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A Tale of Two Blooms: Dynamics of Nitrogen Uptake by Harmful Algae in the Eastern Gulf of Mexico and York River, Virginia, USA

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A Tale of Two Blooms: Dynamics of Nitrogen Uptake by Harmful Algae in the
Eastern Gulf of Mexico and York River, Virginia, USA

A Dissertation

Presented to

The Faculty of the School of Marine Science

The College of William & Mary

In Partial Fulfillment

Of the Requirements for the Degree of

Doctor of Philosophy

By

Lynn M. Killberg-Thoreson

2011

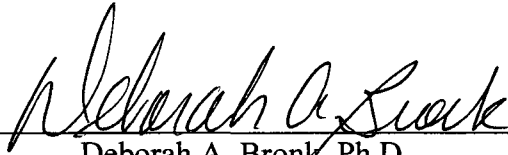
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the requirements for the degree of

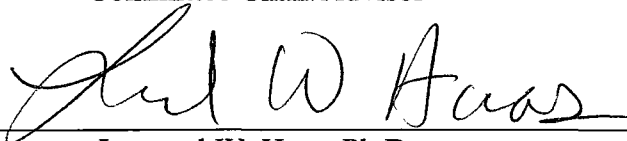
Doctor of Philosophy


Lynn M. Killberg-Thoreson

Approved, by the Committee, April 2011



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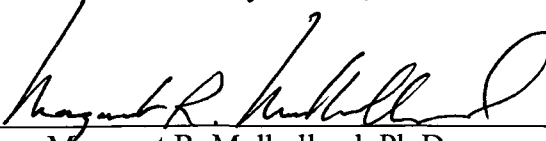
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DEDICATION

To my rock star husband, Tate, you are my sunshine.

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ABSTRACT

The goal of this study was to determine the significance of dissolved inorganic nitrogen (DIN) and dissolved organic nitrogen (DON) to harmful phytoplankton. Two regions that experience frequent and persistent harmful algal blooms (HABs) were examined, the Eastern Gulf of Mexico and York River, Virginia. Nitrogen uptake by harmful algae in these regions was examined using a combination of stable isotopic (^{15}N) tracer techniques and nutrient bioassays.

In the Eastern Gulf of Mexico, kinetic parameters for uptake of N substrates by *K. brevis* were determined and indicated the greatest preference for ammonium (NH_4^+), although all substrates tested were taken up. Investigation of N uptake by *K. brevis* in the light and dark revealed periodicity of diel uptake rates with the maximum and minimum rates measured early in the light and dark periods, respectively. The highest rates of uptake were observed for NH_4^+ . Ratios of NH_4^+ regeneration:uptake were ~ 1 , indicating the importance of regeneration processes to blooms. Three strains of *K. brevis* exhibited significant differences in N uptake rates. The observed nutritional flexibility of *K. brevis* likely helps it flourish under a range of conditions spanning bloom initiation in oligotrophic offshore waters to bloom maintenance inshore.

In the York River, N uptake was dominated by NH_4^+ with the highest uptake rates at all stations, for all size fractions and for all seasons, ranging from 34 to 80% of total absolute uptake. Rates of N uptake by *A. monilatum* are the first reported for this species in the York River, and demonstrate uptake of a diverse suite N substrates. During the *A. monilatum* bloom NH_4^+ regeneration rates equaled those of uptake, indicating the importance of regeneration to blooms. Additionally, three anthropogenic N sources were used to assess their role in exacerbation of a HAB during a 7 day bioassay. Urban parking lot run-off (+ Urban), soil from a construction site (+ Soil) and paper mill run-off (+ Industrial) were added to a natural bloom assemblage. Results indicated the anthropogenic sources had unique N compositions; DIN comprised $\sim 9\%$, 91% and 20% of + Urban, + Soil, and + Industrial, respectively. All N sources stimulated the growth of phytoplankton with the + Urban and + Soil treatments eliciting the greatest response, a doubling in Chl *a* and/or cell concentrations along with nutrient drawdown of both DIN and DON within two days.

The results of this dissertation emphasize the importance of a flexible metabolism to the success of the HAB species investigated here. All harmful phytoplankton studied were able to utilize the variety of DIN and DON sources supplied. Additionally, a universal preference for NH_4^+ was observed in all studies despite the distinct regions examined and unique characteristics of each species.

AUTHOR'S NOTE

The primary research chapters of this dissertation were prepared for submission to the journals listed below. Therefore, these chapters were written in the third person to represent my co-authors. The citations for the chapters are as follows:

Chapter 2:

Killberg-Thoreson, L., Mulholland, M.R., Heil, C.A., Sanderson, M.P., O'Neil, J.M., Bronk, D.A. In Prep. Nitrogen uptake kinetics in field populations and cultured strains of *Karenia brevis*. ***Harmful Algae***

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Chapter 5:

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**A TALE OF TWO BLOOMS: DYNAMICS OF NITROGEN UPTAKE BY
HARMFUL ALGAE IN THE EASTERN GULF OF MEXICO AND
YORK RIVER, VIRGINIA, USA**

CHAPTER 1

INTRODUCTION

Harmful algal blooms

Microscopic algae play a vital role in aquatic systems as the base of the food chain. Some algal blooms, however, can have deleterious effects on surrounding biota and are labeled 'harmful algal blooms'. Despite this moniker, not all harmful algae cause visible blooms and many species are not technically algae. HABs can be placed into various categories based on their production of high biomass accumulations, toxic characteristics, or ability to cause direct physical damage to other organisms, such as HAB species with sharp spines. The high biomass producers are capable of discoloring the water, making it appear shades of red, brown, green or yellow depending on the species present (Glibert *et al.* 2005a). These discolorations can be extensive and are often seen from satellite imagery. One consequence of large accumulations is microbial breakdown of biomass and depletion of oxygen during bloom decomposition, which creates hypoxic or anoxic zones (Anderson *et al.* 2002; Glibert *et al.* 2005a). Other effects include mortality of marine mammals and seabirds as well as fin-fish and shellfish from clogged gills and filter feeding mechanisms, die-offs of sea-grass and submerged aquatic vegetation from benthic shading, as well as formation of foam, scum, noxious odors, and aerosols (GEOHAB 2001; Anderson *et al.* 2002). There are over 300 different species that are considered HABs; the most common are dinoflagellates, however, diatom, raphidophyte, prymnesiophyte and pelagiophyte species have also been shown to cause HABs (Hallegraeff 2003).

Even at low densities in clear water, some HAB species are damaging because of their potent toxins (Glibert *et al.* 2005a). Toxic effects are observed directly, for example being mass fin-fish and shellfish mortality, and also indirectly in the form of economic

hardships. For example, in North Carolina, over a 15 month period between 1987 and 1988, a bloom of the toxic dinoflagellate, *Karenia brevis*, cost an estimated \$8 million in commercial fisheries losses (Tester *et al.* 1991). In Washington state, after detection of HAB toxins and a shellfish recall, two commercial shellfishing companies lost a combined \$1 million in revenue (Hoagland *et al.* 2002). Additional income is lost when tourism and recreational activities cease as unpleasant odors, impaired water quality, dead fish, and mucosal scums deter people from beaches and vacation spots. Another significant problem is the effect of HAB toxins on humans. Humans are directly impacted by inhaling toxic aerosols, causing respiratory distress, in the case of the toxic *Karenia brevis*, or indirectly by eating organisms that have bioaccumulated HAB toxins. Zooplankton, shellfish, and finfish accumulate toxins as they filter harmful phytoplankton from the water; human consumption of toxic organisms can cause a number of potentially life threatening illnesses. A few examples of toxins and their resulting disorders include: brevetoxin, the cause of neurotoxic shellfish poisoning (NSP), domoic acid, the cause of amnesic shellfish poisoning (ASP), and saxitoxin, the cause of paralytic shellfish poisoning (PSP) (Landsberg 2002). HAB toxins can be harmful to other marine animals including: whales (Geraci *et al.* 1989), manatees (Anderson and White 1989), pelicans, and sealions (Scholin *et al.* 2000).

Eutrophication and HABs

Despite recent focus on HABs, historical documents indicate many species have been present for hundreds of years. In 1793 Captain George Vancouver landed in British Columbia and noticed local tribes did not eat shellfish when the water changed color (Dale

and Yentsch 1978), likely to avoid the toxic dinoflagellate responsible for the disease PSP. In the Baltic Sea, paleoecological records have demonstrated the presence of cyanobacterial blooms dating back more than 8,000 years (Bianchi *et al.* 2000)

Over the past few decades, there has been heightened scientific interest in HABs due to perceived increases in their frequency and increasingly larger geographical areas being affected (Hallegraeff 2003). In the United States alone, almost every coastline has experienced HABs when previously they were limited to a few locations (Glibert *et al.* 2005a).

A number of explanations have been proposed for the HAB increase including: global climate change, ballast water discharge, increased scientific awareness (Hallegraeff 2003) and, the most commonly discussed cause, eutrophication (Officer and Ryther 1980; Lam and Ho 1989; Smayda 1990; Richardson and Jorgensen 1996; Richardson 1997; Glibert *et al.* 2005b). Excessive additions of nitrogen (N) and phosphorus (P) in aquatic environments are the result of human activities such as burning fossil fuels and subsequent atmospheric deposition, sewage treatment, aquaculture, farming operations and fertilizer run-off. Food production has been increasing to meet the needs of an ever-growing population. The development of concentrated animal feed operations for cattle, chickens, and other livestock, many located near coastal areas, has led to massive amounts of organic waste produced in small areas of land that cannot absorb it (Mallin 2000). The impact from finfish and shellfish aquaculture ponds can be severe because of the high additions of feed and organic matter recycling that occurs in relatively shallow, enclosed spaces. The amount of nutrients released from these activities will continue to rise as the population grows. In Chesapeake

Bay, since pre-colonial times, human impacts have increased N inputs to the coastal waters six- to eight-fold (Boyton *et al.* 1995).

Nutrient loading in coastal waterways has been suggested as a contributing factor in the perceived global increase in HABs (Officer and Ryther 1980; Lam and Ho 1989; Smayda 1990; Richardson and Jorgensen 1996; Richardson 1997; Glibert *et al.* 2005b). One explanation for this linkage is the nutrient ratio hypothesis in which nutrient additions cause selective enrichment of a single nutrients over other nutrients, favoring domination of one (possibly harmful) species over others depending on their additional nutrient requirements (Tilman 1977, Smayda 1997). For example, atmospheric deposition is an increasingly important source of biologically available N and P to estuarine, coastal and oceanic systems. In general, silica (Si) is less abundant than N or P in atmospheric deposition (GEOHAB 2001). Diatoms require Si to form their cell wall. When the typical P:Si and N:Si nutrient ratios in the water increase in favor of N and P, diatom growth will stop once Si is depleted, but other phytoplankton such as dinoflagellates, which do not have an Si requirement, will continue to grow. Many of these dinoflagellates are HAB species (Smayda 1997). In Hong Kong's Tolo Harbour a 2.5-fold increase in N and P loading from 1976 to 1989 was coincident with an eight-fold increase in red tide blooms (Lam and Ho 1989).

A second explanation is the potential shift in nutrient form; one example is the shift from inorganic to organic N forms such as urea, which may be occurring in conjunction with increased nutrient loading. Urea currently makes up more than 50% of the applied N fertilizer in the world when previously ammonium nitrate was the most common (Glibert *et al.* 2006b). When the nutrient regimes of fertilizer impacted systems switch from nitrate (NO_3^-) to urea based N sources, shifts in phytoplankton species composition often co-occur,

and many regions of the world, with substantially increased urea usage, have corresponding increases in PSP-producing dinoflagellates (Glibert *et al.* 2006b).

While there is evidence to support a link between increasing eutrophication and HAB frequency, this correlation does not necessarily imply causation. The mechanisms underlying HAB proliferation are poorly understood and include a suite of biological, chemical and physical interactions that cannot necessarily be linked to nutrient enrichment and are likely specific to individual HAB species.

Dissolved organic nitrogen

The DON pool is a diverse mixture of compounds, operationally defined as all forms of N passing through a 0.2 to 0.7 μm filter that is not a form of dissolved inorganic N (DIN; ammonium (NH_4^+), nitrate (NO_3^-) and nitrite (NO_2^-)). The pool is often divided by the molecular weights of the compounds; low molecular weight (LMW) compounds include dissolved free and combined amino acids (DFAA, DCAA), urea, nucleic acids, proteins, and amino sugars while high molecular weight (HMW) compounds include humic and fulvic acids, which range in size from 500 to 10^6 Daltons (Thurman 1985; Wershaw and Aiken 1985) and are approximately 3000 times the size of amino acids (Moran and Hodson 1994). Humics are operationally defined by their retention on hydrophobic resins at a pH of 2 and are found in relatively high concentrations in many aquatic systems (reviewed in Hessen and Tranvik 1998). It has been hypothesized that humics contain a central refractory core of humic and fulvic acids but that there are also more labile ions such as ammonium that reside in the humic matrix.

DON can come from a variety of autochthonous sources, including phytoplankton exudation, excretion by zooplankton, and viral infection (reviewed in Bronk 2002). During the final stages of viral infection, the host bacterial or eukaryotic cell bursts open, releasing all of the organic contents inside (Bratbak *et al.* 1998). Another source is “sloppy feeding” by zooplankton that occurs when cells are broken during feeding, releasing their intracellular contents (Dagg *et al.* 1974). Exudation, excretion, viral infection, and zooplankton grazing dominate in open ocean environments, while in freshwater, coastal, and estuarine systems, allochthonous inputs are more substantial. These include upward flux from sediments, anthropogenically influenced run-off, and atmospheric deposition. Atmospheric deposition may be one of the major contributors of DON to the system as 20-70% of the N in rainwater is DON (Seitzinger and Sanders 1999). Due to the great source variations, DON composition and availability can vary between locations as well as on relatively short space and time scales.

While once thought to be composed of refractory compounds, largely unavailable to bacteria and phytoplankton, DON is increasingly recognized as an important component of the marine N cycle (e.g. Berman and Bronk 2003; Mulholland and Lomas 2008). Traditionally, DON was considered recalcitrant due to the high concentrations that persist in marine waters when DIN substrates are low to undetectable (Granéli *et al.* 1999). It was assumed that DON was not utilized because the pool was so consistently large and apparently unchanging. That perception began to shift when high DON cycling rates were measured in freshwater and coastal marine systems (e.g. Bronk *et al.* 1994; Haga *et al.* 2001). Bacteria, phytoplankton, and cyanobacteria have all been shown to utilize DON either directly (or

indirectly (e.g. after photochemical or enzymatic breakdown; reviewed in Antia *et al.* 1991; Lewitus *et al.* 2000; Berman 2001; Bronk 2002; Bronk *et al.* 2007).

Uptake of DON by phytoplankton

DON can be made available to phytoplankton by a number of mechanisms. For small molecules, such as urea and some amino acids, direct uptake can occur by active transport driven by a sodium ion pump or through facilitated diffusion if concentrations outside the cell are high enough (mM) to create a concentration gradient (reviewed in Bronk and Flynn 2006). For larger compounds (>1 kDa) such as proteins, polypeptides, and humic acids, direct uptake through transport proteins cannot occur. One alternative is the use of hydrolytic or oxidative enzymes to break down large polymers into smaller constituent molecules that can subsequently be taken up by the cell. These enzymes are found intracellularly, attached to the outside of the cell, or are released from the cell and found freely in the water column; the latter two are termed extracellular enzymes (Burns 1978, Sinsabaugh *et al.* 1992). Traditionally, extracellular enzyme activity was regarded as a unique characteristic of heterotrophic bacteria and the ability of phytoplankton to obtain nitrogen in this manner has only recently been considered (Sala *et al.* 2001). Two primary types of enzyme activity in phytoplankton are amino acid oxidation and peptide hydrolysis (e.g. Mulholland *et al.* 2003). One estimate is that 20% of the DON utilization in coastal and oceanic environments is due to phytoplankton utilization of amino acid oxidases (Mulholland *et al.* 1998).

In addition to production of their own extracellular enzymes, phytoplankton may benefit from monomers released by bacteria. While some studies have shown bacterial extracellular enzyme hydrolysis and bacterial uptake to be tightly coupled processes (Hoppe *et al.* 1988), other studies with attached bacteria have shown that much more substrate is released than can be assimilated by bacteria (Jacobsen and Rai 1991; Chróst 1990). With this lag, phytoplankton may be able to utilize monomers derived from bacterial enzymatic activity. Another method for phytoplankton N acquisition is liberation of enzymes from bacteria during cell lysis and bacterivory. Free enzymes can continue hydrolyzing larger compounds that could be taken up by phytoplankton (Jacobsen and Rai 1991). High aminopeptidase activity (10-90% of activity) has been found in 0.2 µm filtrates (Jacobsen and Rai 1991), indicating that the enzymes have been liberated from the bacteria.

DON can also be taken up by pinocytosis: a process of ingesting dissolved macromolecules from the medium outside the cell as well as phagocytosis: a process of engulfing particulate matter by the cell. Cells undergoing pinocytosis expand a vesicle from the plasma membrane that engulfs the molecule and pulls it inside the cell for storage inside a vacuole. Pinocytosis is a common method for ingesting dissolved macromolecules in some dinoflagellates, euglenoids and chlorophytes (Kivic and Vest 1974; Klut *et al.* 1987). The harmful dinoflagellate, *A. catenella* was found to directly take up a fluorescently labeled macromolecule, dextran (Legrand and Carlsson 1998). Other similar markers like lectins and peroxidases have demonstrated pinocytosis in the flagellates *Amphidinium carterae* and *Prorocentrum micans* (Klut *et al.* 1987). Phagocytosis by marine and freshwater phytoflagellates has also been well documented (e.g. Sanders and Porter 1988) and has been shown to occur under a wide range of light and nutrient regimes. In phytoplankton,

phagotrophy has also been shown in the photosynthetic bloom-forming dinoflagellates *Heterocapsa triquetra* and *Ceratium furca* (Legrand *et al.* 1998; Smalley *et al.* 1999).

Phytoplankton supplementing their nutrition in this manner would allow for nutrient acquisition even during unfavorable conditions and give a competitive advantage over strictly photosynthetic organisms (Li *et al.* 2000).

Photochemical decomposition followed by uptake of the N photoproducts is another method of N acquisition. DON, particularly isolated humics, can be a source of smaller labile molecules when exposed to sunlight and ultraviolet (UV) radiation. Due to their aromatic nature, humic substances are especially photoreactive when exposed to the UV spectrum (280-400 nm). Humics have been shown to release inorganics such as NH_4^+ and NO_2^- (Bushaw *et al.* 1996; Bushaw-Newton and Moran 1999), as well as small labile organic molecules like urea and amino acids, into the surrounding environment when exposed to UV light (e.g. Amador *et al.* 1989; Jørgensen *et al.* 1998; Bushaw-Newton and Moran 1999).

The DON and HAB link

Specific components of DON may exert selective pressure on phytoplankton community composition (Paerl 1997; Seitzinger and Sanders 1997; Berman and Chava 1999) and phytoplankton capable of DON uptake may have a competitive advantage in organically enriched environments where the DIN supply is limited. Some harmful algae are able to use DON for nutrition and may prefer it to inorganic sources. Specifically, many dinoflagellates have an affinity for organic N (Berg *et al.* 1997).

There are a number of examples to support the DON and HAB link. For instance, the growth of the brown tide organism, *Aureococcus anophagefferens*, commonly found off the coast of Long Island, NY, is stimulated by DON inputs, particularly urea (LaRoche *et al.* 1997; Gobler and Sañudo-Wilhelmy 2001). One study determined that approximately 70% of the total N utilized during an *A. anophagefferens* bloom event was organic (Berg *et al.* 1997). DON from atmospheric deposition has been shown to stimulate coastal phytoplankton production (Seitzinger and Sanders 1999). Ambient urea concentrations were found to potentially provide a large proportion of the N required by the bloom-forming dinoflagellate, *Lingulodinium polyedrum* (Kudela and Cochlan 2000). In a 2003 study, *Prorocentrum minimum* cells made up 93 % of total phytoplankton cells in a bloom in the Chesapeake Bay and had a high affinity for urea (Fan *et al.* 2003). Most recently, studies with harmful dinoflagellates have shown both direct uptake of humics (Doblin *et al.* 2001; See *et al.* 2006) as well as growth stimulation when humic and fulvic acid fractions were added to culture medium (Gangon *et al.* 2005; Heil 2005).

Study Areas and Species of Interest

The research presented in this dissertation is focused in two regions where HABs are prevalent: the Eastern Gulf of Mexico and York River, Virginia, USA.

More than 40 species of toxic microalgae live in the Gulf of Mexico. One of the most common, particularly along the West Florida Shelf is the dinoflagellate *Karenia brevis* (Davis) G. Hansen & Ø. Moestrup (= *Gymnodinium breve* (Davis) or *Ptychodiscus brevis*

(Davis) Steidinger). It is an unarmored cell, typically 18 to 45 μm in size, found year-round throughout the Gulf of Mexico at background concentrations of 1,000 cells L^{-1} . Blooms occur almost every year, generally in the late summer or early fall and generally last three to five months. Occasionally, however, blooms continue for as long as 18 months and may affect thousands of square miles. Reports of *K. brevis* blooms in the Gulf of Mexico date back to the 1530's (Steidinger *et al.* 1998). *K. brevis* produces brevetoxins, a potent neurotoxin, that lead to massive fish kills, marine mammal death, and can cause respiratory distress in humans as well as neurotoxic shellfish poisoning (NSP). NSP is a temporary illness characterized by gastrointestinal, neurological and respiratory distress. Millions of dollars are lost annually to *K. brevis* blooms.

The York River, Virginia, USA is the 5th largest estuary of Chesapeake Bay at ~6900 km^2 (2662 mi^2). It is a partially mixed estuary with a salinity range from polyhaline to freshwater and is considered microtidal. The Mattaponi and Pamunkey Rivers are the two major freshwater tributaries. The wide range of salinities in the York River system is affected by the interactions of freshwater, salt water, tidal energy, and wind (Haas 1975). Common HAB species in the York River include: *Cochlodinium polykrikoides*, *Heterocapsa triquetra*, *Heterocapsa rotundata*, *Scrippsiella trochoidea*, *Karlodinium veneficum*, *Prorocentrum minimum*, *Prorocentrum micans*, and recently, *Alexandrium monilatum*. These dinoflagellate blooms can be dominated by single species but are often found as assemblages of many species and often form blooms in Chesapeake Bay tributaries as well as the Bay itself.

In particular, *C. polykrikoides* (= *Cochlodinium heterolobatum*) has produced annual blooms in the York River (Mackiernan 1968; Zubkoff *et al.* 1979; Marshall 1994).

Cochlodinium polykrikoides is a cosmopolitan species found in warm temperate and tropical waters (Steidinger & Tangen 1996). It is an athecate dinoflagellate that commonly forms chains, most less than eight cells. *Cochlodinium polykrikoides* has formed extensive blooms associated with fish kills in Japan and Korea (Fukuyo *et al.* 1990), however, the strains located in the Chesapeake Bay regions appear to be genetically dissimilar to those found in Asia (Gobler *et al.* 2008).

Alexandrium monilatum (= *Gonyaulax monilata*) is another species of interest. Typically a Gulf coast dweller, found in the same coastal bays as *K. brevis*, there are records of this dinoflagellate in Chesapeake Bay (Morse 1947; Mackiernan 1968), however it first began to bloom extensively in the York River in the summer of 2007. These armored cells (~65 μm in width) exist as single cells or as long chains that resemble stacks of hamburgers. *Alexandrium monilatum* produces the toxin goniiodomin A. In the 2007 bloom, toxic *A. monilatum* caused mortality in the veined rapa whelk (Harding *et al.* 2009). Cells also produce bottom resting cysts that may seed new bloom populations (Walker and Steidinger 1979).

Significance of Research

The research presented here provides insight into the organic N dynamics associated with HABs. First, studies of N uptake by phytoplankton are lacking information regarding uptake of organics. While once thought to be largely refractory, evidence now suggests that DON is an important component of the marine N cycle (reviewed in Berman and Bronk 2003 and Bronk *et al.* 2007) and may stimulate growth of harmful species (e.g. LaRoche *et al.*

1997; Gobler and Sañudo-Wilhelmy 2001). Overall, the issue of HABs is one of worldwide importance as the intensity and frequency of bloom occurrences continues to rise, bringing with it a suite of environmental, economic, and human health impacts (Hallegraeff 2003). Despite their importance, many aspects of HABs are still a mystery. This project provides a framework to highlight the potentially important role of DIN and DON to harmful phytoplankton and advance the understanding of HAB nutrition.

Literature Cited

- Amador, J.A., Alexander, M., Zika, R.G. 1989. Sequential photochemical and microbial degradation of organic molecules bound to humic acid. *App. Environ. Microbiol.* 55, 2843-2849.
- Anderson, D.M., Glibert, P.M., Burkholder, J.M. 2002. Harmful algal blooms and eutrophication: nutrient sources, composition, and consequences. *Estuaries* 25(4b), 704-726.
- Anderson, D., & White, A. 1989. Toxic dinoflagellates and marine mammal mortalities. Woods Hole Oceanographic Institute, Mass, Technical Report WHOI-89-36.
- Antia, N.J., Harrison, P.J., Oliveira, L. 1991. Phycological review: The role of dissolved organic nitrogen in phytoplankton nutrition, cell biology, and ecology. *Phycologia* 30, 1-89.
- Berg, G., Glibert, P.M., Lomas, M.W., Burford, M.A. 1997. Organic nitrogen uptake and growth by the chrysophyte *Aureococcus anophagefferens* during a brown tide event. *Mar. Biol.* 129, 377-387.
- Berman, T. 2001. The role of DON and the effect of N:P ratios on occurrence of cyanobacterial blooms: implications from the outgrowth of *Aphanizomenon* in Lake Kinneret. *Limnol. Oceanogr.* 46, 443-447.
- Berman, T. and Bronk, D.A. 2003. Dissolved organic nitrogen: a dynamic participant in aquatic ecosystems. *Aquat. Microb. Ecol.* 31, 279-305.
- Berman, T.C. and Chava, S. 1999. Algal growth on organic compounds as nitrogen sources. *J. Plankton Res.* 21(8), 1423-1437.
- Bianchi, T.S., Engelhaupt, P., Westman, T., Andren, C. Rolff, Elmgren, R. 2000. Cyanobacterial blooms in the Baltic Sea: Natural or human-induced? *Limnol. Oceanogr.* 45, 716-726.
- Boynton, W.R., Garber, J.H., Summers, R., Kemp, W.M. 1995. Inputs, Transformations, and transport of nitrogen and phosphorus in Chesapeake Bay selected tributaries. *Estuaries.* 18(1B), 285-314.
- Bratbak, G., Jacobsen, A., Heldal, M. 1998. Viral lysis of *Phaeocystis pouchetii* and bacterial secondary production. *Aquat. Microb. Ecol.* 16, 11-16.

- Bronk, D.A. 2002. Dynamics of organic nitrogen. In: Biogeochemistry of marine dissolved organic matter. Hansell, DA, and Carlson, CA [Eds] San Diego, Academic Press, 153-247
- Bronk, D.A. and Flynn, K.J. 2006. Algal cultures as a tool to study the cycling of dissolved organic nitrogen. In: Algal cultures, analogues of blooms and applications. D. V. Subba Rao [Eds.] Dartmouth, Nova Scotia, Canada, Science Publishers, 301-341.
- Bronk, D.A., Glibert, P.M. and Ward, B.B., 1994. Nitrogen uptake, dissolved organic nitrogen release, and new production. *Science*, 265, 1843-1846.
- Bronk, D.A., See, J.H., Bradley, P., Killberg, L. 2007. DON as a source of bioavailable nitrogen for phytoplankton. *Biogeosciences* 4, 238-296.
- Burns, R.G. [Ed.] (1978) *Soil Enzymes*. Academic press, New York.
- Bushaw, K., Zepp, R.G., Tarr, M.A., Schultz-Jander, D., Bourbonniere, R.A., Hodson, R.E., Miller, W.L., Bronk, D.A., Moran, M.A. 1996. Photochemical release of biologically available nitrogen from dissolved organic matter. *Nature* 381, 404- 407.
- Bushaw-Newton, K.L. and Moran, M.A. 1999. Photochemical formation of biologically available nitrogen from dissolved humic substances in coastal marine systems. *Aquat. Microb. Ecol.* 18, 285-292.
- Chróst, R. 1990 Microbial ectoenzymes in aquatic environments. In: Overbeck, J, Chróst, R [Eds]. *Aquatic microbial ecology: biochemical and molecular approaches*. Springer-Verlag, New York, 47-78.
- Daag, M.J. 1974. Loss of prey body contents during feeding by an aquatic predator. *Ecology* 55(4), 903-906.
- Dale, B.Y. and Yentsch, C.M. 1978. Red tide and paralytic shellfish poisoning. *Oceanus* 21(3), 41-49.
- Doblin, M., Carlsson, C., Humme, C., Granéli, E., Hallegraeff, G. 2001. Uptake of humic substances by the toxic dinoflagellate *Alexandrium catenella*. *Harmful Algal Blooms 2000*, 332-335
- Fan, C., Glibert, P., Burkholder, J. 2003. Characterization of the affinity for nitrogen, uptake kinetics, and environmental relationships for *Prorocentrum minimum* in natural blooms and laboratory cultures. *Harmful Algae*. 2, 283-299.
- Fukuyo, Y., Takano, M., Chihara, M., Matsuoka. 1990. Red tide organisms in Japan. An Illustrated Taxonomic Guide. Uchida Rokakuho, Co., Ltd., Tokyo.

