $cell^{-1}$ throughout the day. The total cellular N did not significantly change during the 24 h of the experiment. Values ranged from 71.7 (±3.8) pg $cell^{-1}$ at 06:00 h to 78.1 (±4.0) pg $cell^{-1}$ at 23:30 h with an anomalous drop at 18:00 h to 57.9 (±0.53) pg $cell^{-1}$.

External nitrate concentration was the only environmental variable that significantly influenced uptake rates in the incubations of the Deplete culture (Fig. 3). Uptake rates in the 11 µM $[^{15}N]NO_3^-$ additions averaged 0.52 V h^{-1}, 2.6 × higher than the uptake rate in the 0.5 µM $[^{15}N]NO_3^-$ incubations (0.2 V h^{-1}; $n = 6$, $F = 130$, $p < 0.01$). Nitrate uptake at 25°C was not significantly different from uptake at 22°C. Comparisons during the day between cells incubated at 350 and <60 µmol quanta m^{-2} s^{-1} did not show any significant differences in rate of nitrate uptake. Similarly, uptake during the day, when both light levels were grouped, while slightly higher, did not differ statistically from dark assimilation at night.

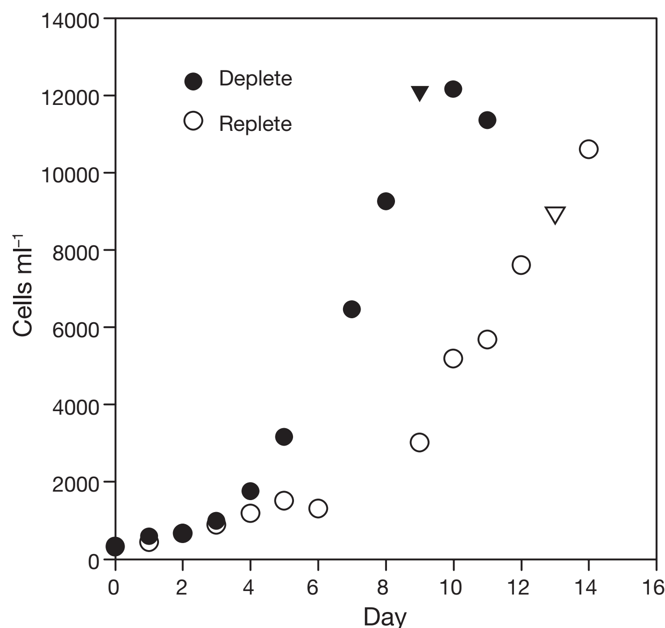

Fig. 2. *Karenia brevis.* Average growth of Deplete and Replete cultures. (∇) points at which each experiment was performed

Replete culture

The Replete culture, acclimated to <60 µmol quanta m^{-2} s^{-1} and 22°C, exhibited growth rates of 0.3 doublings d^{-1} before, during, and after the experiment in the presence of sufficient nitrate in the media. Mean cell diameter during the experiment was 19.86 (±0.05) µm. Intracellular nitrate levels appeared to increase throughout the day to 0.06 (±0.01) pg $cell^{-1}$ before declining to 0.04 (±0.02) pg $cell^{-1}$ at 23:00 h, but sample variation was large compared to the means and did not yield any statistically significant comparisons. Intracellular free amino acids fluctuated throughout the day, increasing to 0.64 (±0.12) pg $cell^{-1}$ by 12:30 h ($n = 6$, $F = 12.9$, $p = 0.02$) before declining to 0.23 (±0.14) pg $cell^{-1}$ at 18:00 h ($n = 6$, $F = 14.0$, $p = 0.02$) and then increasing again to 0.82 (±0.1) pg $cell^{-1}$ at 23:00 h ($n = 6$, $F = 35.7$, $p < 0.01$). The increase in intracellular free amino acids at 23:00 h corresponded to a decreasing trend in intracellular nitrate levels. Total N per cell ranged from a low of 55.5 (±1.8) pg $cell^{-1}$ at 06:00 h after cell division to a maximum of 86.28 (±3.8)

