

ABSTRACT

SINCLAIR, GEOFFREY ALLAN. Physical and Chemical Constraints on the Near-Bottom Ecology of *Karenia brevis*. (Under the direction of Daniel Kamykowski.)

In situ observations of *Karenia brevis* near the sediment-water interface suggests cells derive nutrients from the sediment in oligotrophic water columns. Persistence of near-bottom populations depends on 1) the diel utilization of nutrients, 2) cells interaction with the sediment-water interface, and 3) the effect increased turbulence on cell behavior and physiology. The diurnal uptake of ammonium, nitrate, and urea was similar though *K. brevis* assimilated ammonium and urea faster than nitrate during the day. The nocturnal uptake of ammonium was greater than either urea or nitrate but the nocturnal assimilation of ammonium and urea were similarly greater than nitrate.

Laboratory videos suggested that *K. brevis* migrates into porewaters where elevated nutrients exist. *K. brevis*' ability to migrate into the sediment was tested with chambers divided by a 100 μm mesh overlain with sediment. While the sediment affects the progress of descending cells, migration occurs through thin layers of sediment and increases in response to elevated nutrient concentrations below the sediment. Since all cells found below the sediment had significantly higher C/N ratios than those remaining above the sediment, migration appears related to a cell's internal biochemical state.

Populations of *K. brevis* in proximity to the sediment-water interface may be exposed to periods of elevated turbulence near-bottom. Cells may either, 1) reside above the sediment-water interface and utilize effluxing nutrients *during* shear exposure or, 2) swim through elevated shear rates to access the sediment porewaters to utilize nutrients *after* shear exposure. *K. brevis* is sensitive to shear with both ammonium and nitrate

assimilation decreasing during shear exposure. Cells recover however after exposure to shear. The uptake and assimilation of both ammonium and nitrate was higher in cells after exposure to shear than during exposure to shear. Patterns in uptake and assimilation, during and after shear exposure, suggest that recovery is attributable to both morphological and physiological processes. The behavior and physiology of *K. brevis* near the sediment-water interface alleviates bottom-up controls and promotes population persistence in oligotrophic water columns.

Physical and Chemical Constraints on the Near-Bottom
Ecology of *Karenia brevis*

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DEDICATION

I dedicate this dissertation to my family. My parents, 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 dissertation 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 also dedicate this dissertation to Kristen's family, Andy, Eric and Jill, for accepting me so well into their family. And finally, to Denver, our first and best dog, who made our time in Raleigh NC so special.

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 in 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 fishermen 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. At North Carolina State University, Geoff continued his study of how organisms persist in unlikely places. Geoff and Kristen now live happily in a quiet Cary neighborhood with their 8 month old puppy, Taasi. They are looking forward to their next adventure in the Mississippi delta and starting new careers at the Louisiana Marine Consortium.

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CHAPTER 1. RESEARCH OVERVIEW

The harmful dinoflagellate, *Karenia brevis* (Davis) G. Hansen & Moestrup, 2000, (formerly *Ptychodiscus brevis* (Davis) Steidinger, 1979 and *Gymnodinium breve* Davis), has been studied intensively (Wilson 1966, Vargo & Howard-Shamblott 1990, Steidinger et al. 1998, Van Dolah & Leighfield 1999, Liu et al. 2001a, Walsh et al. 2003a, Janowitz & Kamykowski 2005) due to the socio-economic impacts that blooms have, almost annually, on the west coast of Florida. From January to October 2006, for example Florida red tide killed thousands of fish and numerous manatees and caused human respiratory distress that depressed the local tourist trade. Cell densities greater than 1×10^5 cells l^{-1} near shore are responsible for these problems and often appear suddenly, faster than can be explained by growth rates alone (Tester & Steidinger 1997, Stumpf et al. 2008a). It is therefore unknown where these populations originate. One hypothesis suggests that populations originate offshore in oligotrophic water columns (Tester & Steidinger 1997, Vargo et al. 2008). This hypothesis does not explain, however, why these populations are not detected by satellites (Stumpf et al. 2003) and other monitoring techniques (G. Kirkpatrick, personal communication) or how *K. brevis* acquires the nutrients needed to grow in these oligotrophic water columns (Vargo et al. 2008). Observations of near-bottom populations of *K. brevis* may explain both (Sinclair et al. 2006a). Near-bottom populations may derive nutrients from the sediment either by anticipating the flux of nutrients out of the sediment (Marinelli et al. 1998b) or migrating into the surficial porewaters where higher concentrations likely exist (Jahnke et al. 2005). These populations, if located below the first optical depth, would not be detected by

satellites (Schofield et al. 1999). Models that incorporate the migration behavior of *K. brevis* and therefore allow cells to exist offshore below the first optical depth, have examined population transport in bottom Ekman layers and subsequent accumulation of cells at frontal features and replicate observations of *in situ* aggregation patterns (Liu et al. 2001b, Janowitz & Kamykowski 2006). Determining how the behavior and physiology of *Karenia brevis* interact with the physical and chemical environment is critical to understanding if populations can persist in these near-bottom environments and the mechanisms that promote or constrain this near-bottom niche.

The west Florida shelf (WSF) is typically oligotrophic with nitrogen and phosphorus concentrations in the water column commonly about 0.1 to 0.2 μM within 2 to 4 km of shore in the Gulf of Mexico (Dragovich et al. 1961, Steidinger et al. 1998) and have been observed to be less $<0.01 \mu\text{M}$ for NO_3^- and NH_4^+ (Masserini & Fanning 2000). Nutrient depauperate water columns can lead to transport limitation and reduced growth in phytoplankton (Pasciak & Gavis 1974, Flynn et al. 2002). Identification of potential nutrient sources is important to determine how *K. brevis* persists in oligotrophic water columns since nitrogen is suspected to be one limiting factor to the growth of offshore populations of *K. brevis* (Hecky & Kilham 1988, Vargo et al. 2008).

Potential nutrient sources for *K. brevis* in oligotrophic water columns that occur offshore are spatially and temporally variable and include aerial deposition (Vargo et al. 2008), N-fixation by *Trichodesmium* sp. blooms (Walsh & Steidinger 2001, Mulholland et al. 2006), water column regeneration from grazers (Lester et al. 2008), near-bottom regeneration by diatoms responding to upwelling events (Vargo et al. 2008), and

regenerated nutrients derived from Mississippi River water (Stumpf et al. 2008b). Despite inputs of new nitrogen into the system, nitrate concentrations in both the surface and bottom waters at the 25 m and 50 m isobath are often at or below 0.1 μM and are not able to sustain populations of *K. brevis* (Vargo et al. 2008). Populations of *K. brevis* in close proximity to the sediment were observed during an ECOHAB cruise in fall 2000 by Dr. Kamykowski and colleagues (Figure 1.1). These observations suggest that cells also use nutrients associated with the sediment, whether the nutrients flux out of the sediment or remain in the interstitial porewaters. The diel vertical migration behavior of *K. brevis* may extend the distribution of these near-bottom populations further offshore (Sinclair & Kamykowski 2006), allowing populations to exist below the first optical depth undetected by satellites over broad areas of the WFS (Schofield et al. 1999).

Vertical migration is prominent among the cellular adaptations that *K. brevis* may use to acquire nutrients in oligotrophic water columns (Sinclair et al. 2006b). When increased water-column stratification with decreased large-scale mixing contributes to declines in surface nutrient concentrations, dinoflagellates are thought to dominate (Margalef 1997a; Smayda & Reynolds 2001). The diel vertical migration (DVM) of dinoflagellates to a nutricline confers a competitive advantage over non-motile phytoplankton when surface nutrients are low (Eppley et al. 1968, Cullen & Horrigan 1981, Cullen 1982, Watanabe 1991).

Traditionally, DVM is modeled as ascent during the day to support photosynthetically driven carbon fixation and descent at night to access subsurface nitrogen, phosphorus and assorted trace elements at night to support further biochemical

synthesis (Kamykowski 1974, Heaney & Eppley 1981, Cullen 1985, Smayda & Reynolds 2001). Migration, may however, be controlled by a variety factors including phototaxis/geotaxis (Kamykowski et al. 1998), cellular periodicity (Eppley et al. 1968), or metabolic control (Kamykowski & Yamazaki 1997) which result in migration patterns that differ from simple diurnal ascent and nocturnal descent (Sournia 1974, Ralston et al. 2007). When changes in environmental conditions occur, models that demonstrate migration patterns may also shift within a limited physiological parameter base and have significant implications for the ecology of the organism (Ji & Franks 2007).

If the DVM of *K. brevis* allows cells to maximize exposure to light during the day before returning to higher nutrient concentrations such as the sediment-water interface at night, how migration behavior is coupled to a physiological ability to assimilate nitrogen at night may be critical to cell persistence in an oligotrophic water column. The nocturnal uptake of nitrogen has been found to increase in importance with depth in the water column. At the surface and 30% surface irradiance, nocturnal uptake was 15% and 30% diurnal uptake for nitrate and ammonium, respectively (Cochlan et al. 1991b). This periodicity was not observed in lower light environments like the bottom of the euphotic zone (Cochlan et al. 1991a), where *K. brevis* has been hypothesized to occur. Nocturnal uptake has also been found to become more important as water column nitrogen concentrations decrease (Probyn et al. 1996, Flynn & Fasham 2002) which may be particularly relevant to the oligotrophic regions in the Gulf of Mexico where *K. brevis* is thought to originate (Tester & Steidinger 1997). The behavior and nutrient physiology of

K. brevis in proximity to the sediment may be critical to a populations persistence in an oligotrophic water column.

K. brevis swims about 1 m hr^{-1} (McKay et al. 2007), similar to other dinoflagellates with swimming speeds that range from 0.5 to 2.2 m hr^{-1} (Kamykowski & McCollum 1986). For cells that swim approximately 1 m hr^{-1} , if the sub-surface nutrient source is below 12 m , dinoflagellates are unable to reach the surface during a 12 h ascent before descending. An alternate pattern may be expressed as a response (layering or DVM) to a deep thermocline/nutricline or a sub-thermocline nutricline near the bottom of the euphotic zone in regions of steeply sloping shelves such as those of southern California (Kamykowski 1973; Cullen 1982; Eppley *et al.* 1984). Similar subsurface dinoflagellate aggregations were reported by Nakamura *et al.* (1996) in the Seto Inland Sea, by Pitcher *et al.* (1998) off the west coast of South Africa, and by Townsend *et al.* (2005) in the Gulf of Maine.

Alternatively, on broad gently sloping continental margins like the Gulf of Mexico, the sediment may substitute for more traditional subsurface nutrient sources associated with pycnoclines. Harmful dinoflagellates such as *Alexandrium fundyense* are associated with sediments as cysts that are mixed into the water column as viable cells during storm events (Anderson et al. 2005). In contrast to other *A. fundyense*, there have not been any observations of *K. brevis* cysts in the field. Preservable cysts are produced by relatively few living dinoflagellate species with only six of eighteen orders into which dinoflagellates were classified were known to have a fossil history (Goodman 1987). *K. brevis* may occur near the sediment-water interface primarily as slowly growing

vegetative cells that may substitute for the cyst stage in other dinoflagellates. The area near the sediment presents a complex environment characterized by low light environments, increased current shear near boundaries, and a potential sediment barrier that prevents cells from accessing sediment bound nutrients. The challenge that these environmental factors present to viable, motile *K. brevis* cells have not been previously considered and may be critical to the ecology of the observed near-bottom populations of *K. brevis*.

The sediment characteristics in the regions where near-bottom populations of *K. brevis* have been observed is largely sand/gravel (Twichell et al. 2003), but the nutritive value remain largely unexplored. Both inorganic (Marinelli et al. 1998a, Jahnke et al. 2000) and organic N (Burdige & Martens 1990, Keil & Fogel 2001) have been associated with sandy sediment and result from remineralization of organic matter that is deposited on the sea floor (Rowe et al. 1975, Berg et al. 2003, Darrow et al. 2003). Porewater nutrients often are spatially variable with no clear patterns between the 20 and 40 m isobaths on other continental shelves (Marinelli et al. 1998b) making generalizations about distribution difficult. Porewater nutrient concentrations also vary seasonally in both quantity and quality due to regeneration rates which can be 3-4 times greater during the summer than in winter (Jahnke et al. 2005). Dissolved organic matter in the sediments tends to have higher N content than DOM in the water column and may potentially elevate the levels of bioavailable N in the sediment (Berg et al. 2003) in aerobic conditions (Henriksen & Kemp 1988). In the south Atlantic Bight, ratios of the rates of regeneration for both carbon and nitrogen (C:N) were calculated to be lower (7.2

+/- 2.7) in the summer (>23°C) and higher (11.1 +/- 6.2) in the winter (Jahnke et al. 2005), thus potentially increasing the relative supply of N in the summer when near-bottom populations of *K. brevis* are hypothesized to occur. Shoreward of the 20 m isobath, the sediment on the west Florida shelf also receives nutrient inputs from seepage of groundwater (Cable et al. 1996) and may be enriched in nutrients from anthropogenic land-based sources (Hu et al. 2006).

The across-shelf availability of nutrients from the sediments to *K. brevis* also may be dictated by competition from benthic microalgae when the sediments are in the euphotic zone (Tyler et al. 2003, Porter et al. 2004). One niche where free-swimming *K. brevis* populations may exploit a sediment-derived nutrient source is at water depths beyond the distribution of the microphytobenthos (Cahoon 1999). Alternatively, in the presence of the microphytobenthos, a second opportunity for nocturnal uptake derives from comparisons of both day and night diffusive fluxes of nutrients, which indicate that the uptake of nutrients by benthic microalgae contains a significant light-driven component (Carlton & Wetzel 1988, Sundback et al. 1991, Sundback et al. 2004). Nutrient diffusion/efflux out of the sediment depends on sediment porosity (Curry et al. 2004), permeability (Aller 1983, Meadows & Tait 1989) and ambient water currents (Huettel & Rusch 2000). The development of elevated nutrients in near-bottom waters may result from complex interaction between porewater advection and microbial remineralization and water column dilution. Large patterns in bottom topography such as ripples observed by (Gelfenbaum & Brooks 2003) on the WFS as well as biogenic

influences (benthic invertebrates (Mermillod-Blondin et al. 2005) may also influence the flux of nutrients out of the sediment.

The physical characteristics of the sediment may influence how a cell penetrates the surface layer. When nutrients are absent above the sediment, cells may be able to penetrate into porewater at the sediment surface to access elevated nutrient concentrations by swimming into interstitial spaces similar to benthic dinoflagellates in sandy sediments (Faust 1995). Estimation of interstitial pore space based on the estimated spherical diameter (ESD) of different sieved fractions of sediment, indicated pores widths may range from 73 μm for the 177 μm sieved fraction to 331 μm for the 840 μm size fraction (Hartkamp et al. 1993). The size of *K. brevis* cells range from 17 to 22 μm (personal observation) are significantly smaller than the estimated pore spaces that cells may encounter and suggest that there is potential for *K. brevis* cells to migrate into the sediment porewaters to access higher nutrient concentrations. In coastal environments, similar to those where *K. brevis* is found, the diffusive boundary layers are about 0.12 mm (Santschi et al. 1991). Simple models of the diffusive flux of both nitrate and ammonium out of the sediment and the low nutrient concentrations in close proximity to the sediment (20 μm) suggest that cells must at least interact with the sediment and may derive significant benefit from migrating into the surficial porewaters (Figure 1.2).

Porewater nutrients may be advected to the upper most layers of the sediment during porewater advection (and upwelling) and vary depending on sediment topography (Huettel et al. 1996). Even under elevated current velocities of 10 cm sec⁻¹, which is

high for the WFS (He & Weisberg 2002a), the upwelling water velocities in the sediment would not inhibit the migration of cells into the sediment since they would be less than $2 \mu\text{m sec}^{-1}$ (Huettel & Gust 1992) which is negligible compared to the swimming velocities of *K. brevis* (McKay et al. 2007). While porewater advection may transport nutrients at rates 2-100 times greater than diffusion alone, rates of regeneration rather than advection have been suggested to control surface sediment nutrient concentrations (Jahnke et al. 2005). Penetration of non-motile water-column microalgae into permeable sediment ($k \sim 10^{-11}$) is highest in the upper 10 mm of sediment and decays exponentially with depth (Huettel & Rusch 2000, Shimeta et al. 2003) which would expose cells to significantly greater nutrient concentrations than the overlying water.

Cells in close proximity to the sediment-water interface may also experience periods of elevated turbulence since turbulence tends to increase toward the upper (air) and lower (sediment) boundaries (Sanford 1997). Dinoflagellates have been hypothesized to be very sensitive to small scale turbulence (Peters & Marrase 2000, Stoecker et al. 2006) but responses appear to be species-specific without any clear trends relating to taxonomy or morphology (Berdalet et al. 2007). The persistence of near-bottom populations of *K. brevis* depends on how vertically migrating cells interact with increasing turbulence and associated shear that is encountered toward the sediment boundary (Sanford 1997, Perlin et al. 2005). Since some dinoflagellates are able to sense and respond to elevated levels of shear stress (Maldonado & Latz 2007) understanding how turbulence impacts cell physiology and behavior may be critical to understanding the near-bottom ecology of *K. brevis*.

Turbulence directly influences phytoplankton ecology by influencing growth rates (Gibson & Thomas 1995, Juhl et al. 2000, Sullivan & Swift 2003) and increasing the mass transfer of nutrients (Pasciak & Gavis 1975). Influences of turbulence on photosynthesis (Janowitz & Kamykowski 1991) and swimming trajectories (Yamazaki & Kamykowski 1991) of phytoplankton have been explored in the upper mixed layer of water columns. Yet few studies consider the influences of turbulence on phytoplankton near the bottom of water columns. The fact that responses to turbulence vary differently with growth conditions and physiological state and may particularly be pertinent to populations that grow more slowly near the bottom of coastal water columns like *K. brevis* (Juhl et al. 2000).

K. brevis may encounter different levels of shear depending on whether cells maintain position above the sediment-water interface to anticipate fluxes of nutrients out of the sediment or migrate into the surficial sediment porewaters. The former scenario may expose cells for longer durations to elevated shear rates relative to the overlying water column. Cells that migrate into the sediment, by contrast, may experience a transitory increase in shear rates before reducing exposure to shear by taking refuge in the sediment pore waters with significantly reduced water velocities (Huettel & Rusch 2000). Organism responses like nocturnal nitrogen uptake rates and swimming capabilities in benthic boundary layer may be particularly important to the ecology of near-bottom aggregations of *K. brevis*.

The west coast of Florida has a broad and gently sloping continental shelf with the 100 m isobath situated between 150 and 200 km offshore. Because of the gentle slope,

near bottom light levels at the 30 m isobath are often 10% of surface irradiance that may support benthic microalgae or near-bottom populations of *K. brevis* (Walsh et al. 2003b). Nutrient rich water, associated with the loop current seldom penetrates much beyond the shelf break near the 80 m isobath (Steidinger et al. 1998, He & Weisberg 2003). Waters landward of the shelf break are largely independent of the loop current and driven primarily by winds (He & Weisberg 2003). The near-bottom Eckman layer is therefore responsible for the transport of “ecologically important materials” across the shelf (Weisberg et al. 2001a, Weisberg 2003). Currents on the west Florida shelf (WFS) are also driven by semi-diurnal tides (unlike the rest of the Gulf) with the semi-diurnal constituents increasing in the onshore direction and the diurnal constituents being more uniform across the shelf (He & Weisberg 2002b). Generally the tidal currents are weak, on the order of a few cm sec^{-1} , but the semi-major axis may reach 10 cm sec^{-1} , and may vary geographically. Bottom friction velocities (u_*) range from 0.24 cm sec^{-1} at the 100 m isobath to greater than 0.48 cm sec^{-1} close to shore in non-storm conditions (He & Weisberg 2002b). While levels of near-bottom turbulence may not be as great as the surface wind-mixed layer, the increase near the bottom relative to the turbulence observed in the middle of the water column (Sanford 1997) may be particularly relevant to the diel vertical migration of near-bottom populations of *K. brevis*.

Sediment-associated nutrients may serve as a substitute for the traditional subsurface nitricline and may play an integral role in the ecology of near-bottom aggregations of dinoflagellates. The interaction of *K. brevis* with the sediment may be constrained by cell behavior in proximity to the sediments, cell physiology and ability to

take up nutrients at night when they are in proximity to the sediment, and how cells respond to increasing shear rates they may encounter as they approach the sediment-water interface. In this work I examine three questions critical to elucidate potential mechanisms by which populations of *K. brevis* may persist near the sediment-water interface:

- 1) How does *Karenia brevis* interact with the sediment-water interface at night?
- 2) How does *K. brevis* utilize different forms of nitrogen associated with the sediment?
- 3) How does the near-bottom hydrography influence cell behavior and physiology near the sediment-water interface?

Broader understanding of the physiological and behavior adaptations of *K. brevis* and how they promote or constrain the near-bottom niche will directly contribute to predicting the temporal and spatial distribution of near-bottom populations and how these populations are linked to bloom development on the WFS. The persistence of slowly growing vegetative cells near the sediment-water interface may provide seed populations that are transported shoreward, undetected by satellites (Stumpf et al. 2003), by bottom Eckman transport (Weisberg et al. 2001b, Weisberg 2003).

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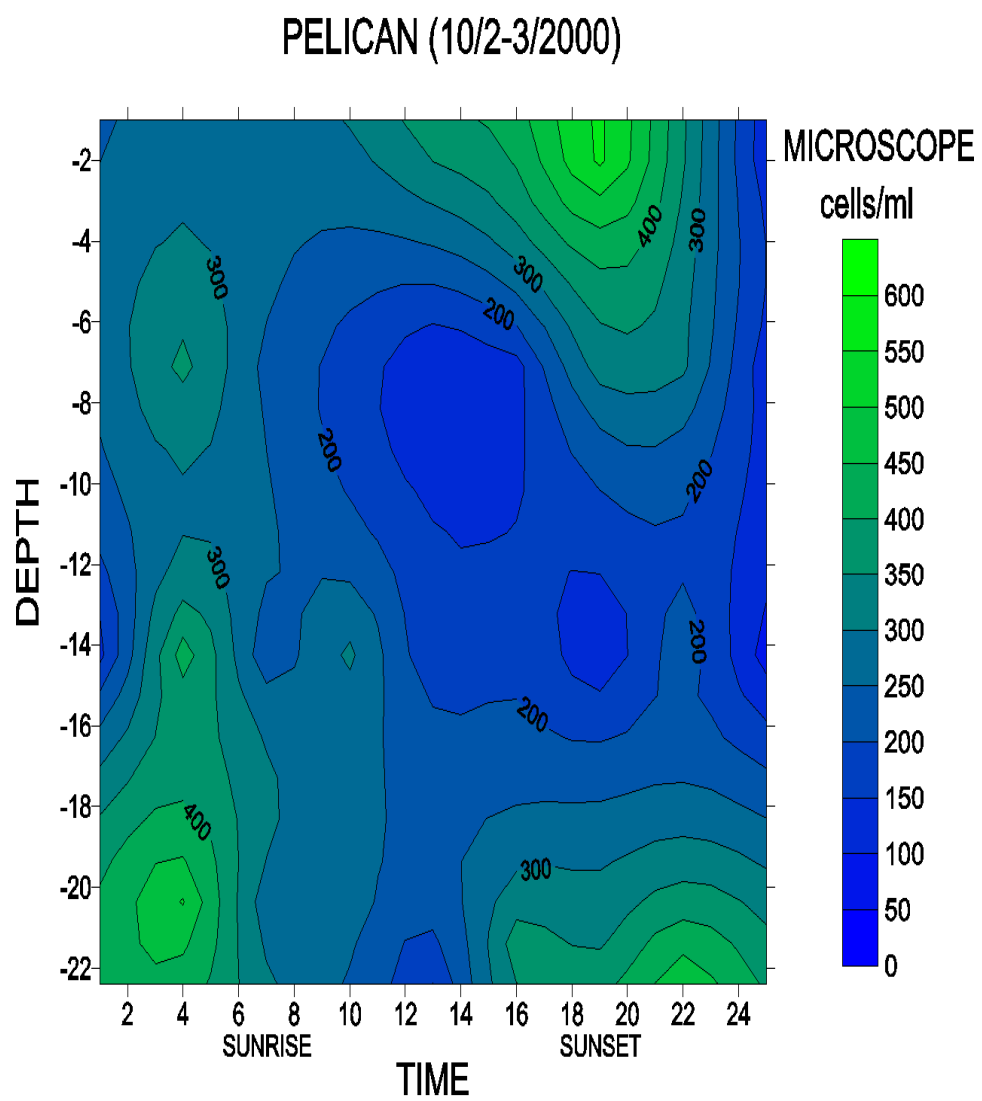


Figure 1.1 Near-bottom aggregations of *K. brevis* observed by Kamykowski *et al.* 2000

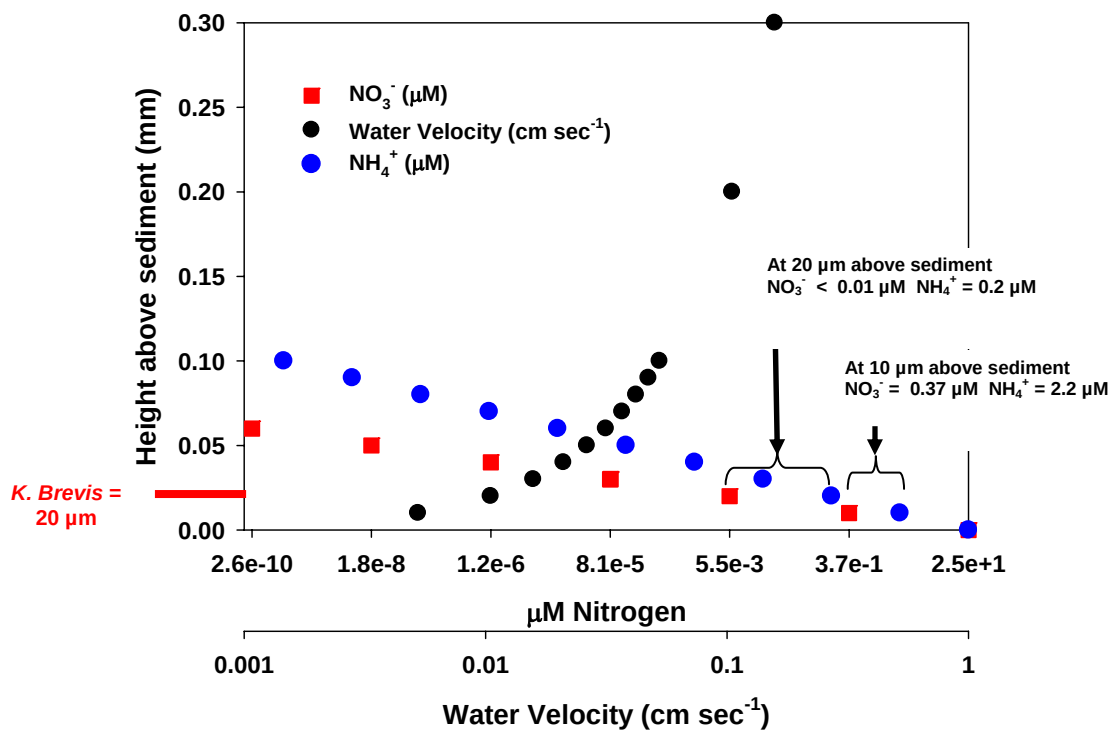


Figure 1.2 Model of the nutrient concentration for ammonium and nitrate in the diffusive boundary layer of coastal environments.