- Gagnon, R., Levasseur, M., Weise, A.M., Fauchot, J. 2005. Growth stimulation of *Alexandrium tamarense* (Dinophyceae) by humic substances from the Manicouagan River (Eastern Canada). *J. of Phycol.* 4, 489-497.
- GEOHAB. 2001. Global ecology and oceanography of harmful algal blooms. Glibert, P., Pitcher, G. [Eds.] Science Plan, SCOR, Baltimore, Maryland and IOC Paris, France: 86.
- Geraci, J., Anderson, D., Timperi, R., St Aubin, D., Early, G., Prescott, J., Mayo, C. 1989. Humpback whales (*Megaptera novaeangliae*) fatally poisoned by dinoflagellate toxin. *Can. J. of Fish. Aquat. Sci.* 46, 1895-1898.
- Glibert, P., Anderson, D., Gentien, P., Granéli, E., Sellner, K. 2005a. The global, complex phenomena of harmful algal blooms. *Oceanography* 18(2), 130-141.
- Glibert, P., Harrison, J., Heil, C., Seitzinger, S. 2006b. Escalating worldwide use of urea – a global change contributing to coastal eutrophication. *Biogeochemistry* 77(3), 441-463.
- Glibert, P., Seitzinger, S., Heil, C., Burkholder, J., Parrow, M., Codispoti, L., Kelly, V. 2005b. The role of eutrophication in the global proliferation of harmful algal blooms: new perspectives and new approaches. *Oceanography* 18(2), 198-209.
- Granéli, E., Carlsson, P., Legrand C. 1999. The role of C, N, P in dissolved and particulate organic matter as a nutrient source for phytoplankton growth, including toxic species. *Aquat. Ecol.* 33, 17-27.
- Gobler, C. & Sañudo-Wilhelmy, S.A. 2001. Temporal variability of groundwater seepage and brown tide blooms in a Long Island embayment. *Mar. Ecol. Prog. Ser.* 217, 299-309.
- Gobler, C.J., D.L. Berry, O.R. Anderson, A. Burson, F. Koch, B.S., Rodgers, L.K. Moore, J.A. Goleski, B. Allam, P. Bowser, Y., Tang, and R. Nuzzi. 2008. Characterization, dynamics, and ecological impacts of harmful *Cochlodinium polykrikoides* blooms on eastern Long Island, NY, USA. *Harmful Algae* 7: 293–307.
- Haas, L.W. 1975. Plankton dynamics in a temperate estuary with observations on a variable hydrographic conditions. Doctoral dissertation. School of Marine Science. The College of William & Mary, Gloucester Point, VA. 202 pp.
- Haga, H., Nagata, T., Sakamoto, M. 2001. Ammonium regeneration within the euphotic zone. *Lake Kizaki: limnology and ecology of a Japanese Lake.* Saijo, Y., Hayashi, H. [Eds.} Backhuys, Leiden, 207-215

- Hallegraeff, G.M. 2003. Monographs on Oceanographic methodology. Harmful Algal Blooms: A Global Overview. Paris, United Nations Educational
- Harding, J.M., Mann, R., Moeller, P., Hsia, M.S. 2009. Mortality of the veined rapa whelk, *Rapana venosa*, in relation to a bloom of *Alexandrium monilatum* in the York River, United States. *J. Shellfish Res.* 28(2), 363-367
- Heil, C.A. 2005. Effects of humic acids on growth, photosynthesis, and respiration of the dinoflagellate *Prorocentrum minimum*. *Harmful Algae* 4, 603-613.
- Hessen, D.O. and Tranvik, L.J. 1998. Aquatic humic matter: from molecular structure to ecosystem stability. *Ecol. Stud.* 133, 333-342.
- Hoagland, P., Anderson, D.M., Kaoru, Y., White, A.W. 2002. The economic effects of harmful algal blooms in the United States: estimates, assessment issues, and information needs. *Estuaries*, 25(4B), 819-837.
- Hoppe, H.G., Kim, S.J., Gocke, K. 1988. Microbial decomposition in aquatic environments: combined processes of extracellular enzyme activity and substrate uptake. *Appl. Environ. Microbiol.* 54, 784-790.
- Jacobsen, T. & Rai, H. 1991. Aminopeptidase activity in lakes of differing eutrophication. Chrost, R. [Ed.] *Microbial Enzymes in Aquatic Environments*. New York, Springer-Verlag, 155-164.
- Jørgensen, N.O.G., Tranvik, L., Edling, H., Granéli, E. and Lindell, M. 1998. Effects of sunlight on occurrence and bacterial turnover of specific carbon and nitrogen compounds in lake waters. *FEMS Microbiol. Ecol.* 25, 217-227.
- Kivic, P.A. & Vesik, M.J. 1974. Pinocytotic uptake of protein from the reservoir in *Euglena*. *Arch. Microbiol.* 96, 155-159.
- Klut, M., Bisalputra, T., Antia, N.J. 1987. Some observations on the structure and function of the dinoflagellate pusule. *Can. J. Bot.* 65, 736-744.
- Kudela, R.M., Cochlan, W.P. 2000. Nitrogen and carbon uptake kinetics and the influence of irradiance for a red tide bloom off southern California. *Aquat. Microb. Ecol.* 21(1), 31-47.
- Lam, C.W.Y. & Ho, K.C. 1989. Red Tides in Tolo Harbour, Hong Kong. *Red Tides: Biology, Environmental Science*. Okaichi, T., Anderson, D.M., Nemoto, T. [Eds.] Elsevier Science, 49-52
- Landsberg, J.H. 2002. The effects of harmful algal blooms on aquatic organisms. *Rev. Fish. Sci.* 10, 113-390.

- LaRoche, J., Nuzzi, R., Wyman, K., Falkowski, P., Wallace, D.W.R., Wallance, D. 1997. Brown tide blooms in Long island's coastal waters linked to interannual variability in groundwater flow. *Global Change Biology* 3(5), 397-410.
- Legrand, C. & Carlsson, P. 1998. Uptake of high molecular weight dextran by the dinoflagellate *Alexandrium catenella*. *Aquat. Microb. Ecol.* 16, 81-86.
- Lewitus, A.J., Koepfler, E.T., Pigg, R.J. 2000. Use of dissolved organic nitrogen by a salt marsh phytoplankton bloom community. *Arch. Hydrobiol. Special Issues on Advanced Limnology* 55, 441-456.
- Li, A., Stoecker, D.K., Coats, D.W. 2000. Mixotrophy in *Gymnodinium galatheanum* (Dinophyceae): grazing responses to light intensity and inorganic nutrients. *J. Phycol.* 36, 33-45.
- Mackiernan, G. 1968. Seasonal distribution of dinoflagellates in the lower York River, Virginia. Thesis. The College of William and Mary. 104 pp.
- Mallin, M.A. 2000. Impacts of industrial-scale swine and poultry production on rivers and estuaries. *American Scientist* 88:26-37.
- Marshall, H.G. 1994. Succession of dinoflagellate blooms in the Chesapeake Bay, U.S.A. In: P. Lassus, et al. [Eds.] *Harmful Marine Algal Blooms*, Intercept Ltd., Andover, Md., pp. 615-620.
- Moran, M. & Hodson, R.E. 1994. Support of bacterioplankton production by dissolved humic substances from three marine environments. *Mar. Ecol. Prog. Ser.* 110, 241-247.
- Morse, D.C. 1947. Some observations on variations in plankton populations, Patuxent River, Maryland 1943-1945. *Chesapeake Biological Laboratory* 65. Solomons, MD. pp. 1-31
- Mulholland, M.R., Glibert, P.M., Berg, G.M., Van Heukelem, L., Pantoja, S., Lee, C. 1998. Extracellular amino acid oxidation by microplankton. *Aquat. Microb. Ecol.* 15, 141-152.
- Mulholland, M.R., Lee, C., Glibert, P.M. 2003. Extracellular enzyme activity and uptake of carbon and nitrogen along an estuarine salinity and nutrient gradient. *Mar. Ecol. Progr. Ser.* 258, 3-17.
- Mulholland, M. R. and M. W. Lomas. 2008. N uptake and assimilation, In: Capone, D. G., D. A. Bronk, M. R. Mulholland and E. J. Carpenter [Eds.], *Nitrogen in the Marine Environment*. Elsevier/Academic. pp. 303-384

- Officer, C.B. & Ryther, J.H. 1980. The possible importance of silicon in marine eutrophication. *Mar. Ecol. Progr. Ser.* 3, 83-91.
- Paerl, H.W. 1997. Coastal eutrophication and harmful algal blooms: importance of atmospheric deposition and groundwater as "new" nitrogen and other nutrient sources. *Limnol. Oceanogr.* 42, 1154-1165.
- Richardson, K. 1997. Harmful or exceptional phytoplankton blooms in the marine ecosystem. Blaxter, J and Southworth, A [Eds.] *Advances in Marine Biology*. San Diego, Academic Press 31, 302-386.
- Richardson, K. & Jorgensen, B. 1996. Eutrophication: definition, history and effects. Jorgensen, B. and Richardson, K. [Eds.] *Eutrophication in Coastal Marine Ecosystems. Coastal and Estuarine Studies*. American Geophysical Union, Washington D.C. 52, 1-19.
- Sala, M., Karner, M., Arin, L., Marassé, C. 2001. Measurement of ectoenzyme activities as an indication of inorganic nutrient imbalance in microbial communities. *Aquat. Microb. Ecol.* 23, 301-311.
- Sanders, R.W. and Porter, K.G. 1988. Phagotrophic phytoflagellates. *Adv. Microb. Ecol.* 10, 167-192.
- Scholin, C., Gulland, F., Doucette, G., Benson, S., Busman, M., Chavez, F., Cordaro, J., DeLong, R., De Vogelaere, A., Harvey, J., Haulena, M., Lefebvre, K., Lipscomb, T., Loscutoff, S., Lowenstine, L., Marin, R. III, Miller, P., McLellan, W., Moeller, P., Powell, C., Rowles, T., Silvagni, P., Silver, M., Spraker, T., Trainer, V., Van Dolah, F. 2000. Mortality of sea lions along the central California coast linked to a toxic diatom bloom. *Nature* 403, 80-84.
- See, J.H., Bronk, D.A., Lewitus, A.J. 2006. Uptake of *Spartina*-derived humic-nitrogen by estuarine phytoplankton in nonaxenic and axenic culture. *Limnol. Oceanogr.* 51(5), 2290-2299
- Seitzinger, S and Sanders, R (1997) Contribution of dissolved organic nitrogen from rivers to estuarine eutrophication. *Marine Ecology Progress Series* 159: 1-12
- Seitzinger, S.P. and Sanders, R.W. 1999. Atmospheric inputs of dissolved organic nitrogen stimulate estuarine bacteria and phytoplankton. *Limnol. Oceanogr.* 44, 721-730.
- Sinsabaugh, R.L., Weiland, T., Linkins, A.E. 1992. Enzymatic and molecular analysis of microbial communities associated with lotic particulate organic matter. *Freshw. Biol.* 28, 393-404.

- Smalley, G.W., Coats, D.W., Stoecker, D.K. 1999. Feeding in the mixotrophic dinoflagellate *Ceratium furca* is influenced by intracellular nutrient concentrations. *Mar. Ecol. Progr. Ser.* 262, 137-151.
- Smayda, T.J. 1990. Novel and nuisance phytoplankton blooms in the sea: evidence for a global epidemic. E., Sundstrom, B., Edler, L., Anderson, D.M. [Eds.] *Toxic Marine Phytoplankton*. New York, Elsevier, 29-40.
- Smayda, T.J. 1997. Harmful algal blooms: Their ecophysiology and general relevance to phytoplankton blooms in the sea. *Limnol. Oceanogr.* 42, 1137-1153.
- Steidinger, K.A. 1999. Historical perspective on *Karenia brevis* red tide research in the Gulf of Mexico. *Harmful Algae* 8(4), 549-561.
- Steidinger, K.A., Tangen, K. 1996. Dinoflagellates. In: C.R. Tomas [Ed.] *Identifying Marine Diatoms and Dinoflagellates*, Elsevier, New York, 534-537.
- Steidinger, K.A., Vargo, G.A., Tester, P.A., Tomas, C.R. 1998. Bloom dynamics and physiology of *Gymnodinium breve* with emphasis on the Gulf of Mexico. In: D.M. Anderson, A.D., Cembella and G.M. Hallegraeef [Eds.] *Physiological Ecology of Harmful Algal Blooms*, 133-153.
- Tester, P.S.R., Vukovich, F., Fowler, P.K., Turner, J. 1991. An Expatriate Red Tide Bloom: Transport, Distribution, and Persistence. *Limnol. Oceanogr.* 36(5), 1053-1061.
- Thurman, E. 1985. Organic Geochemistry of Natural Waters. In: Nijhoff, M, Junk, W [Eds.] *Publishers*. Dordrecht
- Tilman, D. 1977. Resource competition between planktonic algae: An experimental and theoretical approach. *Ecology* 58, 338-348.
- Walker, L.M., Steidinger, K.A. 1979. Sexual reproduction in the toxic dinoflagellate *Gonyaulax monilata*. *J. Phycol.* 15:312-315.
- Wershaw, R. & Aiken, G.R. 1985. Molecular size and weight measurements of humic substances. G, McKnight, D.M., and Wershaw, R.L. [Eds.] *Humic Substances in Soil Sediment and Water: Geochemistry, Isolation, and Characterization*. Aiken, New York, John Wiley and Sons, 477-492.
- Zubkoff, P., Munday, J., Rhodes, R., Warinner, J. 1979. Mesoscale features of summer (1975-1979) dinoflagellate blooms in the York River, Virginia (Chesapeake Bay estuary). In: D. Taylor and H. Seliger [Eds.] *Toxic Dinoflagellate Blooms*, Elsevier, Inc., New York, pp. 279-286.

Structure of Dissertation

This dissertation is separated into four main chapters (2 – 5) and presents results from laboratory experiments examining uptake and utilization of N by species of harmful algae in the Eastern Gulf of Mexico as well as the York River, Virginia, USA. Chapters 2 and 3 explore the N uptake dynamics of the harmful algae, *Karenia brevis*, found in Eastern the Gulf of Mexico and while Chapters 4 and 5 explore the N uptake and utilization by a variety of harmful algae species found in the York River.

In Chapter 2, I investigate N uptake kinetics of *Karenia brevis* in both field populations and with cultured strains using the stable isotope ^{15}N and document uptake of a variety of substrates, including both inorganic and organic N species.

In Chapter 3, I discuss the variability in N uptake rates by *K. brevis* during a diel cycle using a variety of inorganic and organic substrates. The study includes both uptake rates for a field population as well as three cultured strains as well as the importance of uptake in the light and dark is discussed.

In Chapter 4, I examine seasonal variations in ambient conditions and N uptake rates during 2007-2009 in the York River, Virginia. Uptake of both inorganic and organic N sources is examined with a variety of phytoplankton assemblages, including a bloom of the harmful dinoflagellate, *Alexandrium monilatum*.

In Chapter 5, I investigate the role anthropogenic N inputs and eutrophication may play in stimulating harmful algae bloom in the York River, Virginia. The relative importance of these sources to bloom nutrition is discussed as well as future potential impacts due to global climate change.

Finally, in Chapter 6, I conclude with a summary of my results and how they contribute to our knowledge of HAB nitrogen dynamics. I also propose future directions for investigating the connection between nitrogen, eutrophication and HAB proliferation.

CHAPTER 2

Nitrogen uptake kinetics in field populations and cultured strains of *Karenia brevis*

Abstract

The kinetics of nitrogen (N) uptake of ammonium (NH_4^+), nitrate (NO_3^-), urea, an amino acid (AA) mixture, individual amino acids (alanine and glutamate), and humic substrates by the toxic red tide dinoflagellate, *Karenia brevis*, were determined during short term incubations (0.5 to 1 hr) using ^{15}N tracer techniques. Experiments were conducted using field populations collected from extensive blooms during three cruises along the West Florida Shelf in October 2001, 2002, and 2007 as well as with strains in culture from the Florida Fish and Wildlife Institute culture collection (Jacksonville C4 (CCFWC 251) and Texas B4 (CCFWC 267)). Kinetic parameters for the maximum uptake velocity (V_{max}), half-saturation constant (K_s), and the affinity constant (α) were determined and indicate the greatest preference for NH_4^+ followed by NO_3^- , urea, and humics. Rates of AA uptake did not saturate even at the highest substrate additions (50-200 $\mu\text{mol N L}^{-1}$) and, therefore, could not be described according to the Michaelis-Menten function. Although there was a clear preference for uptake of inorganic N sources, *K. brevis* took up all organic substrates tested, including N derived from humic substances. The observed flexibility of *K. brevis* to utilize a variety of inorganic and organic substrates likely helps it flourish under a wide range of conditions from bloom initiation in oligotrophic waters offshore to bloom maintenance near shore where ambient nutrient concentrations may be orders of magnitude greater.

Introduction

Blooms of the red tide dinoflagellate, *Karenia brevis* (C.C. Davis) G. Hansen & Ø. Moestrup (formerly known as *Gymnodinium breve*) have become a nearly annual feature along the West Florida Shelf (WFS) where they can reach populations in excess of 10^6 cells L^{-1} . *K. brevis* produces potent neurotoxins (brevetoxins) that are harmful to fish, seabirds and mammals and can have significant economic and human health impacts (Kusek *et al.* 1999; Kirkpatrick *et al.* 2004). Although typically considered a coastal bloom species (Smayda and Reynolds 2001), *K. brevis* can thrive in a variety of environments and nutrient regimes. Blooms of *K. brevis* are believed to initiate 18-74 km offshore in deep oligotrophic waters (Steidinger 1975; Tester and Steidinger 1997) where concentrations of dissolved inorganic nitrogen (DIN) are relatively low (0.1 - 0.2 μM) (Heil *et al.* 2001) and concentrations of dissolved organic nitrogen (DON) are one to two orders of magnitude greater (5-10 μM) (Heil *et al.* 2001). With the appropriate wind and current conditions, populations of *K. brevis* may be transported inshore and eventually concentrated into larger blooms in more eutrophic bays and inlets (Vargo *et al.* 2001). Given that *K. brevis* blooms persist in such a broad range of environments, numerous and varied inorganic and organic N and P sources have been hypothesized to fuel a bloom during its varied stages of development (Walsh *et al.* 2006; Vargo *et al.* 2008) including upwelling events along the continental shelf (Walsh *et al.* 2006), macro- and microzooplankton grazing and N regeneration (Lester 2005), estuarine outflow (Vargo *et al.* 2004; Vargo *et al.* 2008), decay and remineralization of bottom dwelling diatoms (Vargo *et al.* 2008), atmospheric inputs (Vargo *et al.* 2008), release from the cyanobacterium *Trichodesmium* spp. after N fixation

(Walsh and Steidinger 2001; Lenos *et al.* 2001; Mulholland *et al.* 2004; Mulholland *et al.* 2006), as well as regenerated nutrients released from associated fish kills (Vargo *et al.* 2001, Walsh *et al.* 2006; Walsh *et al.* 2009).

Of particular interest is the role N can play in bloom nutrition, as it is generally limiting in marine systems (Hecky and Kilham 1988). Historically, the inorganic forms ammonium (NH_4^+) and (NO_3^-) were believed to be solely responsible for phytoplankton nutrition, however, DON, particularly urea, may be important to many phytoplankton (e.g. McCarthy 1972; reviewed in Kudela and Cochlan 2000; Berman and Bronk 2003; Bronk *et al.* 2007), and special emphasis has been placed on the potential importance of DON to the growth and toxicity of harmful algal bloom (HAB) species. Early studies determined that *K. brevis* is capable of utilizing organic N (Wilson 1966; Baden and Mende 1979) from a variety of sources including chemically simple forms such as amino acids (Wilson 1966; Baden and Mende 1979; Shimizu and Wrensford 1993; Shimizu *et al.* 1995) and urea (Vargo, reviewed in Steidinger *et al.* 1998; Glibert *et al.* 2009; Sinclair *et al.* 2009) as well as more chemically complex sources such as DON produced *in situ* from *Trichodesmium* (Bronk *et al.* 2004; Mulholland *et al.* 2006; Sipler *et al.* 2009) and humics (C. Heil, unpublished data; Bronk *et al.* unpublished data). Since the typical DIN concentrations along the WFS are extremely low and the DON concentrations high, *K. brevis* may be able to utilize both very low concentrations of inorganic nutrients as well as organics and have a competitive advantage over phytoplankton that rely on DIN alone.

A useful way to evaluate the affinity for a particular nutrient is to examine the nutrient uptake kinetics of that organism. Kinetics experiments can be valuable tools to interpret responses of phytoplankton to varying types and concentrations of N substrates.

Nitrogen kinetics experiments have been conducted on numerous HAB species in culture (e.g., Fan *et al.* 2003; Glibert *et al.* 2006; Sinclair *et al.* 2006; Herndon and Cochlan 2007; Cochlan *et al.* 2008) as well as in field populations (e.g. Kudela and Cochlan 2000; Fan *et al.* 2003; Kudela *et al.* 2008, Li *et al.* 2010). Determining which particular N sources fuel HABs can lead to a better understanding of their development and help model and potentially mitigate future bloom events.

In the case of *K. brevis*, little is known about its N preferences and the extent to which particular N species are utilized. The present study is an extensive examination of uptake of ^{15}N -labeled substrates by *K. brevis* as a function of concentration and provides estimates of the ability of *K. brevis* to utilize inorganics (NH_4^+ and NO_3^-), as well as organics: urea, an amino acid mixture, the individual amino acids alanine and glutamate, and humic-N, under bloom conditions in the field as well as a comparison of strains in culture.

Materials and Methods

Kinetics Experiments

Five sets of kinetics experiments were conducted in the eastern Gulf of Mexico during three cruises aboard the R/V *Suncoaster* in October 2001, the R/V *Walton Smith* in October 2002, and the R/V *Pelican* in October 2007 (Figure 1). Experiments were conducted with near-surface water (~1 m) collected by Niskin bottle at a depth of 1-9 m (2001 and 2002 experiments) or collected using a clean plastic bucket (2007 experiments) or and transferred to an acid cleaned (10% HCl) polyethylene carboy. Sample (10 ml) was preserved in

Lugol's solution for phytoplankton cell counts. Due to a co-occurring *Trichodesmium* spp. bloom during the 2007 experiments, surface water was first gently filtered through a 65 μm Nitex screen to remove *Trichodesmium* spp. from *K. brevis* prior to the initiation of experiments.

At the start of each experiment water was filtered through a pre-combusted Whatman[®] GF/F (450 °C for 2 hr); the filter and filtrate were frozen for subsequent analysis of Chl *a* (in 2007) and ambient concentrations of NH_4^+ , NO_3^- , urea, dissolved primary amines (DPA) and the dissolved free amino acids (DFAA) alanine and glutamate as described below.

Kinetic parameters were determined using ^{15}N -labeled ammonium chloride, ^{15}N potassium nitrate, ^{15}N urea, and a ^{15}N algal amino acid mixture comprised of 16 amino acids or ^{15}N glutamate (glu) or ^{15}N alanine (ala), all obtained from Cambridge Isotope Laboratories (Cambridge, MA) and > 96 atom % enrichment. Experiments were performed in 60-mL PETG bottles that had been amended with increasing amounts of ^{15}N -labeled substrate. Each experiment included a range of eight to ten substrate concentrations: 0.055, 0.10, 0.25, 0.5, 1.0, 5.0, 10, 50, 100, and 200 $\mu\text{mol N L}^{-1}$. The 0.25 $\mu\text{mol N L}^{-1}$ (2001 experiments) or the 0.10 $\mu\text{mol N L}^{-1}$ concentration (2007 experiments) was replicated to provide an estimate of bottle variability. The maximum ^{15}N concentration for all 2007 experiments was 50 $\mu\text{mol N L}^{-1}$. On October 28, 2002 germanium dioxide (~240 mM final concentration) was also added to each experimental bottle. Germanium dioxide in high concentrations leads to diatom death (Lewin 1966) by substituting in place of silica, though it is not generally considered toxic to non-diatomaceous algae (Andersen and Kawachi 2005), and was used to remove competition for N substrate by co-occurring *Skeletonema* spp.

Kinetics experiments were also conducted in 2007 using ^{15}N -labeled humics produced in the laboratory (See *et al.* 2006). The ^{15}N -labeled humics used for these experiments were produced by growing *Spartina alterniflora* with ^{15}N -labeled NH_4^+ in the sediment (See *et al.* 2006). The *Spartina* was cut, dried, and allowed to humify for a period of three months in the dark with coastal bacteria (See *et al.* 2006). The final atom % enrichment of the ^{15}N -labeled humics was (9.8% at. %). The ^{15}N -labeled humics were added according to the protocol described above for the other ^{15}N -labeled substrates. In addition, a duplicate set of samples served as killed controls to correct for abiotic adsorption of the humic label to the filter or the outside of phytoplankton cells (See *et al.* 2006). Killed controls received 10 μL of saturated mercuric chloride (HgCl_2) five minutes prior to the addition of humic label. However, through microscopic examination of *K. brevis* cells in the killed control treatment, it was determined that the added HgCl_2 caused leakage of intracellular contents and burst cells within five to ten minutes. Killed controls, therefore, were not factored into the kinetic rate calculations and rates were determined in the same manner as the other substrates. As a result, humic uptake rates may be slight overestimates.

Samples were incubated for 30 min (2007 experiments) or 1 hr (2001, 2002 experiments) in on-deck incubators cooled with surface seawater under *in situ* conditions under one layer of neutral density screening. Incubations were terminated by filtration onto pre-combusted (450 °C, 2 h) Whatman[®] GF/F filters (2001 and 2002 experiments) or 5 μm Sterlitech[™] Corporation silver membrane filters (2007 experiments) at < 100 mmHg. In 2007 filters were given a final rinse with 2-3 mL of 0.2 μm filtered artificial seawater; no rinse was done in 2001 and 2002. After filtration, filters were placed in polypropylene cryovials and frozen. Prior to analysis of particulate N (PN) concentrations and isotopic

enrichments, filters were thawed and dried overnight in a drying oven at 40°C. Isotopic samples were analyzed on a Europa Automated Nitrogen Carbon Analyzer for Solids and Liquids (ANCA-SL) attached to a GEO 20/20 isotope ratio mass spectrometer.

Laboratory culture experiments were conducted in February 21-24, 2006 using non-axenic cultured strains of *K. brevis* maintained in the Florida Fish and Wildlife Research Institute culture collection in St. Petersburg, FL: Jacksonville C4 (CCFWC 251), and Texas B4 (CCFWC 267), hereafter referred to as Jax and Tex, respectively. Strains were named for the cities near where they were collected: Jacksonville, FL and South Padre Island, Texas. All strains were isolated in October 1999. Strains were maintained in glass carboys at 22°C on a 18:6 light:dark photoperiod at 35.9 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. Cultures (2-3 L) were maintained on GP growth medium (Loeblich and Smith 1968), prepared using seawater (salinity 35) that was collected from the Gulf Stream and filtered through a 0.45 μm filter and autoclaved prior to use. Ammonium was the N-source and cultures were allowed to draw down NH_4^+ prior to the initiation of the experiments. Cellular growth was monitored and cultures were in exponential phase when the experiments began. Uptake kinetics were determined as described above except that 10 mL aliquots of each strain were added to 20 mL borosilicate test tubes to measure kinetic parameters over the range of substrate concentrations. Humics uptake was not investigated during the culture study. Samples were incubated for 0.5 h under the same conditions as those used for culture maintenance. Incubations were terminated by filtration onto pre-combusted (450°C, 2 h) Whatman® GF/F filters and analyzed as described above.

Additionally, on October 29, 2002, a kinetics experiment was also conducted aboard the R/V Walton Smith using an unspecified *K. brevis* cultured strain from the Florida Fish

and Wildlife Research Institute culture collection in St. Petersburg, FL. The experiment was conducted as described previously except 1 mL of well-mixed culture was added to each 60 mL PETG bottle containing 0.2 μm filtered field-collected surface seawater and the 1 h incubation proceeded in the shipboard laboratory where the culture had been maintained.

Analytical methods

Water samples to measure concentrations of NH_4^+ , NO_3^- , and urea were placed in low density polyethylene (LDPE) centrifuge tubes (Corning[®]) while DPA and DFAA samples were stored in high density polyethylene bottles (HDPE) bottles and polypropylene cryovials, respectively. Urea analyses were performed in duplicate while all other analyses were performed in triplicate. Concentrations of NH_4^+ were analyzed manually by the colorimetric phenol-hypochlorite technique of Koroleff (1983). Concentrations of NO_3^- were measured using a Lachat QuikChem 8500 autoanalyzer (Parsons *et al.* 1984). Urea was analyzed according to the diacetyl monoxmine method adapted from Price and Harrison (1987). Total concentrations of amino acids were measured as dissolved primary amines (DPA) using the fluorometric OPA method (Parsons *et al.* 1984) and individual amino acid (glu, ala) concentrations were determined as DFAA using the HPLC method (Cowie and Hedges 1992). Concentrations of Chl *a* were measured fluorometrically on a Turner Design Model 10-AU fluorometer; each sample had been previously filtered onto a pre-combusted GF/F filter, extracted in 90% acetone overnight at 4°C, and analyzed, in duplicate, according to Parsons *et al.* (1984). Samples for phytoplankton species abundance (10 mL) were collected

were fixed with Lugol's solution and 1 mL subsamples were counted on an inverted microscope.

Calculations

Nitrogen specific uptake rates were estimated from the accumulation of ^{15}N in the PN and calculated according to a constant specific uptake model (Dugdale and Wilkerson 1986). Uptake rates were not corrected for isotope dilution (Glibert *et al.* 1982). Due to the short duration of the incubations, the lack of grazers in the experiments, and the high tracer additions used in most incubations, little isotope dilution would be expected. Isotope dilution was likely occurring in the field and resulted in an underestimate of specific uptake rates in the $< 1 \mu\text{m}$ addition incubations.

Curve fitting was completed using a computerized, iterative non-linear least-squares technique (SigmaPlot[®] version 9.0, Systat Software Inc.), which utilizes the Marquardt-Levenberg algorithm (Press *et al.* 1992). The kinetics data were fitted directly to the Michaelis-Menten formula (1).

$$V = V_{max} \cdot S / (K_s + S) \quad (1)$$

Where V is the specific uptake rate (h^{-1}), V_{max} is the maximal specific uptake rate, S is the substrate concentration ($\mu\text{mol N L}^{-1}$), and K_s is the half-saturation constant for the N substrate ($\mu\text{mol N L}^{-1}$). The α value for each plot was also determined as V_{max}/K_s ($\times 10^{-3} \text{ h}^{-1} / \mu\text{mol N L}^{-1}$) and is considered a more robust indicator of substrate affinity at low concentrations (i.e., $S < K_s$) (Button 1978; Healey 1980; Cochlan and Harrison 1991). Elevated ambient concentrations for many experiments violated a basic assumption in the kinetic model that

the nutrient of interest be depleted at the cell surface. In these cases, calculation of kinetic parameters was not possible and those data are not reported.

Results

To assess the nutrient utilization of *K. brevis*, we conducted numerous kinetics experiments under field conditions where *K. brevis* was the dominant organism. During the October 2 and 4, 2001 experiments, *Karenia* spp. accounted for 99.7% and 95.0% of phytoplankton cells, respectively, with a maximum concentration of 9.5×10^6 cells L⁻¹. During the October 28, 2002 field study *Karenia* spp. biomass was dominant but samples also included the diatom *Skeletonema* spp. As noted in the methods, Ge was added to inhibit diatom growth. On October 18 and 22, 2007, bloom densities were 1.3×10^6 and 9.5×10^6 cells L⁻¹, with *K. brevis* cells accounting for 88.6% and 82.6% of total biomass, respectively. Additionally, 3×10^3 and 30×10^3 cells L⁻¹ on October 18 and 22, 2007 were *K. mikimotoi*, respectively, and the remainder of cells were unidentified *Karenia* spp that were likely *K. brevis*. Measurements of Chl *a* for the October 18 and 22, 2007 experiments corresponded to 29.3 and 27.7 $\mu\text{g L}^{-1}$, respectively. In the laboratory culture experiments, cell concentrations were 11.3×10^6 cells L⁻¹ for Jax and 7.8×10^6 cells L⁻¹ for Tex. Cell count data are not available for the October 29, 2002 experiment run using *K. brevis* culture in a field setting.

For the 2001 and 2002 field studies, including the culture experiment conducted in the field, ambient NH₄⁺ measurements were $< 0.2 \mu\text{mol N L}^{-1}$ (Table 1), NO₃⁻ concentrations were $< 0.05 \mu\text{mol N L}^{-1}$. Urea concentrations were comparatively elevated on those dates,

measuring $1.2 \mu\text{mol N L}^{-1}$, except on October 28, 2002 with $0.18 \mu\text{mol N L}^{-1}$.

Concentrations of alanine and glutamate were less than $0.1 \mu\text{mol N L}^{-1}$ for the 2001 and 2002 field sites. For both the 2007 field experiments, NH_4^+ was $< 0.5 \mu\text{mol N L}^{-1}$ (Table 1), NO_3^- and DPA concentrations were $< 0.1 \mu\text{mol N L}^{-1}$. Urea was 0.33 and $1.4 \mu\text{mol N L}^{-1}$ for Oct 18 and Oct 22, 2007 respectively. Concentrations of humics were not measured but due to the oligotrophic nature of the water column at the sampling location, humic concentrations were considered negligible for the purpose of determining uptake rates. Concentrations of humics in the Gulf of Mexico have previously been measured in low concentrations in the fall, comprising $\sim 0.3\%$ of the DOC pool (Harvey *et al.* 1983). Ambient nutrient concentrations of NH_4^+ , NO_3^- in both cultures were $< 1.0 \mu\text{mol N L}^{-1}$.

