ABSTRACT

SINCLAIR, GEOFFREY ALLAN. Environmental and behavioral influences on *Karenia brevis*’ nitrate uptake. (Under the direction of Daniel Kamykowski and Thomas Wolcott.)

I hypothesized that concentrations of *Karenia brevis*, observed near the bottom of a 22 m water column on the West Florida Shelf, might be using sediments as a nutrient source in its oligotrophic environment. In order to optimize cell growth, cells would need to alternately maximize exposure to light, by swimming up into an oligotrophic water column during the day, and nutrients (represented by nitrate), by swimming down at night to the sediment-water interface. Understanding how cell physiology and cell behavior contribute to the acquisition of resources (light and nutrients) that are separated in space is critical to understanding how *K. brevis* populations persist in oligotrophic environments.

To understand how *K. brevis*’ physiology contributes to acquisition of nitrate we tested how *K. brevis*’ uptake of nitrate changes with different prior environmental exposures. We simulated two conditions that cells might encounter: up in an oligotrophic water column and near the sediment-water interface. The first culture represented cells at the apex of their migration away from the sediments and was grown under high light (350 µmol quanta m\(^{-2}\) sec\(^{-1}\)) and reached nitrate-depleted conditions (< 0.5 µM NO\(_3\)) at the time of the experiment. The second culture represented cells that remain near the sediment and was grown under low light (60 µmol quanta m\(^{-2}\) sec\(^{-1}\)) and was under nitrate replete conditions (20 µM NO\(_3\)) at the time of the experiment. Cells exposed to nitrate-depleted environments 12 hours prior to the experiment showed enhanced nocturnal uptake relative to cells exposed to 20 µM NO\(_3\).

*K. brevis* may also use its vertical migration to facilitate nutrient acquisition. In order to understand how vertical migration influenced *K. brevis*’ diel uptake of nitrate we created a 1.5 m deep stratified mesocosm. We examined *K. brevis*’ internal N and uptake rates at three
depths, the surface, middle, and bottom that corresponded to light levels of 350, 125 and 60 µmol quanta m⁻² sec⁻¹. The upper 2/3 of the mesocosm, encompassing the surface and middle samples, was nitrate depleted (< 0.5 µM NO₃) and simulated an oligotrophic water column. The lower 1/3 of the mesocosm (bottom sample) contained 10 µM NO₃ corresponding to elevated nutrients near the sediment. Cell movement and biochemical state support vertical migration as a mechanism for nitrate acquisition. Nocturnal uptake in the mesocosm was significantly less than diurnal uptake. Nocturnal uptake rates in the mesocom were intermediate between cells exposed to nitrate-depleted conditions and nitrate-replete conditions in the first experiment.

*K. brevis* appeared to couple changes in nocturnal uptake with its migration behavior to optimize exposure to light during the day and access to sediments as a nutrient source at night. Migration of *K. brevis* may allow it to exist farther offshore in regions where benthic microalgae are limited by light. The nocturnal uptake rates of nitrate are sufficient to maintain average growth rates of 0.3 div day⁻¹ in near-bottom populations. Near-bottom populations of *K. brevis* may grow undetected in offshore and oligotrophic areas where non-motile phytoplankton would not be able to exploit near-bottom nutrients. Onshore advection of such offshore *K. brevis* populations by upwelling events could account for population aggregations that suddenly appear at the surface.
ENVIRONMENTAL AND BEHAVIORAL INFLUENCES ON KARENIA BREVIS’ NITRATE UPTAKE

By

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DEDICATION

I dedicate this thesis to my family. My parents, Bill and Betty Sinclair, upon whose shoulders I stand, have provided unconditional support in all my endeavors. They encouraged me to learn, grow and keep an open mind about life. My sister, Aimee, has kept me grounded in reality. Our conversations have always helped me gain a different perspective on life, perspectives that I cannot acquire through books or world travels. The thesis would not have been possible without Kristen, my wife and lifelong companion. She has provided tremendous support and encouragement. Her patience and help, often into the wee hours of the night, were invaluable. She has been irreplaceable in this adventure, and indeed, is irreplaceable in life. I would also like to thank Kristen’s family, Andy, Eric and Jill, for accepting me so quickly into their family.

I also dedicate this thesis to my teachers. They were the inspiration for starting down this road in life. They taught me how to observe, question, and seek answers to the unknown. The faculty at North Carolina State University has been especially supportive both financially and intellectually. I look forward to continuing to work with them as I pursue my PhD.
BIOGRAPHY

Geoff grew up in Springfield, Virginia where he attended Ravensworth Farm Elementary School and Annandale High School. He spent much of his childhood outdoors, either playing sports or roaming in the woods and developed a love of nature very early life. After graduating from the University of Virginia, Geoff enlisted in the United States Peace Corps. He lived for two years with subsistence farmers and fisherman where the Alibori and Niger rivers meet, in Kargui, a village in Benin, West Africa. Life here transformed his childhood love of nature to a much deeper appreciation for the natural world and how organisms adapt to it. He decided to return to school to continue studying how organisms adapt to different environments. After a brief stint at the University of California, L.A., studying chemical ecology, he moved to Raleigh, N.C. to be with the love of his life – and now wife – Kristen. Geoff continued his study of how organisms persist in unlikely places at North Carolina State University. Geoff and Kristen now live happily in a quiet Cary neighborhood with their big black dog, Denver.
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Influences of prior environmental exposure and biochemical state on *Karenia brevis*’ diel uptake of nitrate.

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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 1961, Steidinger 1975, Tester & Steidinger 1997). *K. brevis*’ ability to acquire nutrients in these oligotrophic conditions is critical to the growth and development of bloom events, but remains poorly understood.

Nitrogen is often the limiting nutrient in marine systems (Hecky & Kilham 1988). Proposed sources from which *K. brevis* may acquire the nitrogen needed to initiate and sustain population growth in oligotrophic water columns include upwelling events, *Trichodesmium* blooms, and diffusion from the sediment. Upwelled Loop Current water may provide nitrogen necessary to stimulate initiation events in the oligotrophic offshore regions of the West Florida shelf (Tester & Steidinger 1997). Surveys from 1958 to 1961, however, found no changes in biomass associated with upwelling that penetrated to the 40 m isobath (Walsh & Steidinger 2001). Recent analysis of upwelled water does not indicate nitrate enrichment (Walsh 2003). Walsh and Steidinger (2001) hypothesized that if *K. brevis* was deriving nitrogen from upwelled loop current waters, cells would have $\Delta^{15}$NO$_3$ values of 6.7 to 8.3 ppt, similar to Venezuelan slope source water at depths of 200 – 500 m (Lui 1989). Instead *K. brevis* has $\Delta^{15}$N values of 3.6 to 5.1 ppt. The discrepancy between del$^{15}$N of nitrogen source (slope water-originating on the Venezuelan slope) and sink (*K. brevis*) led to the hypothesis that the nitrogen source was “new” nitrogen from co-occurring N-fixing *Trichodesmium* surface blooms. The low $\Delta^{15}$PON signature (-0.08 ppt) (Minagawa 1986) of
*Trichodesmium* may dilute *K. brevis*’ del$^{15}$N signature, causing it to be lower than surrounding waters. Like upwelling events, however, *Trichodesmium* blooms do not always co-occur with surface expressions of *K. brevis*, and the question remains of how *Trichodesmium*-fixed N is transferred to *K. brevis*.

Both surface and near bottom populations of *K. brevis* were identified in a 22 m water column during a 2000 ECOHAB Cruise (Figure 1). The wide vertical distribution suggested another potential source of nitrogen for *K. brevis*: the sediment-water interface. Considerable microphytobenthic primary productivity occurs at depths of 15 to 20 meters in coastal temperate areas (Bodin 1985, Herndl 1989). Other studies report that the majority (30 to 80%) of the phytoplanktonic nitrogen requirement in 5-50 m coastal environments originates from the sediments (Nixon 1981, Blackburn 1983, Boynton 1985). Increased nutrient availability near the sediments relative to that in an oligotrophic water column, combined with algal adaptation to low light, may explain the distribution of benthic micro-algae (Stevenson 1981, Sundback 1988). Unlike benthic microalgae that are constrained to the sediments, *K. brevis* migrates vertically up into the water column at a rate of approximately 1m hr$^{-1}$ in the absence of water motion. *K. brevis* may therefore exploit a niche offshore, where benthic microalgae are light limited and are less able to compete (Cahoon, 1999), using vertical migration to alternate between the nutrients available near the sediment-water interface and the photic zone, accessing adequate light for photosynthesis ~ 10 m above the sediment.

Nutrient availability influences nutrient uptake, which affects growth rate of cells, populations, and communities, and ultimately carrying capacity (Smayda, 1997). Nutrient uptake can be described by the Michaelis-Menten equation $V = (V_{\text{max}}S) / (K_s + S)$, where $V$
is the rate of uptake, $V_{\text{max}}$ is the maximal uptake, $S$ is the substrate concentration and $K_s$ is the half-saturation constant, or affinity constant (Eppley et al. 1969). In low nutrient environments phytoplankton can increase $V$ by decreasing $K_s$ and/or increasing $V_{\text{max}}$.

In oligotrophic environments nutrients are sparse. When a phytoplankter encounters elevated nutrients there is a transitory flux of nutrients to the cell membrane followed by a return to the nutrient deficient environment. A phytoplankter may adapt to short term changes in nutrient availability by modulating its affinity (altering its $K_s$) for a nutrient, thus increasing its uptake efficiency. Early theory described dinoflagellates, typically associated with stable and stratified environments, with low $K_s$ values that enable them to out-compete diatoms in low nutrient environments. Steidinger et al. (1997) reported a nitrate $K_s$ value for $K. \ brevis$ of 0.42, which is consistent with cellular adaptation to oligotrophic environments.

While initiation of $K. \ brevis$ blooms occurs in oligotrophic regions, blooms can be transported to thrive in near-shore environments. A phytoplankter may best adapt to higher nutrients associated with outwelling from land by increasing its uptake capacity (increasing $V_{\text{max}}$).

The strain of $K. \ brevis$ used in this study was isolated from off of Apalachicola Bay, Florida. This estuary exports higher concentrations of dissolved inorganic nitrogen (DIN) to the Gulf of Mexico in the winter than in the summer (Mortazavi et al. 2000). $K. \ brevis$ populations initiated in oligotrophic waters that are offshore in the early fall may then be transported to near shore areas in late fall or early winter when higher levels of DIN are present. The different environments in which $K. \ brevis$ exists, both offshore in oligotrophic environments and near shore, beg reexamination of its nutrient uptake capacities.
Surveys of the nutrient uptake dinoflagellates, raphidophytes, and diatoms (Table 1) found that dinoflagellates generally had higher $K_s$ values than diatoms (Smayda 1997). There are four proposed adaptations to compensate for the potential ecological disadvantages of higher $K_s$ values:

1. Vertical migration for nutrient acquisition.
2. Mixotrophic nutritional tendency.
3. Allelochemically-enhanced interspecific competition.
4. Allelopathic antipredation defense mechanisms (Smayda, 1997).