**CHAPTER 2. GROWTH, UPTAKE, AND ASSIMILATION OF AMMONIUM,
NITRATE, AND UREA, BY THREE STRAINS OF *KARENIA BREVIS***

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2.1 ABSTRACT

Observations of near-bottom populations of *Karenia brevis* suggest that cells derive nutrients from the sediment-water interface. Cells undergoing a metabolic mediated migration may be in close proximity to enhanced concentrations of nutrients associated with the sediment during the day or at night. The growth and diel uptake and assimilation rates *K. brevis* were examined on three nitrogen substrates, ammonium, nitrate, and urea, that are encountered near the sediment in order to better understand the near-bottom ecology of *K. brevis*. Three strains of *K. brevis*, C6, C3, and CCMP229, were grown under 12:12 light dark cycle under $30 \mu\text{mol quanta m}^{-2} \text{sec}^{-1}$ delivered to the surface plain of the culture in order to simulate the near-bottom environment. Nitrogen uptake was evaluated using ^{15}N tracer techniques and TCA extraction was used to evaluate the quantity of N assimilated into cell protein. Growth rates ranged from 0.12 divisions day^{-1} for C6 and C3 grown on nitrate to 0.18 divisions day^{-1} for C3 grown on urea. During the day maximum uptake rates, ρ_{max} , varied from a low of 0.57 $\text{pmol N cell}^{-1} \text{hr}^{-1}$ for CCMP229 grown on nitrate, to a high of 1.66 $\text{pmol N cell}^{-1} \text{hr}^{-1}$ for CCMP229 grown on urea. At night, ρ_{max} was higher for ammonium and urea, ranging from 0.41 $\text{pmol N cell}^{-1} \text{hr}^{-1}$ to 1.36 $\text{pmol N cell}^{-1} \text{hr}^{-1}$, than for nitrate, which ranged from 0.16 $\text{pmol N cell}^{-1} \text{hr}^{-1}$ to 0.38 $\text{pmol N cell}^{-1} \text{hr}^{-1}$. Diel rates nitrate uptake suggest that nitrate does not support populations that persist near the sediment. Only the C6 strain exhibited nocturnal uptake rates sufficient to support observed growth rates on high concentrations of ammonium suggesting that if cells are accessing sediment nutrients, concentrations must be elevated to meet a significant portion of their nutritional demand.

2.2 INTRODUCTION

Populations of *K. brevis* are thought to initiate offshore in low nutrient waters (Tester & Steidinger 1997, Vargo et al. 2008). Concentrations of nitrogen and phosphorus in the water column rarely exceed 0.5 μM and are commonly 0.1 to 0.2 μM within 2 to 4 km of shore in the Gulf of Mexico (Dragovich et al. 1961, Steidinger et al. 1998) and have been observed to be less <0.01 μM for NO_3^- and NH_4^+ (Masserini & Fanning 2000). Potential nutrient sources for *K. brevis* in oligotrophic water columns that occur offshore are spatially and temporally variable and include aerial deposition, N-fixation by *Trichodesmium* sp. blooms (Walsh & Steidinger 2001, Mulholland et al. 2006), water column regeneration from grazers (Lester et al. 2008), near-bottom regeneration by diatoms responding to upwelling events (Vargo et al. 2008), grazing on *Synechococcus* (Glibert et al. *in press*), and regenerated nutrients derived from Mississippi River water (Stumpf et al. 2008). Despite inputs of new nitrogen into the system, nitrate concentrations in both the surface and bottom waters at the 25 m and 50 m isobath are often at or below 0.1 μM (Vargo et al. 2008). Populations of *K. brevis* associated with the sediment (Figure 2.1) suggest that cells may also use nutrients that are remineralized within the sediment.

Mathematical models of the horizontal transport of near-bottom populations simulated the observed aggregation patterns of *K. brevis* and predicted that near-bottom nutrient sources contribute to the maintenance of cell concentrations in frontal regions near-shore (Janowitz & Kamykowski 2006). Once on-shore, *K. brevis* may encounter elevated nutrient concentrations such as from outwelling from estuaries (Vargo et al.

2008), elevated groundwater discharge (Hu et al. 2006), or decaying fish killed by bloom events (Walsh et al. 2006). Due to the multiple nutritional sources that are potentially available to *K. brevis* populations (Glibert et al. *in press*), models attempting to predict bloom transport must integrate biological processes, such as organism physiology, over a wide range of light and nutrient environments, with physical transport processes (Liu et al. 2001a, Janowitz & Kamykowski 2006, Sinclair & Kamykowski 2006). Unfortunately, data concerning the physiological processes of *K. brevis* critical to the growth of both cells and populations in different environments that they are likely to encounter across the west Florida shelf (WFS) are limited (Vargo et al. 2008). Previous biophysical models have primarily relied on how cells take up and grow on oxidized forms of nitrogen (Liu et al. 2001b). *K. brevis* may, however, dynamically regulate its nitrogen physiology and elevate nocturnal rates of nitrate uptake following diurnal exposure to growth saturating levels of light (Sinclair et al. 2006a) or may use nocturnal descent to enhance exposure to nutrients (Sinclair et al. 2006b). Offshore populations that occur near the sediment-water interface may be exposed to higher concentrations of both reduced and organic nutrients (Jahnke et al. 2005, Sinclair & Kamykowski 2008). The broad distribution of *K. brevis*, both within the water column and across the shelf suggests that the nitrogen physiology of *K. brevis* must be understood in a wide variety of environments especially since nitrogen is suspected to be one limiting factor to the growth of offshore populations of *K. brevis* (Hecky & Kilham 1988, Vargo et al. 2008).

Recent field surveys (2005 and 2006) found significantly higher concentrations of nitrate, ammonium, and urea in the surficial sediment compared to both surface waters as

well as water samples taken 50 cm above the sediment (Sinclair & Kamykowski 2008). The low nutrient concentrations found in these surveys were consistent with other surveys of bottom waters from 1998 to 2001 from 10 to 50 m isobath (Vargo et al. 2008). While cell aggregation near the sediment enhances exposure to nutrients in oligotrophic water columns, high concentrations of benthic microalgae may compete for these nutrients during the day (Sundback et al. 2004). The decreased uptake of nutrients by benthic microalgae at night often results in increased nutrient fluxes at night (Carlton & Wetzel 1988). *K. brevis* may compete for sediment-associated nutrients by coupling nocturnal descent with the nocturnal uptake of nutrients. Additionally, *K. brevis* may utilize its ability to vertically ascend in order to enhance light exposure and thus persist further offshore than benthic microalgae (Sinclair & Kamykowski 2006). Thus information concerning both the diurnal and nocturnal uptake rates of *K. brevis* may be relevant depending on whether subsurface populations are initiated in shallower water columns where they may compete with benthic microalgae by taking up nutrients at night, or are initiated in deeper water columns further offshore, beyond the distribution of benthic microalgae which take up sediment-associated nutrients during the day. This work focuses on the near-bottom light environments similar to those encountered by near-bottom populations that occur in deeper water columns offshore but may be applicable to near-shore environments when blooms decrease the attenuation of light at the sediment-water interface and nocturnal uptake is critical to sustain near-bottom populations.

The growing number of isolates of *K. brevis* in culture afford the opportunity to obtain a more representative picture of the growth and uptake capabilities of *K. brevis*. The wide distribution of these isolates, from the coast of Texas to the east coast of Florida, provides an opportunity to evaluate the comparative physiology of different *K. brevis* strains, as different strains of many algae have been shown to exhibit different physiological responses when exposed to similar conditions (Wood et al. 1992, Burkholder & Glibert 2006). In this paper we examine the uptake capabilities of three different strains of *K. brevis* in response to three substrates: ammonium, nitrate, and urea. We hypothesize that cells grown under low light similar to near-bottom aggregations differentially take up and assimilate the different forms of nitrogen. Specifically, the reduced and organic forms of nitrogen may play a critical role in the ecology of these near-bottom populations.

2.3 MATERIALS AND METHODS

The three isolates selected for this study are from different geographic regions. The C6 strain is in closest geographical proximity to the observations of near-bottom populations off of Panama City, FL. The CCMP229 strain was isolated from the central Florida coast, near Manasota, in the same geographic region where nearshore blooms of *K. brevis* have been shown to persist for extended periods of time and also may be a region where near-bottom populations persist (Milroy et al. 2008). The C3 strain was isolated from the east coast of Florida near Jacksonville. The C6 and C3 strains were obtained from the Florida Fish and Wildlife Research Institute while the CCMP229 strain was obtained from Mote Marine Laboratory. Triplicate 1 L batch cultures, of each strain of *Karenia brevis* were grown in L/20 media (Guillard & Hargraves 1993) from which copper and silica were eliminated and modified so that either ammonium, nitrate, or urea was the only nitrogen source. The seawater (salinity = 35) was collected from the Gulf Stream, passed through a 0.2 μm filter, and autoclaved. Each culture was provided with an initial concentration of 20 μM N and 5 μM N was added variably to maintain the initial concentration until cell densities in the cultures permitted experimental manipulation. The cultures were then allowed to deplete the N before the experiments were initiated.

Each strain of *K. brevis* was grown on each nitrogen substrate (ammonium, nitrate, or urea) for 2 generations (~2 weeks each) so that strains were acclimated to the same nitrogen substrate that was used for the uptake experiments. 1 L batch cultures were grown under a 12:12 light/dark cycle with lights on at 06:30 EST and off at 18:30

EST. During the day each culture was grown under light exposures of approximately 30 $\mu\text{mol quanta m}^{-2} \text{ sec}^{-1}$ at the surface plane of the culture and at 22°C for the duration of the experiments.

Changes in cell numbers over time were monitored by counting cells from triplicate batches with a Coulter Multisizer II Particle Analyzer Counter (Beckman-Coulter Inc., Miami, FL) with threshold settings of 13.3 and 30 μm for cell diameter. Since *K. brevis* is not a sphere, cell volumes (μm^3) were approximated using the volume of a cylinder so that the thickness of the cell was dependent upon the ESD using the proportionality constant 0.25 (i. e. $V=0.25*(\text{ESD})^2*\pi*(\text{ESD})$). Net growth rates (cell divisions day^{-1}) of triplicates of each strain on each substrate were determined from the slope of a regression plotting $\ln(N)$ (cells ml^{-1}) vs time (days). Since cells of different strains were different sizes, growth rate was normalized to cell volume. The ammonium and urea in the media was tracked daily following protocols of Holmes et al. (1999) and Mulvenna & Savidge (1992). Nitrate was monitored with an ISUS nitrate analyzer calibrated against Lachat QuikChem 8000 Continuum Series Autoanalyzer.

Upon depletion of the nitrogen in the media, five 60 ml samples were drawn at 12:30 and 23:00 for the uptake and assimilation experiments. The substrate in which the strains were grown corresponded with the ^{15}N -labeled substrate that was evaluated. Thus, strains that were grown on ammonium were inoculated with ^{15}N -ammonium, strains grown on urea were inoculated ^{15}N -urea, and strains that were grown on nitrate were inoculated with ^{15}N -nitrate. The ^{15}N was diluted with ^{14}N to obtain a constant 20% enrichment so that the final percentage of ^{15}N in the media was 20%. The cultures were

inoculated with N concentrations of 0 $\mu\text{mol-N L}^{-1}$ (control), 0.5 $\mu\text{mol-N L}^{-1}$, 1 $\mu\text{mol-N L}^{-1}$, 10 $\mu\text{mol-N L}^{-1}$ and 50 $\mu\text{mol-N L}^{-1}$ for nitrate and ammonium and 0 (control), 0.5 $\mu\text{mol-N L}^{-1}$, 1 $\mu\text{mol-N L}^{-1}$, 10 $\mu\text{mol-N L}^{-1}$ and 80 $\mu\text{mol-N L}^{-1}$ for urea. After 30 minute incubations, the samples were divided and 30 ml of each treatment was filtered onto two precombusted Whatman GF/F 25mm filters. Both filters were washed with isotonic artificial seawater and one filter was immediately frozen at -20°C , while the second filter was treated with 10% ice-cold trichloroacetic acid (TCA) and then frozen. The first filter represented the uptake of nitrogen into the whole cell, while TCA-extracted filter represented only the ^{15}N -(ammonium, nitrate, urea) that was assimilated into proteinaceous material (Lomas & Glibert 1999). Samples for particulate carbon and nitrogen were filtered onto precombusted GF/F filters and dried at 50°C over night and analyzed on a Control Equipment CHN Elemental Analyzer and converted to cellular quotas by dividing by the cell density within the sample (Glibert et al. 1991).

Nitrogen-specific uptake rates of ^{15}N -labeled substrates (V hr^{-1}) were calculated following methods of (Dugdale & Goering 1967).

$$\text{V}(\text{hr}^{-1}) = \frac{^{15}\text{N atom}\% \text{ excess}}{^{15}\text{N atom}\% \text{ enrichment} \times \text{time}}$$

$^{15}\text{N} \%$ excess was the ^{15}N atom % of the particulate fraction minus the ^{15}N atom % normal while ^{15}N atom % enrichment was the initial enrichment of N substrate in the medium. $\text{V}(\text{hr}^{-1})$ was then multiplied by the concentration of particulate nitrogen in the sample and divided by the number of cells in the sample to obtain ρ in $\text{pmol N cell}^{-1} \text{ hr}^{-1}$. Kinetic parameters, ρ_{max} ($\text{pmol N cell}^{-1} \text{ hr}^{-1}$) and k_s ($\mu\text{M N L}^{-1}$), for the three substrates

were determined by curve-fits using the Michaelis-Menten equation (Berges et al. 1994). Kinetic parameters for the individual strains are reported with standard errors generated from the curve fitting model. When uptake did not saturate for the strain comparisons and therefore did not have a fit, the values that were generated are represented in italics. In order to get a representative picture of the three substrates for use in subsequent modeling efforts, the curves for each strain were combined to generate an average Michaelis-Menten equation so that $n = 3$ for each substrate except for nitrate where $n = 2$ (no nitrate data were available for strain C6). Statistical comparisons between groups used one-way ANOVAs followed by a pairwise comparison using t-tests to determine differences between individual groups.

The assimilation efficiency of each substrate for each strain was calculated by dividing the rates of uptake of nitrogen into proteinaceous material ($V_{\text{assimilation}} \text{ (hr}^{-1}\text{)}$) by the rates of uptake of nitrogen into the whole cells ($V_{\text{uptake}} \text{ (hr}^{-1}\text{)}$). Specific uptake rates ($V \text{ hr}^{-1}$) were also compared to specific growth rates ($\mu \text{ hr}^{-1}$) (e.g. Wood et al. 2005).

2.4 RESULTS

Cell Size

There were greater differences in cell size between strains than within strains grown on the different nitrogen substrates. Cell volume of the C3 strain was 18 % larger than the C6 strain ($t = 6.9$, $p = 0.03$) and 20 % larger than the CCMP229 strain (Figure 2.2; $t = 6.2$, $p = 0.02$). When grown on ammonium, the cell volume of C3 was 16% larger than CCMP229 ($t = 3.3$, $p = 0.02$) while the cell volume of C3 was 20 % larger than both CCMP229 ($t = 7.3$, $p < 0.001$) and C6 ($t = 6.9$, $p < 0.001$) when grown on urea. Intra-strain differences in cell volume was only observed in the C3 ($df = 8$, $F = 5.9$, $p = 0.04$) which was 18 % larger when grown on ammonium than when grown on nitrate ($t = 3.4$, $p = 0.01$).

Growth Rates

While there were no inter-specific differences, there were intra-specific differences in growth rates on the different substrates (Table 1.1). Within the strains, the growth of C6 on the three substrates was significantly different ($df = 8$, $F = 33$, $p < 0.001$). On nitrate, C6 grew 20 % slower than it did on urea ($t = 7.8$, $p < 0.01$) and ammonium ($t = 5.8$, $p < 0.01$). C3 seemed to grow faster on urea than ammonium (28%) and nitrate (34%) but these differences were not statistically significant. There were no trends detected in the growth of CCMP229 when grown on the different substrates.

Diurnal Uptake Rates

Michaelis-Menten models of the uptake curves provided kinetic parameters and standard errors for the three strains. While there was considerable scatter in the raw data,

analysis of the kinetic parameters revealed differences between strains for the rates of diurnal uptake as well as the ability of the cells to assimilate the different substrates (Table 2.2, Figure 2.3). No differences were observed among the half saturation constants (k_s) in either strain comparisons or substrate comparisons. There were, however, significant differences in comparisons of the different $\rho_{\max}$ for each substrate within strains. The $\rho_{\max\text{-urea}}$ for CCMP229 was 52% higher than the $\rho_{\max\text{-ammonium}}$ ($t = 6.8$, $p < 0.01$) and 69% higher than the $\rho_{\max\text{-nitrate}}$ ($t = 9.0$, $p < 0.01$). The C6 strain, in contrast, had a 48% higher $\rho_{\max\text{-ammonium}}$ than $\rho_{\max\text{-nitrate}}$ ($t = 4.2$, $p = 0.01$). There were also no differences in the maximum transport into cell ($\rho_{\max}$) within the strains comparing the three substrates. Differences in $\rho_{\max}$ between the three strains were also observed for both the uptake of urea (Figure 2.3 c; $df = 8$, $F = 46.3$, $p < 0.01$) and nitrate (Figure 2.3 e; $df = 8$, $F = 17.4$, $p = 0.003$). For the CCMP229 strain, the $\rho_{\max\text{-urea}}$ was 60 % higher than the C6 strain ($t = 9.1$, $p < 0.01$) and 46% higher than the C3 strain ($t = 7.2$, $p < 0.01$). For the C3 strain, the $\rho_{\max\text{-nitrate}}$ was 56% higher than CCMP229 ($t = 4.6$, $p = 0.003$).

During the day, the assimilation efficiency for the nitrogen substrates was different within the strains but not between the strains. Due to lack of replication, the assimilation efficiencies over all concentration ranges were used to compare the different substrates within a strain. The CCMP229 strain had a 20 % lower assimilation efficiency for nitrate than for ammonium, and 43% lower assimilation efficiency of nitrate than for urea (Figure 2.3 b, d, f; $df = 11$, $F = 11.2$, $p = 0.005$). The assimilation efficiency of nitrate for the C3 strain was 39 % lower than the assimilation efficiency for urea ($t = 3.1$, $p = 0.02$). When the assimilation efficiencies of the strains were averaged together for a

more general comparison of substrate assimilation efficiency, urea was found to be assimilated 64 % more efficiently than nitrate across all substrate concentrations (Figure 2.3 d,f; $t = 3.7$, $p < 0.01$).

Nocturnal Uptake Rates

There were intra-strain differences observed in the nocturnal uptake rates of the three substrates primarily due to the decreased rate of uptake of nitrate at night (Table 2.2, Figure 2.4). The CCMP229 strain had a 70 % higher $\rho_{\text{max-urea}}$ than $\rho_{\text{max-nitrate}}$ (Table 2.2; $t = 3.0$, $p = 0.02$). The C3 strain had a 75 % higher $\rho_{\text{max-ammonium}}$ than $\rho_{\text{max-nitrate}}$ ($t = 4.3$, $p < 0.01$). The C6 strain had the highest nocturnal uptake rates for ammonium which had a 25 % higher $\rho_{\text{max-ammonium}}$ than $\rho_{\text{max-urea}}$ (Table 2.2). The only inter-strain difference in the nocturnal uptake of nitrogen was observed in the $\rho_{\text{max-ammonium}}$ (Figure 2.4a; $df = 8$, $F = 9.5$, $p = 0.01$). The $\rho_{\text{max-ammonium}}$ for the CCMP229 strain was 65% less than either the C6 strain ($t = 3.8$, $p = 0.01$) or the C3 strain ($t = 3.8$, $p = 0.01$). The inter-strain differences in $\rho_{\text{max-urea}}$ and $\rho_{\text{max-nitrate}}$ were not significantly different (Figure 2.4c,e).

When the ρ_{max} of each strain was averaged together to compare nocturnal uptake rates of the different substrates, significant differences were observed (Table 2.3, Figure 2.5c; $F = 23$, $df = 8$, $p = 0.02$). The nocturnal $\rho_{\text{max-ammonium}}$ was 42 % higher than $\rho_{\text{max-urea}}$ ($t = 4.0$, $p < 0.01$) and 70 % higher than $\rho_{\text{max-nitrate}}$ (Figure 2.5c; $t = 6.7$, $p < 0.01$). The nocturnal $\rho_{\text{max-urea}}$ was 49 % higher than $\rho_{\text{max-nitrate}}$ ($t = 2.7$, $p = 0.03$). In general, the assimilation efficiency varied between 35 % for nitrate to 81 % for urea but few significant differences were observed (Figure 2.4b,d,f). The only difference in the ability

to assimilate nitrogen at night was observed in the C6 strain which had a urea assimilation efficiency that was 35 % higher than ammonium ($t = 3.3$, $df = 6$, $p = 0.02$).

Diurnal Uptake Rates vs. Nocturnal Uptake Rates

Significant differences in the diel uptake rates of all substrates were observed when comparing $\rho_{\max}$ within the strains (Table 2.2). When the $\rho_{\max}$ of the strains were averaged for a given substrate, in order to more robustly compare substrates, the only difference observed was nitrate. The CCMP229 strain demonstrated a diel difference in maximum uptake rates ($\rho_{\max}$) with the $\rho_{\max}$ -ammonium 46 % higher during the day than the $\rho_{\max}$ -ammonium at night ($t = 7.3$, $df = 4$, $p < 0.01$). Similarly, the CCMP229 strain had a 70 % higher $\rho_{\max}$ -urea during the day than at night while the C3 strain had a 30 % higher $\rho_{\max}$ -urea during the day than at night ($t = 4.6$, $df = 4$, $p = 0.01$). The CCMP229 strain had a 70 % higher $\rho_{\max}$ -nitrate during the day than at night ($t = 4.4$, $df = 4$, $p = 0.01$) which was the same as the C3 strain which also had a 70 % higher $\rho_{\max}$ -nitrate during the day ($t = 6.3$, $df = 4$, $p = 0.01$). There was no difference between the diurnal and nocturnal $\rho_{\max}$ for any substrate for the C6 strain. When the $\rho_{\max}$ for the strains were averaged, the only diel difference in $\rho_{\max}$ was observed in nitrate which was 70 % higher during the day than at night ($t = 3.4$, $df = 4$, $p = .03$). There were no observed differences in the diel assimilation efficiencies between substrates.

2.5 DISCUSSION

The historical persistency of *K. brevis* in oligotrophic water columns (Walsh et al. 2006) suggests that *K. brevis* may have multiple mechanisms by which to meet nutrient requirements (Glibert et al. *in press*). The observation of populations near the sediment-water interface (Sinclair et al. 2006a, Milroy et al. 2008) as well as laboratory studies (Sinclair & Kamykowski 2008b) suggest that *K. brevis* may derive nutrients from the sediment. The forms and concentrations of nitrogen encountered by populations of *K. brevis* associated with the sediment-water interface are highly variable in space and time (Jahnke et al. 2005). Inputs of nutrients, like ammonium and urea, to the sediment may include inputs of organic matter from the water column (Huettel et al. 2007, Stumpf et al. 2008), or sediment regeneration (Jahnke et al. 2005). Groundwater inputs may increase nitrate concentrations in porewaters (Hu et al. 2006) and supplement nitrate delivered to bottom-waters by upwelling events (He & Weisberg 2003). While urea often constitutes less than 1% of DON pool, it occasionally accounts for up to 20% of the DON pool and up to 50 % of the N used in coastal environments (Harrison et al. 1985, Glibert et al. 1991, Fan et al. 2003, Glibert et al. 2006, Solomon & Glibert 2008) and may play an important role in alleviating nitrogen limitation of near-bottom populations of *K. brevis*. Ammonium, drawn up to the sediment-water interface from more anoxic layers by porewater pumping downstream of topographic features in the sediment (Precht & Huettel 2004) may also be available to cells descending into the surface porewaters. Recycling and remineralization by benthic flora and fauna may contribute to retention of nitrogen within the sediment (Veuger et al. 2007) and provide a variety of nitrogen

substrates with significantly higher concentrations than those of the overlying water column (Sinclair & Kamykowski 2008).

The migration behavior and ability of *K. brevis* to adapt to low light environments (Shanley & Vargo 1993), may extend the distribution of near-bottom populations to the 60 m isobath where water columns are clear and commonly exhibit attenuation constants of about 0.08 (personal observation; Sinclair & Kamykowski 2006). In this study, *K. brevis* was cultured under light levels that simulated a 60 m water column, with an attenuation constant (k) of 0.07, where near-bottom aggregations are hypothesized to originate. Under these light levels, the growth rates of all the strains on the chosen substrates (ammonium, nitrate and urea) varied between 1 division every 6 to 10 days. These growth rates are significantly lower than the commonly cited 1 division every 3 days (Wilson 1966, Shanley & Vargo 1993). The decrease in growth rates at lower light levels results in lower metabolic demands (Geider et al. 1998) that would allow *K. brevis* to persist near the sediment-water interface for extended periods of time. The only distinct difference between the growth rates of the strains under these low light conditions was on ammonium. The C3 strain, isolated on the east coast of Florida, grew about 30 percent slower on ammonium than either the C6 strain or the CCMP229 strain. This difference is probably due to the larger cell size of C3 (Figure 2.3), and the added metabolic requirements derived from photosynthesis that are necessary to divide, rather than from a decreased ability take up and assimilate ammonium relative to the other strains. The larger cell size of C3 may be associated with higher levels of lipids (McKay et al. 2007), which may be one characteristic, in its effect on buoyancy and energy

reserves, that permits populations with similar physiology to advect longer distances and reach the east coast of Florida.

Phytoplankton physiology is up-regulated and down-regulated depending on environmental exposure (Geider et al. 1998, Leonardos & Geider 2004). Surface populations of *K. brevis*, for example, may have higher growth rates and may have higher uptake rates to support these growth rates (Sinclair et al. 2006a). Near-bottom populations, simulated in this study, by contrast, are exposed to lower levels of light, and experience lower growth rates than commonly reported in both field and laboratory studies (Walsh et al. 2006, Sinclair & Kamykowski 2008). If *K. brevis* undergoes a metabolically mediated migration (Kamykowski & Yamazaki 1997, Ralston et al. 2007), cells may be in proximity to sediment-associated nutrients during the day or at night. Thus understanding the regulation of nutrient physiology over the diel period in these near-bottom environments is critical to understanding how cells persist and grow in these near bottom environments.