Uptake kinetics - inorganic nitrogen

Using *K. brevis* collected from the field and grown in cultures, all kinetics experiments with $^{15}\text{NH}_4^+$ as the substrate can be related directly to the Michaelis-Menten kinetics model (Table 1; Figure 2). Estimated maximum rates of uptake (V_{max}) in the field and culture studies exhibited considerable variability and ranged from $27 - 99 \times 10^{-3} \text{ h}^{-1}$ and half saturation constants (K_s) ranged from $0.3 - 1.8 \mu\text{mol N L}^{-1}$. The highest V_{max} obtained was from the October 22, 2007 experiment that also had the highest ambient NH_4^+ concentration ($0.35 \mu\text{mol N L}^{-1}$), while the lowest V_{max} was obtained from the 2002 culture experiment conducted in the field and had the lowest ambient NH_4^+ concentration ($0.04 \mu\text{mol N L}^{-1}$; Table 1). Maximum specific uptake rates versus ambient concentration of NH_4^+ revealed a positive correlation ($r^2 = 0.87$, $P < 0.05$, Figure 3); since K_s is a function of V_{max} ,

a similar relationship was observed for NH_4^+ - K_s ($r^2 = 0.80$, $P < 0.05$, data not shown). Mean V_{max} and K_s values for all experimental dates were $49 \pm 9.9 \times 10^{-3} \text{ h}^{-1}$ and $0.6 \pm 0.4 \mu\text{mol N L}^{-1}$, respectively. Values of α ranged from $53 - 135 \times 10^{-3} \text{ h}^{-1} / \mu\text{mol N L}^{-1}$ with a mean of $79 \times 10^{-3} \text{ h}^{-1} / \mu\text{mol N L}^{-1}$ (Table 1), the highest α value reported for the substrates tested. For the culture studies, experiments with NH_4^+ as the substrate were the only results that yielded curves that fit the Michaelis-Menten formulation, while the other substrates did not, likely a function of the high ambient concentrations of those substrates in the culture media.

For kinetics experiments with $^{15}\text{NO}_3^-$ as the substrate, the October 18 and 22, 2007 experiments fit directly to the Michaelis-Menten formulation (Fig. 4) with a good fit ($r^2 = 0.92$). However, uptake in both the 2001 experiments was non-saturating, even at the highest substrate additions ($200 \mu\text{mol N L}^{-1}$; Figure 4 A, C). For comparison with the 2007 kinetics plots, the 2001 curves were re-plotted according to the maximum concentration used in the 2007 experiments ($50 \mu\text{mol N L}^{-1}$) and a hyperbolic function was obtained with r^2 values of 0.77 and 0.94 for October 2 and 4, 2001 respectively (Figure 4 A, C inserts). The V_{max} for the re-calculated plots were 15 ± 1.3 and $23 \pm 1.6 \times 10^{-3} \text{ h}^{-1}$, which are similar as those for the 2007 experiments (16 and $20 \times 10^{-3} \text{ h}^{-1}$). A mean V_{max} was calculated to be $19.0 \pm 2.7 \times 10^{-3} \text{ h}^{-1}$, which is ~ 2.5 -fold less than the NH_4^+ mean. The mean $\text{NO}_3^- K_s$ value was also less ($0.4 \pm 0.2 \mu\text{mol N L}^{-1}$). The mean α value was $48 \times 10^{-3} \text{ h}^{-1} / \mu\text{mol N L}^{-1}$, slightly less than half the value obtained for the NH_4^+ kinetics.

Uptake kinetics - organic nitrogen

For kinetics experiments with ^{15}N -urea, the October 28, 2002 and October 18, 2007 experiments fit directly to the Michaelis-Menten function (Fig. 5) with r^2 values of 0.77 and 0.62, respectively. The V_{max} parameters matched closely at 24 and $26 \times 10^{-3} \text{ h}^{-1}$ and K_s parameters were 1.6 and $0.3 \mu\text{mol N L}^{-1}$, respectively (Table 1). Values of α were 15 and $43 \times 10^{-3} \text{ h}^{-1} / \mu\text{mol N L}^{-1}$ with a mean of $23 \times 10^{-3} \text{ h}^{-1} / \mu\text{mol N L}^{-1}$ (Table 1).

Specific uptake rates of AA substrates could not be described by the Michaelis-Menten function (Figure 6). The low end of the concentration range of the curves ($< 1.5 \mu\text{mol N L}^{-1}$) appeared more hyperbolic (Figure 6, insert plots), however, the plots were best fit to a linear function of the ambient AA concentration over the range of substrate concentrations examined here. For comparison, if the slopes of the lines were examined (0.18 , 0.34 and $0.13 \times 10^{-3} \text{ h}^{-1} / \mu\text{mol N L}^{-1}$ for AA mix, Ala, and Glu, respectively), the values are small, indicating that the affinity of *K. brevis* for AAs is likely less than the affinity for the other substrates (Table 1).

For humic uptake, both experiments conducted in 2007 fit the hyperbolic function with r^2 values of 0.98 and 0.81. There was variability between the experiments with a ~5-fold difference in V_{max} values and an 11-fold difference in K_s parameters. Humic uptake had the highest observed mean V_{max} (230 ± 86) and K_s ($18 \pm 12 \mu\text{mol N L}^{-1}$). The mean α value was $13 \times 10^{-3} \text{ h}^{-1} / \mu\text{mol N L}^{-1}$. When comparing α parameters for all the substrates tested, the overall trend for *K. brevis* was: $\text{NH}_4^+ > \text{NO}_3^- > \text{urea} > \text{humics} > \text{AA}$. Patterns were similar for absolute uptake parameters (Appendix 1).

Discussion

In kinetic studies, the typical parameters of interest are the V_{max} and K_s . An organism that has a high V_{max} is described as well adapted to utilize a particular nutrient at high uptake rates. For the K_s parameter, the lower the substrate concentration necessary for a given transport rate the greater the adaptation to enhance uptake of that nutrient at low concentrations. Therefore, competitive advantage can come from possessing a high V_{max} or a low K_s . Since the value of K_s is a function of V_{max} , it may not be the most reliable indicator of nutrient preference at low concentrations. The affinity constant α is considered a more robust indicator of substrate affinity at low concentrations as it takes into account both parameters (Button 1978; Healey 1980; Cochlan and Harrison 1991), therefore α values were calculated here.

Uptake kinetics - inorganic nitrogen

Ammonium experiments produced the most curves fitting the Michaelis-Menten hyperbolic function and permitted the determination of a range of parameters spanning a variety of individual bloom events and culture conditions. Of all substrates tested, NH_4^+ exhibited the highest average V_{max} values of inorganic substrates. The calculated V_{max} values were found to vary as a function of ambient concentration with increasing V_{max} as ambient concentration increased ($r^2 = 0.87$, Figure 3). Previous studies have found a similar relationship between $\text{NO}_3^- V_{max}$ and ambient NO_3^- concentrations for natural phytoplankton assemblages in upwelling areas (Dugdale *et al.* 1990; Dugdale and Wilkerson 1991) and the

Ross Sea (Cochlan and Bronk 2001) although not with NH_4^+ . A relationship between the α value of NO_3^- , NH_4^+ , or urea uptake and the percentage of NO_3^- in the media has been observed for *P. minimum* cultures and this observed variability has been related to the nutritional history of the cells (Fan *et al.* 2003). Results from this study indicate that *K. brevis* may modulate its NH_4^+ - V_{max} under variable nutrient settings, allowing them to be more competitive under a broad range of N conditions.

Overall NH_4^+ had the highest affinity constant, 2- to 6-fold greater than the other substrates tested, indicating that *K. brevis* has a high capacity for NH_4^+ uptake as well as a high affinity at low substrate concentrations. A high affinity for NH_4^+ is not unusual for phytoplankton as is the molecule is already in a reduced form, is energetically efficient for cells to use, and requires little additional energy to be assimilated (reviewed in Mulholland and Lomas 2008).

While typically found in relatively low ambient concentrations in samples taken at bloom sites, there are many potential sources of NH_4^+ to blooms such as recent release from *Trichodesmium* spp. (Mulholland *et al.* 2006; Vargo *et al.* 2008); NH_4^+ and DON are the greatest release products (Mulholland *et al.* 2004, 2006) and *Trichodesmium* spp. along the WFS releases an average 52% of recently fixed N (Mulholland *et al.* 2006). Recycled NH_4^+ released from decaying fish may be another important source particularly in coastal and estuarine regions and have been suggested to fuel high biomass blooms (Vargo *et al.* 2001; Walsh *et al.* 2006; Walsh *et al.* 2009). Wilson and Collier (1955) found that additions of fish extracts stimulated growth of *K. brevis* in cultures. It has been estimated that NH_4^+ release from fish may be $\sim 1,000 \mu\text{mol N L}^{-1} \text{d}^{-1} \text{g}^{-1}$ wet fish weight (Appendix 2). Given that fish

kills numbering thousands of fish have been observed and the kinetics parameters presented here, there is the potential for high NH_4^+ release and subsequent utilization by *K. brevis*.

Another potentially important source of N may be regenerated is populations of centric diatoms that develop at near-bottom waters during summer stratification (Heil *et al.* 2001). Decay of diatoms and subsequent remineralization, combined with breakdown of thermal stratification by vertical mixing in the fall may be an NH_4^+ source (Vargo *et al.* 2008). A few benthic flux rates of NH_4^+ have been measured in region in which blooms occur, with rates of $0.002 \mu\text{mol N L}^{-1} \text{d}^{-1}$ (Marinelli *et al.* 1998) and $0.1 \mu\text{mol N L}^{-1} \text{d}^{-1}$ (Darrow *et al.* 2003). More recently, it was determined that at the 10 m isobath along the West Florida Shelf, sediment flux could supply of 3-7% of the N requirement of *K. brevis* (K. Dixon, personal communication)

For NO_3^- , while the 2007 kinetic plots demonstrated a hyperbolic function with increasing substrate concentration, the 2001 plots were unsaturated at the highest substrate levels, despite having lower ambient nutrient concentrations. However, when re-plotted at the maximum substrate concentration added for the 2007 experiments the plots were a hyperbolic function and exhibited similar V_{max} and K_s parameters as determined in the 2007 experiments (Table 1). It is possible that different environmental conditions present at the time of sampling may have induced the non-saturation (Fan *et al.* 2003) or variations in growth rate (not measured here, Dortch *et al.* 1991), however, it is unclear if the same non-saturating trend would have been apparent in the 2007 experiments if the substrate concentrations had been extended beyond $50 \mu\text{mol N L}^{-1}$. Therefore, estimates of kinetics parameters for the 2001 plots may be underestimating the V_{max} and K_s parameters.

Non-saturation of NO_3^- uptake has previously been observed from diatom-dominated populations (Collos *et al.* 1992, 1997, 2005, Lomas and Glibert 1999, 2000; Li *et al.* 2010) and in dinoflagellates (Lomas and Glibert 2000; Li *et al.* 2010). Nitrate uptake by some phytoplankton has been suggested to fit the Michaelis-Menten relationship in concentrations up to approximately $40 \mu\text{mol N L}^{-1}$ (Lomas and Glibert 1999) with diffusional uptake dominating at higher substrate additions, resulting in linear uptake kinetics instead of the Michaelis-Menten relationship (Lomas and Glibert 2000). In diatoms and large cells these linear rates may be the result of an active uptake mechanism induced to fill internal NO_3^- pools (Collos *et al.* 1997; Lomas and Glibert 2000), however, this same relationship may not be applicable to *K brevis* and internal pools of N were not measured here. An alternative hypothesis is that the NO_3^- uptake mechanism may operate as a two-component system including a high-affinity, low-capacity as well as a low-affinity, high-capacity component that is inducible at high concentrations (Lomas and Glibert 2000)

Uptake kinetics - organic nitrogen

Urea is considered an important N source for many HABs including *Lingulodinium polyedrum* (Kudela and Cochlan 2000), *Alexandrium catenella* (Collos *et al.* 2004), and *Aureococcus anophagefferens* (Lomas *et al.* 1996, Berg *et al.* 1997, Gobler *et al.* 2002), *P. minimum* (Fan *et al.* 2003). The increased use of urea as a N-source in fertilizer and run-off into coastal waters is often related to HAB occurrence and selection of dinoflagellates over diatoms (Glibert and Terlizzi 1999, Glibert *et al.* 2006b). Urea has been shown to support growth rates for *K. brevis* ranging from 0.10 to 0.19 divisions d^{-1} (Vargo, in

Steidinger *et al.* 2008; Sinclair *et al.* 2009). Kinetics experiments with urea as a substrate yielded two plots in which the Michaelis-Menten relationship was acquired with the remainder of plots not presented due to a violation of the basic kinetics assumptions (high ambient N concentration, particularly for Jax and Tex cultures $\geq 8 \mu\text{mol N L}^{-1}$).

Overall, rates in this study indicated good agreement for V_{max} values for the two experiments (24 ± 3.3 and $27 \pm 2.3 \times 10^{-3} \text{ h}^{-1}$); K_s values were more variable (1.58 ± 0.97 and $0.63 \pm 0.22 \mu\text{mol N L}^{-1}$) although fall into a similar range as parameters determined in culture studies where hyperbolic Michaelis-Menten relationships were evident (Sinclair *et al.* 2009). The mean α (23 ± 21) for urea was less than the inorganic substrates, but highest of the organic substrates investigated.

Uptake plots for concentration versus specific uptake rates for all AA substrates indicate that *K. brevis* was able to take up all the AA substrates offered although uptake was non-saturable. When linear uptake occurs it is difficult to measure K_s values (Cochlan and Bronk 2001; Lomas and Glibert 2000). Since no V_{max} was determined, non-saturation may be advantageous and indicate a potential adaptation to acquire nutrients at high substrate levels over time (Fan *et al.* 2003). Linear or non-saturable uptake for AAs (Fan *et al.* 2003) and other ^{15}N -labeled substrates (Lomas and Glibert 1999; Fan *et al.* 2003) has previously been observed. It has been hypothesized that linear uptake may simply reflect simple diffusion into the cells (Fan *et al.* 2003). The idea of simple diffusion of AAs is doubtful though considering the ambient low AA concentrations typically observed in nature (Raven 1980; Antia *et al.* 1991); phytoplankton that utilize amino acids require active transport mechanisms to take up AAs due to the high concentrations of intracellular AA pools present within the cells (1-20 mM; Flynn *et al.* 1993). Departures from the hyperbolic function may

also arise from the high variation in K_s values of natural assemblages (McCarthy *et al.* 1981), however, since *K. brevis* was the dominant organism variability due to phytoplankton assemblage is unlikely.

It is possible that observed linearity in uptake could be a function of multiple uptake processes for the same nutrient (McCarthy 1981) or multiple AA transporters on the cell. In phytoplankton, uptake of AAs typically occurs through at least three transport systems related to the charge of the amino acid (reviewed in Antia *et al.* 1991; Bronk and Flynn 2005; Mulholland and Lomas 2008). Since the ^{15}N label used in the 2007 experiment contained a mixture of 26 AAs that included positive, negative, and neutral charges, the uptake demonstrated here likely reflects uptake by multiple transporters. Perhaps that is the reason for the seemingly hyperbolic nature of the curve at very low substrate additions ($< 1.5 \mu\text{mol N L}^{-1}$). While linear uptake was observed in this study, saturating AA (glycine) kinetics have been observed for a population of *K. mikimotoi* in the East China Sea (Table 1, Li *et al.* 2010), although the maximum ^{15}N addition was only $8 \mu\text{mol N L}^{-1}$, much lower than the maximum concentrations added in our experiments. Glutamate is a primary release product of *Trichodesmium* (Capone *et al.* 1994) and *K. brevis* may encounter these transient high concentration pools of AAs within close proximity to a *Trichodesmium* colony. Additionally, it has been estimated that dead fish may release $\sim 100 \mu\text{mol N L}^{-1} \text{ d}^{-1} \text{ g}^{-1}$ wet fish weight (Appendix 3) in the form of DPAs. The high V_{max} values hypothesized for *K. brevis* in this study, in excess of the maximum specific uptake rates observed ($10\text{-}30 \times 10^{-3} \text{ h}^{-1}$), would indicate that *K. brevis* would be able to take advantage of these high AA pools.

While humics were once thought to be refractory substances, recent studies have highlighted the potential importance of humic uptake to coastal and estuarine phytoplankton. Humic acid fractions have been shown to stimulate HABs when added to the culture medium (Gangon *et al.* 2005; Heil 2005) and direct uptake has also been observed (Doblin *et al.* 2001; See *et al.* 2006; C. Heil unpublished data). The *K. brevis* cultures used in this experiment are typically maintained on a culture medium containing humic substances (soil extract). Studies have found that adding soil extract to phytoplankton media promoted culture growth and that this effect is not based on chelation processes alone (Prakash and Rashid 1968; Prakash *et al.* 1973). Direct humic uptake was measured in this study and while results indicate a lower affinity than other N substrates, humic-N uptake by *K. brevis* had the highest average V_{max} measured ($230 \pm 86 \times 10^{-3} \text{ h}^{-1}$) indicating a great capacity for uptake when high concentrations of humics are present. High concentrations of humics can be discharged from Florida rivers on a seasonal basis (Martin *et al.* 1971), therefore, humics could be an important N source during the nearshore maintenance phase of a bloom.

Although the method in which *K. brevis* obtains humic-N is unclear, similar to other phytoplankton, *K. brevis* may be able to access humic-N through phagocytosis, photochemical breakdown or enzymatic cleavage followed by uptake of the freed N (reviewed in Bronk *et al.* 2002, 2007; Mulholland and Lomas 2008). Recently, *K. brevis* has also been shown to graze on whole cells of *Synechococcus* (Procise and Mulholland 2008, Glibert *et al.* 2009), so the ability of *K. brevis* to ingest larger complex molecules has been demonstrated.

Conclusions

Nutrient uptake kinetics are a useful tool to define basic parameters of phytoplankton physiological ecology (Dugdale 1967; Eppley and Coatsworth 1968; McCarthy 1981). Kinetic parameters can assess the nutrient preferences of an organism and allow for comparison amongst different species. In the present study, kinetic parameters were successfully determined for field populations and cultures of the harmful dinoflagellate, *K. brevis*, using a variety of inorganic and organic N substrates including: NH_4^+ , NO_3^- , urea, an amino acid mixture, as well as the individual amino acids alanine and glutamate, and humics. This study represent the most comprehensive assessment of kinetics parameters for *K. brevis* examined in a field setting during three different bloom years and also compares those results to cultured strains. Mean calculated α parameters for the five sets of experiments indicated that the value for NH_4^+ was greatest for all experiments and followed a pattern of: $\text{NH}_4^+ > \text{NO}_3^- > \text{urea} > \text{humics} > \text{AA}$. While *K. brevis* may have a physiological preference to take up NH_4^+ , all inorganic and organic N substrates that were offered were taken up. This highlights the ability of *K. brevis* to utilize a wide variety of nutrient sources and provides greater insight into how populations can thrive under a wide range of nutrient regimes from oligotrophic shelf to eutrophic coastal waters. A better understanding of *K. brevis*'s nutritional preferences will enhance our knowledge of what nutrients are responsible for the initiation and maintenance of large bloom events.

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Literature Cited

- Andersen, R.A. and Kawachi, M. (2005). Traditional microalgae isolation techniques. In R.A. Andersen (Eds). *Algal Culturing Techniques*. pp. 83-99.
- Antia, N., Harrison, P., Oliveira, L. 1991. The role of dissolved organic nitrogen in phytoplankton nutrition, cell biology, and ecology. *Phycologia* 30, 1-89.
- Baden, D.G. and Mende, T.J. 1979. Amino acid utilization by *Gymnodinium breve*. *Phytochemistry* 18, 247-251.
- Berg, G.M., Glibert, P.M., Lomas, M.W., Burford, M.A. 1997. Organic nitrogen uptake and growth by the chrysophyte *Aureococcus anophagefferens* during a brown tide event. *Mar. Biol.* 129, 377-387.
- Berman, T. and Bronk, D.A. 2003. Dissolved organic nitrogen: a dynamic participant in aquatic ecosystems. *Aquat. Microb. Ecol.* 31, 279-305.
- Bronk, D. A. 2002. Dynamics of dissolved organic nitrogen. In: Hansell, D. A. and Carlson, C. A. [Eds.] *Biogeochemistry of Marine Dissolved Organic Matter*. Academic Press, San Diego, CA. pp. 153-247.
- Bronk, D. A., Sanderson, M.P., Mulholland, M.R., Heil, C.A., O'Neil, J.M. 2004. Organic and inorganic nitrogen uptake kinetics in field populations dominated by *Karenia brevis*. In: Steidinger, K. A., J. H. Landsberg, C. R. Tomas and G. A. Vargo [Eds.], *Harmful Algae 2002*. Florida Fish and Wildlife Conservation Commission, Florida Institute of Oceanography and Intergovernmental Oceanographic Commission of UNESCO, St. Petersburg, Florida, USA. pp. 80-82.
- Bronk, D.A. and K.J. Flynn. 2005. Algal cultures as a tool to study the cycling of dissolved organic nitrogen. In: Durvasula, S.R.V. [Eds.] *Algal Cultures, Analogues of Blooms and Applications*. Oxford & IBH Publishing Co. Pvt. Ltd. New Dehli.
- Bronk, D.A., See, J.H., Bradley, P., Killberg, L. 2007. DON as a source of bioavailable nitrogen for phytoplankton. *Biogeosciences Discussions* 3, 1247-1277.
- Button D.K. 1978. On the theory of control of microbial growth kinetics by limiting nutrient concentration. *Deep-Sea. Res.* 25, 113-1177.
- Capone, D., Ferrier, M. and Carpenter E. 1995. Amino Acid Cycling in Colonies of the Planktonic Marine Cyanobacterium *Trichodesmium thiebautii*. *Appl. Environ. Microbiol.* 60, 3989-3995

- Cochlan, W.P. and Bronk, D.A. 2001. Nitrogen uptake kinetics in the Ross Sea, Antarctica. *Deep-Sea Res. II.* 48, 4127-4153.
- Cochlan, W.P. and Harrison P.J. 1991. Kinetics of nitrogen (nitrate, ammonium, urea) uptake by the picoflagellate *Micromonas pusilla* (Prasinophyceae). *J. Exp. Mar. Biol. Ecol.* 153, 129-141.
- Cochlan, W.P., Herndon, J., Kudela, R.M. (2008) Inorganic and organic nitrogen uptake by the toxigenic diatom *Pseudo-nitzschia australis* (Bacillariophyceae). *Harmful Algae* 8(1), 111-118.
- Collos, Y., Siddiqi, M.Y., Wang, M.Y., Glass, A.D.M., Harrison, P.J. 1992. Nitrate uptake kinetics by two marine diatoms using the radioactive tracer ^{13}N . *J. Exp. Mar. Biol. Ecol.* 163, 251-260.
- Collos, Y., Gagne C., Laabir, A., Vaquer, P., Cecchi, P., Souchu, P. 2004. Nitrogenous nutrition of *Alexandrium catenella* (Dinophyceae) in cultures and in Thau Lagoon, Southern France. *J. Phycol.* 40, 96-103.
- Collos, Y., Vacquer, A., Bibent, B., Slawyk, G., Caccia, N., Souchu, P. 1997. Variability in nitrate uptake kinetics of phytoplankton communities in a mediterranean coastal lagoon. *Estuar. Coast. Shelf Sci.* 44, 369-375.
- Collos, Y., Vaquer, A., Souchu, P. 2005. Acclimation of nitrate uptake by phytoplankton to high substrate levels. *J. Phycol.* 41(3), 466-478.
- Conway, H.L., Harrison, P.J., Davies, C.O. 1976. Marine diatoms grown in chemostats under silicate and ammonium limitations. II. Transient response of *Skeletonema costatum* to a single addition of the limiting nutrient. *Mar. Biol.* 35, 187-199.
- Cowie, G.L. and Hedges, J.I. 1992. Sources and reactivities of amino acids in a coastal marine environment. *Limnol. Oceanogr.* 37(4), 703-724.
- Darrow, B.P., Walsh, J.J., Vargo, G.A., Masserini, R.T., Fanning, K.A., Zhang, J.-Z, (2003). A simulation study of the growth of benthic microalgae following the decline of a surface phytoplankton bloom. *Cont. Shelf. Res.* 23:1265-1283.
- Doblin, M, Carlsson, C, Humme, C, Granéli, E, Hallegraeff, G (2001) Uptake of humic substances by the toxic dinoflagellate *Alexandrium catenella*. *Harmful Algal Blooms 2000*: 332-335.
- Dortch, Q., Thompson, P.A, Harrison, P.J. 1991. Variability in nitrate uptake kinetics in *Thalassiosira pseudonana* (Bacillariophyceae). *J. Phycol.* 27, 35-39.
- Dugdale, R. (1967) Nutrient limitation in the sea: dynamics, identification, and significance. *Limnol. Oceanogr.* 12, 685-695.

- Dugdale, R. and Wilkerson, F. 1986. The use of ^{15}N to measure nitrogen uptake in eutrophic oceans: experimental considerations. *Limnol. Oceanogr.* 12, 673-689.
- Dugdale, R.C. and Wilkerson, F.P. 1991. Low specific nitrate uptake rate: a common feature of high-nutrient, low-chlorophyll marine ecosystems. *Limnol. Oceanogr.* 36(8), 1678-1688.
- Dugdale, R.C., Wilkerson, F.P., Morel, A. 1990. Realization of new production in coastal upwelling waters: A means to compare relative performance. *Limnol. Oceanogr.* 35, 822-829.
- Eppley, R.W. and Coatsworth, J. (1968) Uptake of nitrate and nitrite by *Ditylum brightwellii*—kinetics and mechanisms. *J. Phycol.* 4, 151-156.
- Fan, C., Glibert, P., Burkholder, J. 2003. Characterization of the affinity for nitrogen, uptake kinetics, and environmental relationships for *Prorocentrum minimum* in natural blooms and laboratory cultures. *Harmful Algae* 2, 283-299.
- Flynn, K., Flynn, K.J., Jones, K. 1993. Changes in dinoflagellate intracellular amino acids in response to diurnal changes in light and N supply. *Mar Ecol. Prog. Ser.* 100, 245-252.
- Gagnon, R, Levasseur, M, Weise, AM, Fauchot, J (2005) Growth stimulation of *Alexandrium tamarens* (Dinophyceae) by humic substances from the Manicouagan River (Eastern Canada). *Journal of Phycology* 41:489-497
- Glibert, P.M., F. Lipschultz, J.J. McCarthy, Altabet, M.A. 1982. Isotope dilution models of uptake and remineralization of ammonium by marine plankton. *Limnol. Oceanogr.* 27, 639-650.
- Glibert, P., Burkholder, J., Parrow, M., Lewitus, A., Gustafson, D. 2006. Direct uptake of nitrogen by *Pfiesteria piscicida* and *Pfiesteria shumwayae*, and nitrogen nutritional preferences. *Harmful Algae* 5, 380-394.
- Glibert, P.M. and Terlizzi, D.E. 1999. Cooccurrence of elevated urea levels and dinoflagellate blooms in temperate estuarine aquaculture ponds. *Appl. Environ. Microbiol.* 65(12), 5594-5596.
- Glibert, P, Harrison, J, Heil, C, Seitzinger, S. 2006b. Escalating worldwide use of urea – a global change contributing to coastal eutrophication. *Biogeochemistry* 77(3), 441-463.
- Glibert, P.M., Burkholder, J.M., Kana, T M., Alexander, J., Skelton, H., Shilling, C. 2009. Grazing by *Karenia brevis* on *Synechococcus* enhances its growth rate and may help to sustain blooms. *Aquat. Microb. Eco.* 55:17-30.

- Gobler, C.J., Buck, N.J., and Renaghan, M.J. 2002. Impacts of nutrients and grazing mortality on the abundance of *Aureococcus anophagefferens* during a New York Brown Tide bloom. *Limnol. Oceanogr.* 47, 129-141
- Goldman, J.C., Glibert, P.M. 1982. Comparative rapid ammonium uptake by four species of marine phytoplankton. *Limnol. Oceanogr.* 27, 814-827.
- Healey, F.P. 1980. Slope of the Monod equation as an indicator of advantage in nutrient competition. *Microb. Eco.* 15, 281-286.
- Hecky, R. and Kilham P. 1988. Nutrient limitation of phytoplankton in freshwater and marine environments: a review of recent evidence of the effects of enrichment. *Limnol. Oceanogr.* 33, 796-822.
- Heil, C., Vargo, G., Spence, D., Neely, M., Merkt, R., Lester, K., Walsh, J. 2001. Nutrient stoichiometry of a *Gymnodinium breve* Davis (Gymnodiniales: Dinophyceae) bloom: What limits blooms in oligotrophic environments? In: Hallegraeff, G.M., Blackburn, S.I., Bolch, C.J., Lewis, R.J. (Eds.) *Harmful Algal Blooms 2000*, Intergovernmental Oceanographic Commission of UNESCO 2001, Paris, pp.165-168.
- Heil, C.A. 2005. Effects of humic acids on growth, photosynthesis, and respiration of the dinoflagellate *Prorocentrum minimum*. *Harmful Algae* 4, 603-613.
- Herndon, J. and Cochlan, W.P. 2007. Nitrogen utilization by the raphidophyte *Heterosigma akashiwo*: Growth and uptake kinetics in laboratory cultures. *Harmful Algae* 6, 260-270.
- Kirkpatrick, B., Fleming, L.E., Squicciarini, D., Backer, L.C., Clark, R., Abraham, W., Benson, J., Cheng, Y.S., Johnson, D., Pierce, R., Zaias, J., Bossart, G.D., Baden, D.G. 2004. Literature review of Florida red tide: implications for human health effects. *Harmful Algae* 3(2), 99-115
- Koroleff, F. 1983. Simultaneous oxidation of nitrogen and phosphorus compounds by persulfate. In: Grasshoff, K., Eberhardt, M., Kremling, K. (Eds.) *Methods of Seawater Analysis*. 2nd ed., Verlag Chemie, Weinheimer, FRG.
- Kudela, R.M., Ryan, J.P., Blakely, M.D., Lane, J.Q., Peterson, T.D. 2008. Linking the physiology and ecology of *Cochlodinium* to better understand harmful algal bloom events: A comparative approach. *Harmful Algae* 7, 278-292
- Kudela, R.M., Cochlan, W.P., 2000. Nitrogen and carbon uptake kinetics and the influence of irradiance for a red tide bloom off southern California. *Aquat. Microb. Ecol.* 21(1), 31-47.