Nutrient acquisition mechanisms like migration provide a greater competitive advantage than low cellular $K_s$ and high $V_{max}$ (Lieberman et al.1994). *K. brevis*’ behavior of diel vertical migration, migrating up during the day to access light and down at night to access nutrients, provides a mechanism to exploit near-bottom nutrient sources. Cells, however, may experience biological tradeoffs (Cullen & Horrigan 1981, Flynn 1998, Yamazaki & Kamykowski 2000).

Upward migration maximizes exposure to light for photosynthesis. Nitrate uptake in natural populations of marine phytoplankton is generally dependent on light and can often be described by a hyperbolic function, the Michaelis-Menten equation (MacIsaac & Dugdale 1972). The half saturation constants for light’s effect on nitrate and ammonium uptake, or $K_l$, ranged from 1 to 14% of surface light intensity (MacIsaac & Dugdale 1972). Carbon fixation is similarly related to light until the saturation / inhibition level is reached (Platt & Gallegos 1980). The carbon, fixed and stored in the form of carbohydrates and/or lipids, provides energy in the form of ATP and reducing equivalents (NADPH) that contribute to N assimilation (Turpin 1991). Therefore, in eutrophic environments, a cell’s relative ability to
take up nutrients may be a function of its vertical position in the water column and thus its exposure to irradiance at levels below light saturation.

In oligotrophic areas, nutrient uptake is limited by nutrient concentration rather than light. Upward migration of *K. brevis* will increase light exposure but also decrease exposure to nutrients during the day. Downward migration at night could increase nutrient exposure and facilitate uptake in the dark. Dark uptake is generally depressed in most phytoplankton species (Cochlan et al. 1991, Clark 2002). In order to maximize the benefits of a near-bottom nutrient source, *K. brevis* would need to couple migration behavior and consequent changes in exposures to light and nutrients with biochemical storage and physiological capacity to fix carbon in the light and assimilate nutrients in the dark.

This study examined nitrate uptake in light and nutrient regimes similar to those of the near-bottom *K. brevis* population observed on the 2000 ECOHAB cruise (Figure 1). Boundary conditions were defined by the sediment and by the distance a cell could swim upward during a 12 hour period. One culture represented cells at the apex of migration exposed to higher light 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. The importance of behavior in influencing environmental exposures and as a nutrient acquisition mechanism is addressed in a companion paper exploring *K. brevis*’ migration between the light and nutrient regimes examined in this study.

Understanding how *K. brevis*’ internal nitrogen reserves differ in cultures adapted to different environments, and the subsequent physiological responses when environmental
exposure changes, probes the biological tradeoffs that *K. brevis* may encounter during vertical migration. The ability of cells from different prior exposures to take up nitrate at varying light levels during the day and at night may ultimately determine the distribution of cells in the water column and how well cells persist in oligotrophic environments.
METHODS

Two non-axenic 6L batch cultures were started from the same *Karenia brevis* parent culture (strain Apalachicola) and grown in modified L 1/20 media (Guillard 1993) with nitrate as the only nitrogen source. Filtered autoclaved seawater (salinity = 35) was the base. The two batch cultures were grown under a 12:12 light/dark cycle with lights on at 06:30 EST and off at 18:30 EST.

Culture 1, referred to as “Deplete”, represented cells that had undergone a 12 hour upward migration from near the bottom into an oligotrophic water column, where they experience higher light and temperature levels and low nitrate concentrations. The Deplete culture was grown at 25°C and under light delivering approximately 350 µmol quanta m⁻² sec⁻¹ to the surface plane of the culture. Nitrate became depleted in the media 12 hours prior to the experiment (Table 2). Culture 2, referred to as “Replete”, represented cells remaining near the sediment with lower light and temperature levels and higher nitrate concentrations. The Replete culture was grown at 22°C and under light delivering < 60 µmol quanta m⁻² sec⁻¹ to the surface plane of the culture. Nitrate was not limiting (20µM) prior to the experiment.

Nitrate uptake was evaluated both during the day (12:30) and at night (23:00). The 12:30 time was chosen to provide adequate exposure to light prior to the sampling, and 23:00 was chosen to complete the incubation well before cell division, which generally occurs around 03:00 to 04:00 (Kamykowski, 1985). NO₃ uptake was tested with 0.5 µM and 11µM ^1⁵N NO₃ additions to the Deplete culture and 11µM ^1⁵N NO₃ to the Replete culture. The total nitrate in solution in the incubation flasks was 0.5 µM and 11µM ^1⁵N NO₃ for the Deplete culture and 31µM ^1⁵N NO₃ for the Replete culture. The nutrient additions were adjusted so ^1⁵N NO₃ would be 10% of the final concentration of nitrate in solution. The flasks were
incubated in a radial photosynthetron (Babin 1994) 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 μmol quanta m⁻² sec⁻¹ and < 60 μmol quanta m⁻² sec⁻¹) and different temperatures (25°C and 22°C) for two hours (Table 2).

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

Cell counts for all samples were provided by a Coulter Multisizer II Particle Analyzer Counter (Beckman-Coulter Inc., Miami, FL) with threshold settings of 13.3 and 30 μm. Filtrations for intracellular nitrate pool, intracellular free amino acids, and total cellular N used precombusted Whatman GF/C 25mm filters. Whatman GF/C 12mm filters were used for ¹⁵N 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 1984) and a Lachat QuikChem 8000 Continuum Series Autoanalyzer (EPA method 353.2).

Sample volume for intracellular nitrate and intracellular free amino acids was equivalent to 2 X 10⁴ cells. Intracellular nitrate pools were evaluated following the protocol of Thoresen (1982), involving extraction by passing 10 ml boiling DI water through the filters. Filtrate was analyzed with the Lachat QuikChem 8000 Continuum Series
Autoanalyzer (EPA method 353.2). Both intracellular nitrate and intracellular free amino acids were normalized to cell diameter.

Free amino acid assays followed the protein extraction protocol of Sigma Diagnostics (procedure 5656) based on Peterson’s modification of the Lowry method (Diagnostics 1994). 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.

Total cellular N samples were processed on a Carlo Erba nutrient analyzer. The volume filtered corresponded to the approximately 21 µg N filter⁻¹ required 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 ¹⁵N tracer techniques, all incubation vials and storage vials were acid washed with 10% HCl. Nitrate uptake rate on a per cell basis was calculated with the following equation:

\[
V_{\text{Nitrate}} = \left(\frac{\%^{15}\text{N}_{\text{sample}} - \%^{15}\text{N}_{\text{control}} \times \mu\text{g N}_{\text{sample}}}{\%^{15}\text{N}-\text{NO}_3 \times \text{time} \times \text{total cells}}\right)
\]

Uptake rates (pmol cell⁻¹ hr⁻¹) were then normalized to total cellular N by dividing \(V_{\text{Nitrate}}\) by total intracellular N.

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

Deplete Culture

The Deplete culture, acclimated to 350 \( \mu \text{mol quanta m}^{-2} \text{ sec}^{-1} \) and 25 \( ^\circ \text{C} \), exhibited growth rates of 0.5 doublings day\(^{-1} \) prior to the experiment when nitrate in the media was present. The 24 hour experiment started when the nitrate level in the media became undetectable (< 0.5 \( \mu \text{M NO}_3 \) at 6:00 the morning of the experiment). This correlated with the cessation of culture growth (Figure 2). The culture had a mean cell diameter of 20.85 \( \mu \text{m} \) over the 24 hours of the experiment (Figure 3). Both internal nitrate pools (Figure 4) and free amino acids (Figure 5) fluctuated slightly throughout the day. There was a significant decrease in the intracellular nitrate pool at 18:00 compared to 5.5 hours earlier at 12:30 (\( F = 39, \ p < 0.01 \)). The apparent increase 5 hours later at 23:00 was not significant. Fluctuations in intracellular free amino acid levels tended to follow the same diel pattern as intracellular nitrate but were not different from mean levels throughout the day. There was no significant difference between total cellular nitrogen at 12:30 and 23:30 (Figure 6).

Nitrate concentration was the only environmental variable that significantly influenced uptake rates in the incubations of the deplete culture (Figure 7). Uptake rates in the 11 \( \mu \text{M} \) \( ^{15}\text{N NO}_3 \) additions averaged 0.52 \( V_{\text{nitrate}} \ \text{hr}^{-1} \), 2.6 times higher than uptake rates in the 0.5 \( \mu \text{M} \) \( ^{15}\text{N NO}_3 \) incubations (about 0.2 \( V_{\text{nitrate}} \ \text{hr}^{-1} \), \( F = 130, \ p < 0.01 \)). Nitrate uptake at 25 \( ^\circ \text{C} \) was not significantly different than uptake at 22 \( ^\circ \text{C} \). Comparisons during the day between cells incubated at 350 \( \mu \text{mol quanta m}^{-2} \text{ sec}^{-1} \) and <60 \( \mu \text{mol quanta m}^{-2} \text{ sec}^{-1} \) did not show any significant differences in rate of nitrate assimilation. Similarly assimilation at 12:30, across light levels, did not differ significantly from dark assimilation at 23:00.
Replete Culture

The Replete culture, acclimated to < 60 µmol quanta m$^{-2}$ sec$^{-1}$ and 22 °C, exhibited growth rates of 0.3 doublings day$^{-1}$ before, during, and after the experiment due to sufficient nitrate in the media (Figure 2). Mean cell diameter during the experiment was 19.86 µm (Figure 3). Intracellular nitrate levels appeared to increase throughout the day before declining at 23:00 (Figure 4), but sample variation was large and did not yield any statistically significant comparisons. Intracellular free amino acids fluctuated throughout the day (Figure 5), increasing by 12:30 (F = 12.9, p = 0.02) before declining at 18:00 (F = 14.0, p = 0.02) and then increasing again at 23:00 (F = 35.7, p < 0.01). The increase in intracellular free amino acids at 23:00 corresponded to a concurrent decreasing trend in intracellular nitrate levels. There was no significant difference between total intracellular N at 12:30 and 23:00 (Figure 6).

Nitrate uptake (Figure 8) during the day was significantly greater (about 8.5-fold) than nitrate uptake at night (F = 8.6, p < 0.01). No interaction was found between light level and temperature in the incubations. Neither light level nor temperature had any significant impacts on nitrate assimilation.