Kinetic parameters give some insight into how the physiological apparatus may be adapted to different environments (Goldman & Glibert 1983, Morel 1987). A low half saturation constant (K_s), for example, may indicate that cells have high affinities for a nutrient and may be adapted to low nutrient environments (Eppley et al. 1969). If cells access elevated concentrations of nutrients associated with the sediment, the kinetic parameter $\rho_{\max}$ may be more informative of a cell's ability to exploit high nutrient concentrations similar to those that have been observed in the sediment porewaters. The

complexity of inter-strain differences in $\rho_{\max}$ suggests widespread variability in the ability to use different N substrates over a diel cycle.

If *K. brevis* is in close proximity to the sediment during the day, the intra-strain similarities in $\rho_{\max}$ for ammonium, urea and nitrate indicate similar abilities to use the examined nitrogen substrates if encountered in higher concentrations. Unlike other dinoflagellates like *Gymnodinium pulchellum-complex* (Larsen) and *Karlodinium micrum* (Larsen) that took up ammonium faster than urea (Burford 2005), the strains of *K. brevis* exhibited equal ability for diurnal uptake of ammonium and urea. It must be noted, however, that the experiments conducted herein only examined uptake of N sources to which cells were acclimated. How these cells would respond to other N sources under these low light conditions is yet unknown. Nevertheless, the $\rho_{\max}$ between strains for a given substrate was different. The CCMP229 strain had a higher $\rho_{\max\text{-urea}}$ than either the C6 strain or the C3 strain. The observed diurnal assimilation efficiency show similar patterns. Entry of urea into cells seems to be a passive process in phytoplankton (Antia et al. 1991) suggesting that these elevated uptake rates are driven by larger concentration gradient across the cell membrane (Solomon 2006). Correspondingly high assimilation rates for CCMP229 may maintain the concentration gradient by moving urea into biosynthetic pathways and help support relatively higher $\rho_{\max\text{-urea}}$ than the other strains. The urease activity, necessary to maintain this gradient appears to be constitutive and has been observed to vary with growth rate (Fan et al. 2003, Solomon & Glibert 2008). The fact the different strains did not significantly differ in their reported growth rates suggests that either these growth rates were not reflective of the short-term growth and uptake

rates that occurred during these experiments or some strains may possess a higher base level of urease activity. The CCMP229 strain was isolated in a region where harmful algal blooms frequently occur (Walsh et al. 2006) and where near-bottom populations have been observed (Milroy et al. 2008). A differential ability of CCMP229 to use organic substrates, like urea, may help support near-bottom populations in this region.

In contrast to urea, the C3 strain had a higher diurnal $\rho_{\text{max-nitrate}}$ than the CCMP229 strain. The difference in nitrate utilization between C3 and CCMP229 was also observed in their growth rates (Table 2.1). The nitrate assimilation efficiency of C3 was 39 % lower than the assimilation efficiency of urea. The assimilation efficiency of nitrate in CCMP229, by contrast, was 43% lower than the assimilation for urea. This suggests that these differences may be due to inter-strain differences in transport pathways across the cell membrane or in enzyme levels like nitrate reductase. The enhanced ability of the C3 strain to utilize nitrate may help to explain its ability to persist for extended periods of time in water columns like the nitrate-rich loop current waters (He & Weisberg 2003) that have the potential to transport populations to the east coast of Florida where C3 was isolated.

In contrast to diurnal uptake, there were distinct intra-strain differences in the nocturnal ρ_{max} primarily due to the decreases in $\rho_{\text{max-nitrate}}$. Rates of $\rho_{\text{max-nitrate}}$ were 70% lower than rates of $\rho_{\text{max-urea}}$ for CCMP229 and 75% lower than the $\rho_{\text{max-ammonium}}$ for the C3 strain. The decrease in nocturnal uptake rates of nitrate is consistent with the dependence of nitrate uptake on light reactions (Eppley et al. 1971b, MacIsaac & Dugdale 1972, Cochlan et al. 1991). Previous experiments have demonstrated that the C6 strain of *K.*

brevis has the ability to up-regulate its nocturnal nitrate uptake rates after exposure to growth saturating levels of light and prior nitrogen depletion (Sinclair et al. 2006a). Near-bottom populations of *K. brevis* are likely not exposed to sufficient levels of light in order to elevate nocturnal uptake rates to levels similar to those observed for ammonium and urea. If near-bottom populations are only in proximity to elevated nitrate concentrations at night, nitrate is unlikely to be a sufficient source to fulfill their nitrogen requirements.

In contrast to nitrate, ammonium had the highest observed $\rho_{\max}$ of 0.98 pmol N cell⁻¹ hr⁻¹ for both the C3 strain and the C6 strain (Table 2.2). The C6 strain, assimilated ammonium more efficiently at night than either the C3 strain or the CCMP229 strain and also had a high urea assimilation efficiency at night. This pattern may be consistent with the suggestion by McKay *et al.* (2007) that C6 is able to more easily access energy reserves in the dark, necessary for the incorporation of nitrogen into cell protein at night, than C3, which may be more dependent on light reactions (McKay et al. 2007). The ability to utilize reduced and organic forms of nitrogen that occur in porewaters may help support near-bottom populations similar to the C6 strain, which may be representative of near-bottom populations observed in the northern Gulf of Mexico (Figure 2.1).

Overall ammonium and urea appear to be the most likely nitrogen sources, examined in this study, to support aggregations of *K. brevis* at the sediment-water interface at night. The averages of $\rho_{\max}$ of the different strains indicate that the $\rho_{\max}$ -ammonium was 42% higher than the $\rho_{\max}$ -urea and 70% higher than the $\rho_{\max}$ -nitrate. When the assimilation efficiencies of the strains were averaged, little difference in the assimilation

efficiency of the different nitrogen substrates was observed, although urea consistently had the highest assimilation efficiencies of 80%. The high efficiencies for urea result in nearly equivalent amounts of nitrogen being incorporated into proteinaceous material for both ammonium and urea at night.

Patterns in the diel uptake of the different nitrogen substrates indicated that the $\rho_{\text{max-ammonium}}$ was most similar over the diel period and $\rho_{\text{max-nitrate}}$ demonstrated the greatest differences. There were no differences in the diel uptake (ρ_{max}) of ammonium or the assimilation efficiency of ammonium. This pattern is inconsistent with other observations demonstrating the diel pattern in ammonium uptake (Eppley et al. 1971) but do align with how phytoplankton populations exposed to low light levels (1 % surface irradiance) take up ammonium (Cochlan et al. 1991). The $\rho_{\text{max-urea}}$, by contrast, was higher during the day than at night but the assimilation efficiency did not change resulting in nearly equivalent rates of assimilation of ammonium and urea at night. Nitrate uptake and assimilation demonstrated the most significant diel pattern with a significantly lower $\rho_{\text{max-nitrate}}$ at night than during the day due to the strong dependency on light energy (Eppley & Coatsworth 1968, MacIsaac & Dugdale 1972, Lomas & Glibert 2000).

Interpretation of how different strains are selected by different environments may be limited due to the fact that each strain was isolated from the surface aggregations that were presumably better adapted to high light. Despite this constraint, based on observed differences in this study, one may speculate that cells similar to the C3 strain may be better adapted to environments where they encounter oxidized nutrients, similar to what

cells being transported by upwelling plumes, or that reside in the water column. In contrast, cells similar to C6, that have higher rates of nocturnal assimilation of reduced and organic nutrients when under low light during the day, are better adapted to environments where they are in close proximity to high concentrations of reduced nutrients like the sediment-water interface. Overall, it is difficult to detect any consistent trends to determine which strains have the highest maximum uptake rates. Uptake and assimilation may be complicated by short term growth rates (Morel 1987), internal metabolic state (Kamykowski et al. 1998), energy reserves to transport and assimilate N into protein (Geider et al. 1998), and size of the cell (Alwyn & Rees 2007). Since uptake depends not only on the ambient environment but on instantaneous metabolic state the kinetic parameters observed here represent values along a variable spectrum rather than a constant capability (Glibert & Goldman 1981, Goldman & Glibert 1982, Harrison 1983).

Normalizing specific uptake ($V \text{ hr}^{-1}$) to specific growth rates ($\mu \text{ hr}^{-1}$) and plotting them against substrate concentration provides a context in which the kinetics of nutrient uptake may be interpreted relative to growth (Figure 2.6). If V/μ is greater than 1, cells take up nitrogen faster than is needed for growth, whereas if V/μ is less than 1, the specific uptake rates are not sufficient to support growth. During the day, specific uptake rates at low substrate concentrations ($\leq 2 \text{ } \mu\text{M}$ nitrogen) were rarely sufficient to sustain the reported specific growth rates. Only the C3 strain, when exposed to both ammonium and nitrate (Figure 2.6a,e), exhibited specific uptake rates sufficient to support growth at low concentrations. This is consistent with an organism that has adapted to oligotrophic water columns and this capability may enable it to persist long enough to be transported

to the east coast of Florida entrained in the loop current. At higher concentrations of ammonium all strains exhibited specific uptake rates higher than growth rates during the day (Figure 2.6a). Diurnal exposure of cells to urea, however, resulted in more balanced growth ($V/\mu \sim 1$) at 20 μM and higher specific uptake rates than specific growth rates were observed only at higher concentrations of urea (Figure 2.6c). At night, by contrast, only the C6 strain exhibited specific nocturnal uptake rates sufficient to compensate growth from both ammonium and urea at higher nutrient concentrations (Figure 2.6b,d). If the C6 strain represents populations that originated in near-bottom water columns and had to descend at night to maximize exposure to nutrients, these cells may be expected to have nocturnal uptake rates sufficient to support growth. The fact both the CCMP229 and C3 strains had nocturnal specific uptake rates for nitrate (Figure 2.6e) well below those necessary to balance growth indicate that nitrate is not likely to be the dominant contributor to the nitrogen nutrient of cells that descend to the sediment-water interface to increase exposure to nutrients at night.

The relationship between assimilation efficiency to V/μ provides farther insight to the utilization of substrates over the diel cycle (Figure 2.7). During the day, the specific uptake rates of ammonium are larger than those needed to support growth (Figure 2.7a). One would expect that as V/μ decreases, the assimilation efficiency would increase due to the need to assimilate more nitrogen to support growth. This trend is not observed for ammonium during the day since the majority of the specific uptakes rates are higher than those required for growth. At night, *K. brevis* only seems to take up half the ammonium needed for growth while retaining similar assimilation efficiency as those observed

during the day. This suggests that nocturnal uptake rates, especially at low substrate concentrations, alone are not sufficient to support growth at the sediment-water interface.

Unlike ammonium (Figure 2.7a), the majority of points in the urea plot (Figure 2.7c) fall at or below balanced growth irrespective of diel period. Little difference between diel assimilation efficiency and specific uptake rates relative to growth may indicate a more evenly distributed contribution of urea to the incorporation of nitrogen over the diel cycle. The increased efficiency of urea assimilation is observed when specific uptake rates are lowest and fail to compensate for specific growth rates. If descent enhances nutrient exposure in oligotrophic water columns, *K. brevis* may exploit this exposure by enhancing its nocturnal assimilation rates even after exposure to low light environments.

Nitrate was only sufficient to support growth during the day ($V/\mu > 1$) unlike both ammonium and urea in which 1 or more points were ≥ 1 (Figure 2.7b). As V/μ decreased at night, the assimilation rate increased but was not sufficient to compensate for the lower specific uptake rates and supported less than 50 % of growth at night. If near-bottom populations of *K. brevis* that ascend into oligotrophic water columns and are exposed low light, descent to enhanced nitrate concentrations at night does not provide any advantage in terms of nutrient acquisition. Indeed, under the low light levels of these experiments, *K. brevis* requires higher nutrient concentrations than are likely encountered in nature in order for uptake to compensate growth.

The diel vertical migration of *K. brevis* from the sediment-water interface is likely to expose cells to nutrients primarily at night. Nocturnal uptake may become

increasingly important as nutrients become scarce in the upper water column as is commonly observed in the Gulf of Mexico and in low light environments (Probyn et al. 1996). If cells are exposed to higher nutrient concentrations at night, consistent with migrating to and from a subsurface nutrient source, the ability to use nutrients at night becomes increasingly important (Flynn & Fasham 2002). Comparison of the $\rho_{\max}$ for the nocturnal rates of uptake as well as assimilation efficiencies indicate that the reduced and organic forms of N are utilized (taken up and assimilated) more than twice as fast as the oxidized forms. This is similar to other dinoflagellates like *Heterosigma carterae* (Clark & Flynn 2002) and is likely a function of the energetic costs of nocturnal uptake (MacIsaac & Dugdale 1972, Syrett 1981). Additional research is required to assess whether acclimation to other nitrogen sources during one period of the diel cycle may alter their ability to use other nitrogen sources during another part of the diel cycle.

The nocturnal descent of *K. brevis* into the vicinity of the sediment-water interface may significantly increase exposure to reduced and organic forms of nitrogen by *K. brevis* (Sinclair & Kamykowski 2008b). The enhanced ability to use these forms of nitrogen relative to nitrate may play a critical role in the maintenance of subsurface populations in low nutrient offshore environments. Since the energy derived from photosynthesis would be relatively low in subsurface populations, one would expect *K. brevis* to exhibit lower growth rates and uptake rates as observed here (Syrett 1981). *K. brevis* increases assimilation efficiency at night, this increase is not likely to offset the reduced nocturnal uptake rates. If near-bottom populations fulfill their nitrogen

requirements at night other sources like ammonium and urea, which are nearly equivalent in the quantity of nitrogen assimilated at night, are more likely required.

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Table 2.1 Growth rates (divisions day⁻¹) of 3 strains of *K. brevis* the C6 strain, the C3 strain, and the CCMP229 strain.

Strain of <i>Karenia brevis</i>	Ammonia		Nitrate		Urea	
	Growth rate	std dev	Growth rate	std dev	Growth rate	std dev
C6	0.15	0.00	0.12	0.01	0.15	0.01
C3	0.15	0.01	0.13	0.01	0.14	0.01
CCMP229	0.13	0.03	0.12	0.02	0.18	0.03

Table 2.2 Kinetic Summary for Strains normalized to growth rates. Bold indicates that uptake did not saturate. Nitrate uptake for C6 was taken Sinclair et al 2006 and recalculated on a per cell basis. Units for $\rho_{\max}$ in $\text{pmol N cell}^{-1} \text{hr}^{-1}$. k_s values are in μM .

		Diurnal Uptake				Nocturnal Uptake			
		$\rho_{\max}$	SE	k_s	SE	$\rho_{\max}$	SE	k_s	SE
CCMP229	NH_4^+	0.62	0.05	0.37	0.18	0.34	0.04	0.16	0.18
	UREA*	1.29	0.09	32.47	4.55	0.41	0.11	10.97	9.49
	NO_3^-	0.41	0.06	1.32	0.85	0.12	0.01	0.25	0.16
C3	NH_4^+	0.72	0.08	0.17	0.14	0.98	0.20	1.03	0.92
	UREA	0.87	0.02	4.36	0.53	0.62	0.05	0.42	0.23
	NO_3^-	0.85	0.09	0.19	0.15	0.25	0.02	0.39	0.14
C6	NH_4^+	0.89	0.05	0.32	0.11	0.98	0.02	1.02	0.13
	UREA	0.56	0.06	0.37	0.26	0.50	0.03	0.53	0.19
	NO_3^-	0.75	0.13	0.47	0.10	0.22	0.17	0.13	0.05

Table 2.3 Kinetic parameters normalized for growth rates for substrates averaged from 3 *K. brevis* strains. $\rho_{\max}$ in $\text{pmol N cell}^{-1} \text{ hr}^{-1}$. k_s in μM .

	Diurnal Uptake				Nocturnal Uptake			
	$\rho_{\max}$	<i>Std dev</i>	k_s	<i>Std dev</i>	$\rho_{\max}$	<i>Std dev</i>	k_s	<i>Std dev</i>
NH_4^+	0.74	0.14	0.29	0.10	0.77	0.37	0.74	0.50
UREA	0.71	0.22	2.37	17.49	0.51	0.10	0.48	0.08
NO_3^-	0.67	0.23	0.66	0.59	0.20	0.07	0.26	0.13

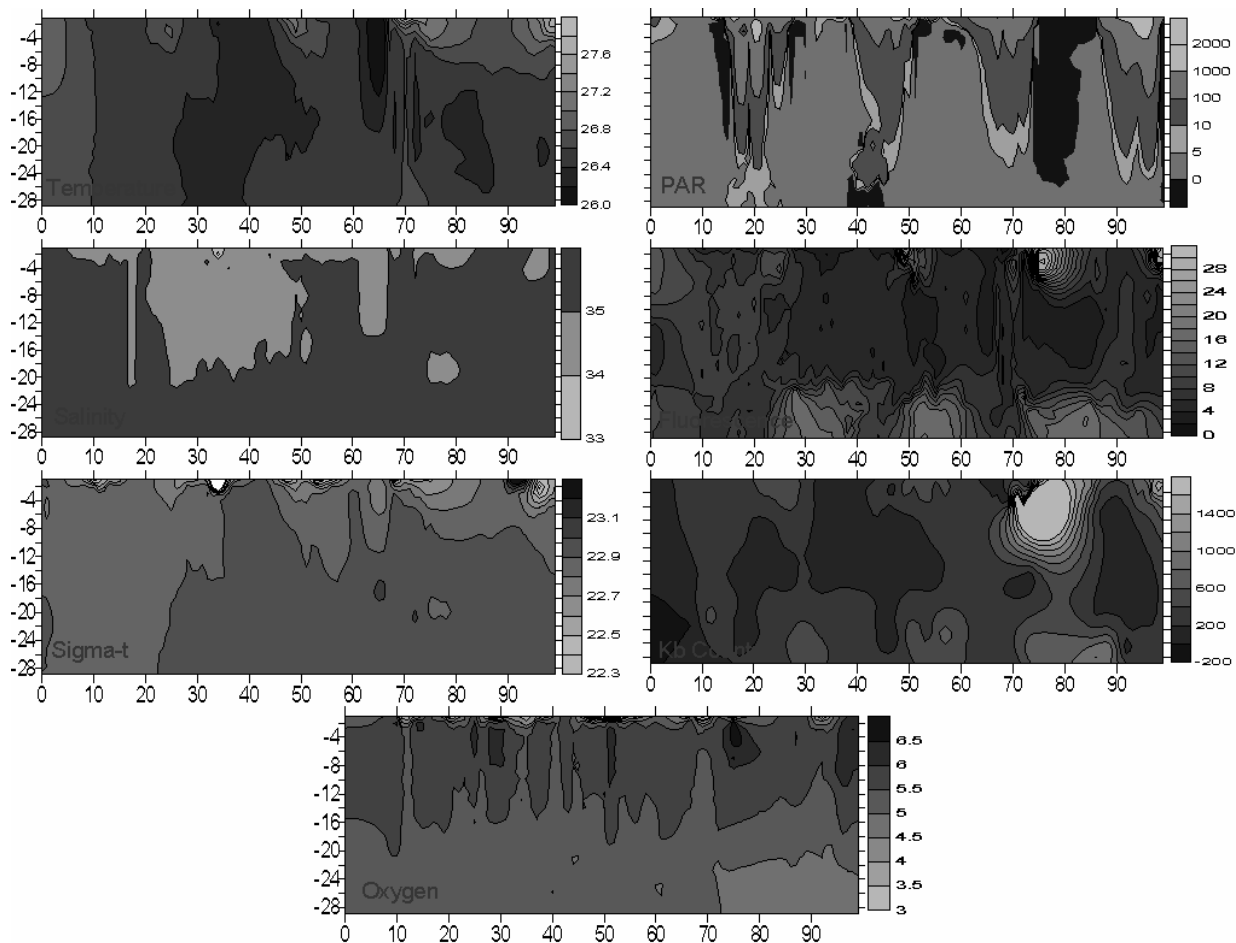


Figure 2.1 Time series of near-bottom populations of *Karenia brevis* off of Pensacola, Florida during the ECOHAB 2000 cruise. Temperature ($^{\circ}\text{C}$), Salinity, Sigma-T, PAR($\mu\text{mol quanta m}^{-1} \text{sec}^{-1}$), Fluorescence, *Karenia brevis* counts (cells L^{-1}), and oxygen are plotted against water column depth (m).

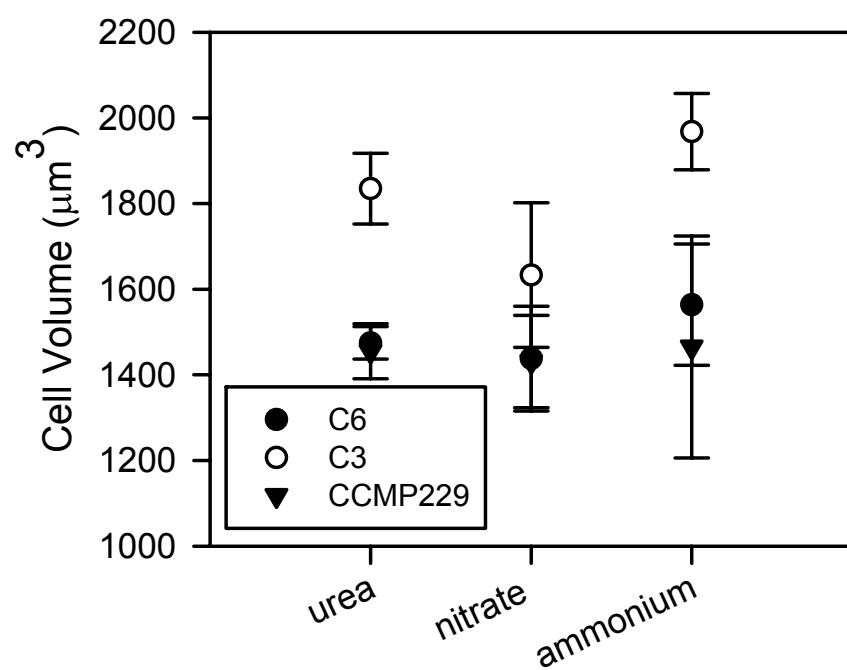


Figure 2.2 Cell volume (μm^3) vs growth nitrogen substrate, urea, nitrate and ammonium

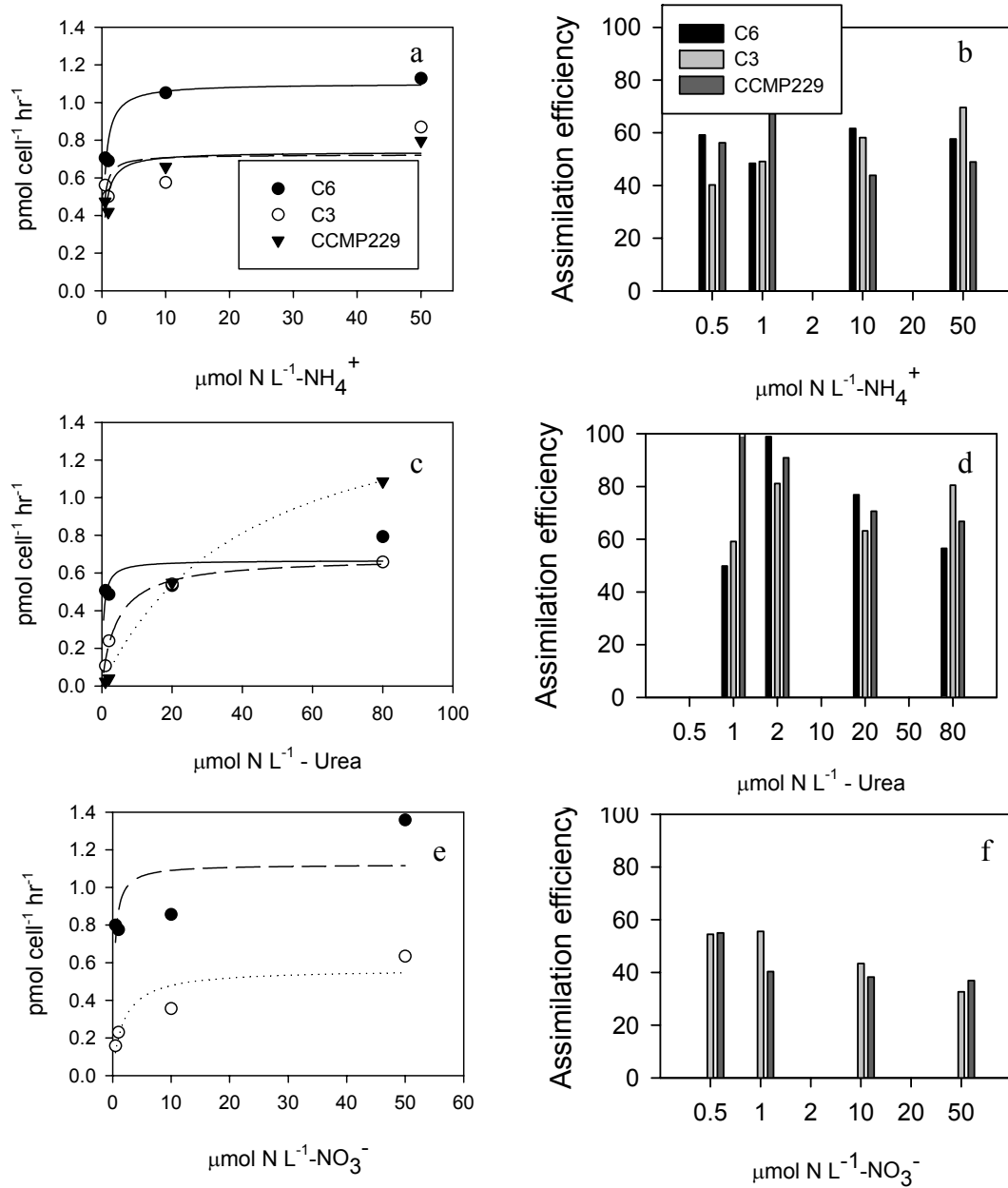


Figure 2.3 Diurnal uptake rates (ρ) in $\text{pmol N cell}^{-1} \text{hr}^{-1}$ and assimilation efficiency ($V_{\text{assimilation}} / V_{\text{uptake}} * 100$) vs substrate concentration.