- Kusek, K.M., G. Vargo, G., Steidinger, K 1999. *Gymnodinium breve* in the field, in the lab, and in the newspaper – a scientific and journalistic analysis of Florida red tides, *Contr. Mar. Sci.* 34, 1–229.
- Lenes, J.M., Darrow, B.P., Cattrall, C., Heil, C.A., Callahan, M., Vargo, G.A., Byrne, R.H., Prospero, J.M., Bates, D.E., Walsh, J.J. 2001. Iron fertilization and the *Trichodesmium* response on the West Florida Shelf. *Limnol. Oceanogr.* 46, 1261-1277.
- Lester, K.M., 2005. Zooplankton of the West Florida Shelf: relationships with *Karenia brevis* blooms. Ph.D. Dissertation, University of South Florida, 199 pp.
- Lewin, J. 1966. Silicon metabolism in diatoms vs germanium dioxide, a specific inhibitor of diatom growth. *Phycologia* 6:1-12.
- Li, J., Glibert, P.M., Zhou, M. 2010. Temporal and spatial variability in nitrogen uptake kinetics during harmful dinoflagellate blooms in the East China Sea. *Harmful Algae* 9(6), 531-539
- Loeblich, A. R., III and Smith, V.E. 1968. Chloroplast pigments of the marine dinoflagellate *Gyrodinium resplendens*. *Lipids* 3(1), 5-13.
- Lomas, M, Glibert, P, Berg, G. 1996. Characterization of nitrogen uptake by natural populations of *Aureococcus anophagefferens* (Chrysophyceae) as a function of incubation duration, substrate concentration, light, and temperature. *Journal of Phycology* 32:907-916
- Lomas, M.W. and Glibert, P.M. 2000. Interactions between NH_4^+ and NO_3^- uptake and assimilation: comparison of diatoms and dinoflagellates at several growth temperatures. *Mar. Biol.* 133, 556-572.
- Lomas, M, Glibert, P. 2000. Temperature regulation of nitrate uptake: a novel hypothesis about nitrate uptake and reduction in cool-water diatoms. *Limnol. Oceanogr.* 44(3), Part 1, 556-572.
- Marinelli, R.L. Jahnke, R.A., Cravens, D.B., Nelson, J.B., Eckman, J.E. 1998. Sediment nutrient dynamics on the South Atlantic Bight continental shelf. *Limnol. Oceanogr.* 43:1305-1320.
- Martin, D.F., Doig, M.T., Pierce, N.H. 1971. Distribution of naturally occurring chelators (humic acids) and selected trace metals in some west coast Florida streams, 1968-1969. Prof. Paper. Ser. 12 Fla. Dept. Nat. Res. 52 p.
- McCarthy, J., Rowland Taylor, W., Taft, J.L. 1977. Nitrogenous nutrition of the plankton in the Chesapeake Bay. 1. Nutrient availability and phytoplankton preferences. *Limnol. Oceanogr.* 22(6), 996-1011.

- McCarthy, J. (1972) The uptake of urea by natural populations of marine phytoplankton. *Limnol. Oceanogr.* 17(5), 738-748
- McCarthy, J. (1981) The kinetics of nutrient utilization. *Can. Bull. Fish. Aquat. Sci.* 21, 211-233.
- Mulholland, M.R., Boneillo, G., Minor, E. 2004. A comparison of N and C uptake during brown tide (*Aureococcus anophagefferens*) blooms from two coastal bays on the east coast of the USA. *Harmful Algae* 3, 361-376.
- Mulholland, M.R., Bernhardt, P.W., Heil, C.A., Bronk, D.A., O'Neil, J.M. 2006. Nitrogen fixation and release of fixed nitrogen by *Trichodesmium* spp. in the Gulf of Mexico. *Limnol. Oceanogr.* 51(4), 1762-1776.
- Mulholland, M. R. and M. W. Lomas. 2008. N uptake and assimilation, In: Capone, D.G., D. A. Bronk, M. R. Mulholland and E. J. Carpenter [Eds.] *Nitrogen in the Marine Environment*. Elsevier/Academic. pp. 303-384.
- Parsons, T., Maita, Y., Lalli, C. 1984. *A manual of chemical and biological methods for seawater analysis*. Pergamon Press, New York.
- Prakash, A and Rashid, M.A. 1968. Influence of humic substances on the growth of marine phytoplankton: dinoflagellates. *Limnol. Oceanogr.* 13, 598-606.
- Prakash A., Rashid, M.A., Jensen, A., Subba, R.D.V. 1973. Influence of humic substances on the growth of marine phytoplankton: diatoms. *Limnol. Oceanogr.* 18, 516-524.
- Press, W.H., Teukolsky, S.A., Vetterling, W.T., Flannery, B.P. 1992. *Numerical recipes*. Cambridge University Press, New York, 994 pp.
- Price, N. and Harrison, P. 1987. A comparison of methods for the measurement of dissolved urea concentrations in seawater. *Mar. Biol.* 94, 307-317.
- Procise, L.A., Mulholland, M.R. 2008. Grazing by the red tide dinoflagellate, *Karenia brevis*: Implications for bloom dynamics. ASLO Ocean Science Meeting, Orlando, FL, March 2008.
- Raven, J. 1980. Nutrient transport in microalgae. *Adv. Micro. Physiol.* 21, pp. 47-226.
- See, J.H., Bronk, D.A., Lewitus, A.J. 2006. Uptake of *Spartina*-derived humic-nitrogen by estuarine phytoplankton in nonaxenic and axenic culture. *Limnol. Oceanogr.* 51(5), 2290-2299.
- Sinclair, G.A., Kamykowski, D., Milligan, E., Schaeffer, B. 2006. Nitrate uptake by *Karenia brevis*. II. Behavior and uptake physiology in a nitrate-depleted mesocosm with a bottom nutrient source. *Mar. Ecol. Prog. Ser.* 328, 125-131.

- Sinclair, G., Kamykowski, D. and Glibert, P.M. 2009. Growth, uptake, and assimilation of ammonium, nitrate, and urea by three strains of *Karenia brevis* grown under low light. *Harmful Algae*. 8:770-780.
- Shimizu, Y. and Wrensford, G. 1993. Peculiarities in the biosynthesis of brevetoxins and metabolism of *Gymnodinium breve*. In: Smayda, T.J., Shimizu, Y. (Eds.), *Toxic Phytoplankton Blooms in the Sea*. Elsevier Science Publishing, Inc. New York, pp. 919-923.
- Shimizu, Y., Watanabe, N., Wrensford, G. 1995. Biosynthesis of brevetoxins and heterotrophic metabolism in *Gymnodinium breve*. In: P., Lassus, G., Arzul, E., Erard, P., Gentien, C., Marcaillou (Eds.). *Harmful Marine Algal Blooms*. Lavoisier Intercept Ltd., Paris. pp. 351-357.
- Sipler, R.E. 2009. The Role of Dissolved Organic Matter in Structuring Microbial Community Composition. Ph.D. Dissertation. Rutgers, N.J. pp. 191.
- Smayda, T.J. and Reynolds, C. 2001. Community assembly in marine phytoplankton: Application of recent models to harmful dinoflagellate blooms. *J. Plankt. Res.* 23, 447-461.
- Steidinger, K.A., Vargo, G.A., Tester, P.A., Tomas, C.R., 1998. Bloom dynamics and physiology of *Gymnodinium breve* with emphasis on the Gulf of Mexico. In: Anderson, D.M., Cembella, A.D., Hallegraeef, G.M. (Eds.). *Physiological ecology of harmful algal blooms*. Springer-Verlag, Berlin, pp. 133-153.
- Steidinger, K.A. 1975. Basic factors influencing red tides. In: V.R. LoCiero (Ed.), *Proceedings of the First International Conference on Toxic Dinoflagellate Blooms*, Massachusetts Science and Technology Foundation, Wakefield, Massachusetts. pp. 153-162.
- Syrett, P. 1981. Nitrogen metabolism in microalgae. *Can. Bull. Fish. Aquat. Sci.* 210, 182-210.
- Tester, P.A., Steidinger, K.A. 1997. *Gymnodinium breve* red tide blooms: Initiation, transport, and consequences of surface circulation. *Limnol. Oceanogr.* 42, 1039-1051.
- Vargo, G.A., Heil, C.A., Ault, D.N., Neely, M.B., Murasko, S., Havnes, J., Lester, K.M., Dixon, L.K., Merkt, R., Walsh, J., Weisberg, R., Steidinger, K.A., 2004. Four *Karenia brevis* blooms: a comparative analysis. In: Steidinger, K.A., Landsberg, J.H., Tomas, C.R., Bargo, G.A. (Eds.), *Harmful Algae 2002. Proceedings of the Xth International Conference on Harmful Algae*. Florida Fish and Wildlife Conservation Commission, Florida Institute of Oceanography and Intergovernmental Oceanographic Commission of UNESCO, Paris, pp.14-16.

- Vargo, G.A., Heil, C.A., Fanning, K.A., Dixon, L.K., Neely, M.B., Lester, K., Ault, D., Murasko, S., Havens, J., Walsh, J., Bell, S. 2008. Nutrient availability in support of *Karenia brevis* blooms on the West Florida Shelf: What keeps *Karenia* blooming? Cont. Shelf Res. 28, 73-98.
- Vargo, G.A., Heil, C.A., Spence, D., Neely, M.B., Merkt, R., Lester, K., Weisberg, R.H., Walsh, J.J., Fanning, K. 2001. The hydrographic regime, nutrient requirements, and transport of a *Gymnodinium breve* Davis red tide on the West Florida shelf. In: Hallegraeff, G.M., Blackburn, S.I., Bolch, C.J., Lewis, R.J. (Eds.) Harmful Algal Blooms 2000. UNESCO, Paris, pp.157-160.
- Walsh, J.J. and Steidinger, K.A. 2001. Saharan dust and Florida red tides: the cyanophyte connection. J. Geophys. Res. 106, 11597-11612.
- Walsh, J.J., Joliff, J.K., Darrow, B.P., Lenes, J.M., Milroy, S.P., Dieterle, D.A., Carder, K.L., Chen, F.R., Vargo, G.A., Weisberg, R.H., Fanning, K.A., Muller-Karger, F.E., Steidinger, K.A., Heil, C.A., Prospero, J.S., Lee, T.N., Kirkpatrick, G.J., Whitley, T.E., Stockwell, D.A., Tomas, C.R., Villareal, T.A., Jochens, A.E., Bontempi, P.S. 2006. Red tides in the Gulf of Mexico: where, when, and why? J. Geophys. Res. 111, C11003.
- Walsh, J.J., Weisberg, J.M., Lenes, J.M., Chen, F.R., Dieterle, D.A., Zheng, L., Carder, K.L., Vargo, G.A., Havens, J.A., Peebles, E., Hollander, D.J., He, R., Heil, C.A., Mahmoudi, B., Landsberg, J.H. (2009) Isotopic evidence for dead fish maintenance of Florida red tides, with implications for coastal fisheries over both source regions of the West Florida shelf and within downstream waters of the South Atlantic Bight. Prog. Oceanogr. 80, 51-73
- Wilson, W.B. 1966. The suitability of sea-water for the survival and growth of *Gymnodinium breve* Davis: and some effects of phosphorus and nitrogen on its growth. FSU Prof. Pap. Ser., 7. pp. 1-42.
- Wilson, W.B. and Collier, A. (1955). Preliminary notes on the culturing of *Gymnodinium breve* Davis. Science, 121, 394-395.

Table 1.

Calculated parameters for uptake kinetics of ammonium (NH_4^+), nitrate (NO_3^-), urea, an amino acid (AA) mixture, individual amino acids (alanine and glutamate), and humics by field populations and cultures of *Karenia brevis*. Ambient concentrations are in units of $\mu\text{mol N L}^{-1}$. Maximum specific rates of uptake (V_{max}) are reported in units of $\times 10^{-3} \text{ h}^{-1}$ and half-saturation constants (K_s) are reported in $\mu\text{mol N L}^{-1}$. Standard error (SE) for estimates of both V_{max} and K_s parameters are given in parentheses. Substrate affinity ($\alpha = V_{max} / K_s$) is reported in units of $(\times 10^{-3} \text{ h}^{-1}) / (\mu\text{mol N L}^{-1})$. n = the number of data points used in each curve. Concentrations below the limit of detection are labeled b.d. while concentrations not determined are labeled n.d. For uptake kinetics that were linear functions of concentration the equation of the line is provided. The symbol ^a denotes curves that were originally non-saturating but were re-plotted to a maximum concentration of $50 \mu\text{mol N L}^{-1}$, from which Michaelis-Menten parameters were calculable. Kinetics parameters determined during previous studies are included for comparison; the ^b symbol denotes parameters that were determined from non-saturating curves. The ^c indicates that this value was re-calculated from the original report of $1.07 \mu\text{g-at N L}^{-1}$ to $\mu\text{mol N L}^{-1}$. Germanium (Ge) was added to the October 28, 2002 experiment to inhibit growth of co-occurring diatoms. Data from Li *et al.* (2010) is from field populations of *K. mikikotoi* in the East China Sea.

Substrate	Experiment	Ambient conc	V_{max}	K_s	α	r^2	n	
NH ₄ ⁺	2 October, 2001	0.042	30.5 (0.97)	0.30 (0.04)	101.7 (13.9)	0.97	10	
	4 October, 2001	0.149	55.3 (0.56)	0.41 (0.02)	134.9 (6.7)	1.00	10	
	28 October, 2002 (Ge added)	0.051	47.5 (1.05)	0.42 (0.04)	114.3 (11.2)	0.99	10	
	18 October, 2007	0.202	54.5 (7.57)	0.63 (0.30)	86.5 (42.9)	0.70	9	
	22 October, 2007	0.351	98.6 (3.15)	1.78 (0.16)	55.4 (5.3)	0.99	9	
	Jacksonville C4 (CCFWC 251)	0.064	33.6 (2.04)	0.32 (0.11)	105.0 (36.7)	0.79	9	
	Texas B4 (CCFWC 267)	0.168	45.9 (4.58)	0.64 (0.31)	71.7 (35.5)	0.72	8	
	Culture incubated in field, 2002	0.042	26.7 (1.96)	0.50 (0.17)	53.4 (18.6)	0.64	8	
	Average for this study			49.1 (9.93)	0.63 (0.41)	78.5 (53.5)		
	Vargo (in Steidinger <i>et al</i> 1998)				0.47			
	Sinclair <i>et al</i> 2009, CCMP 2229				0.37 (0.18)			
	Jacksonville C3				0.17 (0.14)			
	Apalachicola C6				0.32 (0.11)			
L ₁ <i>et al</i> 2010 (<i>K mikimotoi</i>)			500	4.88				
NO ₃	2 October, 2001	0.050	15.2 (1.26) ^a	0.18 (0.06) ^a	84.4 (29.0)	0.77	8	
	4 October, 2001	0.001	22.5 (1.57) ^a	0.50 (0.13) ^a	45.0 (12.1)	0.94	7	
	18 October, 2007	0.106	16.3 (1.03)	0.37 (0.09)	44.1 (11.1)	0.92	8	
	22 October, 2007	0.091	20.4 (1.37)	0.50 (0.12)	40.8 (10.2)	0.92	9	
	Average for this study		18.6 (2.67)	0.39 (0.21)	48.0 (26.7)			
	Vargo (in Steidinger <i>et al</i> 1998)				0.42			
	Sinclair <i>et al</i> 2006	b d			16.2			
	Sinclair <i>et al</i> 2009, CCMP 2229				1.32 (0.85)			
	Jacksonville C3				0.19 (0.15)			
	L ₁ <i>et al</i> 2010 (<i>K mikimotoi</i>)			18.0	43.6			
Urea	28 October, 2002 (Ge added)	0.182	24.2 (3.30)	1.58 (0.97)	15.3 (9.2)	0.77	6	
	18 October, 2007	0.326	26.7 (2.34)	0.63 (0.22)	42.3 (15.2)	0.62	9	
	Average for this study		25.4 (4.04)	1.11 (1.00)	23.0 (21.0)			
	Vargo (in Steidinger <i>et al</i> 1998)				0.535 ^c			
	Sinclair <i>et al</i> 2009, CCMP 2229				32.47 (4.55) ^b			
	Jacksonville C3				4.36 (0.53) ^b			
	Apalachicola C6				0.37 (0.26)			
	L ₁ <i>et al</i> 2010 (<i>K mikimotoi</i>)			20.0	1.35			
	AA mix	18 October, 2007	0.077	y = 0.182x + 0.4458				7
	Alanine	28 October, 2002 (Ge added)	0.011	y = 0.338x + 4.9654				6
Glutamate	2 October, 2001	b d	y = 0.1342x + 2.9439				7	
Glycine	L ₁ <i>et al</i> 2010 (<i>K mikimotoi</i>)		11.0	1.68				
Humic	18 October, 2007	n d	383.2 (81.3)	33.3 (11.4)	11.5 (4.6)	0.98	9	
	22 October, 2007	n d	77.2 (28.1)	2.86 (2.84)	26.9 (28.4)	0.81	8	
	Average for this study		230.2 (86.0)	18.1 (11.7)	12.7 (9.5)			

Figure Legend:

Figure 1. Experimental sites located in the eastern Gulf of Mexico adjacent to Florida.

Figure 2. Specific uptake rates (V , $\times 10^{-3} \text{ h}^{-1}$) of ammonium (NH_4^+) for field populations (A) October 2, 2001, (B) October 18, 2007, (C) October 4, 2001, (D) October 22, 2007, (E) October 28, 2002 and cultures of *K. brevis* (F) culture incubated under field conditions, (G) Jacksonville, (H) Texas. Note the differences in scale for (B and D).

Figure 3. Maximum specific uptake rate (V_{max} , $\times 10^{-3} \text{ h}^{-1}$) as a function of ambient NH_4^+ concentration. Regression for data points is shown ($P < 0.05$).

Figure 4. Specific uptake rates (V , $\times 10^{-3} \text{ h}^{-1}$) of NO_3^- for field populations of *K. brevis*. Plots correspond to the experimental dates: (A) October 2, 2001, (B) October 18, 2007, (C) October 4, 2001, (D) October 22, 2007. Note the differences in scale of the x-axes. The 2001 plots did not fit the Michaelis-Menten formulation at maximum substrate additions of $200 \mu\text{mol N L}^{-1}$ and were re-plotted according to the $50 \mu\text{mol N L}^{-1}$ maximum for the 2007 experiments (insert plots).

Figure 5. Specific uptake rates (V , $\times 10^{-3} \text{ h}^{-1}$) of urea for field populations of *K. brevis*. Plots correspond to the experimental dates: (A) October 28, 2002, (B) October 18, 2007. Note the differences in the x-axes.

Figure 6. Specific uptake rates (V , $\times 10^{-3} \text{ h}^{-1}$) of (A) glutamate, (B) alanine, and an (C) amino acid mixture for field populations of *K. brevis*. Plots correspond to the experimental dates: (A) October 2, 2001, (B) October 28, 2002, and (C) October 18, 2007. Note the differences in the axes. In all plots, the specific uptake rates for the low ^{15}N additions ($< 1.5 \mu\text{mol N L}^{-1}$) are presented as inset graphs.

Figure 7. Specific uptake rates (V , $\times 10^{-3} \text{ h}^{-1}$) of humics for field populations of *K. brevis* on (A) October 18, 2007 and (B) October 22, 2007. Note the variations in the x-axes.

Figure 1.

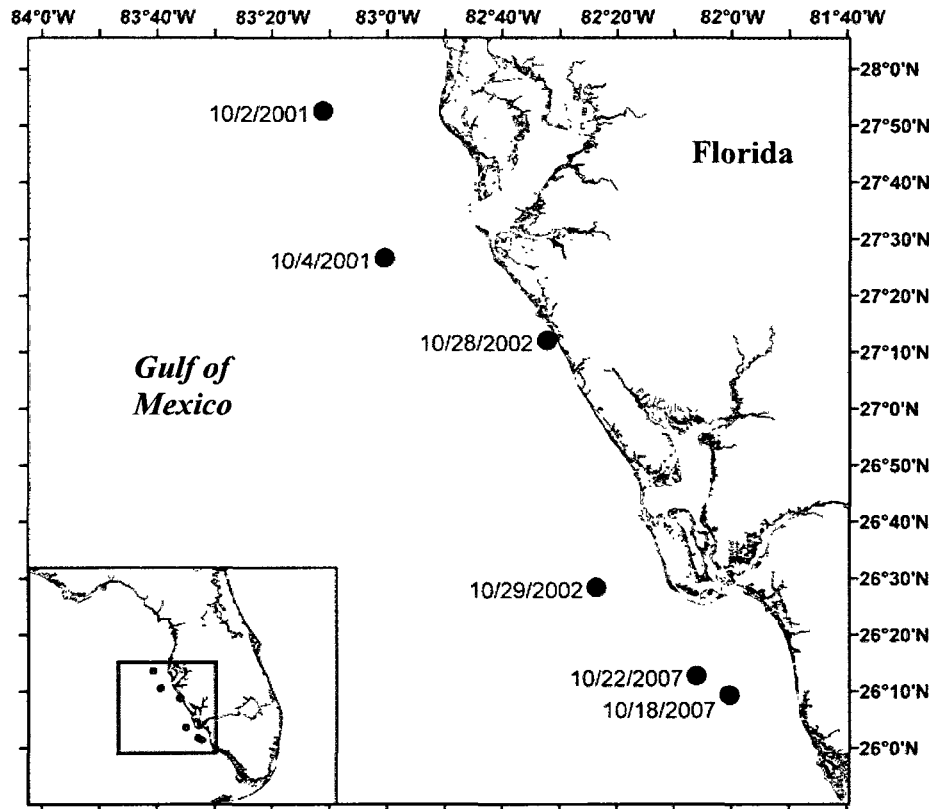


Figure 2.

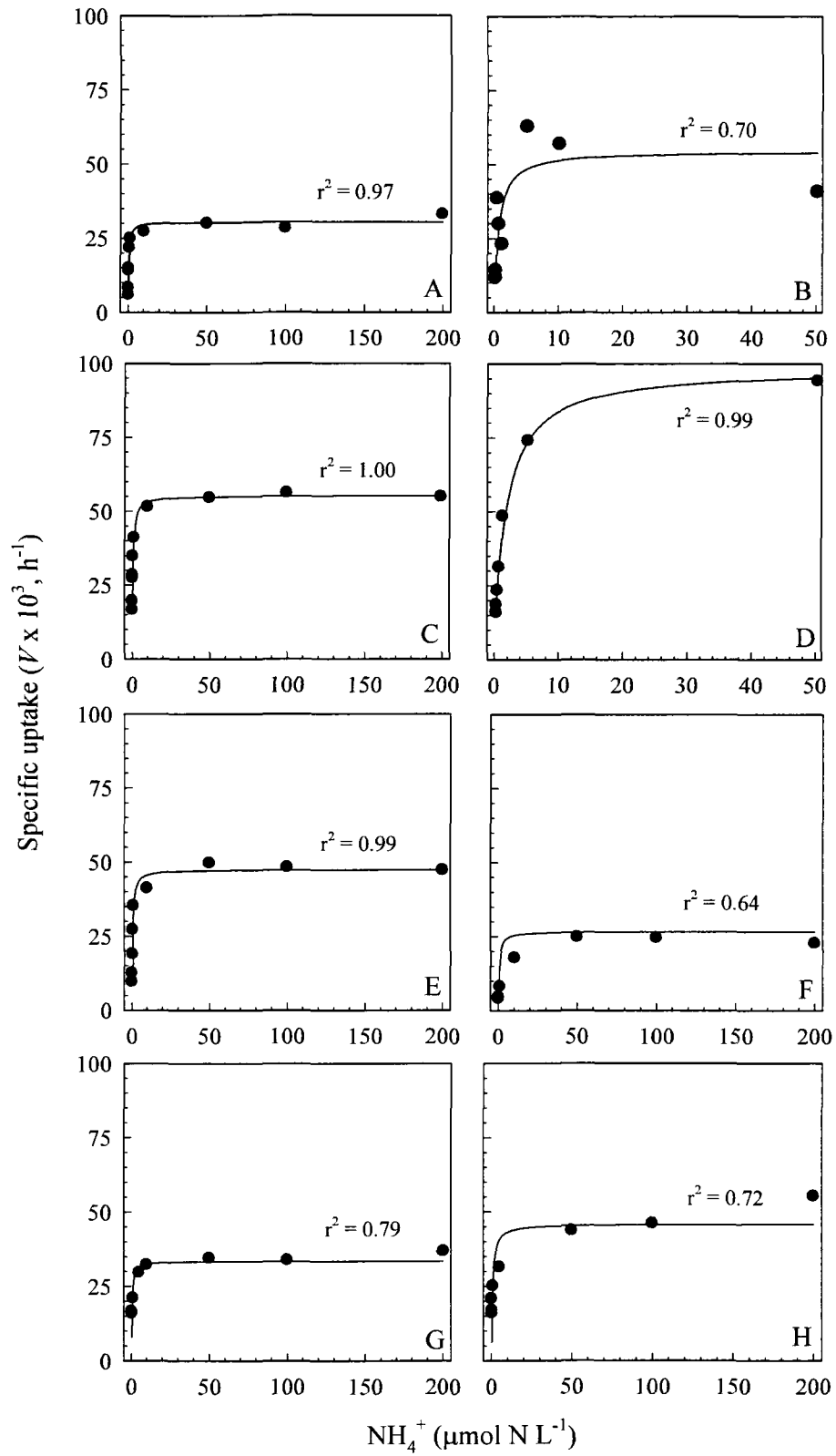


Figure 3.

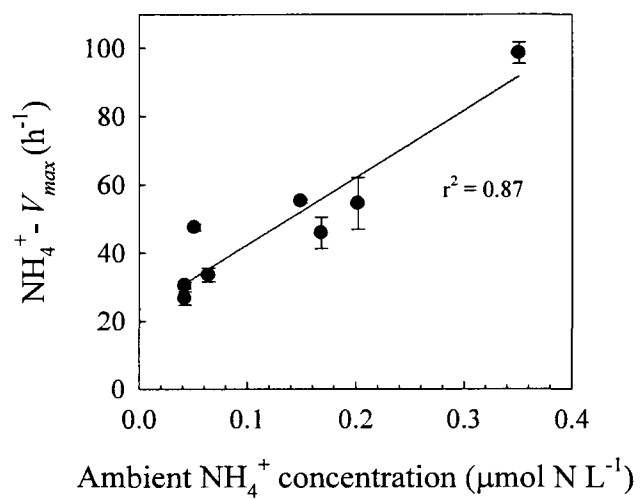


Figure 4.

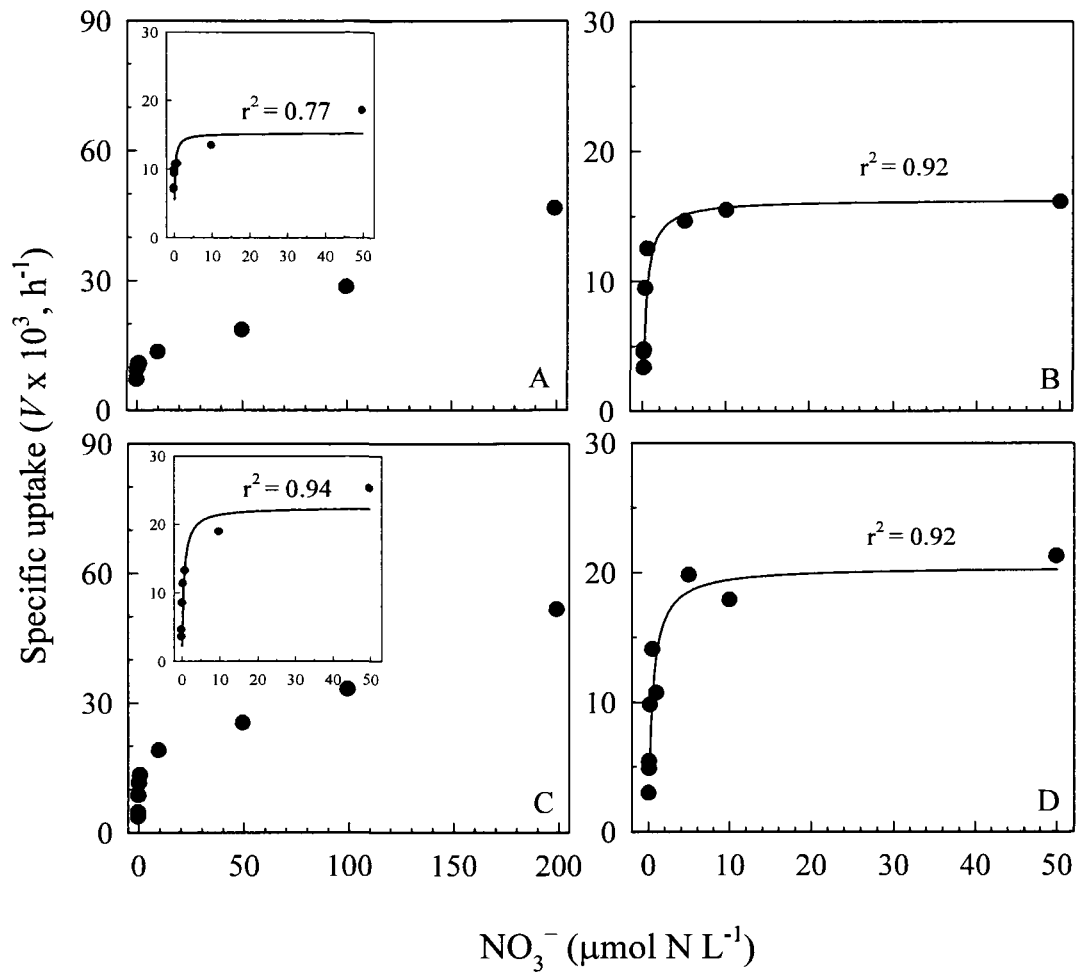


Figure 5.

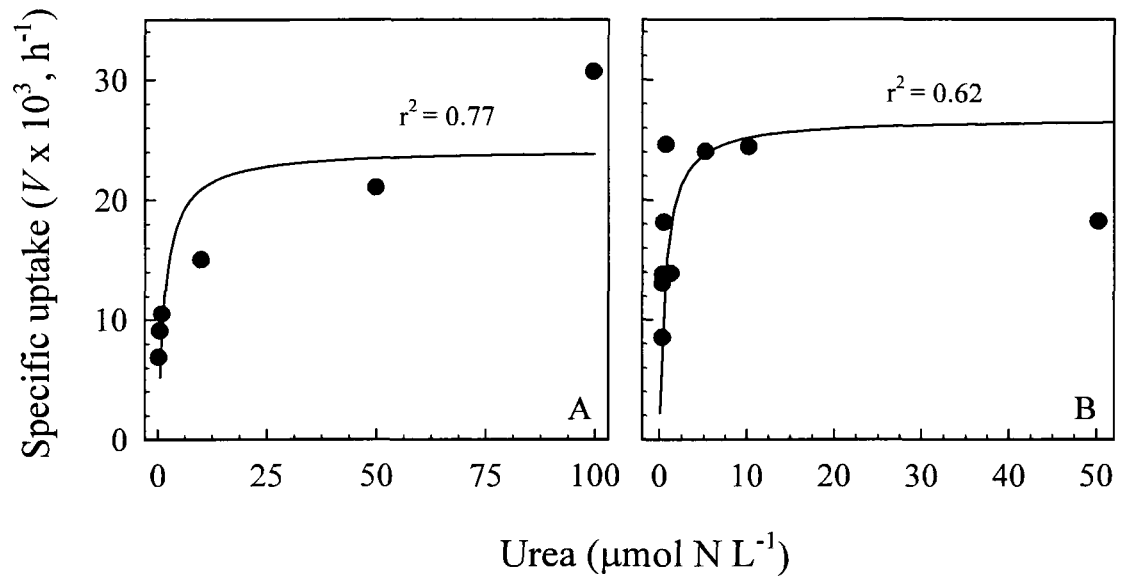


Figure 6.