Deplete vs. Replete

The growth rate of 0.5 divisions day$^{-1}$ of the Deplete culture grown at warmer temperatures and higher light exceeded the 0.3 divisions day$^{-1}$ growth rate of the Replete culture grown at cooler temperatures and lower light during the pre-experiment period when nitrate was present in both cultures. The growth rate of the Replete culture, however, exceeded that of the Deplete culture during the course of the experiment when nitrate was not detectable in the Deplete culture (Figure 2). Average cell diameter (figure 3) in the Deplete
culture (20.85 µm) was about 5% larger than those of cells in the Replete culture (19.86 µm). The average intracellular nitrate level over the diel period of the experiment was significantly greater in the Deplete culture than in the Replete culture (F = 22.8, p < 0.01) (Figure 4). The greatest differences in intracellular nitrate pools occurred at 6:00 when the deplete culture had 1.9 times as much intracellular nitrate as the Replete culture, and at 23:00 when the Deplete culture had 1.8 times as much as Replete culture. Over the diel period, fluctuations in intracellular free amino acids prevented any significant differences between the average levels of the two cultures (Figure 5), but transient differences were evident. Starting at 6:00, the Deplete culture had 1.3 times more intracellular free amino acids than did the Replete culture (F = 18.86, p = 0.012), perhaps a consequence of prior cell division in the Replete culture at 4:00 to 5:00 (Kamykowski, 1985). As the day progressed, the Replete culture accumulated more intracellular free amino acids than the Deplete culture and contained 1.6 times more by 12:30 (F = 12.5, p = 0.02). An apparent increase at 18:00 was not significant due to sample variability. A distinct increase in intracellular free amino acids in the replete culture at 23:00, perhaps in preparation for cell division, again brought its free amino acid content 2.4 times above that of the Deplete culture (F = 55.4, p < 0.01). The replete culture had significantly more total cellular N than the Deplete culture (F = 55.5, p << 0.01) (Figure 6).

There were no significant differences in diurnal nitrate uptake between the Deplete culture in 11 µM 15N NO3 incubations and the Replete culture in the 31 µM 15N NO3 incubations (Figure 9). Nocturnal uptake (Figure 9) in the Deplete culture at 11 µM 15N NO3, however, was 5.6 times greater than that of the Replete culture in the 31 µM 15N NO3 incubations (F = 328, p << 0.01).
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. Morel (1987) describes how cells may adapt to changes in nutrient fields by adjusting uptake rates in relationship to nutrient concentration, reallocating internal biochemical pools, and by changing growth rates. In this study, the K. brevis Deplete culture grown under a low nutrient regime characteristic of an oligotrophic water column in nature was characterized by decreased levels of total cellular N relative to the Replete culture with sufficient N.

While the intracellular biochemical states of these batch cultures may not be the same as those of vertically migrating cells in nature, they may serve as extremes with which to help interpret physiological responses, uncomplicated by behavior and changing environmental conditions. Bronk (1999) asserted that Synechococcus spp. cultures grown in nitrogen-depleted media were analogous to cells in nature that exist in nutrient depleted environments. Similarly, cultures grown in nutrient-replete media conceptually represent the initial condition of cells continuously exposed to a nutrient source (in our scenario a near-bottom nutrient source).

Consistent with the decrease in total cellular N, the Deplete culture began a stationary growth phase during the experiment reflecting a prior depletion of external nitrate. Several studies have demonstrated that growth in the absence of nitrate as a substrate, often in the presence of other nutrient sources such as reduced N forms like NH$_4^+$, results in decreased nitrate assimilation due to lost activity of the enzyme nitrate reductase (Eppley et al. 1969).
While diatoms excrete nitrate in response to higher light levels, dinoflagellates do not (Lomas & Glibert 2000). The Deplete culture (exposed to 350 µmol quanta m\(^{-2}\) sec\(^{-1}\)) retained comparatively higher intracellular nitrate levels than the Replete culture (exposed to <60 µmol quanta m\(^{-2}\) sec\(^{-1}\)).

Intracellular nitrate storage in the short term might seem energetically beneficial when cells are exposed to nitrate-depleted media. Flynn (2002), however, argued that long-term benefits do not exist since reported intracellular nitrate values are not high enough to replenish intracellular organic nitrogen. If intracellular nitrate pools do not confer any significant biochemical advantage in the absence of nitrate-rich media, perhaps the ability to retain intracellular nitrate may be an adaptation to retain nitrate reductase activity. Retention of enzyme activity would allow uptake capacity for nitrate to remain the same over the diel migration until cells return to areas of higher nitrate concentrations.

There were not any nitrate uptake differences during the day within either culture at different light levels (60 and 350 µmol quanta m\(^{-2}\) sec\(^{-1}\)), in contrast to reports of the usual dependence of nitrate uptake on light (MacIsaac & Dugdale 1972). N deficiency in the Deplete culture, reflected in the cessation of growth, may have confounded potential effects of light on nitrate uptake. In general there is a decreasing dependence on light with increasing N deficiency (Raven 1980). The Replete culture, adapted to low light (60 µmol quanta m\(^{-2}\) sec\(^{-1}\)), may have been inhibited by the sudden switch to higher light conditions during the experiment. In nature, *K. brevis’* vertical migration allows gradual acclimation to the ambient light environment.

Whether cells contain sufficient nitrogen or are N-depleted influences the kinetics of N assimilation (Flynn 1998) causing cells to shift short term uptake rates (Morel, 1987).
Comparisons between cultures did not reveal any effect of prior nutrient exposure (and consequent biochemical state) on nitrate uptake capacity in high nitrate (11 µM & 31 µM) incubations. N-deficiency in cells results in increased uptake rates (Raven, 1980) and may also lead to “surge” uptake when nutrient-sufficient environments are encountered (Conway et al. 1976). The saturation of nitrate uptake at the high concentrations (11 µM & 31 µM) and the 2 hour duration of the incubations may explain the non-significant differences between treatments. This result suggests that the hourly uptake capacity (V_{max}) of *K. brevis* at high concentrations does not change with prior light or nutrient exposure. When sufficient nitrate concentrations are encountered (11 µM and greater), cells can acquire nitrate equivalent to the total intracellular N in less than two hours. *K. brevis*’ retention of high uptake capacities in less than ideal conditions (either N-deficient or low light for several hours) may enable it to exploit higher nutrient concentrations upon the breakdown of vertical stratification and subsequent nutrient additions to a previously oligotrophic water column.

Significant differences between cultures were also demonstrated in dark (nocturnal) uptake responses. Nitrate uptake in the Deplete culture was 6 times greater in high nitrate additions than nitrate uptake in the Replete culture. 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 2002). In addition to the influences of changing internal biochemical state on nitrate uptake, there are also inter-species differences on the diel variability of nitrogen uptake (Paasche et al. 1984). Cochlan et al.(1991) reported that nocturnal NO₃ uptake was 50% of diurnal uptake at 30% of surface irradiance (I), while Clark & Flynn (2002) found that nocturnal NO₃ uptake was >50% diurnal NO₃ uptake in N deficient *H. carterae* cultures.
The Deplete culture of *K. brevis* increased its nitrate uptake capacity during the nocturnal dark incubations relative to the Replete culture. While the studies mentioned above reported night assimilation rates >50% of day assimilation rates, we did not find any significant differences between day and night uptake responses in the Deplete culture in either 0.5 µM or 11 µM incubations. Elevated nocturnal uptake responses after exposure to nitrate depleted environments by day may make migration downward, toward a near-bottom nutrient source, biologically and ecologically advantageous (Watanabe 1991, MacIntyre et al. 1997).

The hypothesis that *K. brevis* utilizes near-bottom nutrients presents a biological tradeoff. Cells must migrate up into an oligotrophic water column to optimize light exposure for photosynthesis but must be near the bottom to optimize nutrient uptake. In order to acquire both of these spatially-separated resources, and minimize the tradeoff, they would find it most advantageous to migrate up during the day to photosynthesize and migrate down at night to acquire nutrients. If we assume that doubling cell N is required for cell division, and a net growth rate of 0.3 division day⁻¹ (Wilson 1966), cells must acquire adequate N during 3 downward migrations to the sediment water interface. If cell residency time near the bottom is driven exclusively by demand for N, then based on uptake characteristics we can begin to piece together how long individual cells remain at the bottom and how this compromises exposure to light.

Different nocturnal uptake rates result in different bottom times, different migration distances from the sediment water interface, and consequently different light exposures. If these extreme scenarios are integrated, they may help explain the diurnal dispersion of cells throughout the water column observed in nature (Figure 1). I modeled the potential
migration patterns in a 22 m water column (Figure 10), assuming a 12:12 hour light : dark cycle, and delineated the maximum and minimum amount of time at night cells must spend at the sediment-water interface depending on nocturnal uptake capability, prior nitrate exposure, and ambient nitrate concentrations near the bottom. Cells with nocturnal uptake capacities similar to the N-depleted culture (black lines), in which nocturnal nitrate uptake rates equal diurnal uptake rates, are able to minimize the tradeoff between spatially separated light and nutrients. The bottom time determined for cells to replenish the equivalent of their total cellular N is approximately 3 hours per night in ambient near-bottom nitrate concentrations of 0.5 µM and 1.5 hour in concentrations of 11 µM. In contrast, cells with a nocturnal uptake capacity similar to the Replete culture (red lines) would require 5 hours per night in ambient near-bottom concentrations of 31 µM.

*K. brevis* has a swimming speed “s” of 1 m hr⁻¹. Assuming a closed migration cycle (cells migrate up and down the same distance with the minimum depth at 12:00), the vertical distance (d) traveled upward or downward over the diel period (in the scenarios) can be calculated by

\[ d = (\text{Diel Period} - \text{Bottom Time}) * s / 2 \]

This indicates that N-depleted cells in low near-bottom nitrate concentrations could migrate upward 8.5 m, and depleted cells in high nitrate concentrations could migrate upward 11 m. Replete cells in high nitrate concentrations could migrate only 9.5 m upward. For an aggregation on a 22 m deep bottom, the migration distance (d) must be subtracted from the water column depth to obtain the cells’ z (depth from surface). The potential difference in light exposure can be approximated using Beer’s law: \( I(z) = I_o e^{-kz} \), where \( I_o \) is surface irradiance (2000 µmol quanta m⁻² sec⁻¹), k is the attenuation coefficient, z is depth from
surface, and I(z) is irradiance at depth z. The coefficient k is calculated by Riley’s equation k = 0.04 + 0.0088Chl + 0.054Chl^{2/3}. I used a chlorophyll value of 6 mg Chl a m^{-3}, based on values from a co-occurring surface bloom was observed on the ECOHAB 2000 cruise. This corresponded to a light attenuation constant (k) of 0.2728. I also present attenuation patterns for chlorophyll values of 2 mg Chl a m^{-3} and 4 mg Chl a m^{-3}, corresponding to attenuation constants of 0.1376 and 0.2152 respectively (Table 3). With water turbidity based on Chl a values of 6 mg m^{-3}, cultures with uptake rates equivalent to the Deplete culture are exposed to a maximum of 100 µmol quanta m^{-2} sec^{-1} under high bottom nitrate concentrations and a minimum of 66 µmol quanta m^{-2} sec^{-1} under low nitrate concentrations. Cells with uptake rates equivalent to the Replete culture, by contrast, are exposed to half the light (50 µmol quanta m^{-2} sec^{-1}) under high nitrate concentrations.