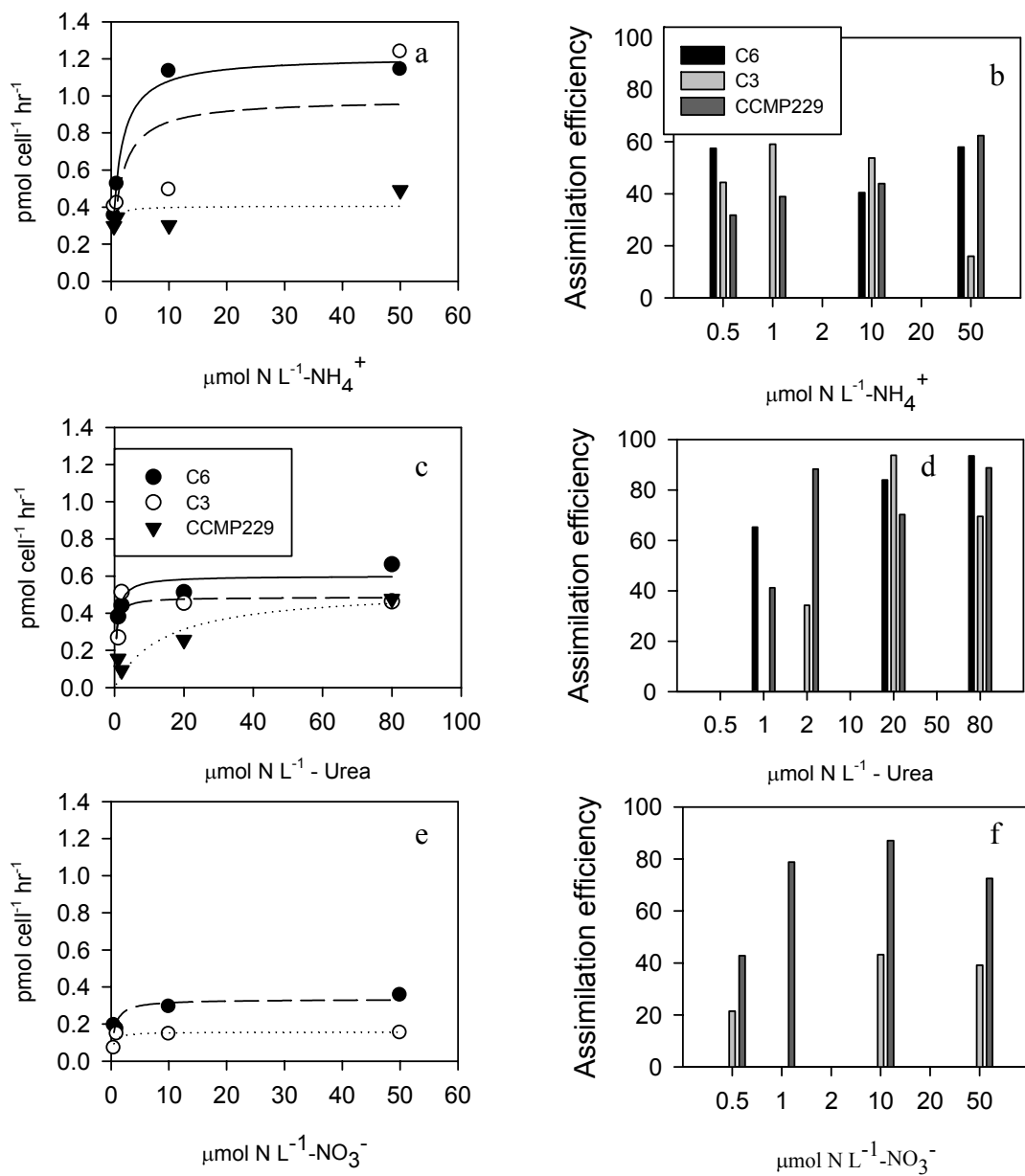


Figure 2.4 Nocturnal uptake rates (ρ) in $\text{pmol N cell}^{-1} \text{ hr}^{-1}$ is in the left column. Assimilation efficiency ($V_{\text{assimilation}} / V_{\text{uptake}} * 100$) vs substrate concentration on the x-axis.

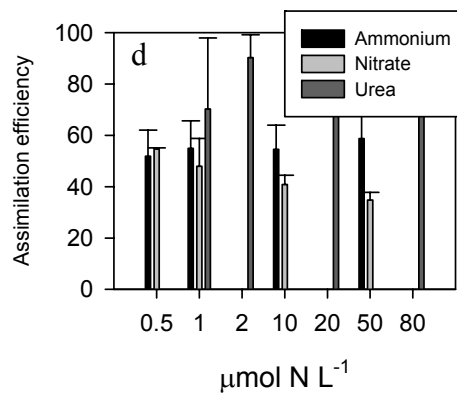
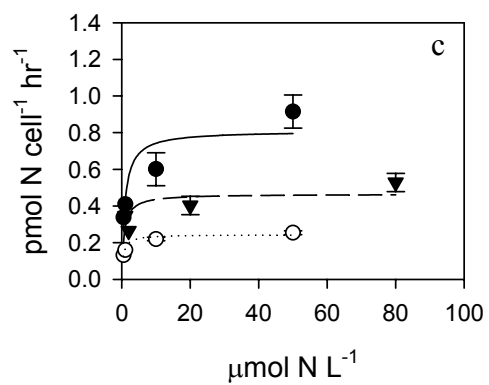
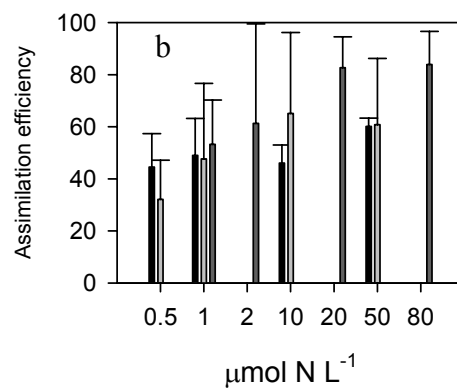
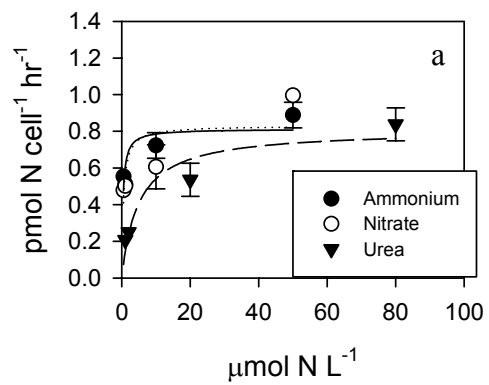


Figure 2.5 Diurnal (a & b) and Nocturnal (c & d) uptake rate ($\text{pmol N cell}^{-1} \text{ hr}^{-1}$) and assimilation efficiency vs substrate concentration

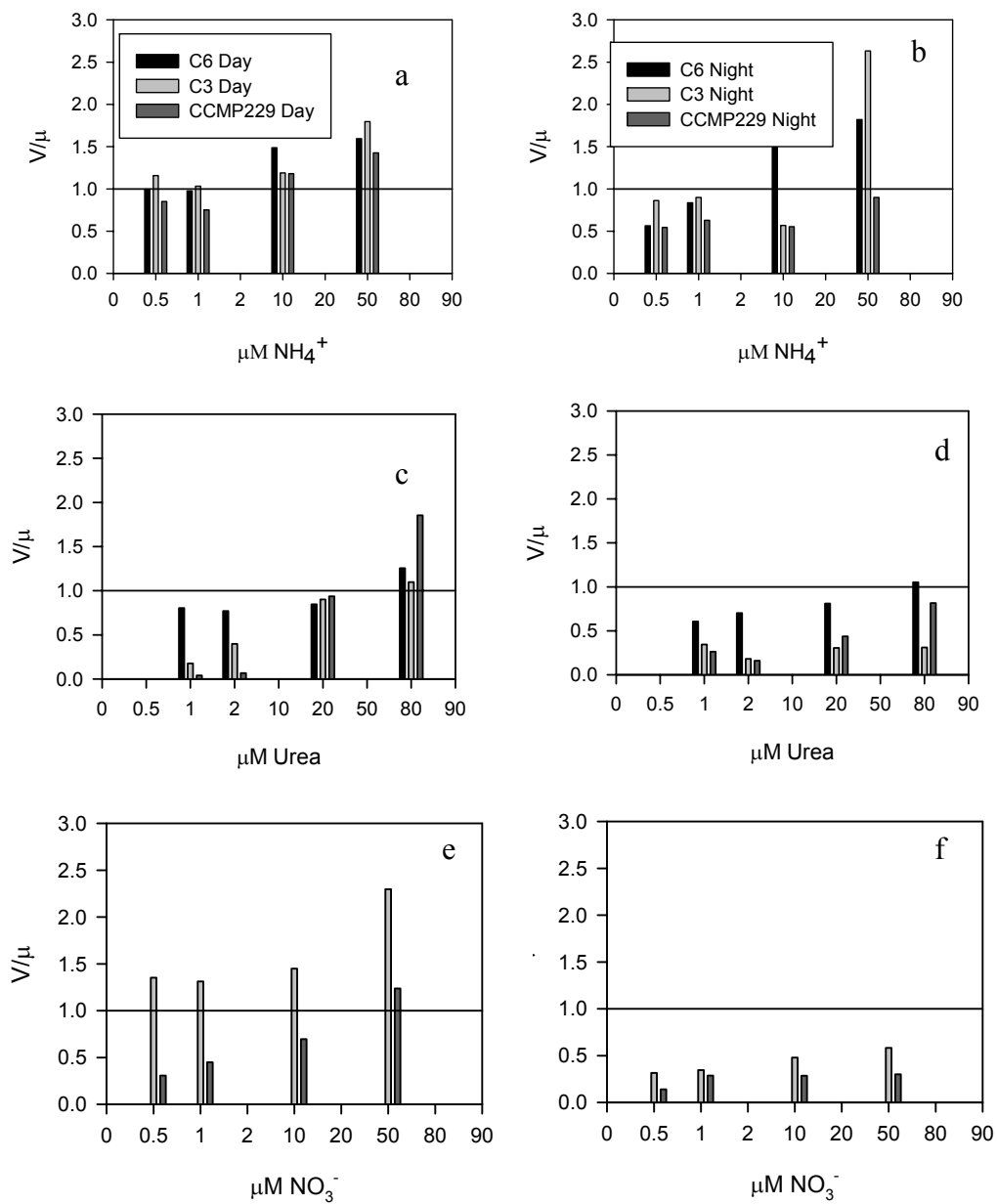


Figure 2.6 Specific uptake rate / specific growth rate (V/μ) vs. substrate concentration of three strains of *Karenia brevis*.

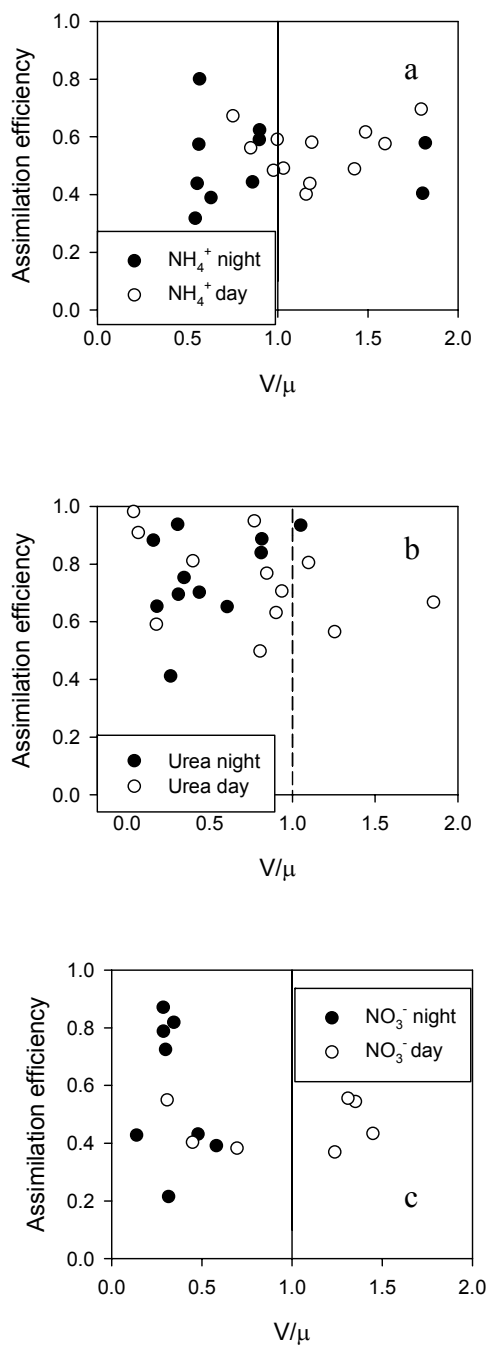


Figure 2.7 Assimilation efficiency vs. Uptake efficiency (specific uptake (V hr^{-1}) / specific growth ($\mu \text{ hr}^{-1}$)).

CHAPTER 3. BENTHIC-PELAGIC COUPLING IN SEDIMENT ASSOCIATED POPULATIONS OF *KARENIA BREVIS*

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3.1 ABSTRACT

Nutrient delivery to populations of *Karenia brevis* in oligotrophic water columns in the Gulf of Mexico remains uncertain. Aggregations of *Karenia brevis* near the sediment-water interface suggest that cells derive nutrients from the sediment. Video of cells near the sediment suggest that cells either access nutrients that flux out of the sediment or migrate into the sediment pores where higher nutrient concentrations exist. Experiments tested *K. brevis*' ability to migrate into the sediment using chambers divided by a 100 μm mesh overlain with a thin layer of sediment. Since the diel vertical migration of *K. brevis* typically displays a nocturnal descent, experiments tested migration response at night in response to sub-sediment nutrient sources. The experiments suggest that while the sediment affects the progress of descending cells, migration occurs through thin layers of sediment and increases in response to elevated nutrient concentrations below the sediment. Since all cells found below the sediment had significantly higher C/N ratios than those remaining above the sediment, migration appears related to a cell's internal biochemical state. The vertical migration behavior of *K. brevis* may help alleviate bottom-up controls and permit populations to persist as vegetative cells near the sediment-water interface.

3.2 INTRODUCTION

The diel vertical migration (DVM) of dinoflagellates to a nutricline confers a competitive advantage over non-motile phytoplankton when surface nutrients are low (Eppley et al. 1968, Cullen & Horrigan 1981, Cullen 1982, Watanabe 1991). Traditionally, DVM is modeled as ascent during the day to support photosynthetically driven carbon fixation and descent at night to access subsurface nitrogen, phosphorus and assorted trace elements at night to support further biochemical synthesis (Kamykowski 1974, Heaney & Eppley 1981, Cullen 1985, Smayda & Reynolds 2001). Migration, may however, be controlled by a variety of factors including phototaxis/geotaxis (Kamykowski et al. 1998b), cellular periodicity (Eppley et al. 1968), or metabolic control (Kamykowski & Yamazaki 1997) that result in migration patterns that differ from simple diurnal ascent and nocturnal descent (Sournia 1974).

In regions of steeply sloping margins like those in southern California, DVM may be oriented to a subsurface nutricline and result in subsurface aggregations of dinoflagellates along a pycnocline (Kamykowski 1980, Cullen 1982, Eppley et al. 1984). Similar subsurface dinoflagellate aggregations were reported in the Seto Inland Sea (Nakamura et al. 1996), off the west coast of South Africa (Pitcher et al. 1998), and in the Gulf of Maine (Townsend et al. 2005). Alternatively, on broad, gently sloping continental shelves, the sediment-water interface may conceptually substitute for the deep nutrient source when nutrients are depleted in the surface waters. *Akashiwo sanguinium* aggregated near the sediment-water interface in a 20 m water column (Kiefer & Lasker 1975), while *Gonyaulux polygramma* displayed a sediment oriented migration in a 32 m

water column (Kamykowski 1980). More recently, near-bottom aggregations of the *Karenia brevis* were observed during an ECOHAB 2000 cruise (Sinclair et al. 2006a).

Since concentrations of N and P, rarely exceed 0.5 μM and are commonly 0.1 to 0.2 μM within 2 to 4 km of shore in the Gulf of Mexico (Dragovich et al. 1961, Steidinger et al. 1998) and NO_3^- and NH_4^+ have been observed to be less <0.01 μM (Masserini & Fanning 2000), the sediments may be an important source of nutrients for these near-bottom aggregations. The sediment characteristics on the West Florida Shelf (WFS) are largely sand/gravel (Twichell et al. 2003) but the nutritive value remain largely unexplored. In other shelf environments both inorganic (Marinelli et al. 1998a, Jahnke et al. 2000a) and organic N (Burdige & Martens 1990, Keil & Fogel 2001) have been associated with sandy sediment. Such sediment is able to provide 30 to 80% of the microalgal N requirement in 5-50 m coastal environments (Nixon 1981, Blackburn & Henriksen 1983, Boynton & Kemp 1985).

The delivery of nutrients to near-bottom populations of motile phytoplankton may be variable. Nutrients may either flux out of the sediment during periods of higher energy (Huettel et al. 1998), or remain in the porewaters when diffusion dominates during periods of low energy (Marinelli et al. 1998b). The microphytobenthos (MPB) also may act as a filter, decreasing the flux of nutrients to the overlying water column, during the day (Sundback et al. 1991, Marinelli et al. 1998b, Jahnke & Jahnke 2000, Jahnke et al. 2000b). If near-bottom populations of *K. brevis* access sediment-derived nutrients, they may do so either at night when diurnal uptake by the MPB is depressed, or in regions further offshore where the concentration of the MPB is lower under light levels

less than 1% surface PAR. The vertical migration behavior of *K. brevis*, coupled with its broad range of photophysiology (Schaeffer et al. 2007), may allow *K. brevis* to access the sediment-water interface in regions farther offshore where light at the sediment interface may be less than 1% of surface PAR and still acquire enough light through vertical migration to ~10 m above the sediment surface to grow (Sinclair & Kamykowski 2006a).

If nutrients remain in the porewater during more quiescent periods (Huettel et al. 1998, Jahnke et al. 2005), when dinoflagellates are hypothesized to dominate (Smayda & Reynolds 2001), near-bottom aggregations of *K. brevis* must migrate into the porewater to fulfill their nutrient requirements. Higher concentrations of nutrients, such as ammonium and iron, may also be transported to the sediment-water interface from deeper anoxic zones (Huettel et al. 1998) and may be available to cells in proximity to the sediment-water interface. This work explores how near-bottom *K. brevis* populations may actively interact with the sediment to acquire nutrients that remain in the porewater during quiescent conditions in offshore regions off the West Coast of Florida.

3.3 MATERIALS AND METHODS

In order to determine the extent to which cells interact with the sediment, we examined the physical and chemical environment to which these populations would be exposed. To do this, we collected sediment samples from the west Florida Shelf at three depths 10m, 15m and 24m (Figure 3.1) during daylight hours. Due to the coarse grain size found in other studies (Twichell et al. 2003), the sediment was collected using SCUBA with mini sediment cores in order to minimize porewater washout that often occurs with larger scoops. The sediment cores were made from modified 25 cc or 60 cc syringes. In addition to grain size, physical sediment characteristics like permeability and porosity that may impact the transport of nutrients within the sediment were determined. Permeability was obtained using a falling-head permeameter (Wise & Myers 2002) while measurements of sediment porosity (Φ), assumed to be equivalent to moisture content, were based on wet vs. dry weight of the sediment (Ullman & Aller 1982).

Porewater also was collected using 5 cc syringes with 18 gauge needles at 5 cm into the sediment and in the top 0.5 cm of the sediment. Water samples were collected at the surface of the water column and 50 cm above the sediment and sampled for nutrients as well. All samples used for nutrient analyses were filtered through a 0.45 μm filter before analysis. All water column and porewater samples were put on ice until return to Mote Marine Laboratory (< 6 hours) where the chemical constituents, NO_3^- , PO_4^{2-} , Urea, dissolved organic nitrogen (DON), and particulate organic nitrogen (PON) were analyzed using techniques outlined in (Parsons et al. 1984). NH_4^+ concentrations were determined immediately on site following the protocol of (Holmes et al. 1999).

The sediment collected was then added to rectangular chambers (30 cm x 15 cm x 5 cm) that were used to observe cell interaction with the sediment in a three dimensional environment (Figure 3.2). Video was taken using the Expert Vision Motion Analysis package and following the experimental set-up of the dissecting microscope and camera that were described in (Kamykowski et al. 1988). All samples were videotaped in the dark with a red measurement light illumination at a magnification of 150. Red light was used because *K. brevis* does not have any known taxis response to this wavelength. Several *Gymnodinium* species exhibit stop responses that peak at 280 nm and at 450–475 nm and show minimal response to red spectrum light (Forward 1970, Forward & Davenport 1970, Forward 1973, 1974, McKay et al. 2007).

In order to test if cells actively migrate into the sediment, we built experimental chambers that could be divided by different sized mesh over which sediment could be laid. The chamber was 30 cm in height and 15 cm in diameter. Mesh screen, with a total area of 706.5 cm², divided the lower 1/3 of the chamber that contained an inlet / outlet port by which cells were sampled or nutrients were added to the system (Figure 3.3). Mesh screen, with pore sizes of 5 µm and 100 µm, were initially used as barriers to test whether the 100 µm mesh chosen for the experimental design inhibited the migration of *K. brevis* cells that had an ESD of 20 µm. Since interstitial pore space, through which cells might hypothetically swim, depends on grain size (Hartkamp et al. 1993), samples of natural sediment were sieved to provide different size class treatments. The range of sizes classes, each larger than the 100 µm pore size of the mesh barrier, included, greater of 840 µm, 380 µm, and 177 µm. Treatments therefore included a 5 µm barrier (control),

100 μm mesh barrier alone, and the 100 μm barrier overlaid with both sieved sediment and natural sediment. Standardization of the thickness of the sediment layer consisted of determining the weight of sediment (3g) necessary to completely cover the 706.5 cm^2 of 100 μm mesh barrier.

Non-axenic 4 L batch cultures were started from the same *K. brevis* parent culture (strain Apalachicola) and grown in L/20 media (Guillard & Hargraves 1993) from which copper was eliminated and modified so that nitrate was the only nitrogen source at 20 μM . To simulate low light environments where offshore populations might occur at the sediment–water interface, cultures were grown with 30 $\mu\text{mol quanta m}^{-2} \text{ sec}^{-1}$ delivered to the surface plane of the culture in a 22 $^{\circ}\text{C}$ temperature control room. Seawater (SW), with salinities (S) of 32, 36 and 38 and passed through a 0.45 μm filter and autoclaved, were used as the base for different cultures to confirm that the salinities chosen for experimental manipulation would not significantly affect the growth or motility of *K. brevis*. All cultures were grown under a 12:12 light/dark cycle with lights-on at 06:30 EST and lights-off at 18:30 EST.

For the experiments, each experimental chamber was filled with 160 mL of seawater (S=36) depleted of nitrogen. This permitted 40 mL of culture grown on a seawater media (S=32) to be gently introduced from the top using a 10 mL syringe so that cells would not pass through the screen barrier from the momentum of the introduction. Fluoresceine dye was used initially to determine that a rate of introduction equivalent to 0.2 mL sec^{-1} was sufficient to keep cells entrained in the less dense upper layer and remain at the top of the water column after introduction. In all experiments cells were

introduced into the top of the chamber 1 hour before lights-off and then each chamber was harvested 5 hours after lights-off, at 23:30, similar to the time cells were observed to aggregate at the sediment-water interface during the ECOHAB 2000 cruise. Fluoresceine dye was used to visually confirm that the stratification was intact beyond the duration of the experiment so that descending cells must have descended by active swimming rather than from break down of water column stratification.

To test the initial hypothesis that cells are able to migrate into the sediment, 9 chambers were used with different barriers. The chambers were divided either with a 5 μm mesh screen, 100 μm mesh screen and a 100 μm mesh on which a thin layer of sediment (< 1.5 cm) was overlaid. Interstitial space (pore size) through which cells must swim is generally a linear relation to grain size with pores being $\sim 40\%$ the size of grain sizes (Hartkamp et al. 1993). In order to determine if there is difference between the pore sizes cells might swim, subsamples of natural sediment were sieved to create 4 categories of sand: 840 μm , 380 μm , 177 μm , and un-seived sand.

The second set of experiments tested whether cells would increase migration into the sediment in response to nutrients found below the sediment. The chamber was set up similar to before, only this time 20 mL of SW (S=38) containing 20 μM of either nitrate or ammonium was introduced from the bottom. Fluoresceine dye was again used to determine that the rate of introduction was sufficient to keep the introduced SW entrained below the sediment. After incubation, cells from the top were harvested first with a 60 mL syringe until water level was below the surficial layer of the sand barrier to prevent the possibility of cells from the upper layer being drawn through the sediment during

sampling. The bottom of the column was subsequently harvested by releasing water from the bottom valve. Samples from both the top and bottom of the chamber were analyzed microscopically and then enumerated using a Coulter Multisizer II Particle Analyzer Counter (Beckman-Coulter Inc., Miami, FL) with lower and upper threshold settings of 13.3 and 30 μm diameter. Filtrations for total cellular C and N used precombusted Whatman GF/D 25 mm filters. 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 an ISUS nitrate analyzer as well as with a Lachat QuikChem 8000 Continuum Series Autoanalyzer. Total cellular carbon and nitrogen samples were processed on a Carlo Erba nutrient (CHN) analyzer. The volume filtered corresponded to approximately $21\ \mu\text{g N filter}^{-1}$ as determined by prior sensitivity analysis.

3.4 RESULTS

The sediments on the West Florida Shelf are comprised primarily of coarse grained sand and shell hash with between 97.3 and 99.6 % of the sediment greater than 177 μm (Figure 3.4). The offshore, 24m site, had the highest concentration of intermediate sized sediment (between 177 μm and 420 μm). This site had the lowest amount of large grain (shell hash) sediment with about 11% greater than 840 μm and also had the lowest amount of grains smaller than 149 μm (0.4%). The 15 m site had the greatest percentage of large grains (62.7 %) but also had the greatest percentage of sediment below 149 μm (2.7%). Grain sizes intermediate between the offshore sites were found in the 10 m site. Sediment permeability of the sites was highly variable and ranged from 6.4×10^{-13} at the 15m site, to 1.8×10^{-11} at the 24m site (Figure 3.5). The porosity at the three sites was not significantly different and ranged from 0.39 ± 0.08 at the 24 m site to 0.44 ± 0.02 at the 15 m site with an average Φ (% volume) of 0.41 ± 0.04 .

In general nutrients, for all species, were more abundant in the sediment than the overlying water column but were spatially variable (Table 4.1). Of the water column nutrients measured, dissolved organic nitrogen was significantly ($t = 3.8$, $n = 12$, $p < 0.01$) greater than the other nutrients. Phosphate was also detected at the surface of the water column but in low concentrations. Nutrients surveyed above the sediment-water interface were generally undetectable. Nutrient concentrations in the sediment were patchy as exemplified by ammonium concentrations that were greater than 50 μM at the 10 and 24 m stations but $2.4 \pm 0.9 \mu\text{M}$ at the 15 m station or by urea concentrations significantly greater at the 10 m station ($F = 42$, $n = 6$, $p < 0.01$).