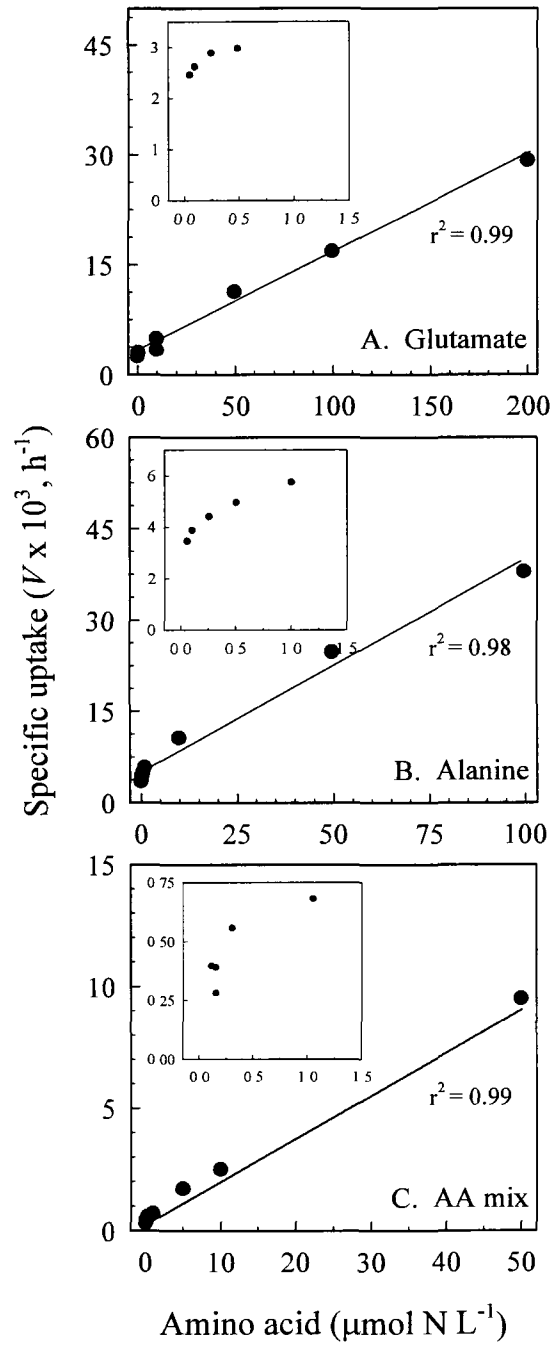
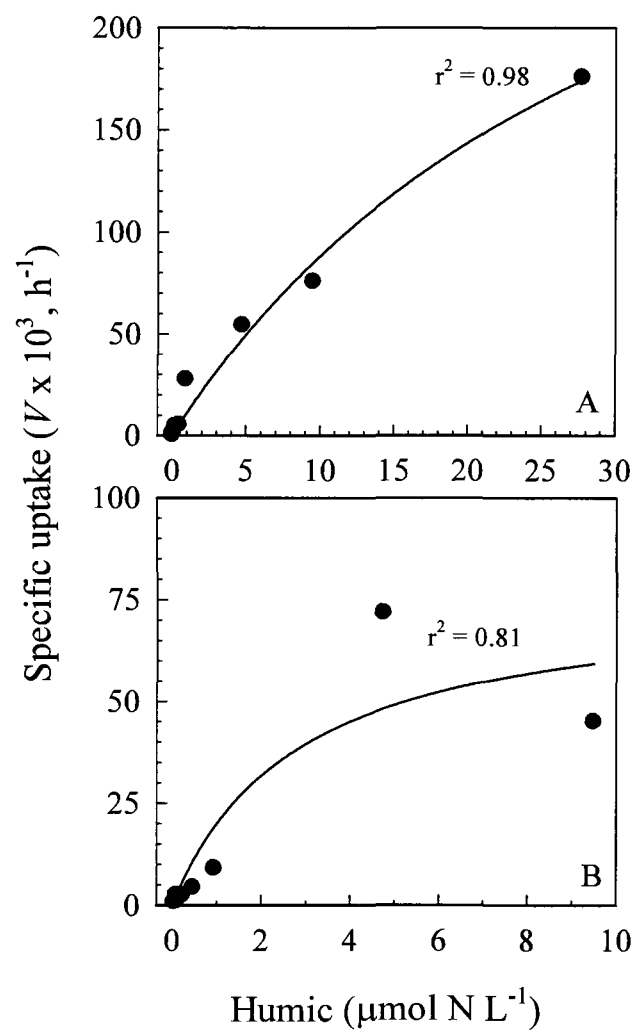


Figure 7.



CHAPTER 3

Diel variability of nitrogen uptake by *Karenia brevis* in a field population and in cultures

Abstract

Blooms of the red tide dinoflagellate, *Karenia brevis*, have become an annual feature along the West Florida Shelf where multiple nitrogen (N) sources likely sustain populations across a variety of nutrient regimes from oligotrophic offshore to enriched estuarine waters. To quantify the ability of *Karenia spp.* to utilize a suite of N substrates in the light and dark, we performed N uptake experiments using water collected at the surface and bottom (ranging from 6.5 to 9 m) of a drogued-tracked water mass at 4 h intervals during a 25 h period. For comparison, similar experiments were performed in the laboratory with three cultured Florida strains of *Karenia brevis* (C.C. Davis) G. Hansen & Ø. Moestrup: Sarasota B3 (CCFWC 254), Jacksonville C4 (CCFWC 251), and Charlotte C2 (CCFWC 257). In both the field and laboratory, short-term (0.5 to 1 h) incubations were performed with ^{15}N -labeled inorganic (NH_4^+ or NO_3^-) and organic (urea or amino acid mixture) substrates. Substrates were added at either trace ($0.1 \mu\text{mol N L}^{-1}$, field and culture) or saturating ($10 \mu\text{mol N L}^{-1}$, culture only) final concentrations. In the culture incubations, trichloroacetic acid (TCA) extractions were also performed at both a light and dark time point to determine the percent assimilation of N substrates into proteins. Results indicate a pronounced periodicity in diel uptake rates with the maximum and minimum rates measured early in the light and dark periods, respectively. Additionally, there was variability in uptake rates among N substrates with the highest rates observed for NH_4^+ . The three strains of *K. brevis* exhibited significant differences in N uptake rates as well as patterns of uptake over the diel cycle. The percent of N assimilated into proteins varied between substrates and strains but remained less than 60% in both light and dark.

Introduction

Karenia brevis (C.C. Davis) G. Hansen & Ø. Moestrup (formerly *Gymnodinium breve* (C.C.Davis) and *Ptychodiscus brevis* (C.C.Davis) Steidinger) is an environmentally and economically devastating harmful alga in the Gulf of Mexico where it forms annual blooms. *K. brevis* is a potent neurotoxin (brevetoxin) producer responsible for extensive fish kills, marine mammal mortalities, human respiratory distress, as well as neurotoxic shellfish poisoning through consumption of contaminated seafood (reviewed in Kirkpatrick *et al.* 2004). Blooms of *K. brevis* initiate offshore (18 to 74 km) (Steidinger 1975; Tester and Steidinger 1997) in deep oligotrophic waters where dissolved inorganic nitrogen (DIN) and phosphorus (DIP) are relatively low (0.1-0.2 μM) compared to concentrations of dissolved organic nitrogen (DON; 5-10 μM) (Heil *et al.* 2001). Cells are then transported, by physical forcing, to increasingly nutrient-rich coastal and estuarine waters (Steidinger and Haddad 1981). Thermal and salinity fronts trap and concentrate *K. brevis* populations (Vargo *et al.* 2001) and the resulting blooms can persist for months (Steidinger and Haddad 1981; Vargo *et al.* 2004; Walsh *et al.* 2006). During that time, *K. brevis* may encounter a variety of external and *in situ* nutrient sources, including upwelling events along the continental shelf (Walsh *et al.* 2006), estuarine outflow (Vargo *et al.* 2004), DON release from the N_2 fixer, *Trichodesmium spp.* (Walsh and Steidinger 2001, Lenos *et al.* 2001, Mulholland *et al.* 2006), nitrogen regeneration from macrozooplankton excretion (Lester 2005), and nutrients released from decaying fish carcasses during brevetoxin-induced fish kills (Vargo *et al.* 2001, Walsh *et al.* 2006). No single nutrient source appears to be adequate to sustain high bloom

concentrations, which are often in excess of 1×10^7 cells L^{-1} (Walsh and Steidinger 2001; Walsh *et al.* 2006; Vargo *et al.* 2008).

Karenia brevis, similar to other bloom-forming dinoflagellates, likely possesses a flexible metabolism. Measurements of δ^{15} particulate organic matter (POM) collected from the West Florida Shelf and *K. brevis* blooms indicate that numerous N sources are utilized by the cells (Havens *et al.* 2004). Early experiments demonstrated the capability of *K. brevis* to utilize ammonium (NH_4^+ ; Wilson 1966; Doig 1973), amino acids (AA; Wilson 1966; Baden and Mende 1979; Shimizu and Wrensford 1993; Shimizu *et al.* 1995) and urea (Shimizu and Wrensford 1993; Shimizu *et al.* 1995) for cellular growth. Direct uptake rates for a variety of individual DIN and DON species have been determined in culture and the field using ^{15}N stable isotope techniques (Vargo, cited in Steidinger *et al.* 1998; Bronk *et al.* 2004, Sinclair *et al.* 2006a and b., Glibert *et al.* 2009, Sinclair *et al.* 2009, Killberg-Thoreson Chapter 2). *Karenia brevis* is also capable of utilizing more chemically complex molecules such as humic acids (Heil, unpublished data), DON released from *Trichodesmium* (Mulholland *et al.* 2006; Sipler *et al.* 2009), and has also been found to feed, mixotrophically, on *Synechococcus* cells (Procise and Mulholland 2008; Glibert *et al.* 2009).

A behavioral mechanism through which dinoflagellates may access a variety of nutrient sources is diel vertical migration (DVM). Many species undergo DVM where cells aggregate in the lighted surface waters during the day to photosynthesize and descend to deeper waters in the dark, where higher concentrations of nutrients may be accessed (e.g. Eppley *et al.* 1968; Eppley and Harrison 1975; Harrison 1976; Heaney and Eppley 1981; Cullen and Horrigan 1981). Therefore, diel periodicities in dinoflagellates may be expected for nutrient uptake kinetics and uptake of inorganic nutrients (Eppley 1975; Hersey

and Swift 1976; MacIsaac 1978; Rivkin and Swift 1982). Uptake in the light versus dark may differ between species (Paashe *et al.* 1984) or between nutrient substrates, including closely related substrates, such as individual AA (Rivkin and Putt 1987).

Karenia brevis displays a behavioral pattern of positive phototaxis (Kamykowski *et al.* 1998 a, b) and negative geotaxis (Heil 1986) where cells aggregate into a 0 to 5 cm surface layer (as great as 1×10^8 cells L^{-1}) during daylight hours and undergo random dispersal at night, rather than a typical downward migration (Heil 1986; Kamykowski *et al.* 1998a). Dark N uptake may be advantageous for phytoplankton, such as *K. brevis*, which typically occur in patchy nutrient environments (Needoba and Harrison 1992). It has been hypothesized that *K. brevis* cells that descend deeper at night would encounter a wider variety of possible nutrient sources (Sinclair *et al.* 2006b) and acquire NO_3^- near the sediments (Sinclair *et al.* 2006 a, b; Sinclair *et al.* 2009). *Trichodesmium spp.* undergo DVM by adjusting their carbohydrate ballast (Walsby 1992; Romans *et al.* 1994) and have been found to release N in both the light and dark (Wannicke *et al.* 2009). It has been hypothesized that *K. brevis* may concurrently migrate with the *Trichodesmium* colonies and could intercept the excreted NH_4^+ and AA (Walsh *et al.* 2006). Dark, as well as light uptake of N substrates by *K. brevis* has been investigated in few culture studies (Sinclair *et al.* 2006a, b; Sinclair *et al.* 2009). The research presented here expands upon previous work by comparing N uptake dynamics among multiple strains of *K. brevis* in culture as well as with a field population followed by a drifting surface drogue.

Specifically, we examined the variability of NH_4^+ , NO_3^- , urea and AA uptake during a diel cycle, and compare the nutritional physiology of *K. brevis* in the field to *K. brevis* strains in culture. Additionally, rates of NH_4^+ and NO_3^- regeneration were measured in the field and

N assimilation was measured in cultures. Given that numerous N sources are hypothesized to initiate and maintain *K. brevis* blooms, a better understanding of the N physiology of *K. brevis* is critical to determining why large blooms form and remain for months.

Materials and Methods

Diel uptake experiments – Field

A diel experiment was conducted aboard the R/V Pelican October 24 to 25, 2007 in the eastern Gulf of Mexico with a natural population of *Karenia spp.* A drogue was deployed in a visible *K. brevis* bloom at the start of the experiment (26° 28' 4.7994", -82° 14' 2.3994") and followed until the completion of the timed experiment (Figure 1). Experiments began ~1 h after dawn (~0800 EST) and were conducted every 4 h for a 25 h period. At each time point, a surface (< 1 m) and deeper water sample, collected 1 m from the bottom (ranging in depth from 6.5 to 9 m), were collected using a series of Niskin bottles mounted on a rosette sampler, followed by immediate transfer into 500 mL polyethylene terephthalate glycol (PETG) bottles. In addition, a 200 mL aliquot was immediately filtered through a pre-combusted (2 h at 450°C) Whatman® GF/F (0.7 µm nominal pore size) filtered at < 100 mm Hg. The filtrate was frozen (-20°C) for subsequent analysis of ambient concentrations of NH₄⁺, NO₃⁻, urea, and AA (measured as dissolved primary amines, DPA). Uptake rates for surface and deep water were measured using ¹⁵N tracer techniques. Tracer additions, defined as ~10% of the ambient N concentration, of ¹⁵N labeled ammonium chloride (98.85 at. %), ¹⁵N potassium nitrate (98% + at. %), ¹⁵N urea (98% at. %), and a

^{15}N algal AA mixture comprised of 16 AA (96-99% at. %) (Cambridge Isotope Laboratories, Cambridge, MA) were added to individual 500 mL PETG bottles in duplicate. The bottles were placed in on-deck, flow-through incubators under simulated *in situ* light conditions; light was attenuated with neutral density screens. *In situ* irradiance measurements taken at the time of sample collection in the light period ranged from 18.5 to 53.9 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ in the surface and 0.28 to 1 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ in the deep samples. Samples were incubated for ~1 h and subsequently terminated by filtration onto pre-combusted (2 h at 450°C) Whatman[®] GF/F filters at < 100 mm Hg. Filters were placed in polypropylene cryovials and frozen (-20°C) for later analysis. The filtrate from the NH_4^+ incubations was collected and frozen for later determination of ^{15}N atom % enrichment of the NH_4^+ pool using solid phase extraction (C_{18} , Dudek *et al.* 1986). Filtrate from the NO_3^- incubations was also collected and frozen for determination of ^{15}N atom % enrichment of the NO_3^- pool using the denitrifying bacteria method (Sigman *et al.* 2001).

For isotopic analysis, filters were thawed and placed in a drying oven overnight (40°C) prior to analysis of particulate N (PN) concentrations and isotopic enrichment. Isotopic samples were analyzed using a Europa Automated Nitrogen Carbon Analyzer for Solids and Liquids (ANCA-SL) attached to a GEO 20/20 isotope ratio mass spectrometer. Nitrogen specific uptake rates (V (h^{-1})) were estimated from the accumulation of ^{15}N in the PN and calculated according to the equations of (Dugdale and Wilkerson 1986).

$$V (\text{h}^{-1}) = \frac{\text{PN } ^{15}\text{N atom\% xs}}{(\text{substrate } ^{15}\text{N atom\% xs})(\text{time})} \quad (1)$$

The PN ^{15}N atom% xs is the ^{15}N atom% of the particulate nitrogen fraction minus the ^{15}N atom% normal atmospheric background, while substrate ^{15}N atom% xs is the enrichment of N substrate in the medium minus the ^{15}N atom% normal atmospheric background. V (h^{-1}) is then multiplied by the concentration of PN to determine the absolute uptake rate. The atom% values of the NH_4^+ and NO_3^- substrate pools were corrected for isotopic dilution (Glibert *et al.* 1982) due to regeneration processes.

Diel uptake experiments – Culture

Diel culture experiments were conducted July 22 to 23, 2008 using three non-axenic cultured strains of *K. brevis* maintained in the Florida Fish and Wildlife Research Institute culture collection in St. Petersburg, FL: Sarasota B3 (CCFWC 254), Jacksonville C4 (CCFWC 251), and Charlotte C2 (CCFWC 257), hereafter referred to as Sara, Jax, and Char, respectively. Strains were named for the cities near which they were collected: Sarasota, Jacksonville, and Charlotte Harbor, FL. Char was isolated in May of 1996 while Sara and Jax were isolated in October of 1999. Strains were maintained in 12 L glass carboys at 22°C on a 12:12 light:dark photoperiod at $35.9 \mu\text{mol photons m}^{-2} \text{s}^{-1}$. Cultures (6-7 L) were maintained on GP growth medium (Loeblich and Smith 1968) with NO_3^- as the N-source. Seawater (salinity 35) used for media preparation was collected from the Gulf of Mexico, filtered through a 0.45 μm filter and autoclaved prior to use. One month before beginning the experiment, cultures were monitored for ambient concentrations and allowed to draw down NO_3^- in an effort to reduce it to the limit of detection.

Uptake experiments began 1 h after the beginning of the light period and were repeated every 4 h during a 25 h period for a total of seven time points. At each time point, carboys of each strain were gently mixed and a 150 ml aliquot was filtered through a pre-combusted Whatman[®] GF/F (2 h at 450°C); the filtrate was frozen for subsequent analysis of ambient concentrations of NH₄⁺, NO₃⁻, urea, and AA, measured as DPA.

Uptake rates were determined as described above with the following modifications. Duplicate 10 ml aliquots of each strain were added to 20 mL borosilicate test tubes to measure uptake with trace (0.10 μmol N L⁻¹ for NH₄⁺, urea, and AA; 1.0 μmol N L⁻¹ for NO₃⁻) and saturated (10 μmol N L⁻¹) additions of ¹⁵N label. Samples were incubated for 0.5 h under identical conditions as those used for culture maintenance. Incubations were terminated by filtration onto pre-combusted (2 h at 450°C) Whatman[®] GF/C filters (1.2 μm nominal pore size) at < 100 mmHg; filters were placed in polypropylene cryovials and frozen. GF/C filters were used to reduce potential bacterial contamination of the filtered sample by retaining *K. brevis* cells but allowing bacteria to pass through. Filters were analyzed as described above and rates were not corrected for isotope dilution (Glibert *et al.* 1982) as little would be expected due to the absence of higher trophic levels and limitations in culture volume available. Statistical analyses (t-tests) were completed using Minitab[®] software version 14.

TCA extraction

During one light (1200) and one dark (2400) time point, the assimilation of NH₄⁺, NO₃⁻, urea and AA into sub-cellular components was measured. Duplicate 10 ml samples of

each strain were amended with $10 \mu\text{mol N L}^{-1} \text{}^{15}\text{N}$ label and incubated under conditions described above. Incubations were terminated by adding a 25 ml aliquot of cold 10% trichloroacetic acid (TCA) to each sample for 10 sec prior to filtration (Glibert and McCarthy 1984). Filters were frozen until analysis by mass spectrometer, as described above. The ^{15}N incorporated into the TCA precipitable fraction represents the assimilation into proteins. The difference between specific uptake rates and ^{15}N assimilated into the TCA fraction is the portion of N taken up that is located in non-proteinaceous cellular components (Glibert and McCarthy 1984). Assimilation was calculated as the quotient of N uptake in the proteinaceous material divided by the N uptake into the whole cells and presented as a percentage.

Analytical methods

Filtered water samples for analysis of NH_4^+ , NO_3^- , and urea were stored in low density polyethylene (LDPE) centrifuge tubes (Corning[®]) and DPA samples were stored in high density polyethylene (HDPE) bottles. Urea and NO_3^- analyses were performed in duplicate and all other analyses in triplicate. Concentrations of NH_4^+ were analyzed manually by the colorometric phenol-hypochlorite technique (Koroleff 1983). Concentrations of NO_3^- were measured using a Lachat QuikChem 8500 autoanalyzer (Parsons *et al.* 1984). Concentrations of urea were analyzed using the diacetyl monoxmine thiosemicarbazide method adapted from Price and Harrison (1987). Total concentrations of AA were measured as DPA using the fluorometric o-phthalaldehyde method (Parsons *et al.* 1984).

Cell counts and identification

Samples for phytoplankton species abundance (10 mL) were collected during the field study, fixed with Lugol's solution, and 1 mL subsamples were counted on an inverted microscope. For the culture studies, cell density was determined using a Beckman Z2 Coulter Particle Count and Size Analyzer (Beckman-Coulter Inc., Miami, FL) with threshold settings of 20 and 40 μm for particle diameter. Since cultures were clonal isolates, it was assumed that one counted particle represented one algal cell.

Results

Cell densities

The initial concentrations of *Karenia spp.* in the field study were 2.1×10^5 cells L^{-1} and 3.0×10^4 cells L^{-1} in the surface and at depth, respectively (Figure 2). In both surface and deep samples *K. brevis* comprised ~78% of total *Karenia spp.*, *Karenia mikimotoi* comprised ~1%, and the remaining cells were *Karenia* that could not be identified to the species level. At the start of the culture experiment cell densities for *K. brevis* were 15.1×10^3 , 4.1×10^3 , and 5.3×10^3 cells L^{-1} for Sara, Jax and Char strains, respectively (data not shown).

Ambient nutrients

Initial ambient nutrient concentrations for the field and culture studies are shown in Table 1. In the field, ambient N concentrations in the surface and deep were similar; the lowest concentrations were NO_3^- ($\sim 0.06 \mu\text{mol N L}^{-1}$) while urea and NH_4^+ concentrations were the highest ranging from 0.24 to $0.35 \mu\text{mol N L}^{-1}$ (Table 1). Cultured *K. brevis* was grown on a NO_3^- -based media, with substantially higher NO_3^- concentrations (28 to $33 \mu\text{mol N L}^{-1}$; Table 1) than other N species ($< 1 \mu\text{mol N L}^{-1}$), and experienced a ~ 2 to $8 \mu\text{mol N L}^{-1}$ decrease during the 25 h period (data not shown). In contrast to NO_3^- , relatively little change in N concentrations were observed for NH_4^+ , urea, or AA during the course of the experiment (data not shown). In both field and culture studies the physiological state of the cells was considered nutrient replete.

Uptake and regeneration rates in the field

In the field, the highest uptake rates were for NH_4^+ for both surface and deep water (Figure 3A, B). When mean absolute uptake rates in the light and dark were compared, there was no significant difference for NH_4^+ , however, there was a significantly greater uptake of urea in the surface in the light ($t = 2.78$, $p = 0.03$) (Table 2). When the absolute uptake rates were examined according to time spent in the light or the dark, there were significant differences between time points, particularly after 1 hour in the light (0800 h) and 1 hour in the dark (2000 h). For instance, the field NH_4^+ and urea uptake rates measured in the surface at the start of the experiment at 0800 h were significantly greater than rates measured at 2000

h ($t = 5.44$, $p = 0.03$ and $t = 29.9$, $p < 0.01$, respectively). Uptake rates of NO_3^- and AA exhibited less variability. NH_4^+ represented the majority of total absolute uptake (43 to 76% for both surface and deep) while AA uptake rates remained consistently lowest at 1 to 18 % of total absolute uptake (Figure 4A, B). There were no significant differences between the surface and deep NH_4^+ or NO_3^- regeneration rates (Figure 5). Although the highest rates of NH_4^+ regeneration were at 0800 and 1200, NO_3^- regenerations experienced little change. NH_4^+ regeneration rates were tightly coupled with their respective uptake rates in both the surface and deep. Ratios of NH_4^+ regeneration:uptake ranged from 0.97-1.8 and 0.93-1.3 in the surface and deep, respectively (data not shown). Ratios of NO_3^- regeneration:uptake were less and ranged from 0.28-1.4 and 0.13-0.47 in the surface and deep, respectively (data not shown).

Nitrogen uptake rates in culture

For the culture experiments, the high ambient NO_3^- concentrations in the media ($>28 \mu\text{mol N L}^{-1}$) for all *K. brevis* strains precluded comparison of NO_3^- tracer data with uptake rates for other substrates. For Sara, saturated uptake was dominated by NH_4^+ which had ~2.5-fold greater absolute uptake rates than all other substrates; moreover, there were less pronounced differences in uptake rate between substrates in the trace experiments (Figure 6A and B). Urea had the lowest uptake rates for both trace and saturating additions, ranging between 0.004 and 0.05 $\text{pmol N L}^{-1} \text{h}^{-1} \text{cell}^{-1}$. There was a significant difference in the absolute uptake rates of the mean light time points versus the mean dark time points for NH_4^+ uptake by Sara for the trace addition ($t = 3.06$, $p = 0.01$) and NO_3^- for the saturated addition

($t = 3.22$, $p = 0.01$; Table 2). When absolute uptake rates were examined according to time spent in the light or the dark, there were significant differences between time points after 1 hour in the light (0800) and 1 hour in the dark (2000) for urea, AA trace and NH_4^+ , for both trace and saturated additions (t -test, $p < 0.05$). In contrast, urea also had significantly greater uptake rates after 9 h in the dark (0400) as opposed to 9 h in the light (1200) for Sara both trace ($t = 5.50$, $p = 0.03$) and saturated ($t = 4.73$, $p = 0.04$). The lowest uptake rates for each individual substrate occurred during the dark (Figure 6A and B). A significant difference between trace versus saturating uptake was observed for NH_4^+ . Rates were ~1.5-fold higher in the saturated treatment, including both light and dark ($t = 5.35$, $p < 0.0001$; Figure 6). No such variation was observed between trace and saturating additions for the other substrates. Overall, Sara had the lowest observed absolute uptake rates per cell.

For Jax, absolute uptake was dominated by NH_4^+ , which had significantly higher rates of uptake than the other substrates for both trace and saturating additions (t -test, $p < 0.001$; Figure 6C, D). Overall, urea absolute uptake rates were the lowest observed in both trace and saturated experiments (Figure 6). Mean NH_4^+ uptake for the light time points were significantly greater than the dark for Jax for both trace ($t = 3.69$, $p < 0.01$) and saturating additions ($t = 3.26$, $p < 0.01$; Table 2). There were also significant differences between time points after 1 hour in the light (0800) and 1 hour in the dark (2000) for NH_4^+ trace ($t = 10.1$, $p = 0.01$) and NO_3^- saturated ($t = 8.5$, $p = 0.01$).

For Char the highest trace and saturating absolute uptake rates observed were for NH_4^+ and AA and the rates were closely coupled over time (Figure 6E, F). Mean NO_3^- uptake for the light time points were significantly greater than the dark for the saturating addition ($t = 2.72$, $p = 0.02$; Table 2) but there were no significant differences between the light and the

dark for the other substrates or the trace additions. There were also significant differences between time points after 1 hour in the light (0800) and 1 hour in the dark (2000) for NH_4^+ both trace and saturated (t-test, $p < 0.05$) as well as AA trace ($t = 9.61$, $p = 0.01$) and NO_3^- saturated ($t = 22.9$, $p < 0.01$). In contrast, urea had significantly greater uptake rates after 9 h in the dark (0400) as opposed to 9 h in the light (1200) for Char saturated ($t = 8.74$, $p = 0.01$). Consistent with the other strains, urea had the lowest rates of uptake for both trace and saturated treatments.

Strain comparison

For all strains, uptake rates exhibited a light:dark periodicity with maximum rates early in the light period and minimal early in the dark. For the inorganic substrates, NH_4^+ uptake accounted for a greater percentage of total uptake in experiments with saturating additions relative to trace additions (Figure 7). Both Sara and Jax, exhibited a similar pattern for saturating NH_4^+ uptake (Figure 7B, D). For all strains, NH_4^+ uptake rates for saturating additions were ~1.5- to 2- fold greater than for trace additions. Urea uptake rates between strains and provided 6-27% of total saturated uptake for all strains (Figure 7B, D, F). Overall, Sara had the lowest uptake rates measured for all strains.

For the organic substrates, there was variability in uptake rates between strains. First, AA uptake by Char in the light and dark was significantly greater than Sara and Jax for both trace and saturated additions (t-test, $p < 0.05$); 21- 53% of total uptake by Char was AA, while AA uptake by Sara and Jax accounted for only 15-27% and 4-26% of total uptake, respectively (Figure 7). Urea generally exhibited the lowest rates of uptake for all strains.

Field vs culture comparison of uptake rates

Absolute uptake rates in the field were measured with trace additions of ^{15}N -labeled substrate and can be compared with the tracer experiments conducted in culture when all calculated in $\mu\text{mol N L}^{-1} \text{h}^{-1}$. The absolute NH_4^+ uptake rates from the field, ranged from 0.3 to $1.4 \mu\text{mol N L}^{-1} \text{h}^{-1}$ and were consistent with rates determined in the culture studies, ranging from 0.2 to $1.0 \mu\text{mol N L}^{-1} \text{h}^{-1}$. As a fraction of total uptake, urea uptake was lowest overall in culture while AA uptake was lowest overall in the field. There was greater uptake variability observed for the culture experiments than the field study.

Nitrogen assimilation

For all strains with saturated additions, the percent assimilation by individual substrates never exceeded 60%. The percent of AA assimilated in the dark was the largest of all substrates, averaging 48%, 42% and 56% for Sara, Jax, and Char, respectively. Also, the percent AA assimilation in the dark was significantly greater than the light for Sara ($t = 2.7$, $p = 0.11$), Jax ($t = 6.8$, $p = 0.02$), and Char ($t = 9.2$, $p = 0.01$; Figure 9). In contrast, the percent urea assimilation in the light was significantly greater than the dark for both Sara ($t = 9.8$, $p = 0.01$) and Jax ($t = 9.5$, $p = 0.01$). Char had significantly less urea assimilation than Sara ($t = 9.3$, $p = 0.01$) and Jax in the light ($t = 9.6$, $p = 0.01$). Sara also exhibited a significantly higher percent assimilation of NH_4^+ in the light than dark ($t = 4.9$, $p = 0.04$) while there were no significant differences in light and dark percent assimilation of NH_4^+ for

Jax or Char. In contrast to NH_4^+ , the percent assimilation of NO_3^- by Sara, was significantly greater in the dark ($t = 5.97, p = 0.03$). Except for AA, the majority of percent assimilation for each substrate by Char was 20% or less.

Discussion

K. brevis blooms occur in a wide range of nutrient environments. Typically, they initiate in nutrient poor offshore waters and are transported to and entrained in increasingly nutrient rich coastal and estuarine waters where they can maintain high cell concentrations for months (Steidinger 1975; Tester and Steidinger 1997; Vargo *et al.* 2004; Walsh *et al.* 2006). This nutrient gradient provides a variety of potential N sources. The variability of available N sources a cell may encounter in the light and dark was examined in this study by the use of multiple ^{15}N tracers, including dissolved inorganic (NH_4^+ , NO_3^-) and organic (urea, AA) species. The variation between tracer (~10% of ambient) ^{15}N additions versus saturated ^{15}N additions were examined in culture; the addition of saturating concentrations of ^{15}N allowed for examination of maximum or potential uptake rates as opposed to the tracer level additions, which are more representative of *in situ* processes.