The hypothetical scenarios imply that prior cell exposure to nitrate availability, as well as cell state, may greatly influence the adaptive advantages of migration behavior, especially comparing N-depleted and N-replete cells under low nutrient environments. Prior cell exposure to nitrate-depleted environments for 12 hours results in decreased total cellular N and increased nocturnal uptake capacity relative to N-replete cells that are continuously exposed to nitrate as an N source. As a result, cells with nocturnal uptake rates similar to those of N-depleted cells may increase exposure to light relative to cells with nocturnal uptake rates like those of N-replete cells when high nitrate concentrations are encountered at the sediment-water interface.

Cells that experience transient changes in nutrient environment, particularly over their vertical migration, are not likely to exhibit uptake responses at either of these extremes. Rather, gradual acclimation over the vertical migration range may place their affinity for
nitrate, and their capacity to take up nitrate, somewhere in between. Future research may better quantify intracellular state as cells approach nitrate depletion, and analyze uptake responses over vertical migration to determine how behavior may influence the biochemical and physiological characteristics described here.
REFERENCES CITED


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Guillard RLPEH (1993) *Stichochrysis immobilis* is a diatom, not a chrysophyte. Research Note 32:234-236


MacIntyre JG, Cullen JJ, Cembella AD (1997) Vertical migration, nutrition and toxicity in the dinoflagellate Alexandrium tamarense. Marine Ecology-Progress Series 148:201-216


U.S. Environmental Protection Agency, Methods fo Chemical Analysis of Water and Waste. Method 353.2


Table 1. K₅ values for dinoflagellates and diatoms. Dinoflagellates tend to have higher K₅ values than diatoms. Modified version of table presented in Smayda (1997)

<table>
<thead>
<tr>
<th></th>
<th>NO₃</th>
<th>Reference</th>
</tr>
</thead>
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<tr>
<td><strong>Dinoflagellates</strong></td>
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<td></td>
</tr>
<tr>
<td><em>Alexandrium tamarense</em></td>
<td>1.5 - 2.8</td>
<td>MacIsaac et al. 1979</td>
</tr>
<tr>
<td><em>Amphidinium carterae</em></td>
<td>~2.0</td>
<td>Deane &amp; O'Brien 1981</td>
</tr>
<tr>
<td><em>Gonyaulax polyedra</em></td>
<td>8.6 - 10.3</td>
<td>Eppley et al. 1969</td>
</tr>
<tr>
<td><em>Gymnodinium breve</em></td>
<td>1.4-16.2</td>
<td>Sinclair 2005</td>
</tr>
<tr>
<td><em>Gymnodinium splendens</em></td>
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</tr>
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<td>18°C</td>
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<td>18°C</td>
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<td>Tomas &amp; Dodson 1974</td>
</tr>
<tr>
<td>25°C</td>
<td>6.55</td>
<td>Tomas &amp; Dodson 1974</td>
</tr>
<tr>
<td><em>Peridinium cinctum</em></td>
<td>29</td>
<td>Sherr et al. 1982</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Diatoms</strong></td>
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</tr>
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<td>Eppley et al. 1969</td>
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<td>Eppley et al. 1969</td>
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<td>Eppley &amp; Coatsworth 1968</td>
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<tr>
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<td>0.6</td>
<td>Eppley et al. 1969</td>
</tr>
<tr>
<td><em>Fragilaria pinnata</em></td>
<td>0.6-1.6</td>
<td>Carpenter &amp; Guillard 1971</td>
</tr>
<tr>
<td><em>Leptocylindrus danicus</em></td>
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<td><em>Thalassiodora pseudonana</em></td>
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Table 2. The experimental design. Culture acclimation of the Deplete culture and the Replete characterize by environmental variables of temperature, light and nutrients on a 12:12 light dark cycle. The treatments outline sampling times (12:30 and 23:00), the temperature, the light exposure, and nitrate additions in the incubations.

<table>
<thead>
<tr>
<th>Culture Acclimation</th>
<th>Deplete Culture</th>
<th>Replete Culture</th>
</tr>
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<tbody>
<tr>
<td>Temperature (°C)</td>
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<td>22</td>
</tr>
<tr>
<td>Light level (µmol m⁻² sec⁻¹)</td>
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<td>60</td>
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<tr>
<td>Nutrient in Media (µM NO₃)</td>
<td>Depleted (&lt; 0.5)</td>
<td>Replete (20)</td>
</tr>
<tr>
<td>Time (Light / Dark)</td>
<td>12:12 Day : Night</td>
<td>12:12 Day : Night</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Treatments</th>
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<tr>
<td>Day / Night</td>
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<td>12:30 / 23:00</td>
</tr>
<tr>
<td>Temperature</td>
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<td>22°C</td>
</tr>
<tr>
<td></td>
<td>25°C</td>
<td>25°C</td>
</tr>
<tr>
<td>Light level (µmol m⁻² sec⁻¹)</td>
<td>350 / dark</td>
<td>350 / dark</td>
</tr>
<tr>
<td></td>
<td>60 / dark</td>
<td>60 / dark</td>
</tr>
<tr>
<td>N15-NO₃ (µM) Additions</td>
<td>0.5 11 0.5 11 0.5 11</td>
<td>0.5 11 0.5 11 0.5 11</td>
</tr>
</tbody>
</table>
Table 3. Light attenuation at different depths achieved by cells under the migration scenario in Figure 15 assuming a surface irradiance of 2000 µmol quanta m⁻² sec⁻¹. Different hypothetical Chl a values resulted in different attenuation (k) values in the water column.

<table>
<thead>
<tr>
<th>Culture</th>
<th>Bottom nitrate (µM)</th>
<th>Depth (m)</th>
<th>Chl a</th>
<th>k (µmol m⁻² sec⁻¹)</th>
<th>I(z) (µmol m⁻² sec⁻¹)</th>
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<tr>
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<td>11</td>
<td>2</td>
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<td>Deplete</td>
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<td>11</td>
<td>4</td>
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<td>Replete</td>
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<tr>
<td><strong>Deplete</strong></td>
<td><strong>11</strong></td>
<td><strong>11</strong></td>
<td><strong>6</strong></td>
<td><strong>0.2728</strong></td>
<td><strong>100</strong></td>
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<td><strong>Deplete</strong></td>
<td><strong>0.5</strong></td>
<td><strong>12.5</strong></td>
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<td><strong>Replete</strong></td>
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<td><strong>6</strong></td>
<td><strong>0.2728</strong></td>
<td><strong>50</strong></td>
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Figure 1. Observations of near-bottom cell aggregations made on the ECOHAB 2000 cruise (Kamykowski pers comm.). Depth (m) is plotted against sampling that began at 00:00 and was done every 2 hours for a 24 hour period.
Figure 2. Average growth of the Deplete and Replete cultures measured by cell counts over time (Days). The blue inverted triangle represents the point at which each experiment was performed.
Figure 3. Cell diameters (µm) of the Deplete and Replete cultures over time (Days). The blue inverted triangle represents the point at which each experiment was preformed.
Figure 4. Diel patterns in intracellular nitrate (iNO₃) in the Deplete and Replete cultures. Values are normalized to cell size for purposes of comparison.
Figure 5. Diel patterns in intracellular free amino acids (Faa) in the Deplete and Replete cultures. Values are normalized to cell size for comparison.
Figure 6. Total cellular N in the Deplete and Replete cultures at 12:30 and 23:00. Values are normalized to cell size.
Figure 7. Uptake of nitrate of the Deplete culture different temperature, nitrate concentrations, and light conditions. Uptake rates are normalized to total cellular N.
Figure 8. Uptake of nitrate in the Replete culture under different temperature and light conditions in 31 µM nitrate. Uptake rates are normalized to total cellular N.
Figure 9. Deplete vs. Replete nitrate uptake. Uptake rates (normalized to total cellular N) are plotted as a function of light, temperature and nitrate concentration (µM).
Figure 10. Theoretical migration scenarios. Nocturnal uptake responses are equivalent to the rates determined for the Deplete and Replete cultures when exposed to either high concentrations of near bottom nitrate or low concentrations of near bottom nitrate. Calculations based on surface irradiance of 2000 µmol quanta m$^{-2}$ sec$^{-1}$ and light attenuation values ($k = 0.2728$).
CHAPTER 2

Influences of behavior and environmental exposure on *Karenia brevis*’ diel uptake of nitrate.

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INTRODUCTION

The diel vertical migration of the toxic dinoflagellate, *Karenia brevis*, permits access to light and nutrients that may be separated in time and space (Kamykowski & Yamazaki 1997, Kamykowski et al. 1999). In the absence of vertical water motion, interactions between behavior and physiology in constantly changing environments determine species’ distributions and persistence in the water column (Cullen 1985, MacIntyre et al. 1997). This study examines *K. brevis*’ nitrate uptake over its vertical migration in different light, temperature, and nutrient conditions.

*Karenia brevis* occurs naturally in background concentrations of 1-1000 cells L\(^{-1}\) across the west Florida shelf in oligotrophic waters (Steidinger, 1975, Haddad and Carder, 1979). Sudden aggregations of *K. brevis* near shore in densities of at least 10\(^5\) cells L\(^{-1}\), have been responsible for massive fish kills, neurolytic shellfish poisoning, mortalities of marine mammals and respiratory irritation in humans (Tester & Steidinger 1997). Increases from background concentrations to fish-killing densities are triggered by a combination of factors including changes in light and nutrients (Steidinger et al 1998). In situ growth rates of 0.2-1 division day\(^{-1}\) alone cannot explain the sudden increases in cell densities to bloom levels (Wilson, 1966, Shanely & Vargo, 1993). Hydrological concentration mechanisms also must contribute to bloom development. Despite their economic and ecological importance, the initiation and subsequent formation of *K. brevis* blooms remain poorly understood.

Initiation regions are found 18 to 74 km offshore in oligotrophic waters 12-37 m deep (Steidinger & Haddad, 1981, Steidinger et al. 1998, Tester & Steidinger, 1997). Low concentrations of N and P are commonly observed (0.1 to 0.2 µM) offshore to within 2-4 km of the shore (Dragovich et al. 1961, Vargo and Shanley 1985). These oligotrophic conditions
suggest nutrients (probably N, Hecky & Kilham 1988) are limiting to growth of phytoplankton. Any additional nutrient inputs would permit growth and possibly initiate red tide events.

There are several proposed sources from which *K. brevis* may acquire the nitrogen needed to initiate and sustain population growth in oligotrophic water columns. These include upwelling events, *Trichodesmium* blooms, and seepage from the sediment-water interface. Upwelled Loop Current water may provide nitrogen necessary to stimulate initiation events in the oligotrophic offshore regions of the West Florida shelf (Tester & Steidinger 1997). There were not, however, any associated biomass increments with upwelling penetrating to the 40 m isobath from 1958 to 1961 (Walsh & Steidinger 2001). Recent analysis of upwelled water does not indicate nitrate enrichment (Walsh et al. 2003). If *K. brevis* cells were deriving the majority of their nitrogen source from the upwelled loop current waters, they should have similar isotopic signatures. The Venezuelan slope waters, the source of upwelled loop current water, have $\Delta^{15}$NO$_3$ values of 6.7 to 8.3 ppt over depths of 200 – 500 m (Lui and Kaplan, 1989). *K. brevis* shows $\Delta^{15}$NO$_3$ values of only 3.6 to 5.1 ppt (Walsh et al. 2003). The discrepancy led to the hypothesis that the nitrogen brought in by upwelled water was diluted by “new” nitrogen from N-fixing *Trichodesmium* blooms (Walsh & Steidinger 2001). *Trichodesmium* has a $\Delta^{15}$PON signature -0.08 ppt (Minagawa and Wada, 1986) and could lower *K. brevis*’ $\Delta^{15}$N signature relative to surrounding waters.