Initial video of cells interacting with the sediment demonstrated that cells maintain position above the sediment as well as actively migrate into the sediment pores (Figure 3.2). The growth rate (div day^{-1}) of the cultures used in subsequent chamber experiments was tracked over several weeks. Seawater media with the salinities (S) of 32, 36 and 38 did not significantly affect the growth rate of *K. brevis*, which is consistent with the wide range of salinity tolerances demonstrated in other studies (Brown et al. 2006). Initial assessment of the chamber design, comparing a 5 μm mesh (control) barrier to the 100 μm barrier, indicated cells freely moved through the latter barrier. There were not any cells found below the 5 μm mesh compared to $62\% \pm 8\%$ of the inoculated cells that were found below the 100 μm mesh barrier at night (Figure 3.6). The addition of a thin layer of natural sediment decreased the percentage of cells found below the 100 μm barrier to $21\% \pm 4\%$ but did not prohibit nocturnal descent of 20 μm *K. brevis* cells. There was no significant difference between the percentage of cells found below the barriers created by the different categories of sieved sand (Figure 3.7). The sieved sand treatments were also similar to that of natural sand and significantly less than the 100 μm mesh without sand control ($F = 62$, $n = 6$, $p < 0.01$). When NO_3^- or NH_4^+ were added below the barrier the percentage of cells found below a barrier of natural sand significantly increased from $21\% \pm 4\%$ to almost $35\% \pm 3\%$ for ammonium and 31 ± 6 for nitrate ($F = 12.8$, $n = 6$, $p < 0.01$). The C:N ratios of cells found below the sediment, in all treatments was significantly greater than those in the parent culture ($t = 6.9$, $n = 6$, $p < 0.01$) as well as those found above the barrier ($t = 6.9$, $n = 6$, $p < 0.01$).

3.5 DISCUSSION

Nutrient delivery to populations of *Karenia brevis* may be spatially variable, originating either from inputs from *Trichodesmium sp.* blooms (Walsh & Steidinger 2001), and upwelling events in offshore areas (Tester & Steidinger 1997), or run off from land in near-shore areas (Cooper et al. 1998, Liu et al. 2001b). Since blooms of *K. brevis* are often thought to initiate offshore in low nutrient waters (Tester & Steidinger 1997), we were particularly concerned with the delivery of nutrients to offshore populations and how the persistence of these populations may serve as inoculum for blooms that are observed near-shore (Janowitz & Kamykowski 2006). The observation of near-bottom aggregations of *K. brevis* suggested another source of nutrients may be the sediment-water interface (Sinclair et al. 2006a). This observation may also help explain why populations of *K. brevis* remain undetected below the first optical depth when they are offshore (Stumpf et al. 2003, Stumpf et al. *in press*).

In a preliminary field survey, inorganic nutrients, while spatially variable, were found to be substantially higher in the sediments than the overlying water column. There were no significant differences found at depth intervals between 0.5 cm and 5 cm depths in the sediment and might suggest significant interstitial exchange (Huettel & Gust 1992). Permeability coefficients (Figure 3.5), while variable, are similar to those of other sandy sediments and tend to promote interstitial exchange, particularly via advective transport (Ehrenhauss et al. 2004). Interestingly, nutrients were undetectable just above the sediment-water interface, contrary to the expected flux of nutrients out of the sediment. The presence of a microphytobenthos at the sampled depths may filter nutrients that flux

out of the sediment (Jahnke et al. 2000b, Sundback et al. 2004a) so that motile cells of *K. brevis* are required to penetrate into the sediment to increase exposure to elevated nutrient concentrations. The composition of surface sediment across the West Florida Shelf (WFS) differs spatially (Brooks et al. 2003) and may grow finer moving offshore (Dalrymple 1992). The sediment in the present sample region of the WFS is largely sand and gravel (Twichell et al. 2003). Estimation of interstitial pore space based on the equivalent spherical diameter (ESD) of different sieved fractions of sediment, indicated pores widths may range from 73 μm for the 177 μm sieved fraction to 331 μm for the 840 μm size fraction (Hartkamp et al. 1993). The size of *K. brevis* cells range from 17 to 22 μm (personal observation) which are significantly smaller than the estimated pore spaces that cells may encounter. Initial tests of both size fractionated sediment and natural non-fractionated sediment indicated that the ability of *K. brevis* to migrate into the surficial pore spaces, assayed as migration through a sediment barrier, did not differ among treatments (Figure 3.7). This suggests that while sediment may be variably distributed, there is a broad range of sandy environments that *K. brevis* may exploit across the shelf.

Coarse grain sediments, characteristic of environments with water velocities that often exceed the transport threshold for sediments, also exist in regions where current velocities are low on the WFS (Harrison et al. 2003). Much of the WFS consists of low energy environments and water velocities unable to achieve sediment transport velocities (Brooks et al. 2003). The typical near bottom current velocities are often below 5 cm s^{-1} (personal observation; (He & Weisberg 2002a). With such low near-bottom current velocities, the interstitial water velocities can be assumed to be less than the 2.8 $\mu\text{m s}^{-1}$

that occurs when currents of 10 cm s^{-1} pass over sandy bottoms with topographic features (Huettel et al. 1998). While such current velocities may be responsible for the burial of non-motile phytoplankton species in the sediment (Huettel & Rusch 2000), *K. brevis* is able to swim up to 100 times faster than these velocities and may be able to escape subsequent burial (McKay et al. 2007).

While spatial heterogeneity is common, the upper few centimeters of carbonate sediment, similar to those found in this survey, are often oxygenated (Sorensen et al. 2007). Two of the 3 sites sampled, however, contained high concentrations of reduced forms of nitrogen near the sediment water interface. These reduced forms of nutrients likely derive from anoxic zones deeper in the sediments that are upwelled to the sediment-water interface when currents flow over rough elements in the bottom topography (Huettel et al. 1998). The upward transport of reduced forms of nitrogen may allow *K. brevis* to access nutrients when migrating into the surficial porewaters of the sediment.

Dissolved organic nitrogen was consistently detected in the water column, though in lower concentrations found in other studies (Heil et al. 2007). Consistent with this survey, however, dissolved organic matter (DOM) in the sediments tends to have higher N content than DOM in the water column and may potentially elevate the levels of bioavailable N in the sediment (Berg et al. 2003a) under aerobic conditions (Henriksen & Kemp 1988). Ratios of regeneration rates for C and N (C:N) were lower, near the redfield ratio, in the summer ($>23^{\circ}\text{C}$), and higher in the winter (Jahnke et al. 2005). This

suggests that relatively more bioavailable N may be associated with the sediment in the summer when near-bottom populations of *K. brevis* are hypothesized to occur.

Migration into the sediment by N deprived cells and subsequent accumulation of cells below the sediment barrier was enhanced by inorganic nitrogen below the sediment (Figure 3.8). Dinoflagellates have been hypothesized (Liu et al. 2001b) and observed (Clegg et al. 2003) to respond to nutrient gradients that result in cell accumulation in the vicinity of nutrients. In the case of this experiment however, the diffusion rate of these nutrients from the dense lower layer (salinity=38) (Mann & Lazier 2006), suggests that there was not sufficient time during the experiment to establish a concentration gradient. Instead, vigorous nocturnal descent may be driven initially in response internal biochemical state.

Cells found below the sediment had significantly higher C:N ratios than cells found above the sediment indicating a significant imbalance in its internal C and N pools (Figure 3.9). The C:N ratios of *K. brevis* can vary significantly, depending on prior environmental exposure and can range from 4 to 15 (Liu et al. 2001b, Sinclair et al. 2006a). The elevated C:N ratios, observed here, suggest that cells found below the barrier are deficient in nitrogen. This observation is consistent with previous hypotheses of metabolic driven migration, where cells ascend to increase light exposure and acquire C, or descend to nutrient rich pools when nutrient deficient (Watanabe 1991, Kamykowski & Yamazaki 1997, Kamykowski et al. 1998a). Cell interaction with the sediment may therefore be a combination of metabolically controlled migration behavior and subsequent responses to the ambient chemical environment.

The sediment-water interface differs from more traditional subsurface nutrient sources in the fact that it may have higher concentrations of reduced forms of N instead of oxidized forms associated with nutriclines (Cullen & Horrigan 1981, Williams et al. 2006). The ability of *K. brevis* to migrate into the surficial porewaters significantly enhances exposure to sediment bound nutrients like ammonium, which is upwelled, through porewater advection, to the sediment-water interface (Huettel et al. 1998). Ammonium may be particularly beneficial since it is often taken up at greater levels at night than oxidized forms of nitrogen (Clark & Flynn 2002). Cells undergoing transient exposure to ammonium, such as those migrating into surficial sediment pores would have a competitive advantage over those cells supplied with a transient supply of nitrate (Wood & Flynn 1995). Differential capabilities to assimilate N in darkness contribute to both cell and population growth and may be important for how near-bottom populations are modeled (Flynn & Fasham 2003). This preliminary work seeks to define a mechanism by which *K. brevis* accesses sediment-bound nutrients. Additional work is required to further characterize the cross-shelf spatial heterogeneity of porewater nutrient concentrations, benthic nutrient fluxes, and the distribution of these near-bottom populations as they relate to models of how blooms are initiated and transported. Blooms of *K. brevis* are characterized by sudden onset where population densities aggregate faster than can be explained by growth rates alone (Steidinger & Tester 1998). One explanation may include the transport of undetected near-bottom populations in upwelling favorable conditions (Tester & Steidinger 1997). Modeling the onshore advection of subsurface populations undergoing diel vertical migration is able to

reproduce the aggregation patterns observed during the ECOHAB 2000 cruise (Janowitz & Kamykowski 2006). The ability of *K. brevis* to actively migrate into sediment pores may increase exposure to nutrients and thereby allow slow growing populations to persist offshore near the sediment-water interface in oligotrophic water columns. These populations may then serve as seed populations for nearly annual bloom events that occur near-shore.

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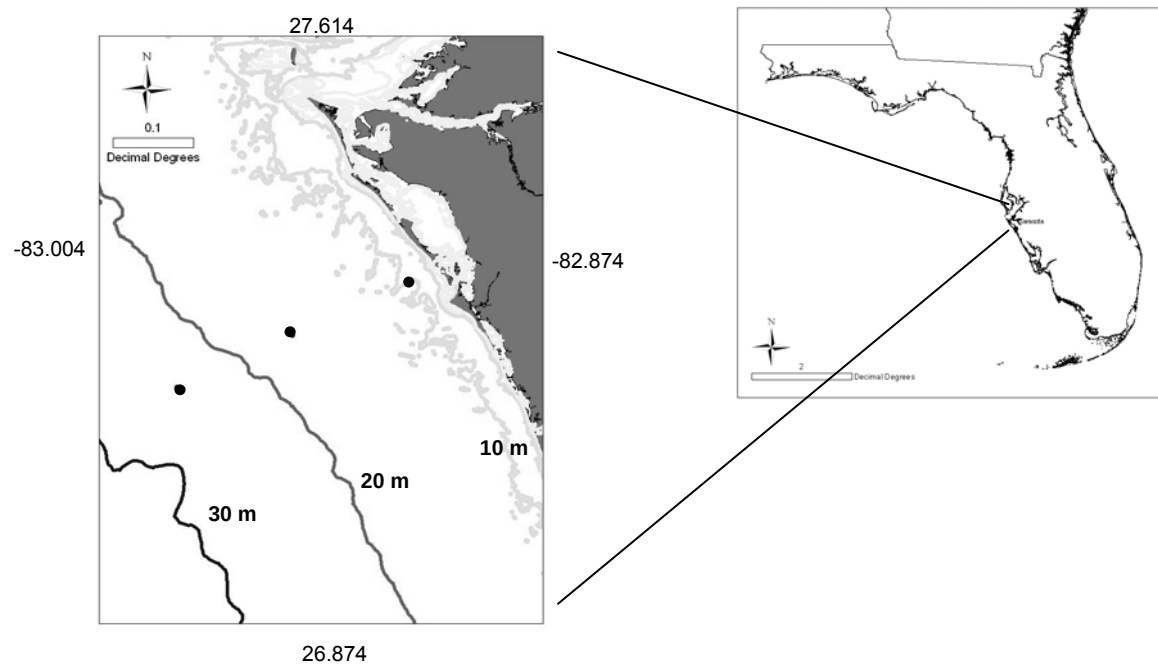


Figure 3.1 The transect for sediment sampling (expanded map) was performed on the West Florida Shelf in water columns that were 10 m, 15 m, and 24 m deep.



Figure 3.2 Laboratory observation of cells in proximity to sediment. The black arrow denotes the sediment-water interface, below which are coarse grain sands upable to be resolved by the microscope. *Karenia brevis* cells above the sediment-water interface denoted by the dotted arrow arrow.

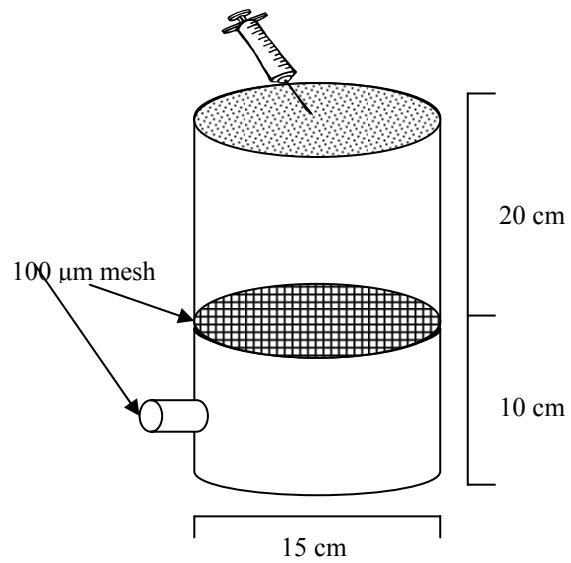


Figure 3.3 The experimental chamber was 30 cm X 15 cm that was divided by a 100 µm nylon mesh screen. Cells were introduced to the top using a 10 ml syringe while nutrients were introduced from below via an outlet port.

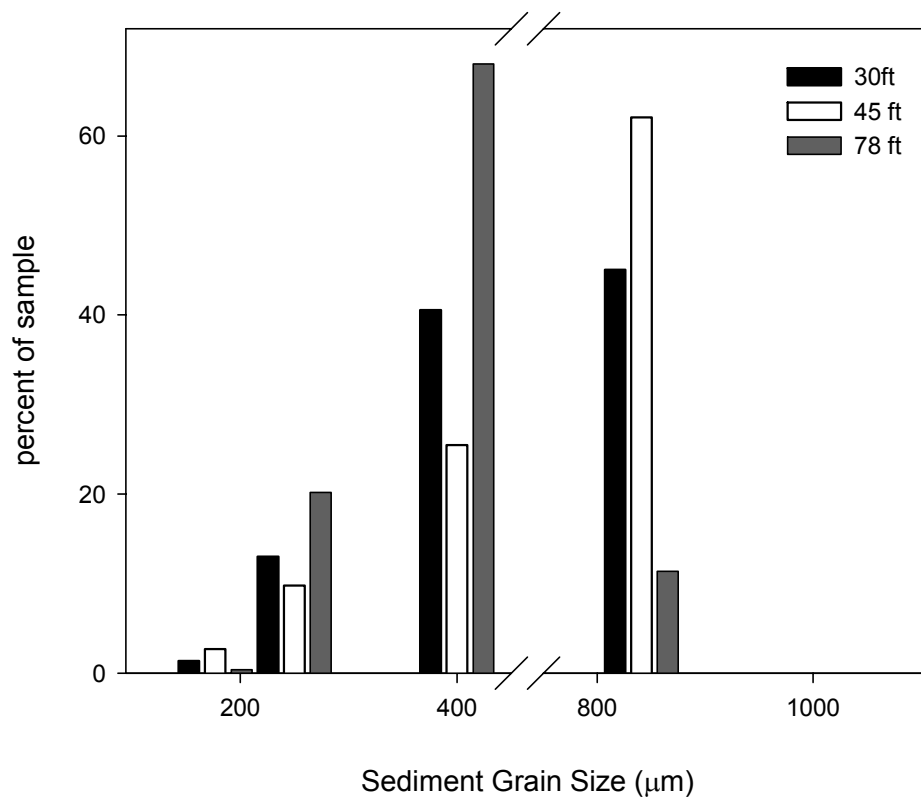


Figure 3.4 Sediment grain size at stations, indicated by depth (10 m, 15 m, 24 m). Percent sample of fraction was determined as fraction dry weight.

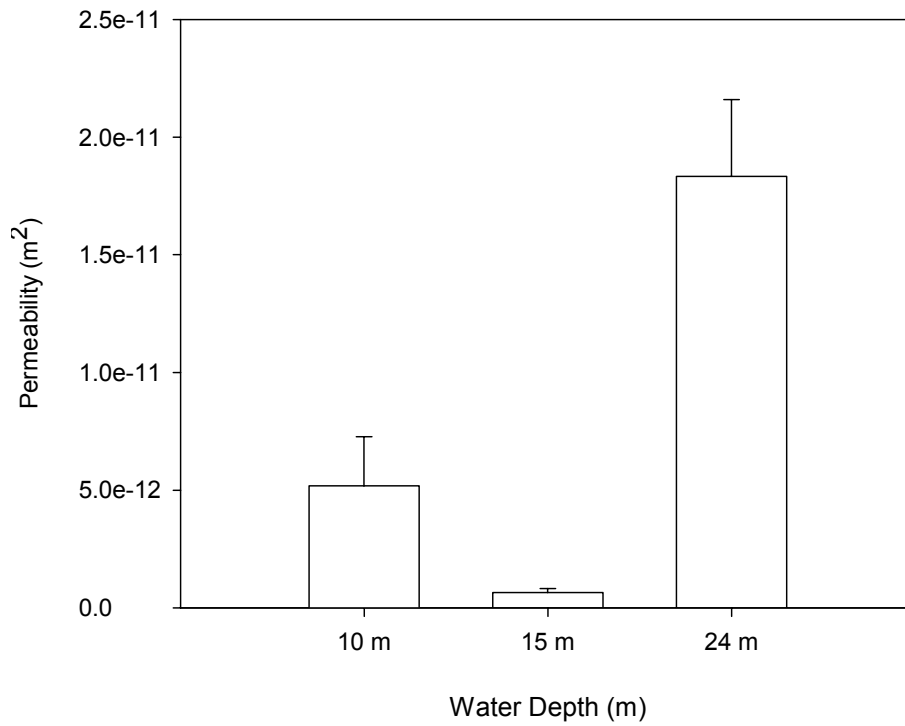


Figure 3.5 Sediment permeability (m^2) vs. Water Depth (m) at the three different depths measured with a falling-head permeameter.

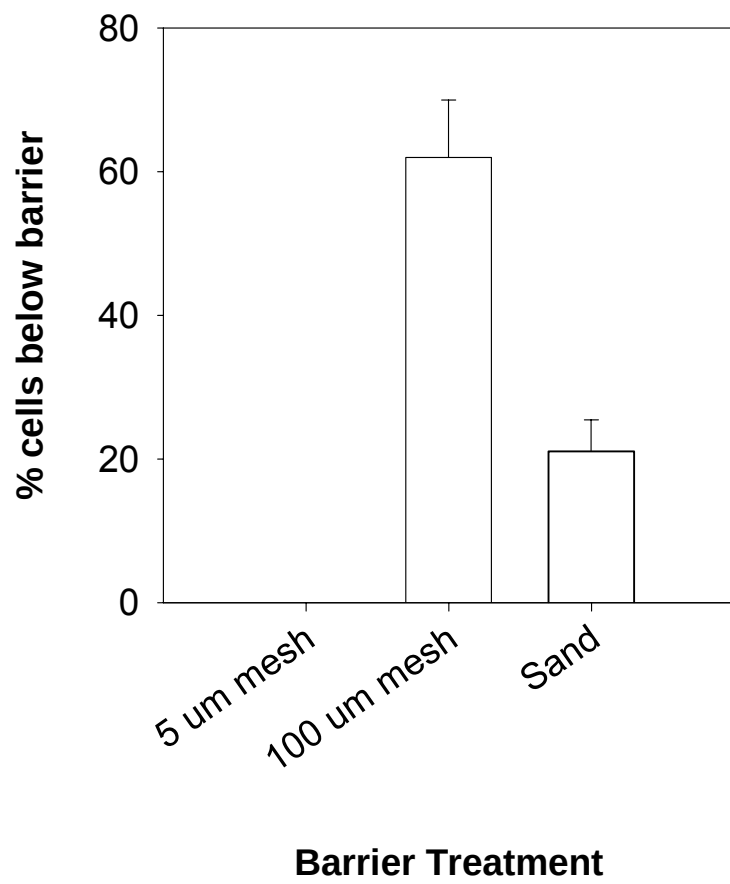


Figure 3.6 Percentage of cells found below barrier with different treatments (5 μm mesh, 100 μm mesh, and 100 μm mesh overlaid with sand).

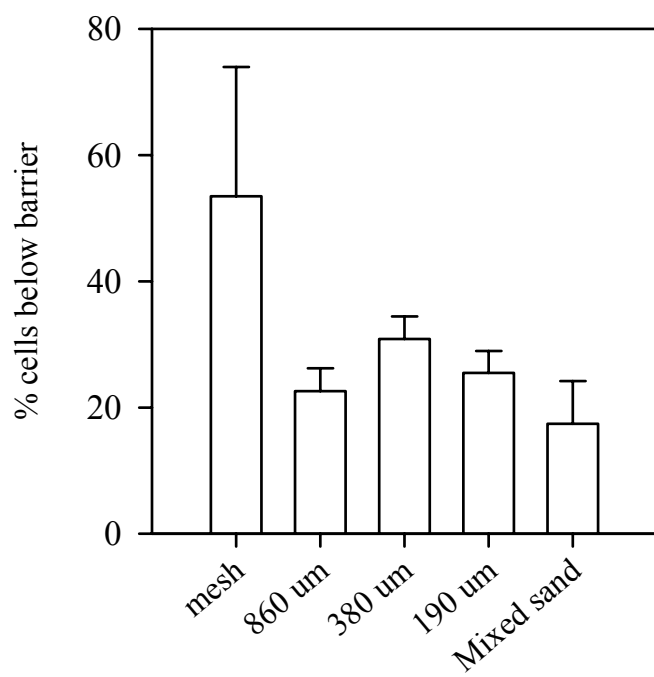


Figure 3.7 Migration different barrier treatments of sieved sand (860 μm , 380 μm , 190 μm), and unsieved sand.

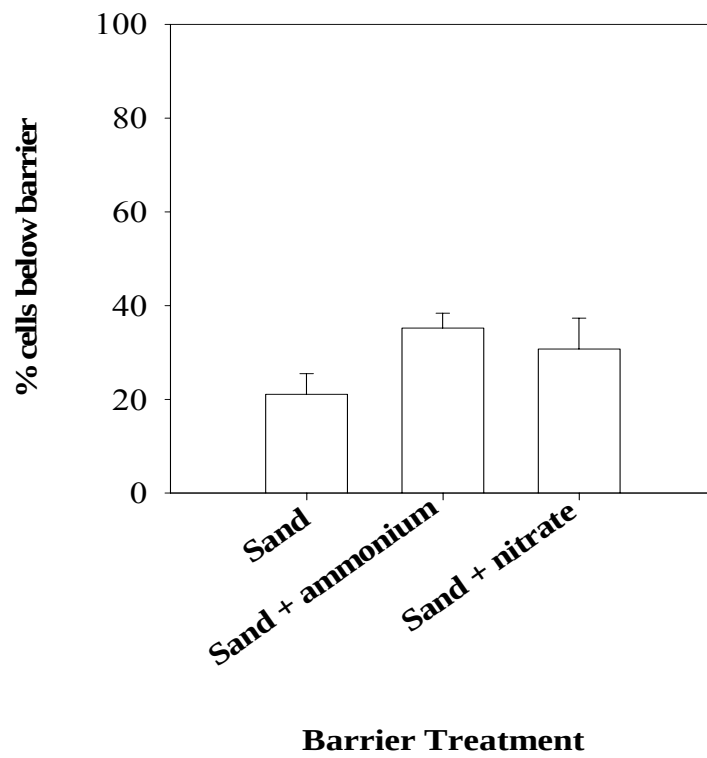


Figure 3.8 No nutrient vs. nutrient treatments (20 μ M nitrogen as either ammonium or nitrate below sand barrier).

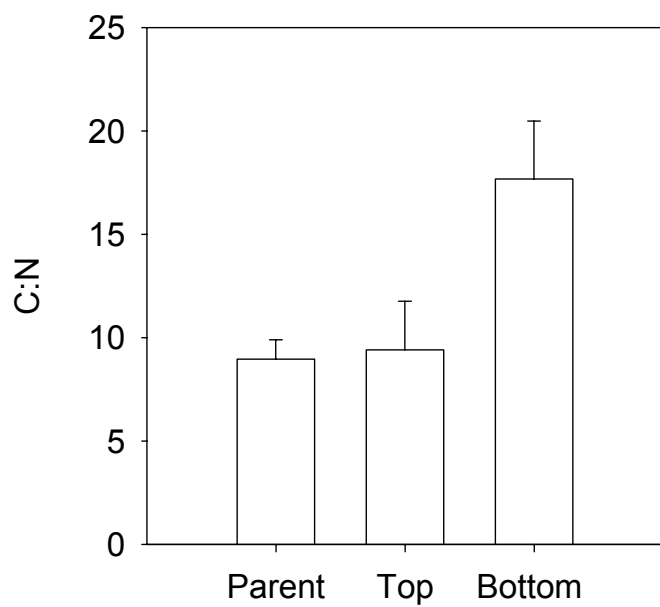


Figure 3.9 C:N of parent culture vs. cells located at top of chamber or bottom of chamber.

**CHAPTER 4. INFLUENCE OF FLUID SHEAR ON THE NITROGEN UPTAKE
AND ASSIMILATION OF *KARENIA BREVIS***

To be submitted to Marine Ecology Progress Series

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4.1 ABSTRACT

In situ observations of *Karenia brevis* near the sediment-water interface as well as laboratory studies of cells interacting with the sediment suggest cells derive nutrients from the sediment in oligotrophic water columns. The persistence of these near-bottom populations may depend on how cells interact with periods of elevated shear that occur within the benthic boundary layer. This study used a high resolution current profiler to examine the water velocities and potential shear rates that *K. brevis* is exposed to in the benthic boundary layer. The effects of different defined shear rates on the uptake and assimilation of ammonium, nitrate and, urea were then determined using Couette flow chambers. The experiments simulated either, 1) cells residing above the sediment-water interface and utilizing nutrients *during* exposure to shear or, 2) cells accessing the sediment porewaters and utilizing nutrients *after* exposure to shear. Shear rates in the benthic boundary layer were observed to vary from 0.02 s^{-1} to close to 3 s^{-1} over 2 hour time scales. The uptake of ammonium was unaffected while the uptake of nitrate decreased during exposure to shear. Assimilation of both ammonium and nitrate decreased during cell exposure to shear. There was no decrease in either the rates of uptake or the assimilation of ammonium after exposure to shear while cells decreased uptake and assimilation for both nitrate and urea. The uptake and assimilation rates of both ammonium and nitrate were higher in cells after exposure to shear than the corresponding uptake and assimilation rates during exposure to shear implying that cells exhibited morphological or physiological recovery during the temporal constraints of these experiments.

4.2 INTRODUCTION

Blooms of the toxic dinoflagellate, *Karenia brevis*, are hypothesized to originate offshore in oligotrophic water-columns (Tester & Steidinger 1997). These populations often appear more suddenly than can be explained by growth rates alone (Stumpf et al. 2003, Walsh et al. 2006, Stumpf et al. 2008) which has led to the hypothesis that these offshore populations exist subsurface (Sinclair & Kamykowski 2006), particularly below the first optical depth detected by satellites (Schofield et al. 2006). The subsurface distribution of these populations place them within the bottom Ekman layers which transport important biological materials across the shelf (Weisberg 2003). Models of the transport of subsurface populations to onshore areas where accumulation occurs in frontal boundaries have not only replicated aggregations patterns observed during bloom events but verified that increases in the concentration of cells in the water-column occur more rapidly than explained by growth rates alone (Janowitz & Kamykowski 2006).