Uptake variations in the light and dark

Results from this study demonstrate significant periodicity and variability in diel uptake rates, with the largest differences observed for NH_4^+ in culture and the field. Absolute uptake rates of NH_4^+ had the highest absolute uptake rates, often exceeding $1 \mu\text{mol N L}^{-1} \text{ h}^{-1}$,

and exhibited significantly higher rates of uptake in the light than dark for all strains and in surface waters in the field. Ammonium is often the most dominant form of dissolved N taken up in marine and estuarine systems (reviewed in Mulholland and Lomas 2008) and may be rapidly taken up by *K. brevis* in the light or the dark since NH_4^+ is already reduced, is energetically efficient for cells to use, and requires little added energy for assimilation (Mulholland and Lomas 2008). An early *K. brevis* study hypothesized that NH_4^+ may be an important N source to blooms (Dragovich *et al.* 1961) and kinetic studies of *K. brevis* have found a high affinity for NH_4^+ (Steidinger 1998; Bronk *et al.* 2004; Killberg-Thoreson Chapter 2).

The high saturated uptake rates for NH_4^+ observed in this study indicate an adaptation of *K. brevis* to take advantage of concentrated transient NH_4^+ pools, such as those released from nearby populations of *Trichodesium spp.* This capability would be advantageous in nutrient-depleted waters (Smayda 1997), such as observed during the offshore bloom initiation phase. Additionally, the tight coupling of NH_4^+ regeneration and uptake rates observed in the field study, in the light and dark as well as the surface and deep (regeneration:uptake rates ~ 1), indicate that uptake by *K. brevis* may be sustained primarily by regeneration processes and highlights the importance of recycled nutrients to *K. brevis* nutrition. While measured at consistently low concentrations within bloom, pools of NH_4^+ are turned over and utilized very rapidly.

A different source of regenerated N may be more important to *K. brevis* inshore. Nutrients released from fish carcasses after brevetoxin-induced fish kills have been hypothesized to fuel high biomass blooms, particularly once cells and fish are concentrated along convergence fronts and in coastal areas (Vargo *et al.* 2001; Walsh *et al.* 2006). The

estimated concentration of N in the water column from a single 90 g (wet weight) fish, taking into consideration dilution from both vertical and horizontal mixing, has been estimated at $5.3 \mu\text{mol N L}^{-1}$ (Walsh et al. 2006; reviewed in Vargo *et al.* 2008). Indeed, a concentration of NH_4^+ , $\sim 6 \mu\text{mol N L}^{-1}$, was measured adjacent to a dead fish while on a research cruise (C. Heil, personal communication) and high concentrations of NH_4^+ release have been measured during fish decay ($\sim 1,000 \mu\text{M d}^{-1} \text{g}^{-1}$ fish wet weight, Appendix 2). It has been hypothesized that an increase of one million *K. brevis* cells could be supported by $1.8\text{-}2.3 \mu\text{mol N L}^{-1}$ (Sipler 2009) or $3 \mu\text{mol N L}^{-1}$ (Odum *et al.* 1955). The high capacity for NH_4^+ uptake coupled with the large NH_4^+ pool present would likely allow for cellular growth and maintenance of high *K. brevis* populations during fish kills.

Within this study we show that NO_3^- uptake in the light was significantly greater than dark uptake for the saturated treatments. Nitrate uptake in the light is likely higher than in the dark (Cochlan *et al.* 1991) because of the energy requirement for nitrate reductase activity (MacIsaac and Dugdale 1972). A bioassay conducted with a field population of *K. brevis* found that in the treatment with added NO_3^- , *K. brevis* experienced a delay in growth compared to other N sources and cell numbers only significantly increased in abundance when light was present (Sipler 2009). Sinclair *et al.* (2006a) found that NO_3^- uptake by *K. brevis* during the day did not differ statistically from dark uptake at night, for deplete culture, however, for replete culture NO_3^- uptake during the day was significantly greater (~ 8.5 fold) than at night. In our study both field and cultures were considered nutrient replete and exhibited an average 2-fold greater uptake rate in the light. It has also been noted that N-limited cells typically enhance nutrient uptake capabilities when N-deficient relative to rates when N-sufficient (e.g. McCarthy and Goldman 1979; Goldman

and Glibert 1982), therefore, our rates may underestimate NO_3^- uptake under N-limited conditions. Sediment-derived sources of NO_3^- in bottom waters have been hypothesized to maintain *K. brevis* blooms during dark uptake, since surface waters are typically N deplete. Results of this study indicate that *K. brevis* populations may take up NO_3^- in the dark, however, these uptake rates are far surpassed in the light when cells are nutrient replete, as well as by other N species if present.

In this study, the organic N species exhibited the least diel variability in uptake rates. Rates of AA uptake generally surpassed those of NO_3^- and urea uptake, although exhibited less variation than other substrates indicating that AA uptake may be a steady source of N to cells during the diel cycle. Darkness can often induce AA uptake likely due to the need for carbon (Lewin and Hellebust 1975). Urea uptake rates were lowest and experienced little change between trace and saturating additions in the culture study indicating uptake may have been saturated even at the tracer addition of substrate. The link between urea and harmful algae has been a topic of much discussion recently as urea is the N-source used in a majority of fertilizer and many HAB species have shown a link between urea uptake and increased toxicity or bloom frequency (Glibert *et al.* 2006). Urea utilization has been shown to be significant particularly for dinoflagellates including *L. polyedrum* in California (Kudela and Cochlan 2000) and *A. catenella* in Thau Lagoon, France (Collos *et al.* 2004). While a previous culture study of urea uptake by *K. brevis* found rates comparable to those of NH_4^+ uptake (Sinclair *et al.* 2009) in the present study urea uptake rates were consistently lower than NH_4^+ in both the field and culture. It is possible that the high concentrations of NO_3^- in the culture media could dampen urea uptake rates as seen in other phytoplankton species (reviewed in Mulholland and Lomas 2008), however, the similarly low rates of urea uptake

in the field when all ambient nutrient concentrations were $< 0.5 \mu\text{mol N L}^{-1}$ indicate this was not the case in this study.

There were oscillations in diel uptake for *K. brevis*. In addition to differences in uptake when averaging all the time points in the light and the dark, there were also variations in uptake between the beginning and the end of the light and dark periods themselves, with uptake rates decreasing during the light period and increasing during the dark. Populations of *K. brevis* begin moving toward the surface prior to the onset of the light period and begin dispersal by randomly swimming from the surface prior to the dark period (Heil 1986) and results from this study indicate that uptake rates concomitantly vary as the cells ascend or descend. A study by Kamykowski *et al.* (1998b) found a similar oscillatory pattern in biochemical composition parameters as observed with our N uptake rates; lipid, carbohydrate, and chl *a*, content were highest at the start of the light period (6:00 h) and decreased until 12:00 h, followed by an increase in the dark from 12:00 to 24:00 h. The large number of time points throughout our 25 h study period provides greater temporal resolution of N uptake by *K. brevis* than studies that examine uptake at a single occasion in the light and dark. The variability in uptake observed both within and between light and dark periods highlights the necessity of frequent sampling over the course of a twenty-four h period. This also has important implications for data comparison and interpretation of *K. brevis* uptake rates collected at different times during the day or night.

Uptake variability between strains

Results from this study indicate variability along the three strains of *K. brevis* investigated. While uptake rates are often a function of the nutrient prehistory of the cell (Mulholland and Lomas 2008), all cultured strains were maintained under similar conditions. Isolates of the same species have been known to vary in physiological responses when grown under identical conditions (Gallagher 1980; Wood and Leatham 1992). Therefore, it is prudent to collect comparative information on intraspecific differences from a variety of strains before extrapolating culture results to field populations. Comparison of multiple strains provides a more comprehensive assessment of what may occur in nature. Studies comparing *K. brevis* strains have documented differences including salinity tolerances (Aldrich and Wilson 1960), swimming speeds (McKay *et al.* 2006), growth rates (McKay *et al.* 2006; Sinclair *et al.* 2009), kinetic parameters (Sinclair *et al.* 2009), photophysiological response (McKay *et al.* 2006), toxin content (Errera *et al.* 2010), toxin production and toxin profiles (Baden and Tomas 1988; LeKan and Tomas 2010). While in this study there was uptake variability between strains, the observed values fit within the range of measurements taken with the field population and are therefore representative of rates that occur within a bloom.

Assimilation of N sources

Less than 60% of the N that was taken up by *K. brevis* was incorporated into proteins. Percent incorporation for all N sources in this study was low compared to values determined

in a previous study (e.g. Sinclair *et al.* 2009) where values for NH_4^+ , NO_3^- and urea ranged from ~40-100% in the light and up to 90% in the dark. Although the end product of N uptake by phytoplankton is primarily proteins, the fate of newly synthesized material is determined by many factors including the nutritional state of the cell and the form of N supplied (Glibert and McCarthy 1984). In N-depleted waters of the Chesapeake Bay, >80% of what was taken up by phytoplankton was incorporated into proteins within ~15 minutes after NH_4^+ addition, while in N-replete waters, a smaller proportion of the N taken up was converted to proteins (40-60%; Wheeler *et al.* 1982). In another study, when cultures were N-sufficient the urea-N that had been taken up was excreted and made little contribution to the N content of the cells; in contrast, N-deficient cells retained all the urea-N they had taken up (Price and Harrison 1988). Considering the N-replete state of the cells at the time of our experiment, excretion is a possible explanation for the lower assimilation rates observed.

Conclusions

This research demonstrated that *K. brevis* is capable of utilizing a wide variety of both inorganic and organic substrates over a diel cycle. The flexible metabolism of *K. brevis* coupled with high uptake rates for many N species may be one of the important reasons that it is able to out-compete other phytoplankton during the initiation phase offshore, under seemingly nutrient poor conditions, and maintain such dense accumulations nearshore in more eutrophied systems. Considerable periodicity and variability was observed between uptake in the light and dark as well as uptake between strains of *K. brevis*, respectively, with the highest rates for NH_4^+ . Results indicate that NH_4^+ uptake and regeneration could be

important factors regulating both bloom initiation as well as bloom maintenance. Knowledge of N sources to *K. brevis* and their uptake variations over the course of a diel cycle are likely important when comparing uptake rates and preparing models of *K. brevis* blooms as the rates may fluctuate dependent on the time the experiment was conducted. A better understanding of *K. brevis*'s nutritional physiology at the cellular level is critical for determining the conditions necessary for large bloom populations to develop and persist.

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Literature Cited

- Aldrich, D.V. & Wilson, W.B. 1960. The effect of salinity on the growth of *Gymnodinium breve* Davis. *Biol. Bull. Woods Hole* 119:57-64.
- Baden, D.G., & Mende, T.J. 1979. Amino acid utilization by *Gymnodinium breve*. *Phytochemistry*. 18: 247-251.
- Baden, D.G. & Tomas, C.R. 1988. Variations in major toxin composition for six clones of *Ptychodiscus brevis*. *Toxicon*, 26:961-963.
- Bronk, D.A., Sanderson, M., Mulholland, M., Heil, C., O'Neil, J. 2004. Organic and inorganic nitrogen uptake kinetics in field populations dominated by *Karenia brevis*. In Steidinger, K.A., Landsberg, J.H., Tomas, C.R. & Vargo, G.A. [Eds]. Proc 10th Int Conf Harmful Algae. Florida Fish and Wildlife Conservation Commission., Florida Institute of Oceanography, Intergovernmental Oceanographic Commission of UNESCO, St. Petersburg, FL. pp. 44-49.
- Cochlan, W.P., Harrison, P.J., Denman, K.L. 1991. Diel periodicity of nitrogen uptake by marine phytoplankton in nitrate-rich environments. *Limnol. Oceanogr.* 36(8):1689-1700.
- Collos, Y., Gagne, C., Laabir, M, Vaquer, A., Cecchi, P, Souchu, P. 2004 Nitrogenous nutrition of *Alexandrium catenella* (Dinophyceae) in cultures and in cultures and in Thau Lagoon, Southern France. *J. Phycol.* 40(1):96-103.
- Cullen, J.J. & Horrigan, S.G. 1981. Effects of nitrate on diurnal vertical migration, carbon to nitrogen ratio, and the photosynthetic capacity of the dinoflagellate *Gymnodinium splendens*. *Mar. Biol.* 62:81-89.
- Doig, M.T. 1973. The growth and toxicity of the Florida red tide organism, *Gymnodinium breve*. PhD Dissertation, Univ. of South Florida, Tampa, 80 pp.
- Dragovich, A., Finucane, J.H. & May, B.Z. 1961. Counts of red tide organisms, *Gymnidinium breve*, and associated oceanographic data from Florida west coast, 1957-1959, U.S. Fish Wildl. Serv. Spec. Sci. Rep. 369, iii, pp 175.
- Dudek, N., Brzezinski, M.A. & Wheeler, P.A. 1986. Recovery of ammonium nitrogen by solvent extraction for the determination of relative ¹⁵N abundance in regeneration experiments. *Mar. Chem.* 18(1):59-69.
- Dugdale R. C. & F.P. Wilkerson, 1986. The use of ¹⁵N to measure nitrogen uptake in eutrophic oceans: experimental considerations. *Limnol. Oceanogr.* 31:673-689.

- Eppley, R.W, Holm-Hansen, O., Strickland, J.D.H. 1968. Some observations on the vertical migration of dinoflagellates. *J. Phycol.* 4:333-340.
- Eppley, R.W. & Harrison, W.G. 1975. Physiological ecology of *Gonyaulax polyedra*, a red water dinoflagellate of southern California. In V.R. LoCicero [Eds.] *Toxic Dinoflagellate Blooms*. Science and Technology Foundation, Wakefield, MA. pp.11-22.
- Errera., R.M., Bourdelais, A., Drennan, M.A., Dodd, E.B., Henrichs, D.W., Campbell, L. 2010. Variation in brevetoxin and brevenal content among clonal cultures of *Karenia brevis* may influence bloom toxicity. *Toxicon.* 55(2-3):195-203.
- Gallagher, J.C. 1980. Population genetics of *Skeletonema costatum* (Bacillariophyceae) in Narragansett Bay, *J. Phycol.* 16:464-474.
- Glibert, P.M., F. Lipschultz, J.J. McCarthy, Altabet, M.A. 1982. Isotope dilution models of uptake and remineralization of ammonium by marine plankton. *Limnol. Oceanogr.* 27:639-650.
- Glibert, P.M. & McCarthy, J.J. 1984. Uptake and assimilation of ammonium and nitrate by phytoplankton: indices of nutritional status for natural assemblages. *J. Plankton Res.* 6(4): 677-696.
- Glibert, P.M., Harrison, J., Heil, C., Seitzinger, S. 2006. Escalating worldwide use of urea – a global change contributing to coastal eutrophication. *Biogeochemistry* 77:441-463.
- Glibert, P.M., Burkholder, J.M., Kana, T M., Alexander, J., Skelton, H., Shilling, C. 2009. Grazing by *Karenia brevis* on *Synechococcus* enhances its growth rate and may help to sustain blooms. *Aquat. Microb. Eco.* 55:17-30.
- Goldman, J.C. & Glibert, P.M. 1982. Comparative rapid ammonium uptake by four species of marine phytoplankton. *Limnol. Oceanogr.* 27:814-827.
- Harrison, W.G. 1976. Nitrate metabolism of the red tide dinoflagellate *Gonyaulax polyedra* Stein. *J. Exp. Mar. Bio. Ecol.* 21:199-209.
- Havens, J.A. 2004. A stable isotopic examination of particulate organic matter during *Karenia brevis* blooms on the central West Florida shelf: Hints at nitrogen sources in oligotrophic waters. Thesis. University of South Florida, Tampa, 124 pp.
- Heaney, S.I. & Eppley, R.W. 1981. Light temperature and nitrogen as interacting factors affecting diel vertical migrations of dinoflagellates in culture. *J. Plankton Res.* 3:331- 344.

- Heil, C.A. 1986. Vertical migration of *Ptycodiscus brevis* (Davis) Steidinger, M.S. Thesis, Univ. of South Florida, Tampa, 118 pp.
- Heil, C., Vargo, G., Spence, D., Neely, M., Merkt, R., Lester, K., Walsh, J. 2001. Nutrient stoichiometry of a *Gymnodinium breve* Davis (Gymnodiniales: Dinophyceae) bloom: What limits blooms in oligotrophic environments? In Hallegraeff, G.M., Blackburn, S.I., Bolch, C.J., Lewis, R.J. [Eds.] Harmful Algal Blooms, Intergovernmental Oceanographic Commission of UNESCO 2001, Paris, pp.165-168.
- Hersey, R.L. & Swift, E. 1976. Nitrate reductase activity of *Amphidium cartearae* and *Cachonina niei* (Dinophyceae) in batch culture: diel periodicity and effects of light intensity and ammonia. *J. Phycol.* 12:29-35.
- Kamykowski, D., Milligan, E.J. & Reed, R.E. 1998a. Relationships between geotaxis/phototaxis and diel vertical migration in autotrophic dinoflagellates. *J. Plankton. Res.* 20:1781-1796.
- Kamykowski, D., Milligan, E.J. & Reed, R.E. 1998b. Biochemical relationships with the orientation of the autotrophic dinoflagellate *Gymnodinium breve* under nutrient replete conditions. *Mar. Eco. Prog. Ser.* 167: 105-117.
- Kirkpatrick, B., Fleming, L.E., Squicciarini, D., Backer, L.C., Clark, R., Abraham, W., Benson, J., Cheng, Y.S., Johnson, D., Pierce, R., Zaias, J., Bossart, G.D., Baden, D.G. 2004. Literature review of Florida red tide: implications for human health effects. *Harmful Algae* 3(2):99-115.
- Koroleff, F. 1983. Simultaneous oxidation of nitrogen and phosphorus compounds by persulfate. In Grasshoff, K., Eberhardt, M., Kremling, K. [Eds.] *Methods of Seawater Analysis*. 2nd ed., Verlag Chemie, Weinheimer, FRG.
- Kudela, R.M. & Cochlan, W.P. 2000. Nitrogen and carbon uptake kinetics and the influence of irradiance for a red tide bloom off Southern California. *Aquat. Microb. Eco.* 21:31-47.
- LeKan, D.K. & Tomas, C.R. 2010. The brevetoxin and brevenal composition of three *Karenia brevis* clones at different salinities and nutrient concentrations. *Harmful Algae* 9(1): 39-47.
- Lenes, J.M., Darrow, B.P., Cattrall, C., Heil, C.A., Callahan, M., Vargo, G.A., Byrne, R.H., Prospero, J.M., Bates, D.E., Walsh, J.J. 2001. Iron fertilization and the *Trichodesmium* response on the West Florida Shelf. *Limnol. Oceanogr.* 46:1261-1277.
- Lester, K.M. 2005. Zooplankton of the West Florida Shelf: relationships with *Karenia brevis* blooms. Ph.D. Dissertation, University of South Florida. 199 pp.

- Lewin, J. & Hellebust, J.A. 1975. Heterotrophic nutrition of the marine pinnate diatom *Navicula pavillardii* Hustedt. *Can. J. Microbiol.* 21:1335-42.
- Loeblich, A.R. & Smith, V.E. 1968. Chloroplast pigments of the marine dinoflagellate *Gymnodinium resplendens*. *Lipids* 3:5-13.
- MacIsaac, J.J. & Dugdale, R.C. 1972. Interactions of light and inorganic nitrogen in controlling nitrogen uptake in the sea. *Deep-Sea Res.* 19:209-232
- McCarthy, J.J. & Goldman, J.C. 1979. Nitrogenous nutrition of marine phytoplankton in nutrient-depleted waters. *Science* 23:670-672.
- McKay, L., Kamykowski, D., Milligan, E., Schaeffer, B., Sinclair, G. 2006. Comparison of swimming speed and photophysiological responses to different external conditions among three *Karenia brevis* strains. *Harmful Algae* 5(6):623-636.
- Mulholland, M.R., Bernhardt, P.W., Heil, C.A., Bronk, D.A., O'Neil, J.M., 2006. Nitrogen fixation and release of fixed nitrogen by *Trichodesmium spp.* in the Gulf of Mexico. *Limnol. Oceanogr.* 51(4):1762-1776.
- Mulholland, M.R. & Lomas M.W. 2008. Nitrogen uptake and assimilation. In Capone, D., Bronk, D., Mulholland, M., Carpenter, E. [Eds.] *Nitrogen in the marine environment*. Academic Press. New York, USA. pp. 303-384.
- Needoba, J.A. & Harrison, P.J. 1992. Influence of low light and a light: dark cycle on NO_3^- uptake, intracellular NO_3^- , and nitrogen isotope fractionation by marine phytoplankton. *J. Phycol.* 40(3):505-516.
- Odum, H.T., Lackey, J.B., Hynes, J., Marshall, N. 1955. Some red tide characteristics during 1952-1954. *Bulletin of Marine Science of the Gulf and the Caribbean* 5:247-258.
- Paashe, E., Bryceson, I. & Tangen, K. 1984. Interspecific variation in dark nitrogen uptake by dinoflagellates. *J. Phycol.* 20(3):394-401.
- Parsons, T., Maita, Y. & Lalli, C. 1984. *A manual of chemical and biological methods for seawater analysis*. Pergamon Press, New York.
- Price, N. & Harrison, P. 1987 A comparison of methods for the measurement of dissolved urea concentrations in seawater. *Mar. Biol.* 94:307-317.
- Price, N.M. & Harrison, P.J. 1988. Uptake of urea C and Urea N by the coastal marine diatom *Thalassiosira pseudonana*. *Limnol. Oceanogr.* 33(4):528-537.
- Procise, L.A., Mulholland, M.R. 2008. Grazing by the red tide dinoflagellate, *Karenia brevis*: Implications for bloom dynamics. ASLO Ocean Science Meeting, Orlando, FL, March 2008.

- Rivkin, R.B. & Putt, M. 1987. Heterotrophy and photoheterotrophy by Antarctic microalgae: light dependent incorporation of amino acids and glucose. *J. Phycol.* 23:442-452.
- Rivkin, R.B. & Swift, E. 1982. Phosphate uptake by the oceanic dinoflagellate *Pyrocystis noctiluca*. *J. Phycol.* 18:113-120.
- Romans, K.M, Carpenter, E.J. & Bergmas, B. 1994. Buoyancy regulation in the colonial diazotrophic cyanobacterium *Trichodesmium tenue*: ultrastructure and storage of carbohydrate, polyphosphate, and nitrogen. *J. Phycol.* 30(6): 935-942.
- Sigman, D.M., Casciotti, K.L., Andreani, M., Barford, C., Galanter, M., and J. K. Böhlke. 2001. A bacterial method for the nitrogen isotopic analysis of nitrate in seawater and freshwater. *Anal. Chem.* 73(17):4145-4153.
- Sinclair, G.A., Kamykowski, D., Milligan, E., Schaeffer, B. 2006a. Nitrate uptake by *Karenia brevis*. II. Behavior and uptake physiology in a nitrate-depleted mesocosm with a bottom nutrient source. *Mar. Ecol. Prog. Ser.* 328:125-131.
- Sinclair, G.A., Kamykowski, D., Milligan, E., Schaeffer, B. 2006b. Nitrate uptake by *Karenia brevis*: Influences of prior environmental exposure and biochemical state on diel uptake of nitrate. *Mar. Ecol. Prog. Ser.* 328:117-124.
- Sinclair, G., Kamykowski, D. & Glibert, P.M. 2009. Growth, uptake, and assimilation of ammonium, nitrate, and urea by three strains of *Karenia brevis* grown under low light. *Harmful Algae.* 8:770-780.
- Sipler, R.E. 2009. The Role of Dissolved Organic Matter in Structuring Microbial Community Composition. Ph.D. Dissertation. Rutgers, N.J. pp. 191.
- Shimizu, Y. & Wrensford, G. 1993. Peculiarities in the biosynthesis of brevetoxins and metabolism of *Gymnodinium breve*. In Smyda, T.J., Shimizu, Y. [Eds.] *Toxic Phytoplankton Blooms in the Sea*. Elsevier Science Publishing, Inc., New York, pp. 919-923.
- Shimizu, Y., Watanabe, N. & Wrensford, G. 1995. Biosynthesis of brevetoxins and heterotrophic metabolism in *Gymnodinium breve*. In Lassus, P., Arzul, G., Erard, E., Gentien, P., Marcaillou, C. [Eds.] *Harmful Marine Algal Blooms*. Lavoisier Intercept Ltd., Paris. pp. 351-357.
- Steidinger, K.A. 1975. Basic factors influencing red tides. In LoCiero, V.R. [Ed.] *Proceedings of the First International Conference on Toxic Dinoflagellate Blooms*, Massachusetts Science and Technology Foundation, Wakefield, MA. pp.153-162.

- Steidinger, K. A. & Haddad, K. 1981. Biologic and hydrographic aspects of red tides. *Bioscience*. 31:814-819.
- Steidinger, K.A., Vargo, G.A., Tester, P.A., Tomas, C.R. 1998. Bloom dynamics and physiology of *Gymnodinium breve* with emphasis on the Gulf of Mexico. In Anderson, D.M., Cembella, A.D., Hallegraeff, G.M., [Eds.] *Physiological Ecology of Harmful Algal Blooms*. Springer, New York. pp. 135–153.
- Tester, P.A. & Steidinger, K.A. 1997. *Gymnodinium breve* red tide blooms: Initiation, transport, and consequences of surface circulation. *Limnol. Oceanogr.* 42:1039-1051.
- Vargo, G.A., Heil, C.A., Ault, D.N., Neely, M.B., Murasko, S., Havnes, J., Lester, K.M., Dixon, L.K., Merkt, R., Walsh, J., Weisberg, R., Steidinger, K.A. 2004. Four *Karenia brevis* blooms: a comparative analysis. In Steidinger, K.A., Landsberg, J.H., Tomas, C.R., Bargo, G.A. [Eds.] *Proceedings of the Xth International Conference on Harmful Algae*. Florida Fish and Wildlife Conservation Commission, Florida Institute of Oceanography and Intergovernmental Oceanographic Commission of UNESCO, Paris, pp.14-16.
- Vargo, G.A., Heil, C.A., Fanning, K.A., Dixon, L.K., Neely, M.B., Lester, K., Ault, D., Murasko, S., Havens, J., Walsh, J., Bell, S. 2008. Nutrient availability in support of *Karenia brevis* blooms on the West Florida Shelf: What keeps *Karenia* blooming? *Cont. Shelf Res.* 28:73-98.
- Vargo, G.A., Heil, C.A., Spence, D., Neely, M.B., Merkt, R., Lester, K., Weisberg, R.H., Walsh, J.J., Fanning, K. 2001. The hydrographic regime, nutrient requirements, and transport of a *Gymnodinium breve* Davis red tide on the West Florida shelf. In Hallegraeff, G.M., Blackburn, S.I., Bolch, C.J., Lewis, R.J. [Eds.] *Harmful Algal Blooms*. UNESCO, Paris, pp.157-160.
- Walsby, A.E. 1992. The gas vesicles and buoyancy of *Trichodesmium*. In Carpenter, E.J., Capone, D.G., Rueter, J.G. [Eds.] *Marine Pelagic Cyanobacteria: Trichodesmium and Other Diazotrophs*. Kluwer, Dordrecht, pp. 141–161.
- Walsh, J.J. & Steidinger, K.A. 2001. Saharan dust and Florida red tides: the cyanophyte connection. *J. Geophys. Res.* 106, 11597-11612.
- Walsh, J.J., Joliff, J.K., Darrow, B.P., Lenes, J.M., Milroy, S.P., Dieterle, D.A., Cerder, K.L., Chen, F.R., Vargo, G.A., Weisberg, R.H., Fanning, K.A., Muller-Karger, F.E., Steidinger, K.A., Heil, C.A., Prospero, J.S., Lee, T.N., Kirkpatrick, G.J., Whitledge, T.E., Stockwell, D.A., Tomas, C.R., Villareal, T.A., Jochens, A.E., Bontempi, P.S. 2006. Red tides in the Gulf of Mexico: where, when, and why? *J. Geophys. Res.* 111:C11003.

- Wheeler P.A., Glibert, P.M. & McCarthy, J.J. 1982 Ammonium uptake and incorporation by Chesapeake Bay phytoplankton: short term uptake kinetics. *Limnol. Oceanogr.* 27(6): 1113-1128.
- Wannicke, N., Koch, B.P. & Voss, M. 2009. Release of fixed N₂ and C as dissolved compounds by *Trichodesmium erythreum* and *Nodularia spumigena* under the influence of high light and high nutrient (P). *Aquat. Microb. Ecol.* 57:175-189.
- Wilson, W.B. 1966 The suitability of sea-water for the survival and growth of *Gymnodinium breve* Davis; and some effects of phosphorus and nitrogen on its growth. FSU Prof. Pap. Ser., 7. pp. 1-42.
- Wood, A.M. & Leatham, T. 1992. The species concept in phytoplankton ecology. *J. Phycol.* 28(6):723-729.

Table 1. Summary of ambient concentrations of ammonium (NH_4^+), nitrate (NO_3^-), urea and amino acids (AA, measured as dissolved primary amines) at the initiation of field and culture uptake experiments. Ambient concentrations are given as ($\mu\text{mol N L}^{-1}$) at the start of each experiment. Values are mean $\mu\text{mol N L}^{-1}$ with ± 1 standard deviation given in parentheses.

	NH_4^+	NO_3^-	Urea	AA
Field -surface	0.35 (0.01)	0.07 (0.01)	0.29 (0.05)	0.12 (0.00)
Field - deep	0.24 (0.02)	0.06 (0.00)	0.26 (0.04)	0.13 (0.01)
Sara	0.98 (0.22)	28.3 (0.00)	0.43 (0.04)	0.69 (0.19)
Jax	0.49 (0.03)	33.2 (0.00)	0.16 (0.00)	0.24 (0.00)
Char	0.46 (0.21)	29.9 (0.00)	0.46 (0.02)	0.49 (0.06)

Table 2. Summary of absolute uptake rates in the field ($\mu\text{mol N L}^{-1} \text{h}^{-1}$) and culture ($\text{pmol N L}^{-1} \text{h}^{-1} \text{cell}^{-1}$) of ammonium (NH_4^+), nitrate (NO_3^-), urea and amino acids (AA) in the light ($n = 4$) and the dark ($n = 3$). Values are means with ± 1 standard deviation given in parentheses. n/a indicates data not available. Light and dark pairs with * indicate a significant difference (t-test) of $p < 0.05$ while ** indicates a significant difference of $p < 0.01$.

Experiment		NH_4^+	NO_3^-	Urea	AA
Field- surface	light	0.74 (0.51)	0.14 (0.08)	0.16 (0.04) *	0.04 (0.00)
	dark	0.37 (0.06)	0.11 (0.01)	0.10 (0.01)	0.05 (0.01)
Field- deep	light	0.40 (0.26)	0.07 (0.03)	0.08 (0.03)	0.04 (0.01)
	dark	0.34 (0.07)	0.11 (0.04)	0.14 (0.07)	0.04 (0.00)
Sara- trace	light	0.044 (0.019)*	n/a	0.023 (0.012)	0.030 (0.009)
	dark	0.019 (0.007)	n/a	0.015 (0.010)	0.025 (0.010)
Sara- saturated	light	0.085 (0.027)	0.026 (0.013) **	0.015 (0.009)	0.032 (0.008)
	dark	0.070 (0.022)	0.008 (0.003)	0.015 (0.010)	0.026 (0.007)
Jax- trace	light	0.190 (0.032) **	n/a	0.034 (0.015)	0.054 (0.017)
	dark	0.127 (0.029)	n/a	0.039 (0.017)	0.063 (0.015)
Jax- saturated	light	0.302 (0.063) **	0.112 (0.051) **	0.034 (0.005)	0.112 (0.032)
	dark	0.171 (0.088)	0.037 (0.012)	0.066 (0.063)	0.085 (0.015)
Char-trace	light	0.096 (0.025)	n/a	0.013 (0.009)	0.113 (0.021)
	dark	0.072 (0.030)	n/a	0.089 (0.025)	0.147 (0.038)
Char- saturated	light	0.185 (0.083)	0.057 (0.028) *	0.015 (0.009)	0.147 (0.038)
	dark	0.115 (0.057)	0.026 (0.015)	0.015 (0.008)	0.121 (0.040)

Figure Legend

Figure 1. Drogue track followed during the field diel study. The symbol (x) denotes where a ^{15}N uptake experiment was conducted.