*Trichodesmium* blooms do not always co-occur with *K. brevis* leaving the question of how N fixed by *Trichodesmium* is transferred to *K. brevis*.

Kamykowski (pers. comm.) sampled both surface and near bottom populations in a 22 m water column during a 2000 ECOHAB Cruise (Figure 1). The surface to bottom cell
distribution suggests another potential source of nitrogen for *K. brevis* at the sediment-water interface. Northeastern (Cable et al. 1996, Corbett et al. 1999) and southeastern (Fanning et al., 1987) areas of the Gulf of Mexico contain groundwater seeps that are transported up through the sediment, demonstrated by both geochemical tracers (\(^{222}\)Rn and \(^{226}\)Ra) and by direct measurement with seepage meters (Rutkowski et al. 1999). Seeps may deliver additional nutrients to near-bottom environments. Deposition from blooms of *Trichodesmium sp.*, or other phytoplankton also may provide both organic matter and nutrients to the sea floor. Better nutrient availability at or near the sediment-water interface in deeper waters, combined with algal adaptation to low light levels, may explain vertical distribution of benthic microalgae (Stevenson & Stoermer 1981, Sundback & Jonsson, 1989).

The vertical distribution of phytoplankton in a water column is limited by light. At a depth of 30 m on the oligotrophic West Florida Shelf, only about 10% of the photosynthetically active radiation (PAR) falling on the surface remains to support benthic microalgae (Walsh & Steidinger 2001). Previous studies have found that considerable microphytobenthic primary productivity occurs in 15 to 20 meters of water in coastal temperate areas (Bodin et al. 1985, Herndl et al. 1989). 30 to 80% of the micro-algal nitrogen requirement in 5-50 m coastal environments is met by seepage from the sediments (Nixon 1981, Blackburn & Henriksen 1983, Boynton & Kemp 1985). *K. brevis* may exist further offshore in regions where it can reach near-sediment nutrients by vertical migration, and benthic microalgae cannot compete because of light limitation.

The ability of *K. brevis* to migrate vertically within the water column at \(\sim 1 \text{ m hr}^{-1}\) (McKay et al. in press) combined with its physiological adaptations may extend *K. brevis*’
near-bottom niche beyond that of other benthic microalgae that use sediments as a nutrient source. *K. brevis* maintains growth under low light conditions (Wilson 1955, Aldrich 1960) and shows a hyperbolic relationship between growth and irradiance over 1.5% to 10% of full sunlight (Shanley 1985). Vertical migration may allow *K. brevis* to persist in near-bottom strata of deeper water columns yet still access higher light intensities similar to those found in shallow water columns closer to shore. A near-bottom offshore niche may allow *K. brevis* to exploit deposition from *Trichodesmium*, or other cyanophyte-primed phytoplankton blooms, with limited competition for organic or inorganic N.

*K. brevis* is adapted for growth in low nutrient environments with low half saturation constants (high affinities) for nitrate (Steidinger et al 1998), and can utilize both inorganic and organic nutrients (Wilson, 1966, Baden & Mende 1979, Vargo and Howard-Shamblott, 1990, Shimizu et al. 1995). Understanding responses of uptake to the different environmental exposures encountered over a diel vertical migration is critical to understanding how *K. brevis* persists in oligotrophic water columns and uses the sediment as a nutrient source. Concentration of nitrate increases with depth in oligotrophic water columns (MacIsaac & Dugdale 1972) and therefore nitrate was the focus of this study.

The relationship between nitrate uptake and light is often adequately described by the Michaelis-Menten equation, \( V = \frac{V_{\text{max}}S}{K_s + S} \), where \( V \) is the rate of uptake, \( V_{\text{max}} \) is the maximal uptake, \( S \) is the substrate concentration and \( K_s \) is the half-saturation constant, or affinity constant (Eppley et al. 1969). Half saturation constants (\( K_l \)) for light dependent uptake for nitrate and ammonium ranged from 1 to 14% of surface light intensity (MacIsaac & Dugdale 1972). Carbon fixation is similarly related to light until some saturation / inhibition level (Platt & Gallegos 1980). The C fixed and stored in the form of carbohydrates
and/or lipids provides energy in the form of ATP and reducing equivalents NADPH that contribute to N assimilation (Turpin 1991). Therefore when a K. brevis population is transported into eutrophic near shore environments, a cell’s ability to take up nutrients may be a function of its vertical position in the water column and thus its exposure to irradiance below saturating levels.

In oligotrophic regions, however, nitrate uptake near the bottom may be greater than at the surface due to deficient nitrate in the upper layers of the water column and to introduction of small quantities of nitrate by seepage up through the sediment-water interface (MacIsaac & Dugdale 1972). Different responses to increased concentrations suggest that other factors control uptake as well. For example, temperature may influence internal biochemical and physiological state (Morel 1987, Clark & Flynn 2002) and result in changes in N-uptake affinity (Eppley 1972, Berges et al. 2002).

Diel periodicity of nitrogen uptake is of particular relevance when cells migrate up during the day to photosynthesize and down at night to access nutrients (Cullen & Horrigan, 1981, Cullen et al. 1985, Kamykowski 1998). Numerous studies have documented periodicity of nitrogen uptake and discussed its ecological relevance to primary production (Epply et al. 1971, Cochlan et al 1991). Nitrate uptake in the dark is generally lower than in light (Cochlan et al. 1991) but is thought to increase in response to N limitation, thereby damping diel periodicity of N uptake (Syrett 1981). If K. brevis utilizes nutrients seeping from the sediments in offshore environments, the diel periodicity of nitrogen uptake may be important in determining its growth rates and vertical distribution in the water column. Understanding uptake rates under different environmental conditions is critical for explaining how K. brevis uses different potential nutrient sources and thus how cells become distributed
vertically. The half saturation constant ($K_s$) is considered an index of a species’ potential competitive ability at low nutrient concentrations (Smayda, 1997). The previously reported $K_s$ value of 0.42 (Steidinger et al. 1998) suggests that cellular affinity may be particularly important to the adaptive success of *K. brevis* in oligotrophic environments, but how this physiological ability interacts with behavior has not been considered. This study examines how the physiology of *K. brevis* interacts with its vertical migration behavior as a nutrient acquisition mechanism in an oligotrophic water column. We used a mesocosm to simulate light and nutrient conditions, that might be encountered by an offshore, near-bottom population of *K. brevis*, similar to conditions observed on the 2000 ECOHAB cruise.
METHODS

Uptake of nitrate by *K. brevis* was examined over its diel vertical migration. We created a 294 L mesocosm, 1.5 m deep X 0.5 m diameter (Figure 2), to permit vertical migration. The mesocosm was filled with 290 L filtered Gulf Stream water (salinity = 35) and modified with L 1/20 medium with nitrate as the only nitrogen source. A 6 L batch culture of non-axenic *K. brevis* (strain Apalachicola), was also grown in a modified L 1/20 medium with nitrate as the only nitrogen source (Guillard 1993) and then added to the mesocosm. The culture was grown until near nitrate depletion. In order to simulate the higher nutrient environment presumably encountered by a near-bottom population, the mesocosm was then thermally stratified by holding the lower 0.5 m at 21.9 °C and the upper 1 m at 25.5 °C. A nutricline was created by slowly inoculating the stable lower layer (below the thermocline) with L 1/20 media. The nitrate in the mesocosm was monitored in the upper 2/3 of the water column until it was depleted to levels undetectable by either manual analysis of nitrate following Parsons (1984) and the Lachat Quikchem 8000 Continuum Series Autoanalyzer. This upper layer simulated low nutrient water columns found in the Gulf of Mexico. The bottom 1/3 of the mesocosm contained 10 µM nitrate simulating elevated nutrients near the sediment water interface. During the diurnal part of the light cycle, irradiances at the surface (~2 cm), middle (~90 cm) and bottom (~125 cm) depths were 350 µmol quanta m\(^{-2}\) sec\(^{-1}\), 125 µmol quanta m\(^{-2}\) sec\(^{-1}\) and < 60 µmol quanta m\(^{-2}\) sec\(^{-1}\) respectively. The culture was grown under these conditions with a 12:12 light : dark cycle for 1 week while nutrients were monitored daily and those below the nutricline were replenished as needed.
Nitrate uptake was examined throughout the mesocosm (at the 3 depths) during the day and at night. The three depths (surface, middle and bottom, serving as three treatments) were sampled at both 12:30 to estimate diurnal nitrate uptake and 23:00 to estimate nocturnal nitrate uptake. The 12:30 time was chosen to provide adequate exposure to light immediately prior to sampling, and the 23:00 time was chosen so cell division which generally occurs around 3:00 to 4:00 (Kamykowski, 1985) would not intervene between the 12:30 and 23:00 sampling. In order to help explain differences in nitrate uptake between the three depths, indicators of internal nitrogen reserves (intracellular nitrate pools, intracellular free amino acids, and total cellular N) were measured in subsamples taken at the same time.

To test for uptake of nitrate were placed into 50 ml flasks. Flasks from the surface (2 cm) and middle (90 cm) were inoculated with $^{15}$N-NO$_3$ in one of the following concentrations: 0 µM (control), 0.25 µM, 0.5 µM, 0.8 µM, 1.2 µM, 2 µM, 4.0µM, 8.0 µM, and 11.0 µM. The bottom samples, both at 12:30 and 23:00, were only inoculated with 11.0 µM $^{15}$N-NO$_3$. The final percentage of $^{15}$N-NO$_3$ was 10% of the total nitrate in the media. The flasks were then incubated under light and temperature conditions similar to those from which they came for two hours in a radial photosynthetron (Babin 1994).