Field observations of *K. brevis* populations aggregating near the sediment at night and dispersing into an oligotrophic water column during the day suggest a significant portion of the ecology of *K. brevis* occurs near the sediment-water interface (Sinclair & Kamykowski 2006, Sinclair et al. 2006a). Laboratory observations suggest that cells may alleviate potential bottom controls of nutrient limitation, both during bloom initiation as well as bloom maintenance, by utilizing the sediments as a nutrient source, either by anticipating fluxes of nutrients out of the sediment, or actively migrating into surficial porewaters (Sinclair & Kamykowski 2008b). The ability of near-bottom populations to interact with the sediment, and thus be sustained by sediment associated nutrients, may

be constrained by the turbulent regimes that may be enhanced near the sediment (Sanford 1997). The biophysical interaction of cells with the benthic boundary layer is an integral part of the bloom initiation repertoire but remains unexplored.

The persistence of near-bottom populations of *K. brevis* depends on how vertically migrating cells interact with increasing turbulence and associated shear that is encountered toward the sediment boundary (Sanford 1997, Perlin et al. 2005). Dinoflagellates have been hypothesized to be very sensitive to small scale turbulence (Peters & Marrase 2000) but responses appear to be species-specific without any clear trends relating to taxonomy or morphology (Stoecker et al. 2006, Berdalet et al. 2007). Since some dinoflagellates are able to sense and respond to elevated levels of shear stress (Maldonado & Latz 2007), understanding how turbulence impacts cell physiology and behavior in close proximity to the sediment may be critical to understanding the near-bottom ecology of *K. brevis*.

Turbulence directly influences phytoplankton ecology by influencing growth rates (Gibson & Thomas 1995a, Juhl et al. 2000, Sullivan & Swift 2003), and increasing the mass transfer of nutrients (Pasciak & Gavis 1975). Influences of turbulence on integrated photosynthesis (Janowitz & Kamykowski 1991) and trajectories (Yamazaki & Kamykowski 1991) of phytoplankton have been explored in the upper mixed layer of water columns. Yet few studies consider the influences of turbulence on phytoplankton near the bottom of water columns. Responses to turbulence vary with growth conditions and physiological state (Juhl et al. 2000) and therefore may be particularly pertinent to

the ecology of slowly growing populations of *K. brevis* that occur near the bottom of coastal water columns.

K. brevis may encounter different levels of shear depending on whether cells maintain position above the sediment-water interface to utilize fluxes of nutrients out of the sediment or migrate into the surficial sediment porewaters. The former scenario may expose cells for longer durations to elevated shear rates relative to the overlying water column. Cells that migrate into the sediment, by contrast may experience a transitory increase in shear rates before reducing exposure to shear by taking refuge in the sediment pore waters with significantly reduced water velocities (Huettel & Rusch 2000). Laboratory experiments documenting active migration into sediments suggest that either scenario is possible (Sinclair & Kamykowski 2008a). It is therefore important to understand how *K. brevis* takes up nutrients both during exposure to shear, similar to what cells might encounter when utilizing the efflux of sediment nutrients, as well as after transitory exposure to shear, similar to what cells might experience migrating through the benthic boundary layer to access sediment-bound nutrients.

Predicting where these near-bottom populations occur, and their subsequent connection to cross-shelf transport vectors, requires associating the distribution of phytoplankton with the turbulent environment on different temporal and spatial scales (Franks 2005). Near-bottom dinoflagellate populations, hypothesized to occur near bottom offshore before advection onshore by upwelling events, may have a broad spatial distribution across the shelf. Vertically migrating populations of *K. brevis* are expected to aggregate at the sediment boundary primarily at night in order to maximize subsequent

exposure to light during diurnal ascent. Depending on light attenuation in the water column the near-bottom niche of *K. brevis* may extend from water columns 20 m deep to 65 m deep (Sinclair & Kamykowski 2006). The bottom stress and therefore the boundary layers to which near-bottom populations may be exposed in this region are variable. Log layer thicknesses have been modeled to range from 0.12 - 0.24 m offshore to > 0.48 m shoreward of the 20 m isobath (He & Weisberg 2002). Due to the inaccessibility of offshore areas, direct measurements of the turbulent fields in the bottom boundary layers, particularly on scales similar to the inertial sub-range (1 cm - 100 cm) that more closely relate to the ecology of dinoflagellates, are few. In order to better correlate the patchiness of phytoplankton with their environment, hydrographic measurements ideally must be made within the inertial subrange of 1-100 cm (Franks 2005). Laboratory experiments in the context of field measurements of near-bottom flow fields can provide valuable insight to the near-bottom ecology of *K. brevis* and suggest temporal and spatial constraints when these seed populations may persist.

4.3 METHODS

The bottom boundary layer off of Pensacola (N 30° 17.577, W 87° 13.238) was characterized for one month in an 18 m water column during the summer when near-bottom populations of *K. brevis* are thought to occur. A high resolution (HR) pulse-coherent firmware version of the 2 MHz Aquadopp Profiler (Nortek's HR Profiler) in a downward facing orientation was used to profile water velocities every 2 cm over 1.56 m above the sediment-water interface (Figure 3.1). Sample averages of 20 sec were taken every 5 minutes for 29.8 days, from 20:54 GMT May 20, 2008 to 16:04 GMT June 19, 2008. Analysis of correlation coefficients of the velocity profiles eliminated six 2 cm cells near both the sensor head and the sediment-water interface that were corrupted due to signal interference. Since *K. brevis* has been observed in close proximity to the sediment, and may migrate through the benthic boundary layer to access sediment associated nutrients, we estimated the potential current shear that cells may experience by calculating the difference in current velocities with depth (du/dz^{-1}) at 2 cm intervals.

In order to test the response of *K. brevis* to different levels of turbulence we created a defined uniform laminar shear using Couette cylinders described by (Stoecker et al. 2006) and similar to those in (Juhl et al. 2000). Couette flow is characterized by a linear velocity gradient across the gap defined by

$$u(r) = \omega_0 r_0^2 (r^2 - r_i^2) / (r_0^2 - r_i^2) r$$

where ω is the angular velocity ($\omega=2\pi N/60$; N is the rotational speed in rpm) and r is the radial position within the gap (Schlichting 1979). In order to produce nearly constant shear across the bottom of a Couette chamber, it is necessary to have a wedge-shaped gap

along the bottom (Shimeta et al. 1995). Shear is estimated as ($\gamma_b = \omega/\theta$) where θ is the bottom angle in radians. The Couette cylinders for this study were made of machine grade polycarbonate with a design that was similar to devices reported by Juhl et al. (2000) and Shimeta et al. (1995). Coette chamber dimensions were an inside radius of the outer cylinder (r_o) of 52.4mm and an outside radius of the inner cylinder (r_i) of 47.6 mm, yielding a wall gap of 4.76 mm and bottom angle (θ) of 5.58. The height of the inner cylinder was approximately 100 mm, such that the chambers could accommodate 150 ml of water.

Synchronous A/C 115 V 60 Hz motors obtained from Allied Electronics, a distributor of Cruzet motors, with motor speeds from 0, 0.2, 0.5, 1, 3, 10 rpm were used to generate shear rates calculated to be 0 (motor off), 0.22, 0.54, 1.1, 3.3, 11.0 s^{-1} , respectively. The shear rate was calculated by the formula

$$\lambda = \frac{2\omega_0 r_o r_i}{r_o^2 - r_i^2}$$

where ω_0 is the relative rotation rate in radians per unit time, r_o is the inside radius of the outer cylinder and r_i is the outside radius of the inner cylinder. Dye experiments on the cylinder with the highest motor speed (10 rpm) confirmed laminar flow (Stoecker et al. 2006).

Triplicate 1 L batch cultures *Karenia brevis*, strain Apalachicola, were grown in L/20 media (Guillard & Hargraves 1993) from which copper and silica were eliminated and modified so that either ammonia, nitrate, or urea was the only nitrogen source. The seawater (salinity = 35) was passed through a 0.2 μm filter, and autoclaved. Each culture

was provided with an initial concentration of 20 μM N and 5 μM N was added variably to maintain the initial concentration until cell densities in the cultures permitted experimental manipulation. In order to prevent preconditioning effects of the media, 2 generations of parent cultures (~2 weeks each) were cultured under the given nitrogen treatment before the 1 L batch cultures were inoculated for the experiment. The batch cultures were grown under a 12:12 light/dark cycle with lights on at 06:30 EST and off at 18:30 EST. During the day each culture was grown under light exposures of approximately 30 $\mu\text{mol quanta m}^{-2} \text{ sec}^{-1}$ at the surface plane of the culture and at 22 °C for the duration of the experiments.

Changes in cell numbers over time were monitored by counting cells from triplicate batches with a Coulter Multisizer II Particle Analyzer Counter (Beckman-Coulter Inc., Miami, FL) with a 200 μm orifice and threshold settings of 13.3 and 30 μm for cell diameter. Net growth rates (cell divisions day^{-1}) of triplicates on each substrate were determined from the slope of a regression plotting $\ln(N)$ (cells ml^{-1}) vs time (days). The ammonia and urea in the media was tracked daily following protocols of Holmes et al. (1999) and (Mulvenna & Savidge 1992). Nitrate was monitored with an ISUS nitrate analyzer calibrated against Lachat QuikChem 8000 Continuum Series Autoanalyzer.

The experimental treatments aimed to simulate cells either maintaining position above the sediment-water interface or migrating through the benthic boundary layer and entering the porewater at night. To test cell response during continuous exposure to shear, five 120 ml samples of cells, upon depletion of the nitrogen in the media, were drawn to be inoculated with 10 $\mu\text{g-at N L}^{-1}$ of either ^{15}N -ammonium, ^{15}N -nitrate, or ^{15}N -

urea, at 23:00 and then added to the Couette cylinders. In order test the effects of prior exposure to shear, cells were added to Couette cylinders for 30 minutes before being removed and inoculated with 10 $\mu\text{g-at N L}^{-1}$ of either ^{15}N -ammonium, ^{15}N -nitrate, or ^{15}N -urea. In all treatments the final percentage of ^{15}N in the media was 20%. The concentration used was 10 $\mu\text{g-at N/L}$ to simulate the elevated concentrations of nitrogen cells might encounter associated with the sediment. After 30 minute incubations, the samples were divided and 60 ml of each treatment was filtered onto two precombusted Whatman GF/F 25mm filters. Both filters were washed with isotonic artificial seawater and one filter was immediately frozen at -20°C , while the second filter was treated with 10% ice-cold trichloroacetic acid (TCA) and then frozen. The first filter represented both uptake and assimilation of the nitrogen form of interest, while the second TCA-extracted filter represented only the ^{15}N -(ammonium, nitrate, urea) that was assimilated into proteinaceous material (Lomas & Glibert 1999). Samples for particulate carbon and nitrogen were filtered onto precombusted GF/F filters and dried at 50°C over night and analyzed on a Control Equipment CHN Elemental Analyzer and converted to cellular quotas by dividing by the cell density within the sample (Glibert et al. 1991).

Nitrogen specific uptake rates of ^{15}N -labeled substrates (V hr^{-1}) were calculated following methods of (Dugdale & Goering 1967).

$$\text{V}(\text{hr}^{-1}) = \frac{{}^{15}\text{N atom}\% \text{ excess}}{{}^{15}\text{N atom}\% \text{ enrichment} \times \text{time}}$$

^{15}N % excess was the ^{15}N atom % of the particulate fraction minus the ^{15}N atom % normal while ^{15}N atom % enrichment was the initial enrichment of N substrate in the

medium. ($V \text{ hr}^{-1}$) was then be multiplied by the particulate weight of nitrogen in the sample and divided by the number of cells in the sample to obtain the uptake rate ρ ($\text{pmol N cell}^{-1} \text{ hr}^{-1}$).

Swimming velocities were determined similar to the protocols of (McKay et al. 2007) using video of cells in a cuvette that provided a three dimensional environment (30 cm X 15 cm X 5 cm) after exposure to different shear rates. Similar to the uptake rate experiments, cells grown under low light and were exposed to shear rates for 30 minutes at night prior to analysis of swimming speeds. All analysis began within 2 mins of exposure. Analysis of cell paths was done using the Expert Vision 1 Motion Analysis package as well as the experimental set-up of the dissecting microscope and camera were like the single cuvette set-up and analysis described in Kamykowski et al. (1988). All samples were videotaped in the dark with a red measurement light illumination at 150 $\times$ magnification. Red light was used because *K. brevis* has no known taxis response to this wavelength as several *Gymnodinium* species stop responses peak at 280 nm and at 450–475 nm and show minimal response to red spectrum light (Forward and Davenport, 1970; Forward, 1970, 1973, 1974). Interpixel distance for the magnification applied (150 $\times$) was determined using the recorded image of a stage micrometer. The average swimming speed of each treatment is calculated by noting the average speed of each cell's path, and, subsequently, averaging the speed of all the cells' average path speeds. 50 cell paths were analyzed for the control (control), 20 paths for 0.22 s^{-1} , 30 paths for 0.54 s^{-1} , 30 paths for 1.1 s^{-1} , and 20 paths for a shear rate of 11.0 s^{-1} .

4.4 RESULTS

Current Profiles

In general the current velocities averaged about 0.05 m sec^{-1} . This translated to shear rates of less than 0.5 sec^{-1} through out the bottom 1.5 m of the water column. While these samples were taken predominantly from a low energy environment as would be hypothesized to support near-bottom *K. brevis* populations, periods existed of significant current shear within the bottom boundary layer. Figure 3.2 represents a 50 h period in which changes in velocity imply increases in shear with decreased distance to the sediment water interface. During this time a selected 2 hour average of shear rates indicate that shear rates increased from $0.1 \pm 0.02 \text{ s}^{-1}$ to $1.7 \pm 1.1 \text{ s}^{-1}$ 20 cm above the sediment-water interface (Figure 3.3). This is in contrast to more quiescent periods when the shear rates throughout the bottom boundary layer averaged $0.06 \pm 0.03 \text{ s}^{-1}$ (Figure 3.4).

Swimming speed after shear

Cells swimming speed seemed to decrease with increasing shear rate though the overall differences between treatments and the control were not significant ($F = 1.99$, $df = 149$, $p = 0.09$; Figure 3.5). Comparisons among the treatments indicated cells exposed to a shear rate of 1.1 s^{-1} swam 13 % slower relative to the control ($t = 2.3$, $df = 78$, $p = 0.03$) and 12 % slower than cells exposed to shear rates of 11.0 s^{-1} .

Exposure during shear

There was no difference between the control of zero shear and treatments exposed to different shear rates for the uptake of ammonium. There was, however, a difference in

the uptake of ammonium between different shear rates ($F = 6.8$, $df = 18$, $p < 0.01$; Figure 3.6). The ammonium uptake rates at a shear rate of 0.54 s^{-1} was 35 % greater than at 0.22 s^{-1} ($t=4.6$, $p < 0.01$), 1.1 s^{-1} ($t = 4.9$, $p < 0.01$), and 3.3 s^{-1} ($t = 4.8$, $p < 0.01$). For nitrate, there was no difference between the rates of nitrate uptake during exposure to different rates of shear (Figure 3.7). However, the uptake rates of nitrate in the zero shear treatment was 48 % to 60 % higher than the rates of nitrate uptake in the treatments exposed to shear ($F = 5.2$, $df = 18$, $p = 0.02$). Cells in the zero shear treatment took up nitrate 60 % faster than cells exposed to shear rates of 0.22 s^{-1} ($t = 4.2$, $p < 0.01$), 54 % faster than cells exposed to 0.54 s^{-1} ($t = 6.3$, $p < 0.01$), and 48 % faster than cells exposed to 1.1 s^{-1} ($t = 6.5$, $p < 0.01$) or 11.0 s^{-1} ($t = 4.2$, $p < 0.01$; Figure 3.7).

Cells that were not exposed to shear had 28 % higher rates of ammonium assimilation into protein than cells exposed to 0.22 s^{-1} ($df = 4$, $t = 4.0$, $p = 0.02$) and 18 % greater rates of assimilation into protein than cells exposed to 1.1 s^{-1} ($df = 4$, $t = 2.8$, $p = 0.04$; Figure 3.6). The assimilation rates of nitrate appeared to be particularly sensitive to shear exposure. Cells exposed to no shear had 60 % higher nitrate assimilation rates in protein than all the treatments when cells that were exposed to shear and nitrate ($t > 27$, $p < 0.01$ for all comparisons; Figure 3.7).

The data for the uptake of urea during exposure to shear was lost and not reported.

Exposure after shear

Unlike the differences in the rates of ammonium uptake during exposure to shear, there were not any significant differences in the rates of uptake or assimilation of ammonium after exposure to shear (Figure 3.6). The rates of nitrate uptake after shear

were, by contrast, different between treatments ($F = 4.0$, $df = 18$, $p = 0.04$; Figure 3.7). The rates of nitrate uptake when cells were exposed to zero shear (control) was not different from the shear rate treatment of 0.22 s^{-1} , but was twice as large as the rate of nitrate uptake of cells exposed to a shear rate of 0.54 s^{-1} ($t = 3.1$, $p = 0.01$) and 47 % larger than rate of uptake when exposed to a shear rate of 3.3 s^{-1} ($t = 3.0$, $p = 0.02$). The rate of nitrate assimilation after exposure to shear however was 51 % greater when exposed to zero shear than when exposed to a shear rate of 0.22 s^{-1} ($t = 3.2$, $p = 0.01$), 60 % greater than when exposed to a shear rate of 0.54 s^{-1} ($t = 3.8$, $p < 0.01$) or a shear rate of 3.3 s^{-1} ($t = 3.7$, $p < 0.01$), and 40 % greater than when exposed to a shear rate of 11.0 s^{-1} ($t = 2.5$, $p = 0.04$; Figure 3.7). There was between a 60 % and 75 % difference in the rates of urea uptake after exposure to shear cells exposed to zero shear and those exposed to the different shear rate treatments (Figure 3.8). For cells not exposed to shear, the rates of urea uptake were 64% greater than cells exposed to shear rates of 0.22 s^{-1} ($t = 6.4$, $p < 0.01$), 60% greater than cells exposed to shear rates of 0.54 s^{-1} ($t = 6.9$, $p < 0.01$) or 1.1 s^{-1} ($t = 8.0$, $p < 0.01$), 68 % greater than cells exposed to shear rates of 3.3 s^{-1} ($t = 9.1$, $p < 0.01$), and 75 % greater than cells exposed to shear rates of 11.0 s^{-1} ($t = 10.0$, $p < 0.01$). Additionally there was a decline in uptake rates as shear increased. Cells exposed to shear rates 11.0 s^{-1} exhibited rates of urea uptake between 47 % and 53 % less than the uptake rates when cells were exposed to lower shear like 0.22 s^{-1} ($t = 3.3$, $p = 0.01$), 0.54 s^{-1} ($t = 2.8$, $p = 0.02$), and 1.1 s^{-1} ($t = 2.9$, $p = 0.02$). The rates of urea assimilation into protein were greater in cells not exposed to shear than in cells exposed to shear prior to incubation. The assimilation rates of urea into protein of cells not exposed to shear were

between 48% and 78% greater than the treatments when cells were exposed to shear rates of 0.22 s^{-1} ($t = 2.4$, $p = 0.04$), 0.54 s^{-1} ($t = 2.5$, $p = 0.04$), 1.1 s^{-1} ($t = 3.4$, $p < 0.01$), 3.3 s^{-1} ($t = 2.9$, $p = 0.02$), 11.0 s^{-1} ($t = 3.9$, $p < 0.01$; Figure 3.8).

During vs. after shear

There were significant differences between the ammonium uptake and assimilation experiments exposing cells to shear during the ^{15}N incubations and prior to the ^{15}N incubations. Cells exposed to shear during the ^{15}N incubations exhibited 23% lower rates of ammonium uptake than cells that were exposed to shear prior to incubations ($F = 9.9$, $df = 18$, $p = 0.03$; Figure 3.6). Cells incubated with ^{15}N after exposure to shear demonstrated a 27% higher rate of ammonium assimilation relative to cells incubated to ^{15}N during shear ($F = 129.3$, $df = 18$, $p < 0.001$). There were no significant differences between the rates of nitrate uptake when cells were incubated with ^{15}N during exposure to shear and cells incubated with ^{15}N after exposure to shear. The rates of assimilation of nitrate into protein were, however, different between cells exposed to ^{15}N during shear and after shear ($F = 11.0$, $df = 11$, $p = 0.01$). Cells exposed to shear during ^{15}N incubation demonstrated 37% lower rates of assimilation of nitrate into protein than cells exposed to ^{15}N after exposure to shear ($t = 2.9$, $p = 0.03$; Figure 3.7).

Substrate differences

In cells that were not exposed to shear, the uptake rate of ammonium was, on average, 55% greater than the uptake rate of nitrate ($t = 5.1$, $df = 4$, $p < 0.01$; Figure 3.9). Similarly, cells that were not exposed to shear had assimilation rates of ammonium into

protein that were 55 % higher than the rates of nitrate assimilation into protein ($t = 8.3$, $df = 4$, $p < 0.01$). The differences in the rates of uptake and assimilation of ammonium and nitrate became more marked when cells were exposed to ^{15}N during shear ($F = 68$, $df = 11$, $p < 0.001$; Figure 3.9). Cells exposed to ^{15}N during shear, on average, had ammonium uptake rates that were 74 % higher than the rates of nitrate uptake ($t = 18$, $df = 11$, $p < 0.01$). Cells exposed to ^{15}N during shear had ammonium assimilation rates into protein that were 82 % higher than nitrate assimilation rates into protein ($t = 17.8$, $df = 11$, $p < 0.01$; Figure 3.9).

In experiments where cells were exposed to ^{15}N after exposure to shear, there were differences between substrates in both the uptake ($F = 57$, $df = 8$, $p < 0.001$) and assimilation ($F = 52$, $df = 8$, $p < 0.001$) of nitrogen. Overall, the uptake rates of ammonium were 60 % greater than the uptake rates of nitrate ($t = 10.2$, $p < 0.01$) and 48 % greater than the uptake rates of urea ($t = 7.8$, $p < 0.01$). In cells that were not exposed to shear (control), the ammonium assimilation rates into protein were 60 higher than the assimilation rates of nitrate into protein ($t = 10.2$, $p < 0.01$) and 30 % higher than the assimilation of urea into protein ($t = 4.3$, $p < 0.01$). The assimilation rates of urea into protein were 50 % greater than the assimilations rates of nitrate into protein in cells that were not exposed to shear ($t = 5.8$, $p < 0.01$). Again, when cells were exposed to shear prior to incubation with ^{15}N , the differences in the rates of uptake ($F = 139$, $df = 17$, $p < 0.001$) and rates of assimilation ($F = 215$, $df = 17$, $p < 0.001$; Figure 3.10) of the substrates were more greater. In cells exposed to shear prior to incubation with ^{15}N , the rates of ammonium uptake were 75 % higher than the rates of nitrate uptake ($t = 13.9$, $p <$

0.01) and 80 % higher than the rates of urea uptake ($t = 15$, $p < 0.01$). The assimilation rates of the different substrates likewise showed greater differences between the treatments that were exposed to shear before inoculation. The assimilation rates of ammonium into protein were 82 % greater than the assimilation rates of nitrate into protein ($t = 19.2$, $p < 0.01$) and 67 % greater than the assimilation rates of urea into protein ($t = 16.3$, $p < 0.01$). The average assimilation rates of urea into protein were 37 % greater than the assimilation rates of nitrate into protein ($t = 2.9$, $p = 0.01$).

4.5 DISCUSSION

Observations of near-bottom populations and laboratory experiments that examined near-bottom behavior and nutrient dynamics suggest that *K. brevis* may derive nutrients from the sediment-water interface either by maintaining position above the sediment or migrating into surficial porewaters where higher nutrient concentrations exist. Cell persistence near the sediment-water interface may be constrained by elevated levels of turbulence that typically occur towards water column boundaries such as the sediment-water interface (Sanford 1997). Turbulence may differentially affect cells depending on whether they reside for prolonged periods in an area of elevated turbulence in proximity to the sediment or whether they experience this turbulence in a transitory manner similar to what cells experience as they descend into surficial porewaters in order to acquire nutrients at night. As a first approximation of what cells might experience as they descend to the sediment-water interface, we measured the change in water velocities with depth in the benthic boundary layer on length scales approaching what might be expected for the smallest eddies (Osborn 1978). We observed that water velocities are characteristically low in the study region in agreement with previous studies on the Florida shelf (He & Weisberg 2002). While the calculated shear rates near the bottom in this region are generally low ($0.06 \pm 0.03 \text{ s}^{-1}$), they are higher than those typically reported for the water column (MacKenzie & Leggett 1993, Mann & Lazier 2006). Additionally there are distinct periods where near-bottom shear rates are considerably higher (Figure 3.3) and may begin to influence dinoflagellate ecology.

K. brevis swims approximately 1 m hr^{-1} and has been observed to descend in order to acquire nutrients (Sinclair et al. 2006b, McKay et al. 2007). Cells descending into the sediment may experience a transitory exposure to elevated shear rates. Similar to other dinoflagellates (Tynan 1993, Karp-Boss et al. 2000), *K. brevis* seems to decrease swimming velocities slightly after exposure to increasing levels of water shear as applied in this near-bottom simulation though treatment results were not appreciably different over the range of applied shear (Figure 3.5). Directionality of cell paths was not determined in this study, but other dinoflagellates have been found to be able to reorient themselves under shear rates as high as 1 s^{-1} (Karp-Boss et al. 2000). This suggests that cells, even as they pass through regions of enhanced shear that begin to occur about 20 cm above sediment-water interface, will still be capable of orienting themselves and directed swimming (Karp-Boss et al. 2000). The fact that swimming velocities were maintained at higher shear rates (10 s^{-1}) suggests that *K. brevis* may be able to migrate through bottom boundary layers during periods of elevated shear in order to reach the sediment-water interface.

Alternatively, if *K. brevis* maintains position above the sediment in order to utilize the flux of nutrients out of the sediment, cells would increase the duration of exposure to elevated shear rates. Dinoflagellates are often thought to be sensitive to elevated levels of turbulence (Peters & Marrase 2000) though studies show a wide range of responses and recovery rates (Berdalet 1992, Sullivan & Swift 2003, Stoecker et al. 2006). One explanation is that dinoflagellate sensitivity depends on the physiological state of the cell (Juhl et al. 2000, Juhl & Latz 2002). The physiological state of *K. brevis* has been shown

to change with time of day, as well as with prior light and nutrient exposure which may change depending on the diel vertical migration of *K. brevis* (Van Dolah & Leighfield 1999, Liu et al. 2001b, Schaeffer 2006, Sinclair et al. 2006a). Descending cells are thought to be motivated by cellular state and particularly by low levels of cellular nitrogen (Liu et al. 2001a, Sinclair & Kamykowski 2008a). The cells in these experiments were deprived of N for at least 12 hours simulating a 12 hour ascent into an oligotrophic water column. Cells in these experiments may therefore respond similarly upon introduction to elevated shear rates as cells descending into regions of increased turbulence near the sediment-water interface. If cells are nutrient stressed after migrating into an oligotrophic water column, their sensitivity to elevated shear, encountered in close proximity to the sediment-water interface, may increase. How these cells respond to increasing levels of shear, and how they specifically take up and assimilate nutrients may be of critical importance to understanding the ecology of near-bottom populations of *K. brevis*.