Figure 2. Cell counts of *Karenia spp.* in the field study for the surface (open circles) and deep (closed circles) samples in units of cell L^{-1} . The shaded area represents the dark period.

Figure 3. Absolute uptake rates ($\mu\text{mol N L}^{-1} \text{h}^{-1}$) of NH_4^+ (solid circles), NO_3^- (solid triangles), urea (open squares), and amino acids (open diamonds) for *K. brevis* in the field (surface, deep). The field study used only trace levels of ^{15}N . Error bars represent ± 1 standard deviation of the mean (of duplicate incubations), and are frequently smaller than the symbols. The shaded area represent the dark period.

Figure 4. Absolute uptake rates ($\mu\text{mol N L}^{-1} \text{h}^{-1}$) of NH_4^+ (solid circles), NO_3^- (solid triangles), urea (open squares), and amino acids (open diamonds) for *K. brevis* in both culture (Sara, Jax, Char). Data for the culture studies represent both trace (A, C, E) and saturated (B, D, F) ^{15}N additions. High ambient NO_3^- concentrations in the culture study did not allow tracer level rates to be measured (A, C, E). Error bars represent ± 1 standard deviation of the mean (of duplicate incubations), and are frequently smaller than the symbols. The shaded area represent the dark period. Note the variations in the y-axes.

Figure 5. Total absolute nitrogen uptake ($\mu\text{mol N L}^{-1} \text{h}^{-1}$) made up of ammonium (NH_4^+ ; gray hatched), nitrate (NO_3^- ; black), urea (white), and amino acids (AA; gray) for *K. brevis* in the field (surface, deep). The shaded area represents the dark period.

Figure 6. Total absolute nitrogen uptake ($\mu\text{mol N L}^{-1} \text{h}^{-1}$) made up of ammonium (NH_4^+ ; gray hatched), nitrate (NO_3^- ; black), urea (white), and amino acids (AA; gray) in culture (Sara, Jax, Char). Data for the culture studies represent both trace and saturated ^{15}N additions while the field study used only trace levels of ^{15}N . High ambient NO_3^- concentrations in the culture study did not allow tracer level rates to be measured. The shaded area represents the dark period. Note the variations in the y-axes.

Figure 7. Rates of NH_4^+ regeneration (A) and NO_3^- regeneration (B) in the field, in surface and deep water. Error bars represent ± 1 standard deviation of the mean (of duplicate incubations), and are frequently smaller than the symbols. The shaded area represents the dark period.

Figure 8. Percentage of nitrogen assimilated into the TCA precipitable fraction for ammonium (NH_4^+), nitrate (NO_3^-), urea, and amino acids (AA). This represents the average percentage of nitrogen uptake assimilated into proteins \pm standard deviation, for the *K. brevis* culture study with saturated ^{15}N additions at one mid light (1200 h) and one mid dark (2400 h) time point. Open and shaded bars along the x-axis represent the light and dark periods, respectively.

Figure 1.

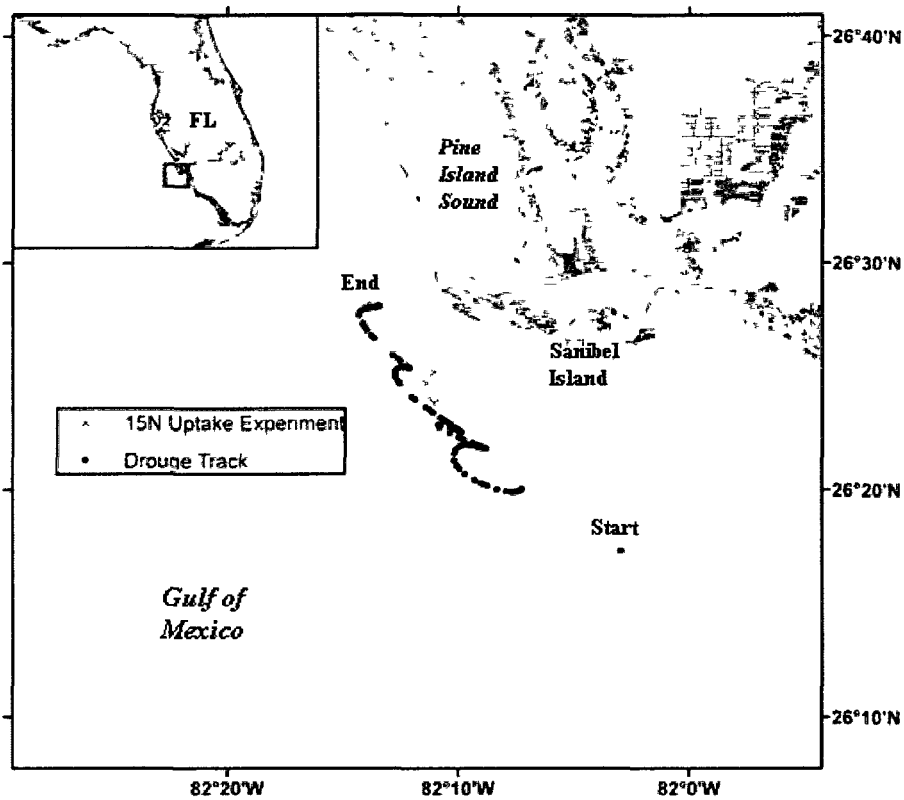


Figure 2.

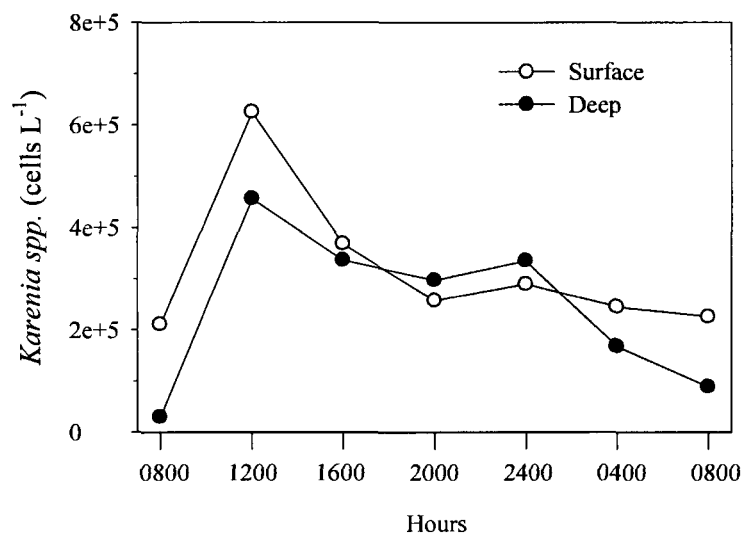


Figure 3.

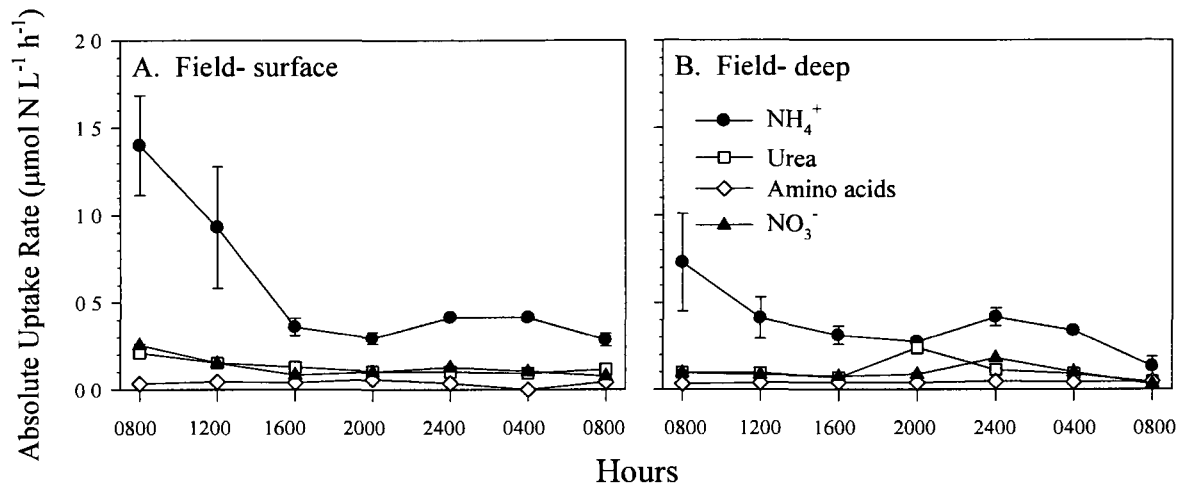


Figure 4.

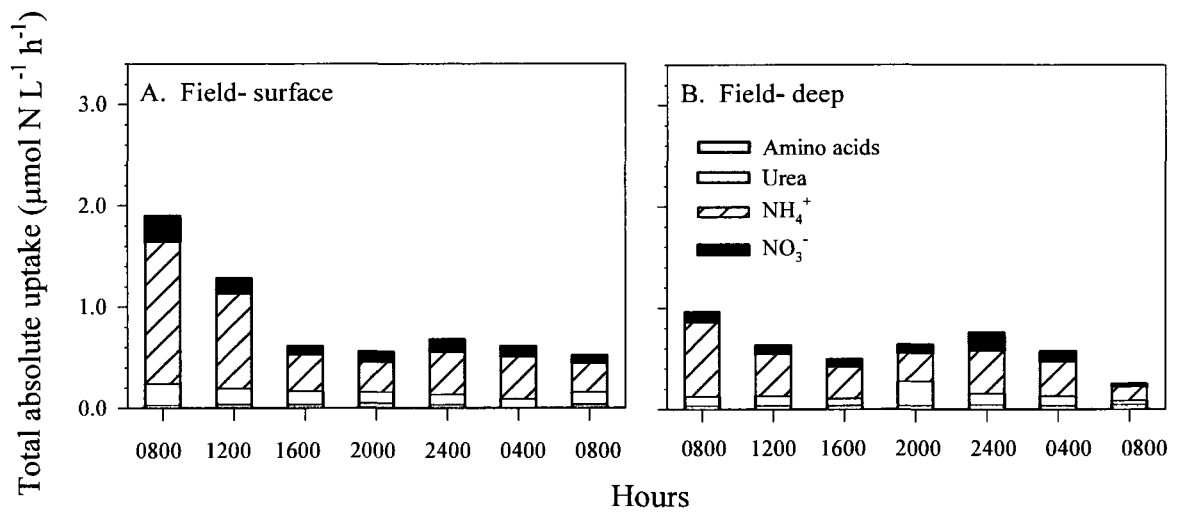


Figure 5.

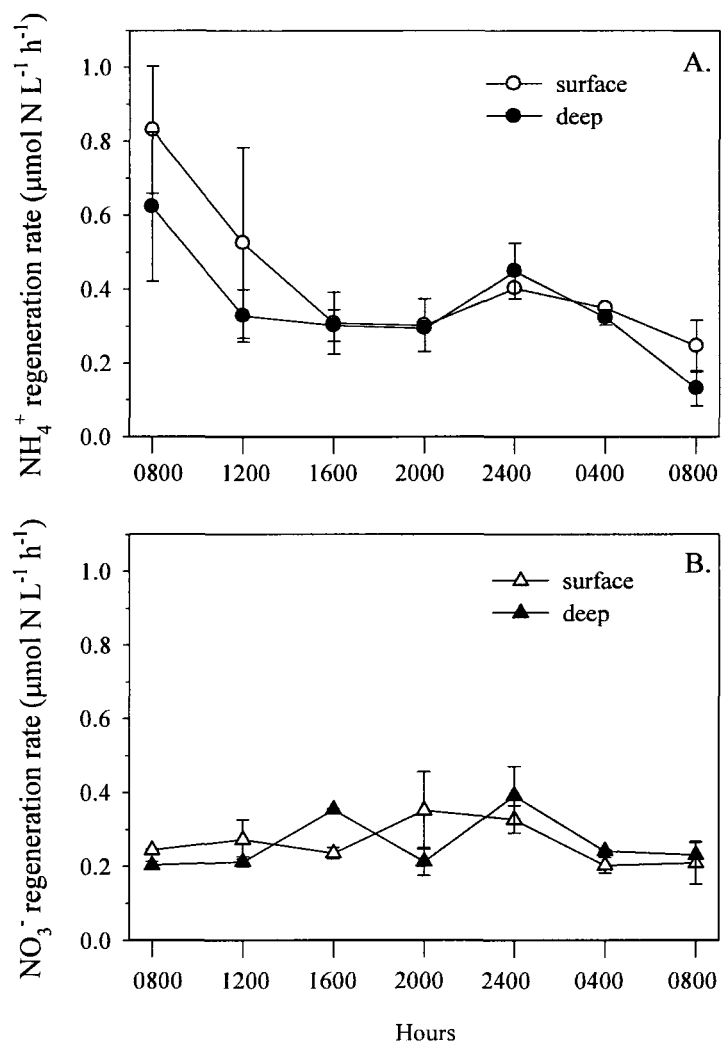


Figure 6.

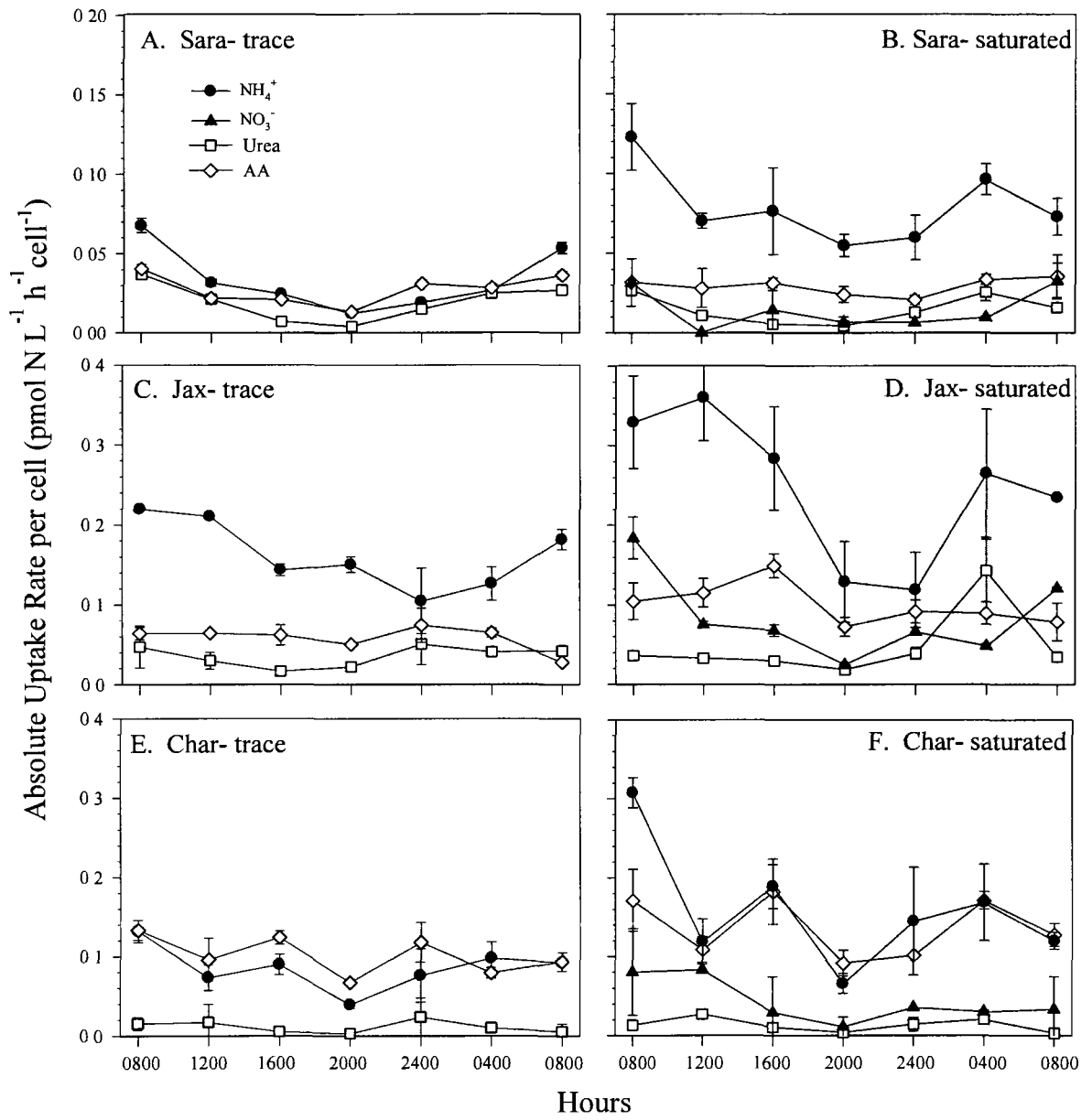


Figure 7.

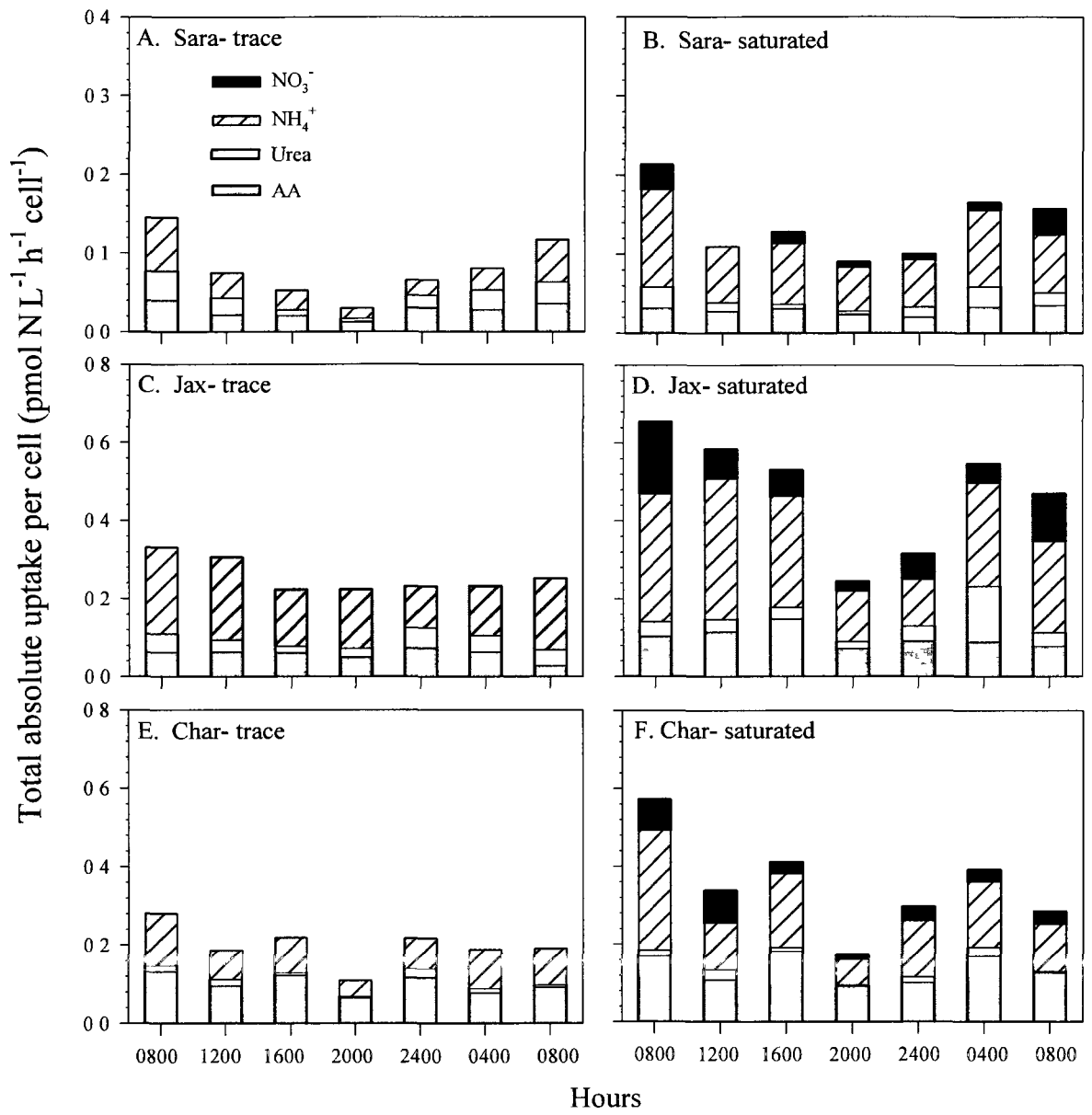
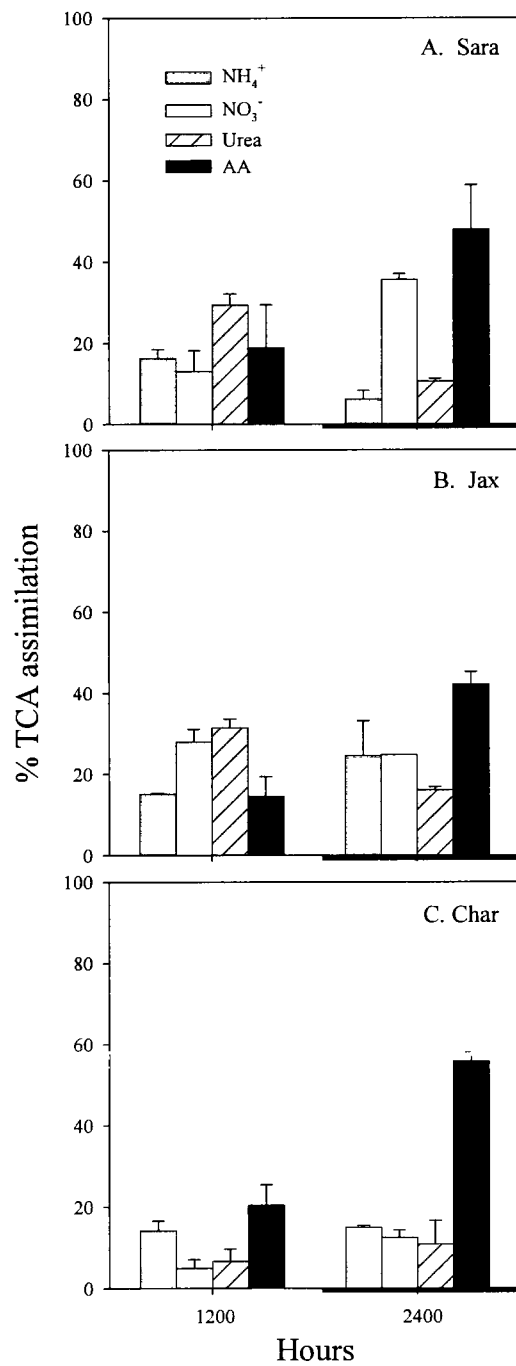


Figure 8.



CHAPTER 4

Seasonal nitrogen uptake dynamics and harmful algal blooms in the York River, Virginia

Abstract

Three stations in the York River, Virginia were sampled seasonally from 2007-2009 to investigate the nitrogenous nutrition of the phytoplankton present with the most frequent sampling focused on the late summer/early fall when harmful algal blooms typically occur. The goals of this study were to examine spatial and temporal variability in the York River for a suite of parameters at each station including: 1) ambient nutrient concentrations, 2) uptake rates of ammonium (NH_4^+), nitrate (NO_3^-), nitrite (NO_2^-), urea and amino acids in three size fractions (GF/F ($>0.7 \mu\text{m}$), $> 5 \mu\text{m}$, and $5 \mu\text{m} - 0.2 \mu\text{m}$), and rates of ^{13}C -bicarbonate uptake, and 3) ammonium regeneration and nitrification rates. There were decreasing nutrient concentrations from the upriver to downriver sites. High concentrations of dissolved inorganic nitrogen (DIN) were detected during the late summer and fall 2008; concentrations of NH_4^+ and NO_2^- reached maximums of 9.9 and 7.6 $\mu\text{mol N L}^{-1}$, respectively. Ratios of DIN:DIP indicated the York River was relatively N-limited during the summer and fall and P-limited in the winter and spring. Rates of NH_4^+ and NO_3^- regeneration measured via isotope dilution were often far greater than uptake rates. The highest uptake rates were for NH_4^+ at all stations, for all size fractions, and for all seasons sampled, ranging from 34 to 80% of total absolute uptake. The pattern of uptake was $\text{NH}_4^+ > \text{NO}_3^- > \text{urea} > \text{AA} > \text{NO}_2^-$ in the GF/F fraction at each station, however, urea uptake was often utilized at equal or greater rates than NH_4^+ in the $> 5 \mu\text{m}$ and $5 \mu\text{m} - 0.2 \mu\text{m}$ size fractions. Nitrogen uptake in blooms of *Alexandrium monilatum* were the first rates measured for this species in the York River estuary and

demonstrated uptake of a diverse suite of inorganic and organic N substrates, dominated by NH_4^+ .

Based on the results, a sequence of events that initiate and maintain HABs in the York River was determined. High concentrations of NH_4^+ and NO_2^- were observed, likely caused by regeneration processes or freshwater run-off due to rainfall events. High concentrations of NH_4^+ were then oxidized to NO_2^- and NO_3^- via nitrification. Nitrogen accumulated in the water column and was transported downstream. A combination of physical conditions and high N pulse may have stimulated harmful algae, allowing for large blooms to form, which were then maintained by N supplied by regeneration.

Introduction

Estuaries are dynamic systems often characterized by strong gradients in freshwater flow, circulation, sediment loads, nutrient inputs, and temperature. These ever-changing conditions can cause large temporal and spatial heterogeneity in the supply of nutrients to the microbial community. The patchiness in nutrient supply and variability of nutrient flux play a key role in determining phytoplankton community composition. Specifically, nitrogen (N) is considered to be the nutrient limiting primary production in estuarine and coastal marine systems (Ryther and Dunstan 1971; D'Elia *et al.* 1986; Hecky and Kilham 1988) therefore, understanding sources and transformations of N in estuarine systems is key to understanding phytoplankton production. Nitrogen dynamics in estuaries, however, are often complex.

Nitrogen sources to coastal waters are vast and varied and include both natural and anthropogenic origins (reviewed in Paerl and Piehler 2008; Boynton and Kemp 2008). Overland runoff, riverine delivery, groundwater discharge, atmospheric deposition, *in situ* biotic processes, as well as sediment remineralization may all provide significant sources of N to an estuary (Capone 2000). The diversity of N sources is reflected in the numerous dissolved inorganic and organic species that make up the N pool. Most measurements and models of N loading in estuaries have focused on dissolved inorganic N (DIN) species including ammonium (NH_4^+), nitrate (NO_3^-) and nitrite (NO_2^-), however, DIN is often only a small component of total dissolved nitrogen (TDN). The majority of N in coastal and oceanic surface waters is dissolved organic N (DON), typically 60-70% (Antia *et al.* 1991; Bronk 2002). Dissolved organic N encompasses a largely uncharacterized pool of compounds as well as urea, amino acids (AA), and larger more complex humic and fulvic acids (reviewed in Antia *et al.* 1991; Bronk. 2002).

While N is a vital element for phytoplankton production, concentrations in estuaries are often found in overabundance. In the Chesapeake Bay alone, N loads nearly doubled between 1950 and 2001 (Hagy *et al.* 2004), caused by anthropogenic N loading from an increasingly large population residing in the Chesapeake Bay watershed. Nutrient overload can have deleterious consequences including increases in total phytoplankton biomass. Eutrophication may cause selective enrichment of a specific nutrient, which can cause shifts in phytoplankton abundance and often favor domination of harmful species over others (Ryther and Dunstan 1971; Tilman 1977, Officer and Ryther 1980; Smayda 1990; Hallegraeff 2003; Smayda 1990; Smayda 1997). Linking

HAB proliferation with a particular nutrient form may enhance our understanding of bloom dynamics.

Like many estuaries, the York River has experienced eutrophication. According to nutrient water quality status reports for the York River and Chesapeake Bay, conditions are considered degraded (reviewed in Reay 2009) and poor water clarity is a persistent and widespread problem in the York River (Dauer *et al.* 2005). Increases in the occurrences of HABs in the lower Chesapeake Bay have been ascribed to these changes in water quality (Paerl 1988, Glibert *et al.* 2001), specifically, estuarine enrichment of dissolved organic matter (DOM) relative to inorganic nutrients (Paerl 1988, Lewitus *et al.* 1999, Glibert *et al.* 2001). Numerous phytoplankton species can utilize DON sources as part of their N nutrition (Berg *et al.* 1997, Lewitus *et al.* 1999, Mulholland *et al.* 2002). Phytoplankton that can utilize both inorganic and organic nutrients may have a competitive advantage when either concentrations of inorganic nutrients are low or when organic nutrients are high (reviewed in Glibert and Legrand 2006). One nutrient found to be increasingly important to HAB nutrition is urea. The increased use of urea as the N source in fertilizer, and subsequent run-off into streams and rivers, has made it an important nutrient to consider for stimulating eutrophication and HAB frequency (Glibert *et al.* 2006).

In addition to the typical diatom bloom every spring, initiated by increased freshwater flow, destratification, and nutrient input, the York River experiences nearly annual HABs in the late summer and early fall (Ho and Zubkoff 1979, Marshall 1994). These blooms dominate in the lower York River and are composed primarily of dinoflagellate species (Marshall 1994). Blooms can be composed of a single species but

are often found as multi-species assemblages. Specifically, *Cochlodinium polykrikoides* has produced extensive blooms annually (Mackiernan 1968; Zubkoff *et al.* 1979; Marshall 1994). Once constrained to the York River, these blooms have recently appeared in the lower Chesapeake Bay proper and its adjacent tributaries (Mulholland *et al.* 2009a).

Alexandrium monilatum is another species of interest. Typically a Gulf coast dweller, there are records of this dinoflagellate in the Chesapeake Bay (Morse 1947; Mackiernan 1968), however, it first began to bloom extensively in the York River in the summer of 2007. *Alexandrium monilatum* cells produce the toxin goniiodomin A (Hsia *et al.* 2006) and the 2007 bloom caused mortality in the veined rapa whelk (Harding *et al.* 2009). Little is known about the ecophysiology of either harmful alga with regard to N nutrition. Nitrogen uptake by HABs has been investigated in other Chesapeake Bay tributaries (e.g. Glibert *et al.* 2001; Fan *et al.* 2003; Mulholland *et al.* 2004; Mulholland *et al.* 2009a, b), however, little research has focused on the York River estuary and no information is available regarding *A. monilatum* in the region. Early observations of *A. monilatum* blooms in Florida implicated the high nutrient content in sewage in bloom initiation and maintenance (Connell and Cross 1950). Understanding triggers to bloom formation are key as both *A. monilatum* and *C. polykrikoides* form a resting cyst stage that sinks to the sediment and “seed” new bloom populations under favorable conditions, making future blooms likely.