Cell migration in the mesocosm changes cell distribution with time. Therefore subsequent depth samples at 23:00 are not the same populations of cells as those sampled at 12:30. In order to control for this behavioral component and more accurately contrast diurnal vs. nocturnal nitrate uptake, a subset from each diurnal treatment was trapped to prevent movement from conditions to which they were exposed during the day. These samples, referred to as migration control groups, were then inoculated along with the nocturnal treatments.
Triplicates of the 0.5 µM and 11.0 µM incubations were used to determine variability of the technique at high and low nitrate concentrations. Volume limitations, incubator size, and financial constraints did not permit more triplicates at other concentrations. Incubations were terminated by filtration onto ashed Whatman GF/C 12mm filters. Nocturnal filtering of samples was done under low intensity green light to minimize the photosynthetic response. NO₃ uptake cell⁻¹ hour⁻¹ was calculated by correcting for the final percentage ¹⁵N in the sample, dividing by the number of cells in the sample and dividing by the incubation time thus:

\[ V_{nitrate} = \frac{((%^{15}N_{sample} - %^{15}N_{control}) \times \mu g \text{ N}_{sample})}{(%^{15}N_{-NO₃} \times \text{ time} \times \text{ total cells})} \]

Nitrate uptake velocity (\( V_{nitrate} \), units = (pmol cell⁻¹ hr⁻¹) was corrected for total cellular N in each sample in order to more accurately compare uptake velocity between depths. Uptake rates were normalized to total cellular N by dividing \( V_{Nitrate} \) by total cellular N.

Cell counts for all samples were obtained from the Coulter Multisizer II Particle Analyzer Counter (Beckman-Coulter Inc., Miami, FL) adjusted to count particles between 13.3 µm and 30 µm in diameter. Cell counts permitted calculation on a per cell basis of nitrate uptake as well as intracellular nitrate, intracellular free amino acids, and total cellular N.

Filtrations for intracellular nitrate pool, intracellular free amino acids, and total cellular N used precombusted Whatman GF/C 25mm filters. Nitrate in the media prior to experiments was tracked using both manual techniques (Parsons 1984) and a Lachat Quikchem 8000 Continuum Series Autoanalyzer. Similarly, internal nitrate pools were estimated following the protocol evaluated by Thoresen (1982). Samples were filtered, washed with artificial seawater and frozen. Internal nitrate pools were extracted by passing
25 ml boiling DI water through filters. Filtrate was analyzed with the Lachat Quikchem 8000 Continuum Series Autoanalyzer (EPA method 353.2).

Free amino acid assays followed the protein extraction protocol of Sigma Diagnostics (procedure 5656) based on Peterson’s modification of the Lowry method (Diagnostics 1994). Fluorescence was measured at 390 nm excitation and 475 nm emission with a Turner fluorometer. Amino acid concentration was determined from a standard curve.

Total cellular N samples were processed on a Carlo Erba CHN analyzer. The volume filtered corresponded to the approximately 21µg N filter⁻¹ required as determined by prior sensitivity analysis. Nitrate uptake was evaluated using a mass spectrometer aligned with the Carlo Erba nutrient analyzer. According to clean O5N tracer techniques all incubation vials and storage vials were acid washed with 10% HCl.

Uptake coefficients were determined by non-linear regression based on the Michaelis-Menten equation using SAS systems software. Comparisons were done by one-way ANOVAs and two-way ANOVAs when testing for significance of responses and interactions between variables.
RESULTS

Day

*K. brevis* exhibited a strong surface aggregation with ~ 45,400 cells ml\(^{-1}\) in the upper
2 cm of the mesocosm at 12:30. Cell densities just above the nutricline were about 7,900
cells ml\(^{-1}\) while densities below the nutricline were about 15,200 cells ml\(^{-1}\) (Figure 3). Cell
diameter increased significantly with depth; surface cells averaged 19.14 µm, middle cells
averaged 20.14 µm, and bottom cells averaged 20.86 µm (Figure 4). In general, cells above
the nutricline were significantly smaller than cells below the nutricline (F = 13.9, p < 0.01).
Despite differences in cell diameter and external nitrate concentrations, intracellular nitrate
level did not vary significantly with depth, even though there appeared to be a decreasing
trend (surface 0.002 pg cell\(^{-1}\) µm\(^{-1}\), middle 0.001 pg cell\(^{-1}\) µm\(^{-1}\) and bottom 0.0005 pg cell\(^{-1}\)
µm\(^{-1}\) normalized to cell diameter) with depth (Figure 5). Intracellular free amino acids varied
with depth (Figure 6). Cells from the surface (0.012 pmol cell\(^{-1}\) µm\(^{-1}\)) and middle (0.013 pg
cell\(^{-1}\) µm\(^{-1}\)), both in the nutrient depleted layer, had significantly less free amino acid than
cells below the nutricline (0.018 pg cell\(^{-1}\) µm\(^{-1}\); F = 15.3, p < 0.02 and F= 17.0, p < 0.02
respectively). Total cellular nitrogen (Figure 7) was significantly greater in cells from the
middle (1.86 pmol cell\(^{-1}\) µm\(^{-1}\)) and bottom (1.66 pmol cell\(^{-1}\) µm\(^{-1}\)) than in those from the
surface (1.35 pmol cell\(^{-1}\) µm\(^{-1}\); F = 24.4, p < 0.01 and F= 14.9, p < 0.01 respectively). There
was no difference between cells in the middle (just above the nutricline), and those from
below the nutricline. Figure 8 summarizes the diurnal patterns observed in the mesocosm.

Uptake increased with nitrate concentration in both the surface and middle layers
(Figure 9). Comparisons of uptake in high substrate concentrations (11 µM with cells from
the surface and middle, and 21 µM with cells from the bottom) revealed that cells from both
the surface ($F = 389, p < 0.01$) and the middle ($F = 11.3, p = 0.02$) of the mesocosm took up nitrate significantly faster than cells at the bottom. At high nitrate concentrations in the top and middle layers (11 µM), cells had similar capacities and were capable of replenishing > 90% of their cellular N in one hour. Regressions based on the Michaelis-Menten equation yielded different estimates for $V_{\text{max}}$ (Table 1): 2.3 at the surface and 1.14 in the middle. The difference in these numbers may be due to the uptake of the surface cells not reaching an asymptote at the nitrate concentrations which would render calculating a true value impossible. Cells from below the nutricline were, by contrast, able to replenish > 30% of their total cellular N per hour.

Cells from the middle took up significantly more nitrate per hour than did cells from the surface when provided 0.5 µM nitrate additions ($F = 13.9, p = 0.02$). In 1 hour under these low nitrate concentrations, cells from the middle were able to take up ~15% of their total cellular N while cells from the surface only took up 7-8%. The $K_s$ values derived from the curves likewise indicated that the middle cells had greater affinity (3.5) than did surface cells (16.2) (Table 1).

Night

The sampling at 23:00 showed a distinct increase in cell concentration below the nutricline (Figure 3). The cell concentration at the surface was ~5,300 cells ml$^{-1}$, while that in the middle (just above the nutricline) was ~4200 cells ml$^{-1}$. The cell concentration at the bottom (below the nutricline) was ~18,200 cells ml$^{-1}$. Cell diameter (Figure 4) increased significantly with depth (Figure 4). Cells at the surface and middle of the mesocosm (19.56 µm and 19.94 µm) were significantly smaller on average than cells at the bottom (20.67 µm; $F = 19.6, p < 0.01$).
Patterns in vertical distribution of the intracellular nitrate pools differed markedly from those observed during the day (Figure 5). Cells in the nitrate-depleted media above the nutricline, both at the surface (0.005 pg cell\(^{-1}\) µm\(^{-1}\)) and in the middle (0.005 pg cell\(^{-1}\) µm\(^{-1}\)), had significantly greater intracellular nitrate pools than cells below the nutricline (0.001 pg cell\(^{-1}\) µm\(^{-1}\); \(F = 11.5, p < 0.03\) and \(F = 17.5, p < 0.01\) respectively). Levels of intracellular free amino acids (Figure 6) tended to increase with depth but the only significant difference was between cells at the surface (0.24 pg cell\(^{-1}\) µm\(^{-1}\)), which had significantly fewer free amino acids than cells at the bottom (0.35 pmol cell\(^{-1}\)) (\(F = 9.6, p < 0.04\)). The cells at the surface also had significantly less total cellular N (41 pmol cell\(^{-1}\)) (Figure 7) than did cells in the middle (52 pmol cell\(^{-1}\); \(F = 31.8, p < 0.01\)) or at the bottom (50 pmol cell\(^{-1}\); \(F = 6.4, p < 0.03\)). There was no difference in total intracellular N between cells in the middle and at the bottom of the mesocosm. Figure 10 summarizes the diurnal patterns observed in the mesocosm.

Nocturnal uptake of nitrate increased with nitrate concentration at a given depth but did not differ between the three depths in the 11 µM incubations (Figure 11). Cells at all levels were able to take up at least 17% of their total cellular N in one hour at 11 µM. The estimated \(V_{max}\) values for cells from the surface and middle are 0.24 and 0.28 respectively (Table 1). Similar values may be inferred for the bottom cells since uptake rates of all groups in the 11 µM additions were similar.

Cells from the middle took up significantly more nitrate in the 0.5 µM additions than did cells from the surface (\(F = 63.2, p < 0.01\)). Cells in the middle of the mesocosm were able to take up \(\sim 7\%\) of their intracellular N while surface cells were only able to take up \(\sim 3\%\) (Figure 11). There was no significant difference in the rate of nitrate uptake between
depths in the 11 µM incubations. The affinity of cells from the middle was greater than that of those from the surface (Ks = 1.43 µM and 4.62 µM respectively, Table 1).

The migration control groups from each depth (surface and middle), trapped during the day and incubated at night, did not show any apparent differences in maximal uptake rates when compared to the nocturnal samples (Figure12). These groups did however show lower Ks than cells at the surface at night (Table 1).

**Day vs. Night**

The vertical distribution of cells changed from 12:30 to 23:00 (Figure 3). The dense surface aggregation found during the day dispersed downward at night with highest concentration at 23:00 found below the nutricline. With this redistribution, the average cell size (Figure 4) in the surface layer increased significantly (F = 205, p < 0.01) and the average cell diameter in the middle and below the nutricline decreased (F = 18.4 p = 0.013; F = 10.8, p = 0.029). Intracellular nitrate levels (Figure 5) were greater at all depths at 23:00 than at 12:30 (F = 9.8, p < 0.01). Intracellular free amino acid content did not differ among cells at the three depths (Figure 6). Total cellular N (Figure 7) was, however, significantly greater at 23:00 than at 12:30 in cells located at surface (F = 102, p < 0.01) and middle (F = 30.0, p < 0.01) of the column (in the nitrate depleted water). The comparison of cells at the bottom between 12:30 and 23:00 showed no significant difference, again due to the high variability in the measurements.

Nitrate uptake was significantly greater at 12:30 than at 23:00 for all depths (F = 20.9, p << 0.01) (Figure 13). Estimates for Vmax indicate that cellular capacity to take up nitrate is 4 - 8 times greater in the day than at night (Table 1). Affinity for nitrate apparently is greater at night than during the day; nocturnal Ks values were lower by a factor of 2-3 (Table
1). The kinetics of nitrate uptake were not measured for the bottom population, so comparisons of $K_s$ values cannot be extended to that layer of the mesocosm.
DISCUSSION

The hypothesis that *K. brevis* uses a near-bottom nutrient source in stable and stratified low nutrient offshore waters depends on its behavioral and physiological adaptations. Cell dispersion from the sediments during the day, and aggregation near the sediments at night, observed on the ECOHAB 2000 cruise, suggest that *K. brevis* migrates up into an oligotrophic water column before returning to strata of higher nutrients at night. The diel vertical migration (DVM) of *K. brevis* as a nitrate acquisition mechanism may optimize both photosynthesis during the day and nitrate uptake at night.