The effect of exposure to fluid shear on the nitrogen physiology of *K. brevis* appears to be complicated depending on a combination of shear rate, substrate type, and physiological response. The uptake of ammonium during exposure to shear did not increase compared to the control treatments (Figure 3.6). This is contrary to models suggesting that increasing shear decreases the diffusive boundary layer around cells and increases the diffusive flux of nutrients to a cell (Pasciak & Gavis 1974). Theoretically shear rates increase nutrient delivery by decreasing the thickness of the diffusive boundary layer (δ_{eff} ; DBL) thereby decreasing the time a nutrient diffuses through the

DBL. The average shear rates experienced by *K. brevis* migrating between low shear rates in the water column and elevated shear rates near the sediment were used to estimate potential changes in a 20 µm cell's DBL using the equation:

$$Sh = \frac{r_0}{\delta_{eff}} = 1$$

where the Sherwood number

$$(Sh) = 1 + 0.29x(Pe)^{\frac{1}{2}}$$

since the Reynolds number (Re) << 1 for *K. brevis* and the Pectlet number (Pe) < 0.01 (Ploug et al. 1999). An Alternative calculation for the Sherwood number is $Sh = 1 + 45Re^{0.57}$

(Kiorboe 1993). As a cell descends through the water column, the increase in (average) shear rates a cell experiences, from 0.016 sec⁻¹ to 0.2 sec⁻¹, decreases the DBL by 7%. Under strong currents when near bottom shear rates approach 1 sec⁻¹, the DBL may decrease as much as 20%. Increasing shear rates may theoretically interact with physiological state and ambient nutrient environments to optimize uptake in near bottom environments.

We did not observe the predicted increase in ammonium uptake by *K. brevis* as expected from modeling studies (Table 3.1). One explanation is that enhanced shear rates may deform carrier proteins that are likely involved in ammonium transport into the cell (Wheeler 1980, Balch 1986, Mader et al. 2006, Vitkova et al. 2007). This hypothesis is consistent with other reports that demonstrate non-attached dinoflagellates respond to mechanical stress (Maldonado & Latz 2007). Cellular deformation that may occur during

exposure to shear stress may also increase the “leakiness” of cells thereby offsetting the potential increases in nutrient delivery. The observed assimilation of ammonium into protein decreased with shear rate during exposure to shear. This implies that while shear stress may directly impact nutrient delivery, stress responses may continue to affect down-stream reactions such as the incorporation of N into cellular protein.

The difference in uptake and assimilation rates during shear exposure is more striking when examining substrates that require more energy in order to incorporate nitrogen into cellular protein. The uptake of nitrate decreased relative to the control and the assimilation of this nitrate was less than 60 % of the control. The difference between the decrease in uptake and assimilation of nitrate vs. ammonium may be due to how cellular stress influences metabolic pathways. Nocturnal uptake and assimilation of nitrate requires more energy than does the uptake of ammonium and is expected to be different especially in low light adapted cells (MacIsaac & Dugdale 1972, Flynn 1998, Sinclair & Kamykowski 2008b). Cells stressed by an increasing fluid shear may further decrease costly metabolic processes similar to cells decreasing growth rates in response to turbulence (Berdalet et al. 2007). Thus increased shear rates may have a synergistic impact on the morphology and the physiology of *K. brevis*.

Previous evidence suggests that as the intermittency of shear increases, from 2.5 hours to 0.25 hours, the growth rates of dinoflagellates declines (Gibson & Thomas 1995b). The experiments performed here were similar to the more intermittent regimes used by Gibson & Thomas (1995b) and may indicate that shorter temporal intermittency is stressful to *K. brevis*. While the present Couette chamber study did not examine shear

rates representative of the water column (0.02 s^{-1}), the shear rates used were more representative of what cells encounter in close proximity to the sediment-water interface (Figure 3.3). Modeling the diffusive flux of ammonium or nitrate out of the sediment suggests that cells must be within 10 to 20 μm of the sediment to derive any appreciable benefit from elevated nutrient concentrations (Sinclair & Kamykowski 2007). Unfortunately, due to a 2 cm profiling cells and bottom interference, potential shear rates derived from the current measurements are difficult to extrapolate to similar distances above sediment-water interface. Using the formulation du/dz , shear rates would increase from 0.5 s^{-1} to greater than 40 s^{-1} by substituting length scales in the μm range near the sediment-water interface (clearly not realistic). Due to bottom roughness, modeling the decay in water velocity near the sediment-water interface is also difficult (Mann & Lazier 2006). Nevertheless, the ability of cells to take up nutrients when exposed to shear rates representative of 0.5 m above the sediment may still depend on the type of nutrients the flux out of the sediment as well as the duration of cell exposure to elevated shear rates.

To simulate cells migrating through a boundary layer of 0.5 m before entering sediment porewaters, cells were exposed to shear for 30 minutes before being removed from the enhanced shear rates and exposed to nutrients. We did not observe any differences in the uptake or assimilation rates of ammonium after exposure to shear relative to the control. The 24 % increase in uptake of ammonium after exposure to shear relative to uptake during shear may be related to the cell recovery from the deformation experienced during shear exposure (Vitkova et al. 2007). The 27 % increase in cell

assimilation may result from an additive effect of the decreasing the deformation and recovery of metabolic pathways incorporating ammonium into cellular protein.

The rates of nitrate uptake after exposure to shear decreased by 50 % in two treatments relative to cells not exposed to shear. Because of this, the overall rate of nocturnal uptake of nitrate after exposure to shear did not differ from rates of nitrate uptake during exposure to shear. The rates of nitrate assimilation into protein after exposure to shear, by contrast, were 36% higher than the rates of nitrate assimilation into protein during exposure to shear. Thus, while recovery occurred, the extent of this recovery is lower compared to the recovery of rates observed in the assimilation of ammonium. This suggests that recovery may be linked to substrate type. The effects of shear exposure on nitrate uptake during and after exposure may be less pronounced due to the decreased activity of nitrate assimilation at night.

The uptake of urea after exposure to shear decreased from 60 % - 75 % relative to the control. There was also an average 50 % decrease in uptake by cells previously exposed to elevated shear rates of 11.0 s^{-1} relative to lower shear rates of 0.22 s^{-1} and 0.54 s^{-1} . Reflective of the patterns in urea uptake, *K. brevis* decreased its overall nocturnal assimilation rates of urea 48 % - 78 % relative to the control. The uptake of urea has a passive transport pathway more similar to ammonium uptake as well as an active transport pathway more analogous to nitrate (Solomon 2006). These patterns in uptake and assimilation after exposure to elevated shear are more consistent with the uptake and assimilation of nitrate rather than ammonium. These similarities suggest that transitory

exposure to elevated fluid shear may have a larger affect on more complicated biochemical pathways and inhibit short-term nutrient uptake.

If shear rates do indeed affect metabolic pathways differently, one would expect different recovery rates of cell uptake and assimilation after exposure to shear. Cells exposed to ammonium increased their uptake rates by 24% after exposure to shear relative to those rates measured during shear. Cells assimilated ammonium 27 % faster after shear exposure than during shear exposure. Cells exposed to nitrate, by contrast increased their uptake rates by 28 % and assimilation rates by 36 % after shear exposure relative to during exposure to shear. The 10% difference between the two changes in assimilation rates suggests exposure during shear had a greater effect on the assimilation for nitrate, a more complicated metabolic pathway, than on the assimilation of ammonium.

The migration of *K. brevis* through the benthic boundary layer into surficial porewaters potentially exposes cells to a variety of nitrogen substrates (Marinelli et al. 1998, Jahnke et al. 2005). The utilization of different substrates detected in the porewaters may determine how *K. brevis* meets its nitrogen requirements when nitrogen is insufficient in the water column. The nocturnal uptake of the ammonium control was 60% greater than the nitrate control and 40 % greater than the urea control. The assimilation patterns of the control groups were similar with cells assimilating 82 % more ammonium than nitrate and 30 % more than urea at night. The urea control, exhibited a relatively high efficiency of nocturnal assimilation and was 30 % greater than nitrate control. Exposure to shear prior to uptake rates seems to strengthen an already strong

trend in the ability to utilize different nutrient substrates at the sediment-water interface observed in other studies (Sinclair & Kamkowski, *submitted*). *K. brevis* utilized ammonium more proficiently than urea which was utilized more proficiently than nitrate.

In field surveys, ammonium and urea were often found in higher concentrations than nitrate in the porewater (Sinclair & Kamykowski 2008b). While nutrient interactions are complicated (Dortch & Conway 1984) ammonium generally decreases the uptake of both nitrate (Lomas & Glibert 1999) and urea (Horrigan & McCarthy 1982). Likewise urea generally decreases the rate of nitrate uptake (Cochlan & Harrison 1991). If multiple nutrient sources were present in the porewater, the enhanced affect of shear exposure on the proficiency of *K. brevis* to utilize the different substrates may have important implications for how the nutrient kinetics and quotas of these near-bottom populations are modeled.

One example of how these uptake differences may contribute to the environmental exposure of migrating near-bottom populations and ultimately cell and population growth is illustrated in the model described in Sinclair & Kamykowski (2006). This work suggests that near-bottom populations exposed to elevated shear rates near the sediment-water interface grow more slowly, with a 7 day division cycle in contrast to a 3 day division cycle, and have lower uptake rates than anticipated by Sinclair & Kamykowski (2006). The different capabilities of cells to assimilate nitrogen (in this case ammonium) under different hydrographic conditions that cells may encounter near the sediment-water interface result in different levels of light to which cells are exposed to over a diel vertical migration (Figure 3.11). In deeper water columns

such as 60 m, cells that have not been exposed to shear during the nitrogen assimilation are exposed to 12 % more light per day than cells assimilating N after exposure to shear and 26 % more light than cells assimilating nitrogen during exposure to shear. Cells that migrate into the surficial porewaters, and therefore assimilate nutrients after exposure to shear, are exposed to 15 % more light per day than cells maintaining position above the sediment (assuming constant nutrient concentrations in both locations.) This difference becomes more striking in shallower water columns as attenuation of light increases and migration time into the water column becomes more important. Cells with assimilation rates like those not exposed to any shear are exposed to 20 % more light than those cells that elect to migrate into the sediment and 40 % more than those cells that are continuously exposed to shear during nocturnal uptake and assimilation. Cells that migrate into the sediment are exposed to 24 % more light per day than cells that maintain position above the sediment, and have decreased assimilation rates. This simple model illustrates the potential importance of exposure to elevated rates of fluid shear in the ecology of near-bottom populations of *K. brevis*.

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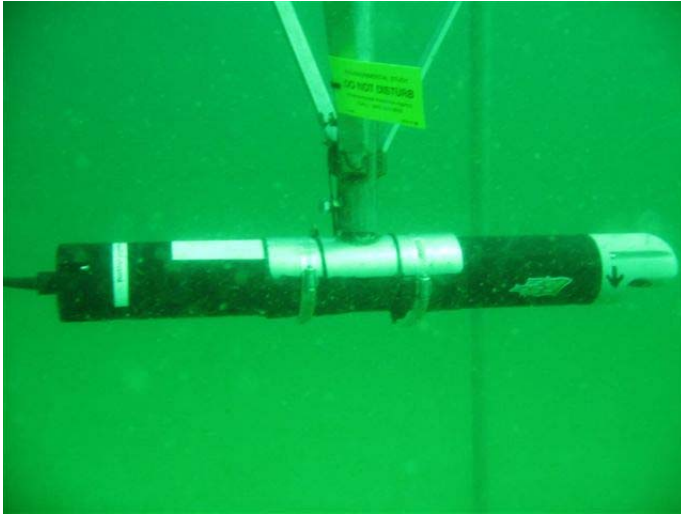
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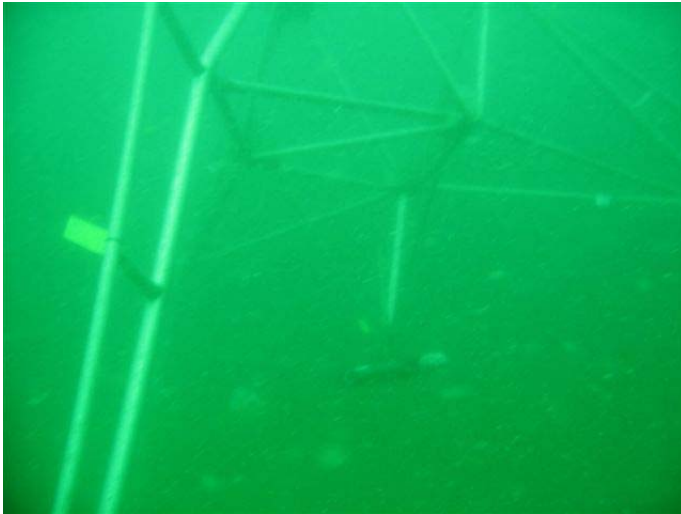
Table 4.1 Changes in nutrient flux to cell with increasing shear rates assuming constant nutrient concentration.

Influence of shear rate on nutrient delivery to the surface of a cell

Change in Shear Rate	% Decrease in Diffusive Boundary Layer	% Increase of Nitrate Flux to Cell
.01 to .2	6%	7%
.2 to 1	6%	25%
.01 to 1	12%	30%



a



b

Figure 4.1 A downward facing AquaDopp (a) attached to a tripod (b) was used to characterize the current velocities in the bottom 1.50 m of the water column.

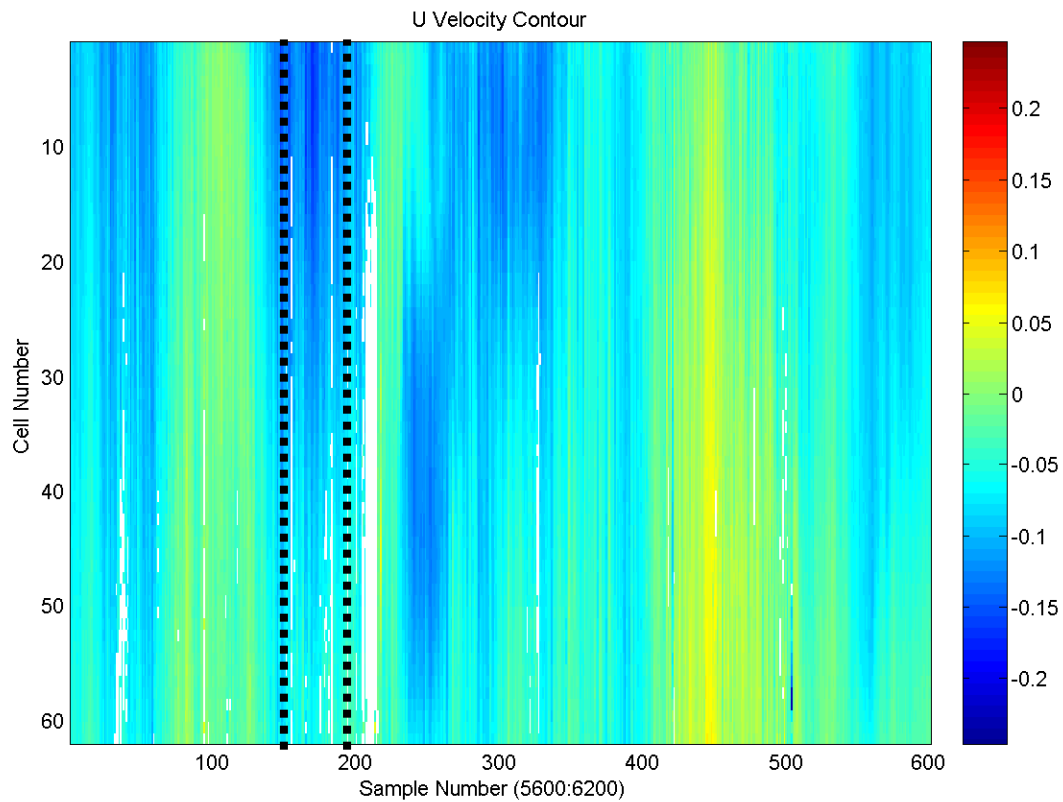


Figure 4.2 Fifty hour subsection of one month of current velocity profiles near the sediment-water interface. Samples (sample number) were taken in 5 minute increments represented on the x-axis. Height above the sediment-water interface is on the y-axis. Readings were taken every 2 cm (= 1 cell). Cell 0 is closest to the AquaDopp sensors and cell 60 closest to the sediment-water interface. The dotted lines roughly demarcates a 2 hour period with increasing water shear toward the sediment water interface illustrated in Figure 4.3.

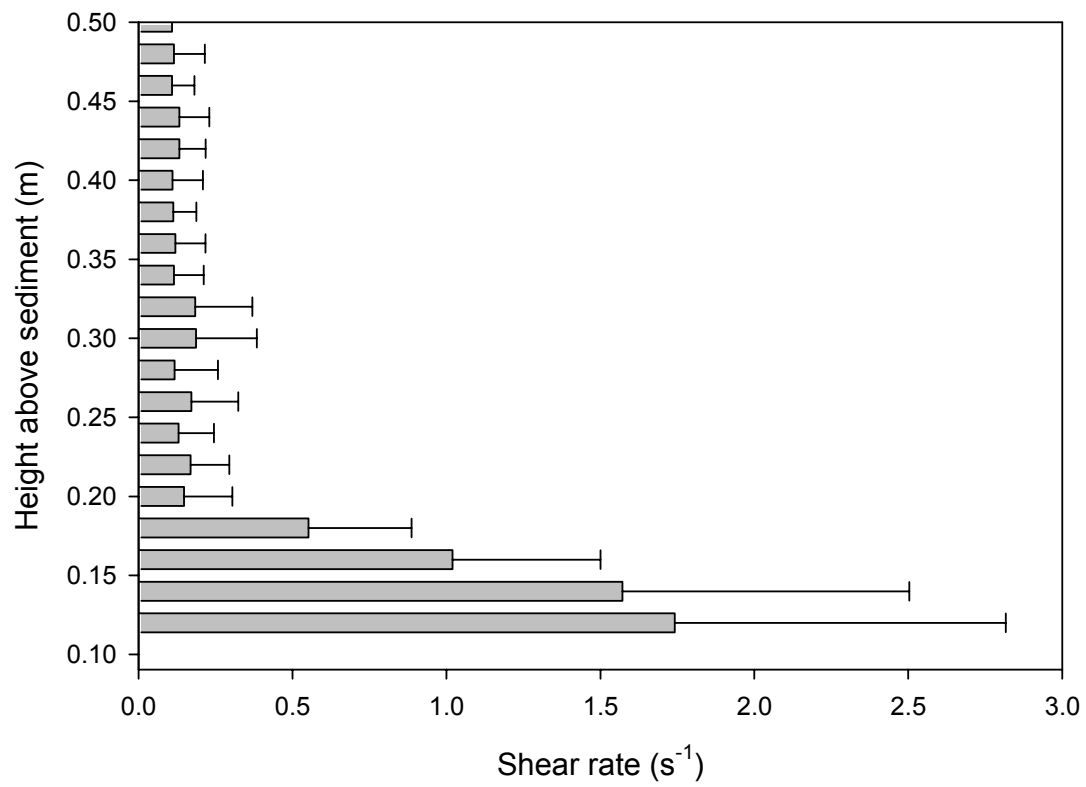


Figure 4.3 This is a representative 2 hour average of shear rate (du/dz) vs. height above the sediment (m), calculated from the contour of water velocity in Figure 4.2.

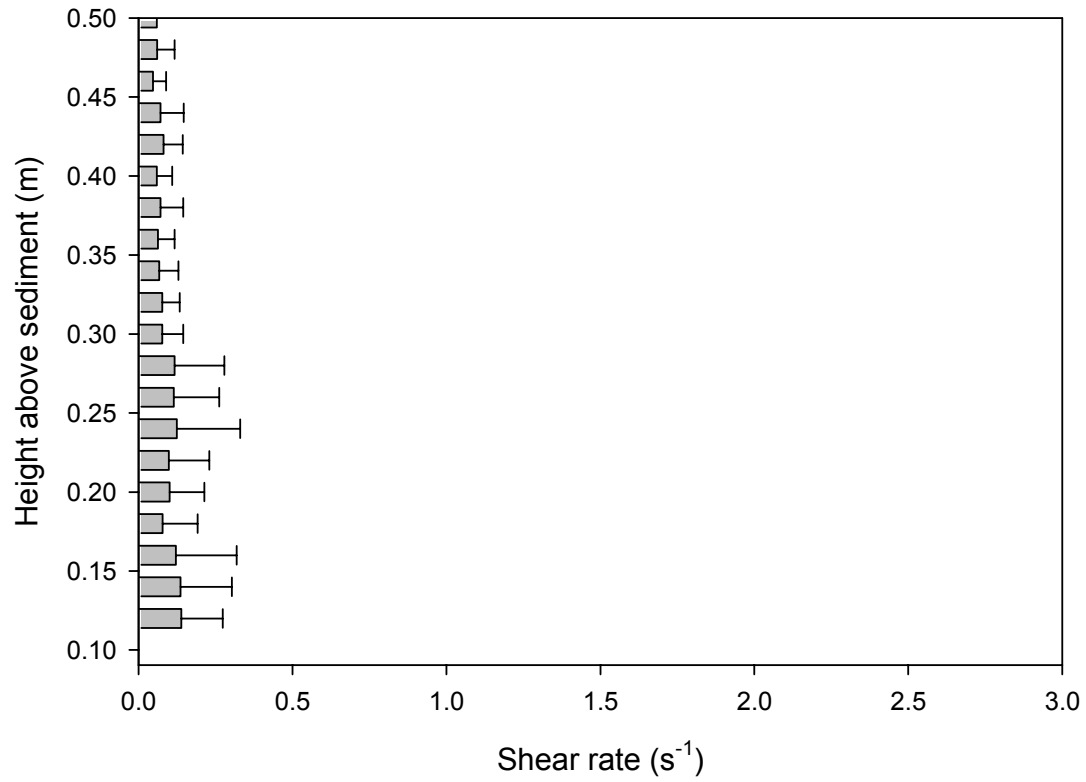


Figure 4.4 Two hour average of shear rate (du/dz^{-1}) vs height above the sediment (m) during a period of decreased shear 3 days prior to the contour in Figure 4.2.

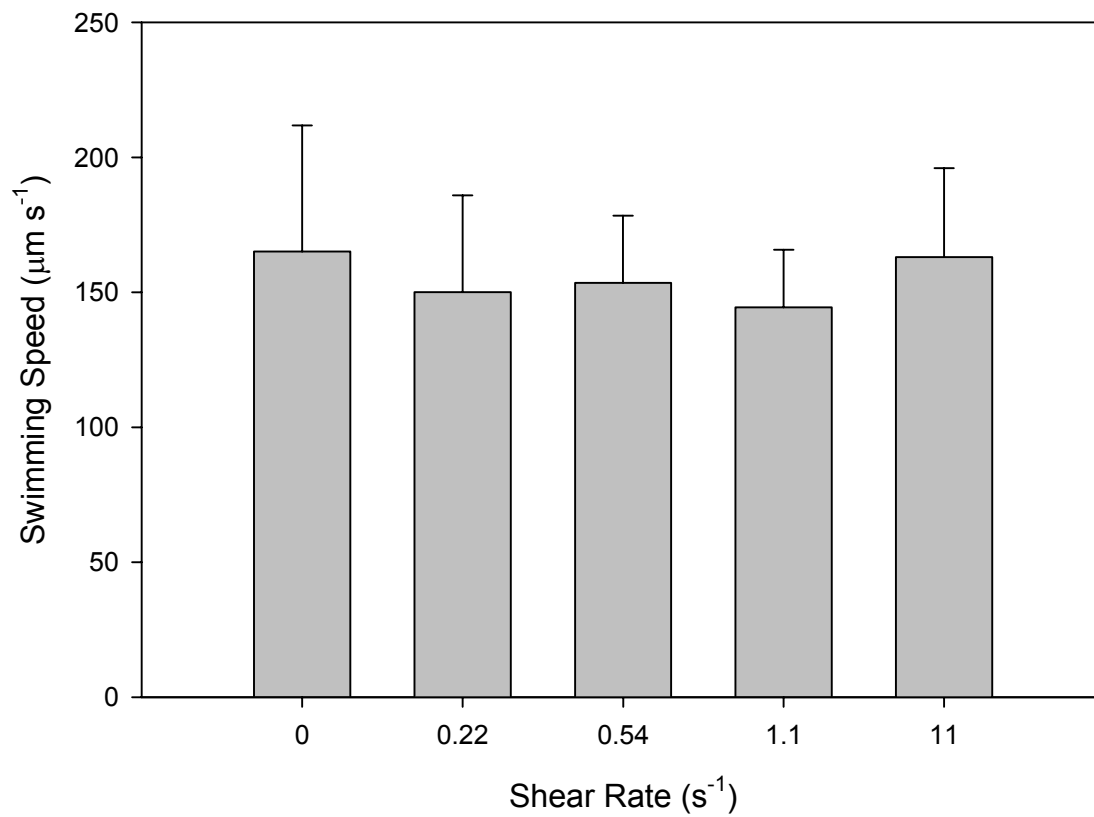


Figure 4.5 Swimming speed vs. shear rate. Swimming speed was assessed within 2 minutes after exposure to shear rate.