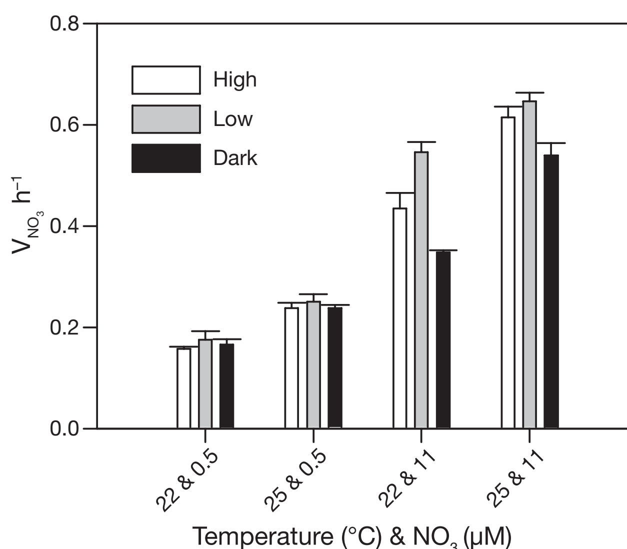

Fig. 3. *Karenia brevis.* Nitrate uptake by Deplete culture under various combinations of temperature, nitrate concentration, and light exposure. Uptake rates normalized to total cellular N

pg cell^{-1} at 23:00 h. The N content per cell increased significantly to 79.4 (±3.4) pg cell^{-1} at 12:30 h and remained high for the rest of the experiment (n = 6, F = 39.0, $p < 0.01$).

Nitrate uptake (Fig. 4) during the day was significantly greater (about 8.5-fold) than at night (F = 8.6, $p < 0.01$). No interaction was found between light level and temperature in the incubations. Neither light level, except for the nocturnal dark treatments, nor temperature had any significant impacts on nitrate assimilation.

Deplete vs. Replete

Prior to the experiment, the previously depleted culture exposed to higher light and temperature conditions had slightly higher growth rates than the replete culture exposed to low light and sufficient nitrate. During the course of the experiment, however, the growth rate of the Replete culture exceeded that of the Deplete culture. On the morning of the experiment about 15% of the cells were newly divided in the Deplete culture, whereas in the Replete culture only about 10% of the cells were newly divided. 10% of the cells in the Replete culture divided the night of the experiment whereas <0.5% of the cells divided in the Deplete culture. The Replete culture continued to grow after the experiment, while the number of cells in the deplete culture declined by ~800 cells.

Average cell diameter in the Deplete culture (20.85 ± 0.05 µm) was about 5% larger than those of cells in the Replete culture (19.86 ± 0.05 µm) resulting in approximate spherical equivalent volumes of 4753 and 4104 µm^{-3}, respectively. The average intracellular nitrate level per cell over the diel period of the experiment was significantly greater in the Deplete culture than in the Replete culture (n = 24, F = 22.8, $p < 0.01$). The greatest differences in intracellular nitrate pools occurred at 06:00 h when the deplete culture had 1.9× as much intracellular nitrate as the Replete culture, and at 23:00 h when the Deplete culture had 1.8× as much as Replete culture. Over the diel period, fluctuations in intracellular free amino acids prevented the detection of any significant differences between the average levels of the 2 cultures, but transient differences were evident. Starting at 06:00 h, the Deplete culture had 1.3× more intracellular free amino acids per cell than did the Replete culture (n = 6, F = 18.86, p = 0.012), perhaps a consequence of prior cell division in the Replete culture between 04:00 and 05:00 h (Van Dolah & Leighfield 1999). As the day progressed, the Replete culture accumulated more intracellular free amino acids than the Deplete culture and contained 1.6× more by 12:30 h (n = 6, F = 12.5, p = 0.02). An apparent increase at 18:00 h was not significant due to sample variability. A distinct increase in intracellular free amino acids in the Replete culture at 23:00 h, perhaps in preparation for cell division, again brought its free amino acid content 2.4× above that of the Deplete culture (n = 6, F = 55.4, $p < 0.01$). Interestingly, comparisons of total N cell^{-1} at 06:00 h indicated that total N cell^{-1} in the Deplete culture was significantly greater than in the Replete culture (n = 6, F = 14.3, p = 0.02) which may have been due to the larger cell size of the Deplete culture and because the Deplete culture had sufficient nutrients for growth the night before the experiment. When the data was normalized to cell volume (µm^{-3}) the significant difference at 06:00 h disappeared and the total N cell^{-1} was significantly greater in the Replete culture than the Deplete culture during the rest of the experiment prior to uptake incubation (n = 18, F = 30.7, $p < 0.001$, Fig. 5).