The migration inferred from the field observation is supported by the strong diurnal aggregations at the surface of the mesocosm and the subsequent redistribution of cells at night into the nitrate-replete lower layer of the mesocosm. The patterns in cell densities also confirm the insignificant effect of the thermocline provided here on vertical migration behavior (Kamykowski 1981). Changes in average cell diameter also indicate cell movement. Day vs. night patterns suggest that larger, more replete cells either move into the surface layer (or remain in the surface layer) in anticipation of the next light cycle, while smaller cells descend, leading to lower average cell size at deeper depths at night.

The patterns in internal nitrogen suggest that vertical migration may be in response to the limiting resource. At 12:30, small cells with less total cellular N than those cells lower in the mesocosm aggregated at the surface rather than descending to increase cellular N. The enhanced exposure to light would increase carbohydrate stores that may subsequently enhance uptake of nitrate (Turpin 1981). Larger cells lower in the water column contained more total cellular nitrogen, by contrast, perhaps continuing to accumulate organic N in preparation of cell division at 03:00 to 04:00.
At night, cells throughout the water column exhibited an increase in internal N relative to levels during the day, consistent with accumulating N in preparation for cell division. There was a distinct increase in intracellular nitrate pools in cells in the nitrate depleted region of the water column. The increased levels of the intracellular nitrate most probably resulted from the decreased rate of conversion of inorganic nitrate to organic N in the absence of light. If these cells no longer possessed enough energy to convert nitrate into organic forms, they may begin upward migration in anticipation of the next light cycle in order to optimize exposure to light needed for subsequent N uptake.

The mechanism of diel vertical migration to both acquire nutrients and optimize photosynthesis is contingent upon sufficient residence time near the sediment to take up nutrients adequate to support growth. The time required to take up enough nutrients to support a given growth rate depends on nocturnal uptake rates. Cells in this experiment showed a 3-fold decrease in uptake from day to night in the surface and middle layers, and a 2-fold decrease in the bottom layers. Nitrate uptake rates typically decline at night in plankton assemblages (Cochlan et al. 1991) as well as within a species (Clark & Flynn 2002).

While the declining maximum uptake rates ($V_{max}$) of the surface and middle cells demonstrate decreased nocturnal uptake capacity, cells’ nitrate affinities increase at night (Table 1). The greatly enhanced affinities of the surface control group (that did not exchange cells with other layers before the nocturnal incubations) relative to those of freely-moving cells sampled at the surface suggest that cells that remained at the surface became increasingly N deficient throughout the day. The apparent increase in affinity at night in the middle layer may be due to more depleted surface cells dispersing deeper in the mesocosm.
At night, without energy from light, cells may increase the efficiency with which they take up nitrate in order to meet cellular demands.

While the affinities for nitrate found in the upper layer of the mesocosm are not consistent with values reported previously ($K_s = 0.42 \mu M$, Tester et al. 1997), they are consistent with those of other dinoflagellates (Table 2). The discrepancy may be due to different environmental conditions, different $K. brevis$ strains or to different techniques. The value ($K_s = 0.42 \mu M$) reported in Tester et al. (1997) was obtained using the Wilson strain and measuring the disappearance of nitrate from the media.

If nocturnal uptake responses provide sufficient N for growth and division, $K. brevis$ may minimize tradeoffs between accessing light during the day and nutrients at night with different migration strategies similar to those calculated in Sinclair (2005). Cell growth averages 0.3 divisions day$^{-1}$, ranging from 0.2 to 0.5 divisions day$^{-1}$ (Wilson 1966, Shanley & Vargo 1993, Sinclair, 2005). At these average growth rates (0.3 divisions day$^{-1}$), cells typically experience a minimum of 2 downward migrations, and may be exposed to the near sediment stratum, at least 2 times between cell divisions. Conservatively assuming that cells need to double intracellular N in order to divide, one may begin to piece together how long cells must reside at the sediment-water interface in order to acquire sufficient nutrients. Figure 13 is a cartoon of potential migration patterns in a 22 m water column based upon the nocturnal uptake capabilities observed in this study assuming that maximal light exposure occurs around 12:00. The model delineates the maximum and minimum amount of time cells must spend at the sediment water interface during the night, depending on whether high (solid line) or low (dotted line) nitrate concentrations are encountered. Cells at night in low ($\leq 0.5 \mu M$) nitrate concentrations take up the equivalent of $\sim 5\%$ of their total cellular N per
hour and must reside at the sediment water interface for at least 10 hours per night in order to double their total cellular N. Cells in an 11 µM nitrate environment take up the equivalent of 20% of their total cellular N per hour and would only have to spend 2.5 hours per night.

Assuming cells migrate up and down the same distance at a speed “s” of 1 m sec⁻¹, the 2 scenarios yield a vertical distance (d) traveled upward or downward over the diel period, calculated by

\[ d = (\text{Diel Period} - \text{Bottom Time}) \times s / 2 \]

Consequently, low near-bottom nitrate concentrations (≤ 0.5 µM) limit upward vertical migration to ~ 7 m, while high near-bottom nitrate levels (≥ 11 µM) permit vertical migration up to ~ 11 m from the bottom. Subtraction of this migration distance from the bottom depth yields z, or the distance from the surface. If nitrate concentration in near-bottom water is low, cells only reach a depth of 15 m. If nitrate concentration in near bottom water is higher, cells may ascend to within 11 m of the surface.

The potential difference in light exposure during migration can be approximated using Beer’s law: \( I(z) = I_o e^{-kz} \) where I is irradiance, k is the attenuation coefficient and z is depth from surface. The coefficient k is calculated by Riley’s equation:

\[ k = 0.04 + 0.0088\text{Chl} + 0.054\text{Chl}^{2/3} \]

where Chl is chlorophyll a in mg m⁻³. Factors such as CDOM and chlorophyll a change light attenuation and ultimately the quantity and quality of light reaching phytoplankton in deeper waters. In order to simulate a variety of water column conditions I chose three Chl a concentrations: 2 mg Chl a m⁻³ for less dense surface blooms, 4 mg Chl a m⁻³ for moderate surface blooms and 6 mg Chl a m⁻³ for high cell concentrations. These correspond to attenuation constants of 0.136, 0.2152, and 0.2728 respectively (Table 3). With Chl a
concentrations of 4 mg Chl a m\(^{-3}\), cultures exposed to low near-bottom nitrate concentrations encounter a maximum of 79 µmol quanta m\(^{-2}\) sec\(^{-1}\). Cells exposed to high near-bottom nitrate concentrations, by contrast, have a maximum exposure of 188 µmol quanta m\(^{-2}\) sec\(^{-1}\). While the 4 m difference between the apex of the different migrations does not seem great, the cells migrating up to within 11 m of the surface are exposed to light intensities significantly greater than 79 µmol quanta m\(^{-2}\) sec\(^{-1}\) for 8 hours. The fact that *K. brevis*’ growth has been found to saturate at light intensities of 125 µmol quanta m\(^{-2}\) sec\(^{-1}\) suggests that in more turbid waters (with light attenuation equivalent to waters with net values of 4 mg Chl a m\(^{-3}\)) vertical migration to areas of higher nutrients may significantly increase C fixation relative to that of cells that only migrate up to 15 m depths.

The theoretical migration strategies indicate that cells must anticipate daybreak in order to reach the apex of the migration apex when irradiance is greatest, probably around ~12:30. Kamykowski (1981) determined that theoretical migration strategies to maximize photosynthesis coincided with observed cell migration phases in waters of varying turbidity. Both the models and observed cell migration anticipated sunrise with cells often beginning upward migration just after midnight. Time spent at the lowest depths also tended to be less than 4 hours, consistent with the scenarios presented in this paper.

*K. brevis* increased its affinity for nitrate as light levels decreased. In nature, near-bottom cells that use sediments as a nutrient source in offshore and oligotrophic environments may derive less energy (needed for uptake of nitrate) from low light exposures. It may be more advantageous for *K. brevis*, therefore, to take up nitrate more efficiently in these near-bottom areas. As populations are transported near shore by upwelling events, however, light and nutrient levels may increase, favoring adaptations that enhance uptake.
capacity to maintain *K. brevis*’ competitive advantage until it reaches densities that permit the exclusion of other phytoplankton by allelochemically enhanced competition (Smayda, 1997).

Onshore transport of blooms may result from the different vertical cell distributions and thus different horizontal advection (Lui et al. 2001). *K. brevis* blooms often are associated with vertically well-mixed water columns, most likely due to a combination of wind mixing and onshore transport of water as a result of upwelling-favorable winds (Vargo, 2002). This upwelled water, originating midshelf, moves onshore and can form a thermal front seaward of the 10 m isobath (Lui et al. 2001, Reed 2002). Near-bottom populations of *K. brevis* would be entrained in this water mass and delivered unobserved to areas of accumulation, such as thermal or salinity fronts (Reed, 2000, Weisberg et al., 2001), commonly associated with surface expressions.

As cells move onshore to higher-nutrient environments the dependence of nitrate uptake on light may become increasingly important. The patterns observed in this study suggest that uptake rates of nitrate are consistent with both the vertical distribution of cellular N characteristics and the environmental variables of nitrate, light, and temperature. During the day, surface cells with lower levels of total cellular N had significantly higher nitrate uptake rates than bottom cells at the same ambient nitrate concentration. This greater capability is consistent with the increased uptake associated with a cell’s decreased intracellular N content (Flynn 1998, Morel 1987). Cells in the middle took up nitrate significantly faster than did cells at the bottom, suggesting a strong light dependence of nitrate uptake. Increased uptake with increasing light agrees with general theory (McIsaac & Dugdale, 1972).
Average net growth rates of cultures adapted to low light (0.3 divisions day\(^{-1}\); Wilson, 1966, Sinclair 2005), and growth rate saturation at 125 μmol quanta m\(^{-2}\) sec\(^{-1}\) (Shanley, 1985) indicate that \(K\). brevis populations can be adapted to irradiances much lower than those encountered at the surface. Cells may be adapting to low light levels in near-bottom populations, such as those observed on the ECOHAB 2000 cruise. Increased biomass (CDOM) associated with breakdown of vertical stratification may decrease light attenuation sufficiently to permit \(K\). brevis to reach its maximum nitrate uptake rate (where light \(\geq\) 18% surface irradiance). Higher uptake rates might permit \(K\). brevis to effectively compete with other phytoplankton that grow faster at higher irradiances. \(K\). brevis' uptake rates and adaptation to low light levels may permit existence both near the sediment in offshore and oligotrophic regions as well as near shore in higher light and nutrient environments.