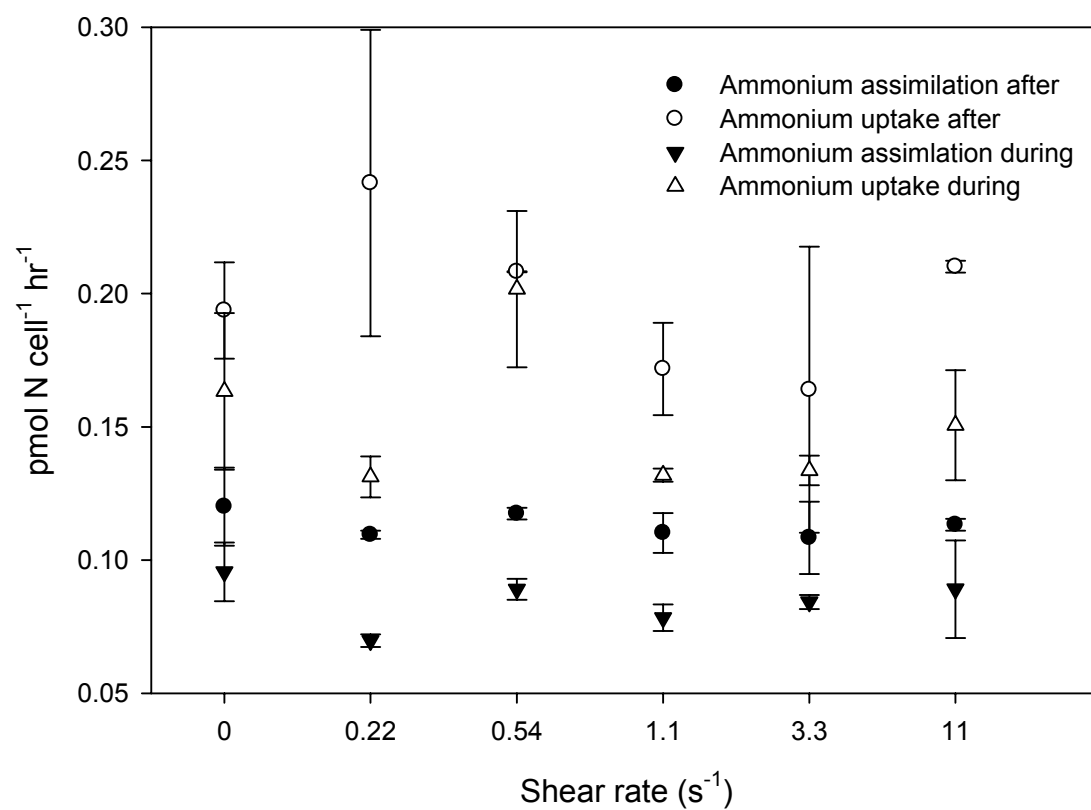


Figure 4.6 Uptake and assimilation of ammonium during exposure to shear and after exposure to shear. Uptake and assimilation rate is on the y-axis in $\text{pmol N cell}^{-1} \text{ hr}^{-1}$ and shear rate (s^{-1}) is on the x-axis.

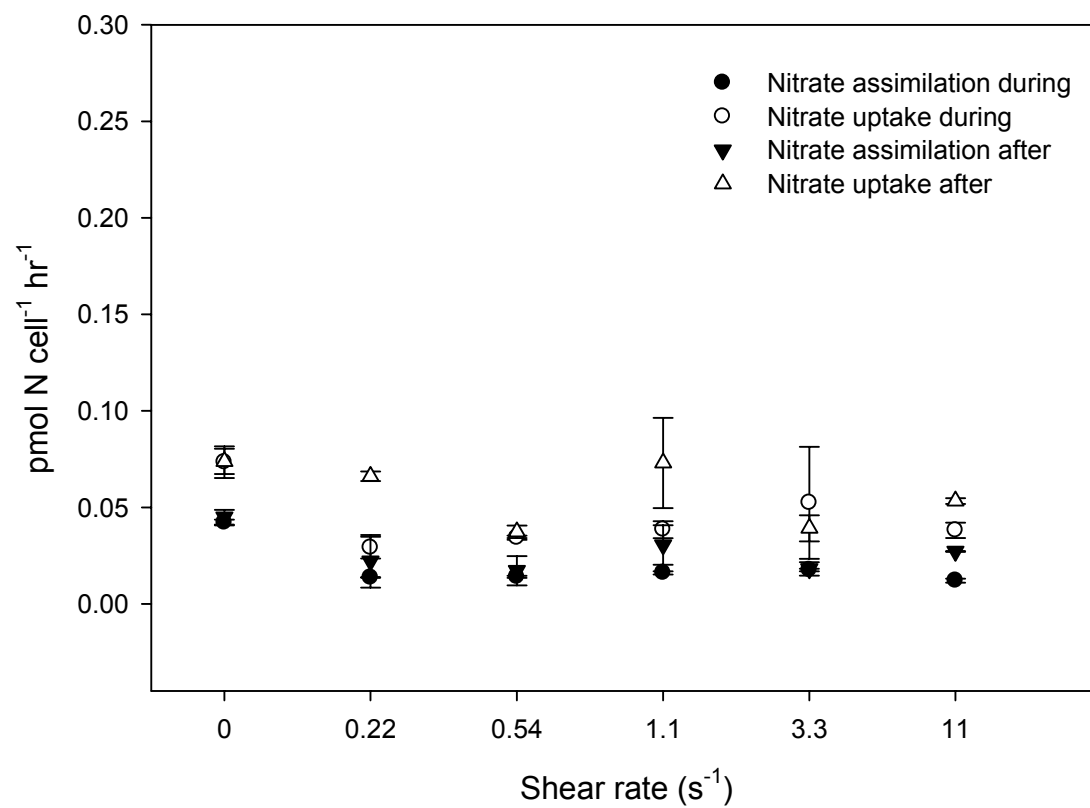


Figure 4.7 Uptake and assimilation of nitrate during exposure to shear and after exposure to shear. Uptake and assimilation rate is on the y-axis in $\text{pmol N cell}^{-1} \text{ hr}^{-1}$ and shear rate (s^{-1}) is on the x-axis.

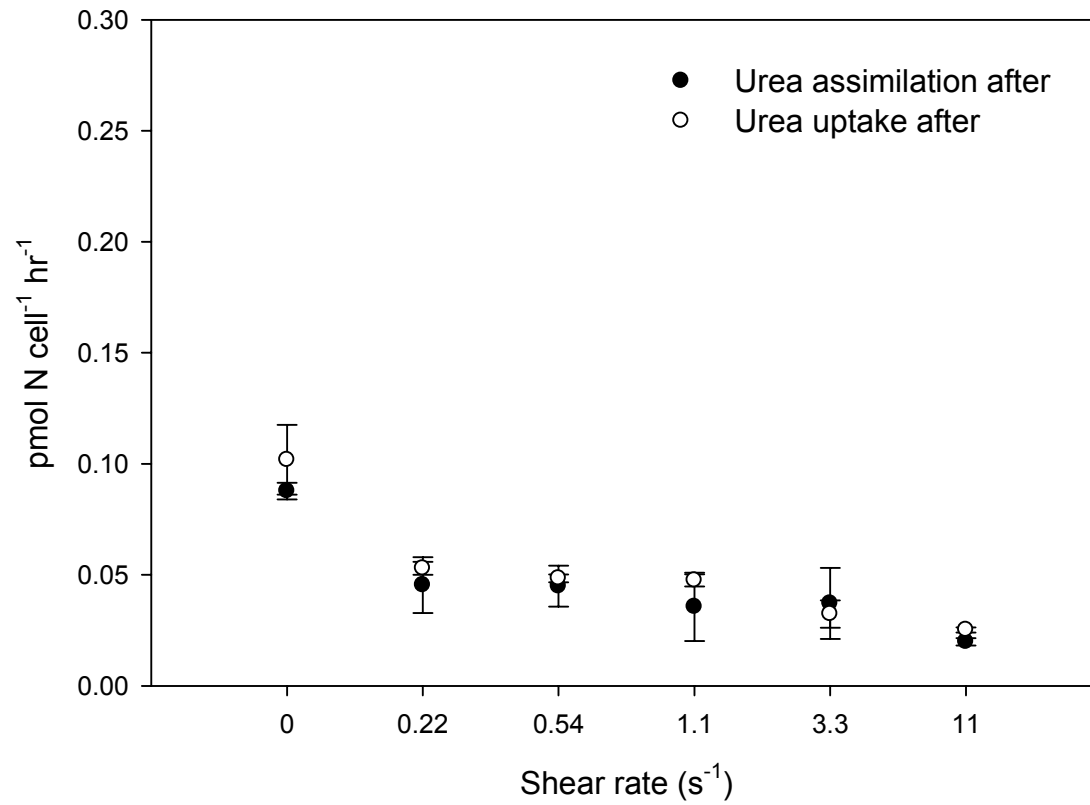


Figure 4.8 Uptake and Assimilation of urea after exposure to shear. Urea uptake decreases after exposure to higher shear rates. Uptake and assimilation rate is on the y-axis in pmol N cell⁻¹ hr⁻¹ and shear rate (s⁻¹) is on the x-axis.

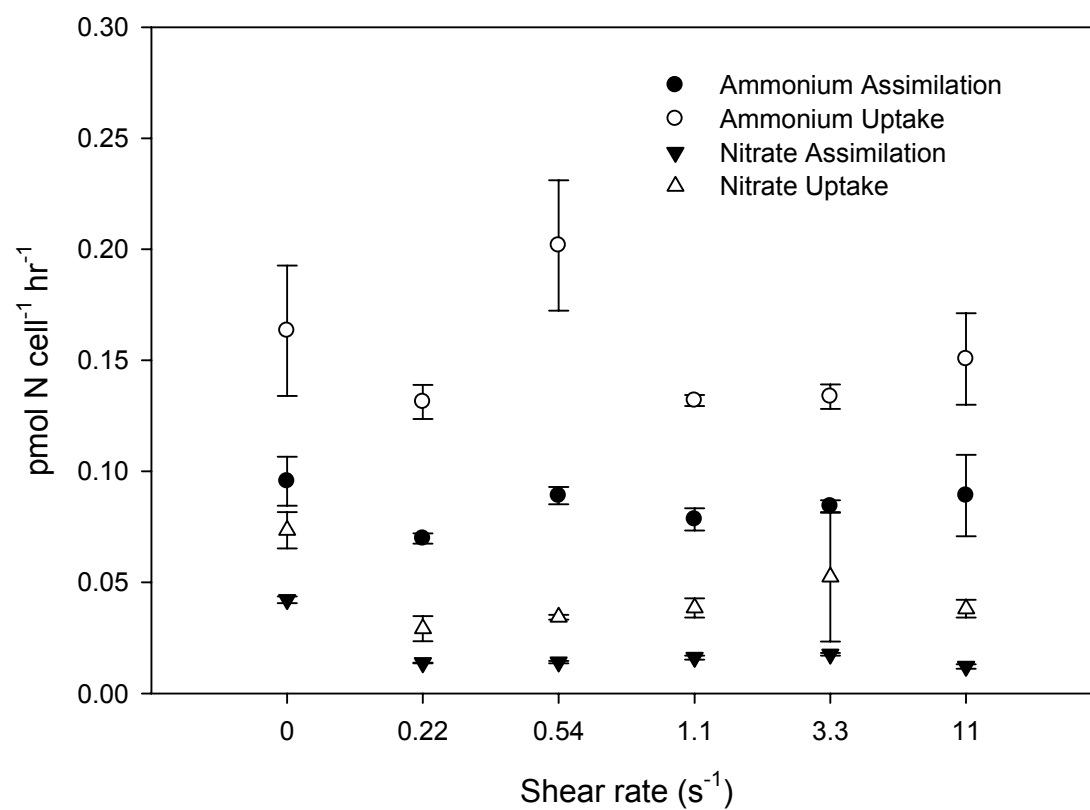


Figure 4.9 Uptake and assimilation of ammonium and nitrate during shear. Uptake and assimilation rate is on the y-axis in $\text{pmol N cell}^{-1} \text{ hr}^{-1}$ and shear rate (s^{-1}) is on the x-axis.

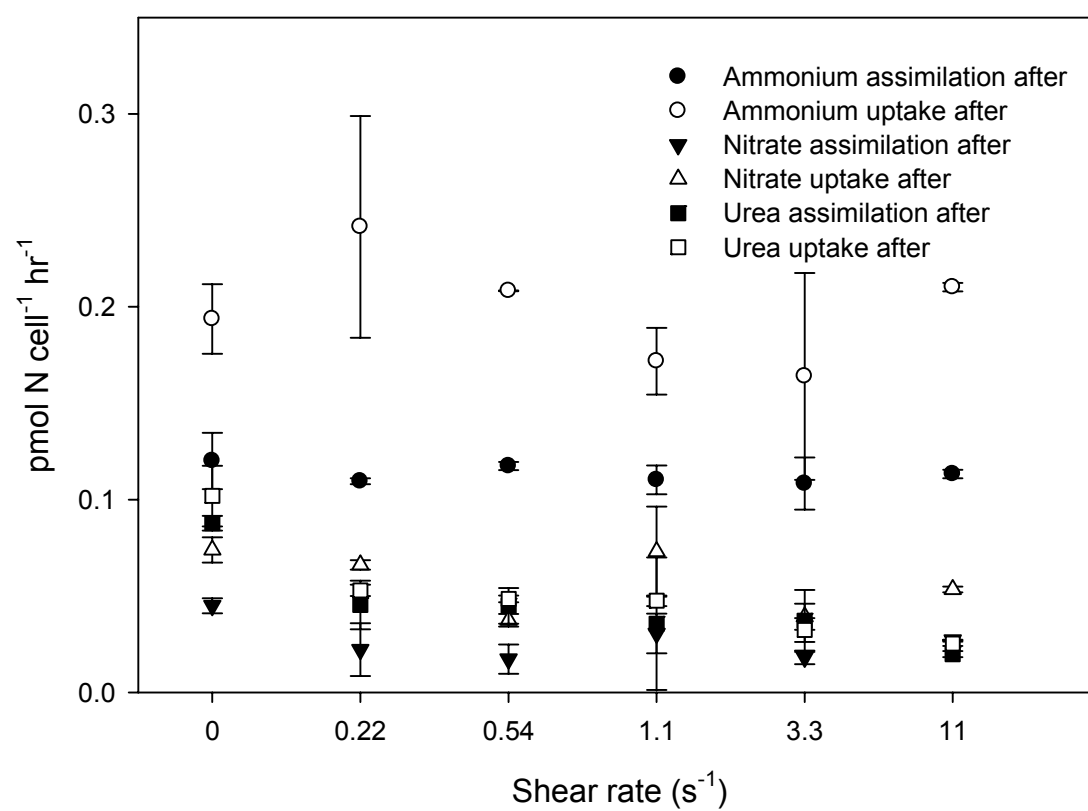


Figure 4.10 Uptake and assimilation of ammonium, nitrate, and urea after exposure to shear. Uptake and assimilation rate is on the y-axis in $\text{pmol N cell}^{-1} \text{ hr}^{-1}$ and shear rate (s^{-1}) is on the x-axis.

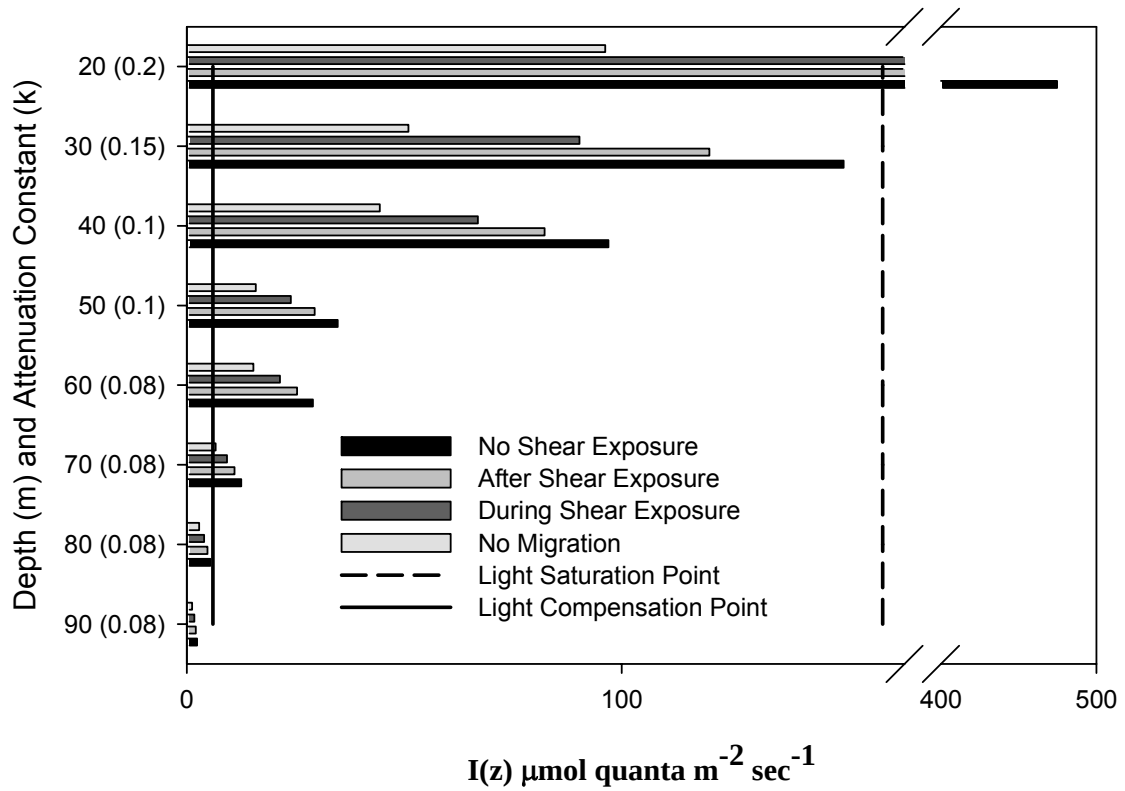


Figure 4.11 Mean light exposure of migrating cells with different nocturnal uptake rates. Nocturnal uptake rate directly impacts time spent in proximity to the sediment-water interface and the consequent ascent. The vertical lines denote the points at which cells are compensated for growth (solid line) and the point at which growth is saturated (dashed line). The integrated light exposure is on the x-axis in $\mu\text{mol quanta m}^{-2} \text{sec}^{-1}$ and depth in meters is on the y-axis.

CHAPTER 5. CONCLUSIONS AND FUTURE DIRECTIONS

5.1 GROWTH, UPTAKE, AND ASSIMILATION OF AMMONIUM, NITRATE, AND UREA BY THREE STRAINS OF *KARENIA BREVIS*

The nitrogen nutrition of *Karenia brevis* is poorly understood. Multiple sources of nitrogen may be accessible to populations so understanding how *K. brevis* takes up, assimilates, and grows on a wide variety of nitrogen substrates is critically important for understanding its ecology in oligotrophic water columns of the west Florida Shelf. The growth and uptake of ammonium, nitrate and urea were performed under low light ($30 \mu\text{mol quanta m}^{-2} \text{ sec}^{-1}$) in order to simulate the near-bottom environment where populations of *K. brevis* have been observed. *K. brevis* demonstrated growth rates lower than typically reported and demonstrated a diverse ability to utilize the different nitrogen substrates. During the day *K. brevis* demonstrated similar capability in the uptake of all substrates. The assimilation of ammonium and urea during the day were however greater than the assimilation of nitrate. At night, the uptake of ammonium was greater than urea and the uptake of urea was greater than that of nitrate. Nocturnal assimilation of both ammonium and urea were greater than nitrate. If near-bottom populations are in proximity to the sediment at night, cells may derive significant advantage from accessing the reduced and organic nutrients present in the sediment pore-waters.

While this study indicated there was potential of broad variability in uptake capacities between strains, replication was clearly insufficient to draw robust conclusions. Additional work remains in addressing the inter-specific variability of different strains of *K. brevis* especially since they may influence how population growth is modeled in

different regions in the Gulf of Mexico (Liu et al. 2001, Villareal et al. 2001, Janowitz & Kamykowski 2006, Hetland & Campbell 2007). *K. brevis* encounters changing light and nutrient regimes both over its diel vertical migration (Sinclair et al. 2006) as well as during transport across the shelf (Janowitz & Kamykowski 2006). Therefore examining nutrient physiology over a wider range of environmental exposures such as higher light is critical to more complete understanding of the nutrient physiology of *K. brevis*.

Ultimately, additional work on field populations is needed. Some work has been done but has focused primarily on surface populations (Mulholland et al. 2006). Comparative physiological experiments with both near-bottom populations as well as surface populations would provide valuable insight into how both surface and near-bottom populations are fulfilling their nutrient requirements and may contribute to understanding how blooms develop. *K. brevis* has demonstrated a wide variety of potential nutritional strategies that remain unexplored especially in how they impact growth (Jeong et al. 2005, Glibert et al. 2008). Nitrogen in these studies may be thought of as a model nutrient, and one potential limiting nutrient for which to examine the nutrient physiology of *K. brevis*. Other candidates depending on ambient nutrient environment also include more complex forms of N (Bronk et al. 2006), perhaps from *Trichodesmium* (Mulholland et al. 2006), phosphorus (Vargo 1990, Shanley & Vargo 1993), iron (Walsh & Steidinger 2001) and other assorted micro-nutrients (Eppley et al. 1971). Since one or more nutrients may be limiting to phytoplankton growth (Saito et al. 2008), and nutrient interaction may differentially affect uptake (Timmermans et al. 1994, Lomas & Glibert 1999), future work should also focus on nutrient combinations.

Ultimately the ability to culture *K. brevis* in axenic cultures will undoubtedly aid in isolating how *K. brevis* meets its nutritional needs in oligotrophic water columns (Skelton et al. 2007).

5.2 BENTHIC-PELAGIC COUPLING IN SEDIMENT ASSOCIATED POPULATIONS OF *KARENIA BREVIS*

In situ observations of aggregations of *Karenia brevis* near the sediment-water interface and laboratory videos of cells in close proximity to the sediment suggest that cells derive nutrients from the sediment. Experiments tested *K. brevis*' ability to migrate into the sediment using chambers divided by a 100 μm mesh overlain with a thin layer of sediment. Since the diel vertical migration of *K. brevis* typically displays a nocturnal descent, experiments tested migration response at night in response to sub-sediment nutrient sources. The experiments suggest that while the sediment affects the progress of descending cells, migration occurs through thin layers of sediment and increases in response to elevated nutrient concentrations below the sediment. Since all cells found below the sediment had significantly higher C/N ratios than those remaining above the sediment, migration appears related to a cell's internal biochemical state.

These simple experiments support a dynamic interaction of cells with the sediment. One of the limitations of the experimental design was that it was necessary to sacrifice a chamber every time cells were sampled. Therefore questions pertaining to cell and population growth or finer metabolic processes such as enzyme activity were difficult to achieve due to sample volume. Slight modification of the chamber design such as increasing the chamber size and adding additional sampling ports may provide a platform to perform more complicated experiments. Future work involving larger chambers on longer time scales may allow examination of cell and population growth as well as enzyme activity and uptake rates. Additionally the apparent separation of cells above and below the sediment barrier yielded striking biochemical patterns that may permit further

analysis of genetic control of the physiology of *K. brevis* by analyzing gene activity (Van Dolah, *personal communication*).

The near-bottom niche of *K. brevis* has been hypothesized to sustain offshore populations in oligotrophic water columns. As populations are advected onshore, populations are thought to encounter enhanced nutrients associated with runoff (Brand & Compton 2007). Interesting questions remain however if these near-shore populations also access regenerated nutrients in the sediment and help maintain populations like those that persisted for the better part of 2005.

5.3 INFLUENCE OF FLUID SHEAR ON THE GROWTH AND NUTRIENT UPTAKE OF *KARENIA BREVIS*

In order to begin to define the hydrographic environment *K. brevis* encounters near the sediment-water interface, a high resolution current profiler was used to examine the water velocities and potential shear rates in the benthic boundary layer. Shear rates in the benthic boundary layer varied from 0.02 s^{-1} to close to 3 s^{-1} over 2 hour time scales. While these shear rates were not taken on length scales similar to *K. brevis* important patterns can be inferred from the location and magnitude of the shear rates. The fact that velocity fluctuations intensified near the sediment-water interface suggests that *K. brevis* will encounter increased turbulence as it approaches the sediment-water interface. The effect of different defined shear rates on the uptake and assimilation of ammonium, nitrate and, urea was determined using Couette flow chambers. The experiments simulated either 1) cells residing above the sediment-water interface and utilizing nutrients *during* exposure to shear or, 2) cells accessing the sediment porewaters and utilizing nutrients *after* exposure to shear. The uptake rate of ammonium was unaffected while the uptake of nitrate decreased during exposure to shear. Assimilation rates of both ammonium and nitrate decreased during cell exposure to shear. There was no decrease in either the uptake or the assimilation rates of ammonium after exposure to shear while cells decreased rates of uptake and assimilation for both nitrate and urea. The uptake and assimilation rates of both ammonium and nitrate were higher in cells after exposure to shear than the uptake and assimilation during exposure to shear implying that cells exhibited morphological or physiological recovery during the temporal constraints of these experiments.

Obtaining meaningful experimental results depends on the ability to scale the energetic condition to those observed in the ocean. In this way one may better determine the role of turbulence on increasing or decreasing different biological rates, such as growth, nutrient uptake, or swimming rates (Peters & Marrase 2000). While the temporal scale of shear exposure was on the order of those used in other studies (Gibson & Thomas 1995), shear fields that influence small phytoplankton change randomly in both direction and intensity (Mann & Lazier 2006). Improvements to this work could be to use variable speed motors in order to start to randomize the duration and magnitude of shear to which cells are exposed to been to approximate the spectrum of shear cells may encounter in nature (Peters & Redondo 1997).

If *K. brevis* is in proximity to the sediment for extended periods of time, it is critical to understand how the potentially elevated shear rates that cells encounter affect growth rates both on an individual cellular basis as well as on the population level. One may start to quantify growth of cells by temporally tracking cellular parameters such as cell size or DNA content or population growth as changing cell numbers. Use of Couette chambers can be supplemented by other experimental designs with stir bars or orbital shakers for which turbulent energy dissipation rates have been calculated (Sanford 1997). Understanding how the near-bottom turbulent environment varies across the shelf and how *K. brevis* may respond to this turbulence may help predict when and where elusive near-bottom populations occur.

5.4 FINAL THOUGHTS

The work in this dissertation sought to explore the physical and chemical constraints on the near-bottom ecology of the toxic dinoflagellate *Karenia brevis*. The persistence of *K. brevis* near the bottom of the water column is but one facet in the complex ecology of *K. brevis* but may serve as an important starting point to understand the spectrum of environments in which *K. brevis* is capable of living. While the results presented are specific to the near-bottom ecology of *K. brevis*, the results may also be relevant in a larger context of the transport of these populations and the surface aggregations near the coast.

Slow growing vegetative cells may serve as an alternative life history strategy associated with the sediment. Near-bottom populations growing at rates of approximately $0.1 \text{ division day}^{-1}$ in the presence of modest grazing may take months to achieve even modest cell densities (Kubanek et al. 2007). The accumulation of *K. brevis* near shore both off the west coast of Florida (Stumpf et al. 2008) as well as off the coast of Texas (Hetland & Campbell 2007) at a rate faster than can be explained by cell division has led to the conclusion that bloom onset is due primarily to hydrodynamic factors. The persistence of slow growing vegetative cells may provide seed populations that are transported shoreward, undetected by satellites (Schofield et al. 1999, Stumpf et al. 2003), by bottom Eckman transport (Weisberg et al. 2001, Weisberg 2003). Off the west coast of Florida, modeling the transport of subsurface populations have replicated aggregations observed during the ECOHAB 2000 cruise (Janowitz & Kamykowski 2006). Upwelling events occur during the summer months off the coast of Texas and

may coincide with the growth of near-bottom populations. If the nutrient controls on growth are relaxed with shoreward transport, these near-bottom populations may ascend and become entrained in downwelling currents during the fall transition (Hetland & Campbell 2007). The near-bottom ecology *K. brevis* may therefore have a broad influence throughout the Gulf of Mexico.

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