There were no significant differences in daytime nitrate uptake between the Deplete culture in 11 µM [^{15}N]NO_3^- incubations and the Replete culture in the 31 µM [^{15}N]NO_3^- incubations. Nocturnal uptake in the Deplete culture at 11 µM [^{15}N]NO_3^-, however, was 5.6× greater than that of the Replete culture in the 31 µM [^{15}N]NO_3^- incubations (n = 6, F = 328, $p < 0.01$).

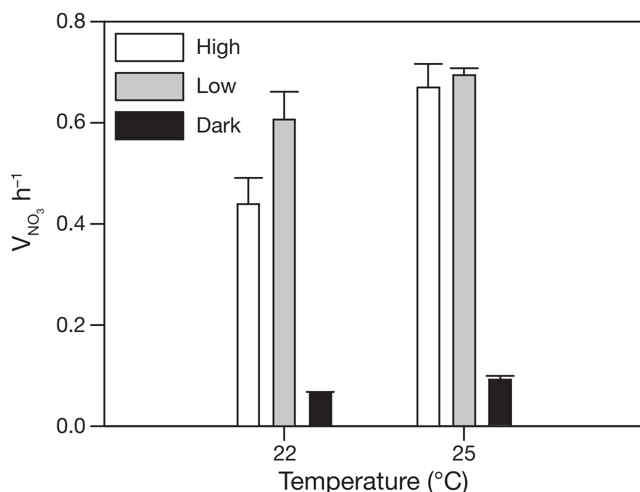

Fig. 4. *Karenia brevis*. Nitrate uptake by Replete culture under various combinations of temperature and light exposure in 31 µM nitrate. Uptake rates normalized to total cellular N

DISCUSSION

The persistence of dinoflagellates in nutrient-poor or patchy environments necessitates physiological mechanisms to adapt to periods of low nutrients, while maintaining the ability to quickly exploit pulses of more concentrated nutrients when they are encountered. Cells adapt to changes in nutrient fields by

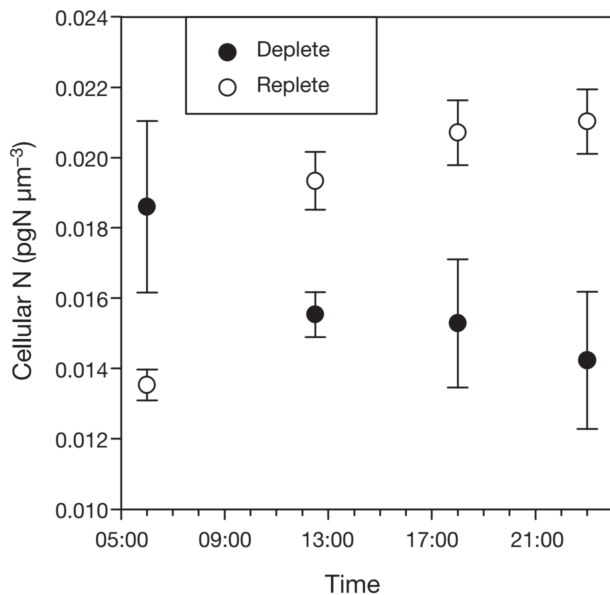

Fig. 5. *Karenia brevis*. Total cellular N (pg) normalized to cell volume in Deplete and Replete cultures at 6:00, 12:30, 18:00 and 23:00 h

adjusting uptake rates in relationship to nutrient concentration, by re-allocating internal biochemical pools, and by changing growth rates (Morel 1987). In the present context, cells undergoing a regular DVM to and from nutrient sources should react both biochemically and physiologically over the diel cycle to the different light and nutrient conditions that are encountered.

In this study, the Deplete *Karenia brevis* culture was exposed to nitrate-depleted media the morning the experiment began. The timing of this depletion simulated at least 12 h of ascent into an oligotrophic water column before a return to a near bottom nutrient source. The observed trends in biochemical pools (internal nitrate, free amino acids, and total cellular N), as well as in growth rates, suggest that cells respond to relatively short term and persistent depletion of nitrate in the media (as would be encountered during a 12 h migration into oligotrophic regions of the water column.) Declining trends in internal nitrate throughout the day suggest cells continued to assimilate what N was left in internal nitrate pools. In agreement with other studies, which have argued that long term benefits do not exist with regard to internal nitrate pools (Flynn & Fasham 2002), the relative abundance of nitrate pools relative to amino acids and total cellular N were not sufficient to prevent declining cellular N (as indicated by declining trends in both internal free amino acids and total cellular N) during the duration of the experiment. No division was detected after nitrate was depleted from the media suggesting that increasing N stress of the cells decreased growth rates.