The behavior and physiology of \(K\). brevis permit growth and persistence in both, offshore oligotrophic environments, and nearshore higher-nutrient environments. Offshore near-bottom populations are able to minimize the biological tradeoff presented by resources separated in time and space by migrating up to access light during the day and migrating down to take up nitrate at night. The nocturnal increase in cell affinity may contribute to \(K\). brevis ability to take up nutrients at night. As \(K\). brevis populations move onshore to higher-nutrient environments, they are able to increase nitrate uptake capacity in response to increased light levels during the day. This increased capacity may contribute to increased growth rates. Increased growth rates, along with physical concentration mechanisms, can lead to cell densities that, through allelochemical interspecific competition, become monospecific \(K\). brevis aggregations. Better understanding of \(K\). brevis' physiology, and how it relates to behavior, is critical to understanding how cells grow and persist in different
environments. This understanding, in turn, will contribute to better prediction of how population aggregations of *K. brevis* are formed.
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Table 1. Vmax and Ks values predicted from nonlinear regression fits using the Michaelis-Menten equation.

<table>
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<th>$V_{\text{max}}$</th>
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Table 2. Kₘ values for dinoflagellates and diatoms. Dinoflagellates tend to have higher Kₘ values than diatoms. Modified version of table presented in Smayda (1997).

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<td><em>Alexandrium tamarense</em></td>
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<td>Tomas &amp; Dodson 1974</td>
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<tr>
<td><em>Peridinium cinctum</em></td>
<td>29</td>
<td>Sherr et al. 1982</td>
</tr>
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<td>Diatoms</td>
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<tr>
<td><em>Asterionellopsis glacialis</em></td>
<td>0.7-1.3</td>
<td>Eppley et al. 1969</td>
</tr>
<tr>
<td><em>Asterionellopsis glacialis</em></td>
<td>0.9-1.1</td>
<td>Romeo &amp; Fisher 1982</td>
</tr>
<tr>
<td><em>Chaetoceros gracilis</em></td>
<td>0.1-0.3</td>
<td>Eppley et al. 1969</td>
</tr>
<tr>
<td><em>Coscinodiscus lineatus</em></td>
<td>2.4-2.8</td>
<td>Eppley et al. 1969</td>
</tr>
<tr>
<td><em>Coscinodiscus wailesii</em></td>
<td>2.1-5.1</td>
<td>Eppley et al. 1969</td>
</tr>
<tr>
<td><em>Ditylum brightwellii</em></td>
<td>2</td>
<td>Eppley &amp; Coatsworth 1968</td>
</tr>
<tr>
<td><em>Ditylum brightwellii</em></td>
<td>0.6</td>
<td>Eppley et al. 1969</td>
</tr>
<tr>
<td><em>Fragilaria pinnata</em></td>
<td>0.6-1.6</td>
<td>Carpenter &amp; Guillard 1971</td>
</tr>
<tr>
<td><em>Leptocylindrus danicus</em></td>
<td>1.2-1.3</td>
<td>Eppley et al. 1969</td>
</tr>
<tr>
<td><em>Rhizosolenia stolterfothii</em></td>
<td>1.7</td>
<td>Eppley et al. 1969</td>
</tr>
<tr>
<td><em>Skeletonema costatum</em></td>
<td>0.4-0.5</td>
<td>Eppley et al. 1969</td>
</tr>
<tr>
<td><em>Skeletonema costatum</em></td>
<td>2.1</td>
<td>Romeo &amp; Fisher 1982</td>
</tr>
<tr>
<td><em>Thalassiosira oceanica</em></td>
<td>0.3-0.7</td>
<td>Eppley et al. 1969</td>
</tr>
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<td><em>Thalassiosira oceanica</em></td>
<td>0.5-1.0</td>
<td>Eppley &amp; Renger 1974</td>
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<td><em>Thalassiorira pseudonana</em></td>
<td>1.87</td>
<td>Carpenter &amp; Guillard 1971</td>
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</tbody>
</table>
Table 3. Light attenuation at different depths achieved by cells under the migration scenario in Figure 13 assuming a surface irradiance of 2000 µmol quanta m\(^{-2}\) sec\(^{-1}\). Different hypothetical Chl a values resulted in different attenuation (k) values in the water column.

<table>
<thead>
<tr>
<th>Bottom nitrate (µM)</th>
<th>Chl a (mg m(^{-3}))</th>
<th>z depth (m)</th>
<th>k</th>
<th>l(z)</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>2</td>
<td>11</td>
<td>0.1376</td>
<td>440</td>
</tr>
<tr>
<td>0.5</td>
<td>2</td>
<td>15</td>
<td>0.1376</td>
<td>254</td>
</tr>
<tr>
<td>11</td>
<td>4</td>
<td>11</td>
<td><strong>0.2152</strong></td>
<td>188</td>
</tr>
<tr>
<td><strong>0.5</strong></td>
<td><strong>4</strong></td>
<td><strong>15</strong></td>
<td><strong>0.2152</strong></td>
<td><strong>79.27</strong></td>
</tr>
<tr>
<td>11</td>
<td>6</td>
<td>11</td>
<td>0.2728</td>
<td>100</td>
</tr>
<tr>
<td>0.5</td>
<td>6</td>
<td>15</td>
<td>0.2728</td>
<td>33</td>
</tr>
</tbody>
</table>
Figure 1. Observations of near-bottom cell aggregations made on the ECOHAB 2000 cruise (Kamykowski pers comm.). Depth (m) is plotted against sampling that began at 00:00 and was done every 2 hours for a 24 hour period.
Figure 2. Schematic of the 294 L mesocosm. Samples were pulled from the Surface, Middle, and Bottom at 12:30 and 23:00. The thermal stratification permitted a nitrate depleted surface layer and a nitrate rich bottom layer.
Figure 3. Vertical cell distribution in the mesocosm. The surface aggregation (2 cm) and the middle aggregation (90 cm) were both in the nitrate depleted upper layer. The bottom aggregation (125 cm) was in the nitrate replete lower layer.
Figure 4. Patterns of cell diameter with depth. The surface aggregation (2 cm) and the middle aggregation (90 cm) were both in the nitrate depleted upper layer. The bottom aggregation (125 cm) was in the nitrate replete lower layer.
Figure 5. Patterns of intracellular nitrate versus depth. The surface aggregation (2cm) and the middle aggregation (90 cm) were both in the nitrate depleted upper layer. The bottom aggregation (125 cm) was in the nitrate replete lower layer.
Figure 6. Patterns of intracellular free amino acids as function of time and depth in the mesocosm. The surface aggregation (2cm) and the middle aggregation (90 cm) were both in the nitrate depleted upper layer. The bottom aggregation (125 cm) was in the nitrate replete lower layer.
Figure 7. Patterns of total cellular N as a function to time and depth in the mesocosm. The surface aggregation (2 cm) and the middle aggregation (90 cm) were both in the nitrate depleted upper layer. The bottom aggregation (125 cm) was in the nitrate replete lower layer.
<table>
<thead>
<tr>
<th>Cell #</th>
<th>Diameter (µM)</th>
<th>iNO₃ (pmol cell⁻¹)</th>
<th>FAA (pmol cell⁻¹)</th>
<th>TN (µM)</th>
<th>Vmax (hr⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>45,400</td>
<td>19.14</td>
<td>0.002</td>
<td>0.012</td>
<td>1.35</td>
<td>0.94</td>
</tr>
<tr>
<td>± 1086</td>
<td>± 0.05</td>
<td>± 0.0009</td>
<td>± 0.003</td>
<td>± 0.19</td>
<td>± 0.04</td>
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<td>7,900</td>
<td>20.14</td>
<td>0.001</td>
<td>0.013</td>
<td>1.86</td>
<td>0.83</td>
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<tr>
<td>± 240</td>
<td>± 0.05</td>
<td>± 0.001</td>
<td>± 0.002</td>
<td>± 0.36</td>
<td>± 0.24</td>
</tr>
<tr>
<td>14,524</td>
<td>20.86</td>
<td>0.0005</td>
<td>0.018</td>
<td>1.66</td>
<td>0.36</td>
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<tr>
<td>± 221</td>
<td>± 0.05</td>
<td>± 0.0003</td>
<td>± 0.001</td>
<td>± 0.08</td>
<td>± 0.02</td>
</tr>
</tbody>
</table>

Figure 8. Summary of diurnal patterns in mesocosm: population distribution, cell size, intracellular nitrate (iNO₃), intracellular free amino acids (FAA), total cellular N (TN), uptake rate (V_max).
Figure 9. Diurnal nitrate uptake normalized to cell N as a function of nitrate concentration and depth and light level at 12:30.
<table>
<thead>
<tr>
<th>Cell #</th>
<th>Diameter (µM)</th>
<th>iNO$_3$ (pmol cell$^{-1}$)</th>
<th>FAA (pmol cell$^{-1}$)</th>
<th>TN</th>
<th>V$_{max}$ (hr$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5,300</td>
<td>19.56 ± 0.02</td>
<td>0.005 ± 0.002</td>
<td>0.012 ± 0.003</td>
<td>2.1</td>
<td>0.18 ± 0.07</td>
</tr>
<tr>
<td>4,200</td>
<td>19.94 ± 0.07</td>
<td>0.005 ± 0.002</td>
<td>0.016 ± 0.005</td>
<td>2.64</td>
<td>0.22 ± 0.03</td>
</tr>
<tr>
<td>18,200</td>
<td>20.67 ± 0.08</td>
<td>0.001 ± 0.0001</td>
<td>0.017 ± 0.001</td>
<td>2.4</td>
<td>0.18 ± 0.03</td>
</tr>
</tbody>
</table>

Figure 10. Summary of nocturnal patterns in mesocosm: population distribution, cell size, intracellular nitrate (iNO$_3$), intracellular free amino acids (FAA), total cellular N (TN), uptake rate (V$_{max}$).
Figure 11. Nocturnal nitrate uptake normalized to cell N as a function of nitrate concentration and depth at 23:00.
Figure 12. Nocturnal uptake Surface and Middle samples and their controls. Samples were drawn out of mesocosm at night while controls were trapped during the day, held at constant light off, and incubated at 23:00.
Figure 13. Diurnal vs Nocturnal uptake from all depths, surface (2cm), middle (90 cm) and bottom (125 cm) as a function nitrate concentration, light level and time of day.
Figure 14. Theoretical migration scenarios. Physiological differences may enable behavioral plasticity. Uptake rates are equivalent to nocturnal uptake rates found in mesocosm in different nitrate concentrations. The black is representative of what was observed in the ECOHAB 2000 cruise. The color lines are potential changes in vertical migration (arrows) and bottom time (boxes) based on bottom nutrient field. Green represents cell migration to high nitrate concentrations (11 µM), which correspond to shorter bottom time and increased proximity and time exposed to light. Red represents cells migrating to low nitrate concentrations (0.5 µM), corresponding to longer bottom time and decreased time exposed to light. Calculations based on surface irradiance of 2000 µmol quanta m^{-2} sec^{-1} and light attenuation values (k = 0.2125).