By contrast, the observed trends in the Replete culture did not indicate N stress. Fluctuations in internal nitrate pools and free amino acids suggested N assimilation with the start of the light period but without the decreasing trends in the internal nitrate pool observed in the Deplete culture. The increasing trend of total cellular N throughout the day as well as maintenance of growth rates with 10 % of the cells dividing the night after the experiment confirmed that these cells were not N-stressed during the experiment.

The uptake of nitrate follows a general hyperbolic curve in its dependence on light (MacIsaac & Dugdale 1972). Increased light translates to enhanced energy that contributes to the active uptake and assimilation of nitrate (Turpin 1991). No differences in the uptake of nitrate during the day were observed within either culture at the different light levels (60 and 350 µmol quanta m^{-2} s^{-1}) used here. The most probable explanation for this is that both light intensities fell on the asymptote of a hyperbolic curve for the light-dependent nitrate uptake by *Karenia brevis*. While no such curve for *K. brevis* has been described, previous research suggests that *K. brevis* may maximise growth with as little as 45 µmol quanta m^{-2} s^{-1} (Shanley 1985, Shanley & Vargo 1993) and thus may maximise nitrate uptake at similar light levels. In addition, daytime nitrate uptake may be maximal under both nitrate concentrations (11 and 31 µM) used to inoculate the cultures. Both concentrations were significantly greater than those encountered in an oligotrophic water column, but may be analogous to concentrations of N derived from the sediment (Jahnke et al. 2005).

Though there are significant inter-species differences in the diel variability of nitrogen uptake (Paasche et al. 1984), uptake rates are often depressed in the dark. Cochlan et al. (1991) reported that nocturnal NO_3^- uptake was 50 % of diurnal uptake at 30 % of surface irradiance. While DVM by *Karenia brevis* allows cells to access the sediment surface, this behavior is only advantageous if it is coupled with elevated levels of nocturnal uptake.

The *Karenia brevis* cultures used here showed significant differences in their uptake of nitrate in the dark during the incubations at 23:00 h. Nitrate uptake in the culture previously depleted of nitrate was almost 6× greater in high nitrate additions than nitrate uptake in the culture previously exposed to sufficient nitrate. A similar pattern occurs in the dinoflagellate, *Heterosigma carterae*, which assimilates N in darkness as the C:N ratio increases concurrent with an increase in water soluble carbohydrate (Flynn & Fasham 2002). In comparison to nocturnal nitrate uptake rates that were found to be >50 % diurnal nitrate uptake in N deficient *H. carterae* cultures, the Deplete *K. brevis* cultures had nocturnal uptake rates that were nearly equivalent to

daytime rates in both the 0.5 µM and 11 µM incubations. This elevated uptake rate may be due to 'surge' uptake when N-deficient cells first encounter N-sufficient environments (Conway et al. 1976). Elevated nocturnal uptake responses after exposure to nitrate-depleted environments by day may make nocturnal descent to a near-bottom nutrient source biologically and ecologically advantageous (Watanabe & Kimura 1991, MacIntyre et al. 1997).

Previous modeling efforts by Liu et al. (2001a) elaborated on the metabolically-influenced vertical migration behavior of *Karenia brevis* introduced by Kamykowski & Yamazaki (1997). Liu et al. (2001b) explored how different nutrient sources might influence the population growth of *K. brevis*, and how vertical position, determined by biochemical, behavioral, and environmental factors, influenced transport trajectories on the West Florida Shelf. This work expands upon Liu et al. (2001b) by defining the nocturnal uptake capacity of *K. brevis* for nitrate and by suggesting that an additional near-bottom nutrient source on the West Florida Shelf may be the sediment-water interface.

Recently, elevated nitrate concentrations were not found to be associated with upwelling events (Walsh et al. 2003). This suggests that near-bottom populations may be utilizing an alternate source. The nitrogen form selected in this study may result from the flux of regenerated organic matter in an oxygenated water column. However, the nutrient exposure of cells descending to the sediment-water interface may consist of multiple species of N depending upon seasonal and spatial variability (Jahnke et al. 2005) as well as pore-water advection (Heuttel & Rusch 2000). The ability of *Karenia brevis* to utilize both reduced and organic sources of N has been established in the lab (Baden & Mende 1979, Gomperts 2003) and in the field for aggregations near the surface of water column (Bronk et al. 2004). If *K. brevis* uses its DVM to descend to the sediments to access nitrogenous nutrients, additional knowledge on the nocturnal uptake under different levels of N stress is needed for other N species.

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