The goals of this study were to examine spatial and temporal variability in the York River for a suite of N parameters. Sampling was conducted at three stations spanning the mesohaline and polyhaline regions of the York River, from 2007-2009, we

determined: ambient nutrient concentrations at each station, rates of nitrogen uptake including NH_4^+ , NO_3^- , NO_2^- , urea and AA in size fractions GF/F ($> 0.7 \mu\text{m}$), $5 \mu\text{m}$, and $5 \mu\text{m} - 0.2 \mu\text{m}$, as well as rates of NH_4^+ and NO_3^- regeneration and ^{13}C uptake rates. Sampling was most frequent during the summer and fall, when HABs are prevalent, to determine potential nutrient triggers to bloom initiation as well as patterns of N utilization during a bloom.

Materials and Methods

Sampling stations

The York River, VA is the 5th largest tributary of Chesapeake Bay at $\sim 6900 \text{ km}^2$ (2662 mi^2). It is considered a microtidal, partially mixed estuary with a salinity range from polyhaline to freshwater. The York has two freshwater tributaries, the Mattaponi and Pamunkey Rivers, whose confluence is at West Point, $\sim 48 \text{ km}$ from the mouth of the river. The residence time in the York is dependent on freshwater flow; it has been estimated between 45 and 90 days for material discharged at the headwaters of the Mattaponi and Pamunkey rivers during high and mean flow, respectively (Shen and Haas 2004). The salinity distribution in the York River system is affected by the interactions of freshwater, salt water, tidal energy, and wind (Haas 1975).

For the monitoring portion of the study, field sampling was conducted at three stations in the York River from August 2007-2009. The Chesapeake Bay National Estuarine Research Reserve in Virginia (CBNERRVA) maintains shallow water

monitoring stations along the York River; three of these stations were sampled for this study: Clay Bank (upper York mesohaline), Gloucester Point (lower York mesohaline), and Goodwin Islands (polyhaline) (Figure 1). The stations were most intensively sampled during August and September to capture conditions during typical harmful algal bloom seasons (late summer/early fall). The Clay Bank station (N 37° 20' 49.5" W 76° 36' 41.94") is located northeast of the York River channel, approximately 28 km upstream from the mouth of the river. It experiences salinity ranges from > 5 - 18. The sampling station is influenced by a secondary turbidity maximum that moves back and forth in a region of about 20 to 40 km from the mouth of the York River estuary. Note that the 9/9/08 sampling occurred slightly downriver of the Clay Bank station due to the threat of approaching storms. The Gloucester Point station (N 37° 14' 53.82" W 76° 29' 47.46") is located north of the York River channel, approximately 10 km upstream from the River's mouth in the polyhaline region (> 18). The Goodwin Islands station (N 37° 13' 01.2" W 76° 23' 19.2") is located on the southern side of the York River, near the mouth with salinities typically greater than 18. On 9/12/07 during an extensive bloom, sampling was conducted with water collected from N 37° 13' 0.923" W 76° 27' 0.02" near a power plant and oil refinery, referred to as PWP (Figure 1).

During the summer of 2007 there was an extensive bloom of *A. monilatum* in the York River. The bloom was characterized by the Virginia Institute of Marine Science (VIMS) using DATAFLOW surface water quality mapping system during cruises are carried out on a monthly basis. The DATAFLOW system utilizes a YSI 6600 multiparameter sonde equipped with a flow-through chamber. The Virginia Estuarine and Coastal Observing System (VECOS) website (<http://www2.vims.edu/vecos>) contains

all DATAFLOW data, collection information, and methods. Additionally, rainfall data for Taskinas Creek (TSK000.23), a York River tributary and fixed shallow water monitoring site, located at N 37° 24' 54.79" W 76° 42' 52.74", was obtained (Figure 2). The station is located ~ 23 miles upstream from the mouth of the York River. This data was made available through a partnership with the NOAA National Estuarine Research Reserve, Centralized Data Management Office, with help from the National Weather Service (NWS) and the Hydrometeorological Automated Data Service (HADS) and is available at (<http://cdmo.baruch.sc.edu/>). Pamunkey River discharge data was obtained from the US Geological Survey (USGS) station 01673000 near Hanover, Virginia (Figure 3; <http://va.water.usgs.gov>). The Pamunkey River is responsible for ~80% of the freshwater input to the York River.

Uptake experiments

At each station, temperature, salinity, and dissolved oxygen were measured using a YSI 6600 multiparameter sonde equipped with a flow-through chamber as described above. Surface water samples were collected using an acid-cleaned bucket, stored in 20 L acid-cleaned HDPE carboys, and immediately transferred to the laboratory. A 200 mL sub-sample was filtered through a pre-combusted (450°C, 2 h) Whatman[®] GF/F filter at < 100 mm Hg; the filtrate and filter were frozen (-20°C) for future analysis of ambient nutrients and Chlorophyll *a*, respectively. Whole water samples were also preserved using 1% glutaraldehyde (final concentration) for phytoplankton enumeration (10 mL) and 100 mL of sample was placed in a polycarbonate bottle for examination of

phytoplankton community composition and q-PCR analysis as part of an on-going HAB monitoring program.

Stable isotope tracer techniques were used to quantify uptake rates of inorganic and organic N and carbon (C). Ten 500 mL polyethylene terephthalate (PETG) bottles were filled using water collected from each station. Estimated tracer levels additions, (~10% of the ambient N concentration), of the following substrates were added separately to duplicate bottles: $^{15}\text{NH}_4\text{Cl}$ (98.85 at. %), K^{15}NO_3 (98%+ at. %), $\text{Na}^{15}\text{NO}_2$, dually labeled ^{15}N and ^{13}C -urea (98% at. %), and ^{15}N and ^{13}C -labeled algal mixture of 16 AA (96-99%+ at. %), as well as ^{13}C -labeled bicarbonate (HCO_3^-) (Cambridge Isotope Laboratories, Andover, MA, USA). After substrate additions, the bottles were placed in an incubator for 1 h under simulated *in situ* light and temperature conditions.

For all August and September experiments in 2008 and 2009, incubations were terminated by filtration onto a range of filters to examine three size fractions of the microbial community. From each bottle, 150 to 200 mL of whole water was filtered (< 100 mmHg) onto a pre-combusted (450°C, 2 h) Whatman[®] GF/F filter (0.7 μm nominal pore size), a second aliquot (150 mL) of whole water was filtered onto a 5 μm (Sterlitech[™] Corporation) silver membrane filter, and 25 to 50 mL of that filtrate was then passed through a 0.2 μm (Sterlitech[™] Corporation) silver membrane filter. For the January and May experiments, samples were only filtered onto GF/F filters. While GF/F filters are typically used for estimates of uptake rates, with the purpose to collect the entire phytoplankton fraction, over 50% of the bacterial community may also be retained on GF/F filters, particularly in estuarine waters (Gasol and Moran 1999; Lee and Fuhrman 1987); in the York River, GF/F filters retained 53-71% of the bacteria (Bradley

2008). The purpose of additional size fractionation, therefore, was to separate the micro- (> 20 μm) and nano- (3-20 μm) plankton, in which the harmful species common to the York River belong, from the picoplankton (0.2- 3 μm), including bacteria. After filtration, each filter was placed in a polypropylene cryovial and frozen (-20°C) until analysis.

Filtrate from the NH_4^+ incubations was retained for determination of ^{15}N atom % enrichment of the NH_4^+ pool using the solid phase extraction technique (C₁₈, Dudek *et al.* 1986). Filtrate from the NO_3^- incubations was also collected and used to determine the ^{15}N atom % enrichment of the NO_3^- pool using the denitrifying bacteria method (Sigman *et al.* 2001).

To determine the isotopic enrichment of filters as well as particulate N (PN) and particulate C (PC) concentrations, filters were thawed and placed in a drying oven overnight (40°C) and then analyzed using a Europa Automated Nitrogen Carbon Analyzer for Solids and Liquids (ANCA-SL) attached to a GEO 20/20 isotope ratio mass spectrometer. Nitrogen and C uptake rates were estimated from the accumulation of ^{15}N or ^{13}C in the PN or PC and calculated according to the equations of Dugdale and Goering (1967). Ambient HCO_3^- concentrations were estimated based on salinity and assumed that the water column was saturated with respect to dissolved oxygen (Parsons *et al.* 1984). The atom % values of the NH_4^+ and NO_3^- substrate pools were corrected for isotopic dilution according to principles in Glibert *et al.* (1982).

During the 2007 bloom (9/7/07) a ^{15}N uptake experiment was conducted as described above except all samples were filtered onto 5 μm silver membrane filters; no additional size fractions were examined and $\text{H}^{13}\text{CO}_3^-$ uptake was not measured. On

9/12/07 kinetic parameters were determined using all ^{15}N substrates listed above except NO_2^- . Experiments were performed with 25 mL samples added to borosilicate test tubes that had been amended with increasing amounts of ^{15}N -labeled substrate. The experiment included a range of eight substrate concentrations: 0.055, 0.10, 0.25, 0.5, 1.0, 5.0, 10, and 50 $\mu\text{mol N L}^{-1}$. Samples were incubated for 0.5 h under *in situ* light and temperature conditions. Incubations were terminated by filtration onto 5 μm silver membrane filters; filters were analyzed as described above. Rates were not corrected for isotope dilution (Glibert *et al.* 1982) as little would be expected due to the short duration of the incubations and the large size of most of the additions. Curve fitting was done using a computerized, iterative non-linear least-squares technique (SigmaPlot[®] version 9.0, Systat Software Inc.), which utilizes the Marquardt-Levenberg algorithm (Press *et al.* 1992). The kinetics data were fitted directly to the Michaelis-Menten formula (1).

$$V = V_{max} \cdot S / (K_s + S) \quad (1)$$

where V is the specific uptake rate (h^{-1}), V_{max} is the maximal specific uptake rate, S is the substrate concentration ($\mu\text{mol N L}^{-1}$), and K_s is the half-saturation constant for the N substrate ($\mu\text{mol N L}^{-1}$). Elevated ambient concentrations for most experiments violated a basic assumption in the kinetic model that the nutrient of interest be depleted at the cell surface. In these cases, calculation of kinetic parameters was not possible and those data are not presented. For the sampling on 8/28/09 only ambient nutrient concentrations were measured (as described above) and no N uptake was measured.

Sample analysis

Water samples collected were during the experiments to be analyzed for TDN, NH_4^+ , NO_3^- , urea, phosphate (PO_4^{3-}), silica (SiO_2) and were stored in low density polyethylene (LDPE) centrifuge tubes (Corning®). Amino acid samples were stored in high density polyethylene bottles (HDPE) bottles. Analyses were performed in either duplicate (urea, NO_3^- , NO_2^-) or triplicate (all others). Concentrations of NH_4^+ were analyzed manually using the colorimetric phenol-hypochlorite method (Koroleff 1983). A Lachat QuikChem 8500 autoanalyzer was used to measure concentrations of NO_3^- , NO_2^- , PO_4^{3-} , and SiO_2 (Parsons *et al.* 1984). The persulfate oxidation technique was used to analyze TDN concentrations (Bronk *et al.* 2000). Urea was analyzed using the manual diacetyl monoxmine thiosemicarbizide method adapted from Price and Harrison (1987). Total AA were measured as dissolved primary amines (DPA) using the fluorometric o-phthalaldehyde method (Parsons *et al.* 1984). Dissolved organic nitrogen concentrations were determined as the difference between the TDN and DIN; the errors from the TDN, NH_4^+ , NO_3^- and NO_2^- measurements were propagated to provide a standard error for DON. A Shimadzu TOC-V was used to measure dissolved organic carbon (DOC) concentrations (Hansell *et al.* 1997). Concentrations of Chl *a* were measured on a Turner Design Model 10-AU fluorometer; each sample had been previously filtered onto a pre-combusted GF/F filter, in duplicate, extracted in 90% acetone overnight at 4°C, and analyzed according to Parsons *et al.* (1984).

Results

Community composition and biomass

Based on non-quantitative microscopic observations, numerous dinoflagellate species were present during the sampling (Table 1). In particular, *Prorocentrum micans* was ubiquitous and observed on nearly every sampling date. Numerous harmful dinoflagellates were observed including *A. monilatum*, *Akashiwo sanguinea*, *C. polykrikoides*, *Gyrodinium striatum*, *Karlodinium veneficum*, *Prorocentrum minimum*, and *Pfiesteria*-like organisms (PLOs). Centric diatoms and *Chaetoceros* spp. were commonly present as well as the ciliate *Myrionecta rubra*.

On 9/7/07 there was a monospecific bloom of *A. monilatum* (99%) with cell concentrations 1.9×10^6 cells L⁻¹. This bloom reached Chl *a* concentrations in excess of 60 µg L⁻¹ across the entire lower York River (data not shown). The bloom included denser patches and a discrete sample of Chl *a* was > 300 µg L⁻¹. On 9/12/07 the bloom had dissipated and cell concentrations decreased (9.3×10^5 cells L⁻¹) with Chl *a* 38.3 µg L⁻¹ at PWP.

Due to preservation problems, quantitative measurements from visual phytoplankton cell counts could not be determined for most samples. However, based on q-PCR analysis, the presence of *A. monilatum* was quantified on multiple instances as reported in Reece (2009, 2010). On 8/29/08, *A. monilatum* cells were detected at all three stations, with the counts decreasing with distance from the river mouth, 1.3×10^3 , 250, and 20 cells L⁻¹ at Goodwin Islands, Gloucester Point and Clay Bank, respectively (Reece

2009; Table 1). The largest accumulation of *A. monilatum* detected in 2008 and 2009 was at Goodwin Islands on 9/9/08 (2.8×10^6 cells L^{-1} , Reece 2009). Blooms of *A. monilatum* are often patchy in distribution. For example, at Gloucester Point cell counts were much lower (250 cells L^{-1}), and no *A. monilatum* cells were detected at Clay Bank on that date. The *A. monilatum* bloom at Goodwin Islands was not monospecific and also contained *P. micans*, *C. furca*, PLOs, as well as *K. veneficum*. Additionally, the PLO known as “Lucy” was quantified by q-PCR on 8/18/09 with 1.57×10^5 and 4.98×10^4 cells L^{-1} at Goodwin Islands and Gloucester Point, respectively (Reece 2010). Visual observation of those samples revealed *C. polykrikoides*, *C. furca*, and *P. micans* at both sites as well (Table 1).

Chlorophyll *a* concentrations on 9/9/08 at Goodwin Islands during the *A. monilatum* bloom were 17.5 ± 0.9 $\mu g L^{-1}$, three-fold higher than the other stations and PN and PC concentrations from the GF/F fraction were the highest measured at 22.8 ± 0.9 and 200 ± 10 $\mu mol N$ or $C L^{-1}$, respectively (Table 1). In general, Chl *a* concentrations were variable and ranged from 4 to 23 $\mu g L^{-1}$ at all stations with the lowest values in late August (8/29/08) and winter (1/22/09). Other than the *A. monilatum* bloom station, PN concentrations exhibited a decreasing trend in August and September 2008 at all stations (Table 1). Concentrations of PC were approximately an order of magnitude greater than PN but exhibited similar trends (Table 1). Ratios of PC:PN from the GF/F fraction, steadily increased during 2008-2009 at all stations with maximum ratios (>11) in the winter (1/22/09) and spring (5/26/09; Table 1).

On 8/28/09, a bloom became visually apparent with brown discolored water at Gloucester Point. This bloom of 4.2×10^6 cells L^{-1} , was dominated by *C. polykrikoides*

(57%) although it also contained *A. monilatum*, *P. micans*, *C. furca*, *G. instriatum*, *Phaeopolykrikos hartmanni*, *Scrippsiella. trochoidea* with a Chl *a* concentration of 119 $\mu\text{g L}^{-1}$ (Table 1).

Nutrient dynamics

Nutrient concentrations varied spatially with the highest at the upriver site (Clay Bank) and decreased progressively toward the river mouth with the lowest concentrations at Goodwin Islands (Table 2). This trend was observed for concentrations of TDN, the individual constituents that make up the pool, as well as for PO_4^{3-} and SiO_2 (Table 2). For instance, during August and September 2008 concentrations of NO_2^- at Clay Bank were 2- to 6-fold greater than at Gloucester Point or Goodwin Islands (Table 2); Clay Bank NO_2^- concentrations even exceeded ambient NO_3^- concentrations.

Nutrient concentrations also varied temporally. The highest concentrations and greatest variability in ambient nutrients among stations were observed for the August and September 2008 samplings. In contrast, concentrations in the winter (1/22/09), spring (5/26/09), and summer (8/18/09) sampling were similar amongst all stations and low ($< 1 \mu\text{mol N L}^{-1}$; Table 2)

At all stations the majority of TDN was comprised of DON (55 to 98 %), although the majority of this DON (mean $81 \pm 8\%$) was uncharacterized (i.e., not urea or AA). Overall, concentrations of DON were fairly uniform both temporally and spatially with mean concentrations ranging from 17.6 to 21.0 $\mu\text{mol N L}^{-1}$ (Table 2).

Concentrations of DON species quantified (AA, urea) were generally lower than DIN

species throughout the year, never exceeding $2 \mu\text{mol N L}^{-1}$, which corresponds to $< 5\%$ of the TDN pool. Ratios of DON:DIN peaked in the winter/spring 2009 (Table 2). The DIN pool was dominated by NH_4^+ at all stations, which was most pronounced concentrations were greatest in late August and September 2008, with concentrations reaching ~ 6 to $10 \mu\text{mol N L}^{-1}$ (Table 2). Concentrations of NO_3^- and NO_2^- also increased during August and September 2008.

Similar to N, SiO_2 and PO_4^{3-} concentrations were highest during the summer and fall of 2008, although were below the limit of detection in the winter and spring of 2009 (Table 2). TDN concentrations were tightly coupled with SiO_2 at Gloucester Point and Goodwin Islands during August and September 2008 (Table 2). Overall, SiO_2 was highest upriver at Clay Bank. Ratios of DIN:DIP indicate N was limiting during the summer and fall months at all stations while P was limiting in winter and spring (Table 2). Concentrations of DOC were highest at Clay Bank ($283\text{-}328 \mu\text{mol C L}^{-1}$), and found at similar levels at Gloucester Point and Goodwin Islands (Table 2). Ratios of DOC:DON at all stations ranged from 10.8 to 16.4 (Table 2)

The Goodwin Islands bloom on 9/9/08 was associated with DIN concentrations $< 1 \mu\text{mol N L}^{-1}$. Most dramatic was the 7.5 to 9-fold lower NH_4^+ concentration than concurrently measured at Clay Bank and Gloucester Point, respectively (Table 2), likely a function of utilization by the high *A. monilatum* biomass. The ratio of DON:DIN was slightly elevated (9.4) compared to other stations (~ 1.3) while DIN:DIP was depressed (7.0) compared to the Goodwin Islands sampling dates before and after (13.8, 17.2, respectively). Concentrations of SiO_2 and PO_4^{3-} declined at Goodwin Islands on 9/9/08

during the *A. monilatum* bloom; the drawdown of SiO₂ indicates diatoms were also likely present despite no notice of them in microscopic observations.

For, Gloucester Point 9/7/07, 8/28/09 and PWP 9/12/07, all individual inorganic and organic N species measured were at low concentrations (< 1.0 μmol N L⁻¹; Table 2), while DON was elevated (23.2 and 27.0 μmol N L⁻¹ on 9/7/07 and 8/28/09, respectively) compared to other occasions throughout the monitoring study. This provided high ratios of DON:DIN (68.2 and 27.0), which were 4- to 7-fold greater than during the 9/9/08 bloom (9.4), likely a function of the higher biomass present on those dates. Ratios of DIN:DIP during the 2007 bloom events were low (< 2.0) and indicated N limitation. Conversely, during the Gloucester Point bloom on 8/28/09 the water appeared to be P limited (DIN:DIP = 53.8).

Nitrogen uptake rates > GF/F size fraction

Ambient nutrient concentrations were often variable, therefore, efforts to keep ¹⁵N tracer additions under 10% of ambient concentrations (Dugdale and Goering 1967) also varied from a low of ~1% for the inorganic N sources examined to as high as ~40% for the organics. Additionally, due to the exceptionally low NO₂⁻ concentrations on some occasions, atom percent enrichments of the substrate were as great as 98%. High enrichments could cause concern regarding artificial stimulation of uptake rates due to the great amount of substrate available. Low uptake rates for those substrates, however, particularly compared to NH₄⁺, indicated they would not affect our conclusions. Measurements of uptake rates at varying atom percent enrichments of the ambient

nutrient pool were similar even at low (0.5%) and high (100%) enrichments (Mulholland *et al.* 2009b).

For the > GF/F fraction, the overarching pattern of uptake at each station was $\text{NH}_4^+ > \text{NO}_3^- > \text{urea} > \text{AA} > \text{NO}_2^-$. Total absolute uptake of all substrates followed the opposite spatial pattern as trends in nutrient concentration, with the highest rates occurring at the station farthest down river (Goodwin Islands) that continuously exhibited the lowest nutrient concentrations (Figure 4). At Goodwin Islands, high total absolute uptake rates also occurred on 9/9/08, in the midst of the *A. monilatum* bloom where NH_4^+ uptake was dominant followed by NO_3^- , NO_2^- and urea uptake and finally AA with the lowest uptake rates (Figure 4). Generally, NH_4^+ uptake was dominant at all stations, across all sampling dates, and accounted for the largest proportion of total uptake (34 to 80%, Figure 5). When concentrations of NH_4^+ dominated the DIN pool from 8/29/08 to 9/22/08, NH_4^+ uptake encompassed 60 to 79% of total uptake at all stations with relatively little contribution from the other substrates (Figure 5).

Of all stations, Clay Bank had the highest observed rates of NO_2^- uptake (Figure 4). For instance, on 8/7/08 and 8/18/09 absolute uptake rates of NO_2^- at Clay Bank exceeded those at Gloucester Point and Goodwin Islands by 7- to 20-fold (Figure 4). At Clay Bank on 8/7/08, there was the greatest percentage of total uptake attributed to NO_2^- among all stations and all sampling dates (2 to 19 %; Figure 5). Overall NO_3^- contributed up to 40 % of total absolute uptake at all stations (Figure 5), however, when NO_2^- and NO_3^- concentrations at Clay Bank were at their highest (9/9/08 and 9/22/08), the corresponding uptake rates were some of the lowest measured (Figure 4).

Urea and AA uptake was more variable with no single station experiencing predominance in absolute uptake rates (Figure 4). The highest urea and AA absolute uptake rates were on 8/7/08 and ranged from 0.18 to 0.25 $\mu\text{mol N L}^{-1} \text{h}^{-1}$ at all stations. The highest percent contributions of urea and AA to total absolute uptake, however, were lowest in 2008 and steadily increased during 2009 (Figure 5). The percentage of total absolute uptake of DIN exceeded that of DON at all stations and for all time points with ~55 to 88% of total absolute uptake (Figure 5).

> 5 μm size fraction

The $> 5 \mu\text{m}$ fraction encompassed the size range of all major bloom-forming dinoflagellates in the York River, while excluding most bacteria, and therefore is the fraction that best represents dinoflagellate N uptake. Overall, the pattern of uptake for the $> 5 \mu\text{m}$ and GF/F fractions were similar except urea contributed a greater percentage of total uptake in the $> 5 \mu\text{m}$ fraction. Rates of urea uptake often matched or exceeded those of NO_3^- . Ammonium uptake was dominant at all stations, contributing 31-88 % of total absolute uptake (Figure 5). Total absolute uptake by the $> 5 \mu\text{m}$ size fraction accounted for 35-95%, 21-57% and 19-52%, of total absolute uptake in the $> \text{GF/F}$ fraction for stations Clay Bank, Gloucester Point, and Goodwin Islands, respectively. The lower percentage of GF/F uptake represented by large cells in the Gloucester Point and Goodwin Islands fractions is likely a function of increased heterotrophic activity commonly found downriver. During the 9/9/08 bloom, total absolute uptake by the $> 5 \mu\text{m}$ fraction, in which *A. monilatum* cells were dominant, accounted for only 19% of total

absolute uptake in the > GF/F fraction, indicating that uptake by cells < 5 μm was very important during the bloom.

Comparison of the smaller 9/9/08 bloom with the extensive 2007 bloom revealed similarities in proportions of N species taken up. Ammonium uptake was more dominant in 2009 (57%) compared to 2007 (35%), however urea and NO_3^- were both taken up in equal proportions, accounting for 27-30% and 19% of total uptake in 2009 and 2007, respectively (Figure 5). Amino acid and nitrite uptake accounted for ~6% and 1%, respectively, of total uptake during both blooms (Figure 5).

Nitrogen uptake rates, 5 μm – 0.2 μm size fraction

Similar to the > 5 μm fraction, the overall uptake pattern observed was $\text{NH}_4^+ > \text{NO}_3^- \geq \text{urea} > \text{AA} \geq \text{NO}_2^-$ (Figure 4). NH_4^+ was the dominant substrate taken up on all sampling dates contributing 20 to 75% of total absolute uptake (Figure 5). High total absolute uptake ($1.2 \mu\text{mol N L}^{-1} \text{h}^{-1}$) was observed at Goodwin Islands on 9/9/08 coinciding with the *A. monilatum* bloom. Total absolute uptake for the 5 μm – 0.2 μm fraction often exceeded the total absolute uptake rates observed for the > 5 μm fraction. This was observed not only for total absolute uptake of all substrates but also for absolute uptake of individual substrates. Total absolute uptake in the > 5 μm and 5 μm – 0.2 μm size fractions combined were generally greater than or equal to total absolute uptake in the GF/F fraction, averaging $163 \pm 51\%$, $111 \pm 14\%$, $100 \pm 17\%$, of total GF/F uptake at Clay Bank, Gloucester Point, and Goodwin Islands, respectively.

Nitrogen uptake kinetics

Nitrogen uptake kinetics were examined in the 2007 bloom from water collected at PWP (Figure 6). Due to the high concentrations of ambient N present (e.g. $1 \mu\text{mol N L}^{-1}$ measured for urea) a hyperbolic function of uptake rates versus increasing substrate concentration could only be determined for NH_4^+ . The calculated V_{max} and K_s parameters were $33.7 \pm 2.7 \times 10^{-3} \text{ h}^{-1}$ and $7.3 \pm 1.8 \mu\text{mol N L}^{-1}$, respectively (Figure 6).

Ammonium and nitrate regeneration

This study represents the first determination of NO_3^- and NH_4^+ regeneration rates within in the York River, which is prone to very high NO_2^- concentrations (McCarthy *et al.* 1977; Sin *et al.* 2000). Regeneration rates varied throughout the monitoring period and ranged from a low of $0.1 \mu\text{mol N L}^{-1} \text{ h}^{-1}$ in the winter/spring and summer 2009 to highs of 4.7 and $7.5 \mu\text{mol N L}^{-1} \text{ h}^{-1}$ for NO_3^- and NH_4^+ regeneration, respectively in 2008 (Table 3). At Clay Bank, NO_3^- regeneration was dominant in the late summer/fall 2008, often exceeding rates of NH_4^+ regeneration; the opposite was observed at Gloucester Point, which had the most variable and highest measured NH_4^+ regeneration rates (Table 3). Rates of NO_3^- and NH_4^+ regeneration were similar at Goodwin Islands and the lowest rates measured at all stations. Ratios of regeneration: absolute uptake revealed that regeneration processes were dominant during late August and September 2008 (Table 3).

Regeneration in excess of uptake likely allowed for accumulation of those nutrients within the water column (Table 3)

Carbon absolute uptake rates

Rates of C uptake were investigated using ^{13}C -labeled urea, AA, and HCO_3^- . No significant ^{13}C enrichment was found in incubations with urea, therefore rates were not determined. It is likely that the urea-C was cleaved via urease and quickly respired. For all fractions, the highest rates of C uptake from AA occurred on 8/7/08, similar to what was observed for the N uptake rates (Figure 7), however, most C uptake rates were low compared to N, averaging 0 to $0.05 \mu\text{mol C L}^{-1} \text{h}^{-1}$ at all stations during the monitoring period.

Photosynthetic HCO_3^- uptake comprised the majority of C assimilation. In contrast to N uptake rates, HCO_3^- uptake rates were higher in the $> 5 \mu\text{m}$ fraction than $5 - 0.2 \mu\text{m}$ with the highest rates measured during 2008. The highest uptake rates were observed on 9/9/08, coinciding with the bloom of *A. monilatum* at Goodwin Islands (Figure 7). Uptake rates in the GF/F, $> 5 \mu\text{m}$, and $0.2 - 5 \mu\text{m}$ size fractions were 6.1, 3.4, and $1.0 \mu\text{mol C L}^{-1} \text{h}^{-1}$, respectively. During the 9/9/08 bloom, HCO_3^- : total N uptake in the $> 5 \mu\text{m}$ size fraction was 7.8 (Table 3). There is one caveat regarding the high rates observed in the bloom. Since HCO_3^- uptake rates were determined using an estimated ambient DIC concentration based on salinity, the rates may have overestimated the ambient concentrations and, therefore, uptake rates. DIC drawdown is often observed by high algal biomass and no actual measurements of DIC were taken to verify the estimates

Discussion

One of the primary goals of this study was to quantify a variety of N species spatially and temporally along the York River estuary. Estuaries, by definition, are transitional environments and that variability was observed in the spatial and temporal changes in ambient nutrient concentrations during the period sampled. Spatially, nutrient concentrations increased with distance from the river mouth with the greatest concentrations measured at Clay Bank. This trend has been commonly observed in the Chesapeake Bay (McCarthy *et al.* 1977) and the York River in particular, especially for TDN, while DON typically exhibits little variation (Reay 2009). Total dissolved N loads have displayed a strong positive correlation with rainfall and stream flow, increasing from April to August with highest loads in August/September (Reay 2009). Recently, the lowest nutrient loads have occurred during the regions historic dry year (2002) and peak N loads were associated with a historic wet year (2003; Reay 2009). The measured increases in TDN and SiO₂ indicate that rainfall and increased runoff are likely responsible for the concentration increases observed in our study during the summer/fall 2008. The increase in nutrients coincided with increased rainfall and freshwater flow mid August through September (Figures 2, 3). One event in particular, Tropical Storm Hanna moved through the area September 6, 2008 and provided rainfall as well as increased river flow, with maximum wind gusts > 40 mph (Figures 2, 3).

Concentrations of NH₄⁺, NO₃⁻, and NO₂⁻ exhibited the largest variability during the 2008 sampling period, ranging from < 1 to 10 μmol N L⁻¹, with the greatest changes observed upriver at Clay Bank (Table 2). Ammonium and NO₂⁻ accumulations have been

documented in previous Chesapeake Bay studies, particularly in the late summer and early fall (McCarthy *et al.* 1977; McCarthy *et al.* 1984; Bronk *et al.* 1998).

Concentrations of NH_4^+ and NO_2^- of $4 \mu\text{mol N L}^{-1}$ and $\geq 10 \mu\text{mol N L}^{-1}$, respectively, have been reported and represented up to 95% of the TDN pool (McCarthy *et al.* 1977). These high concentrations may be the result of terrestrial run-off or water column nitrification (e.g. Kemp *et al.* 1982). Bottom water NH_4^+ concentrations of 10-12 $\mu\text{mol N L}^{-1}$ in the York River have been observed, concurrently with $\sim 2 \mu\text{mol N L}^{-1}$ in surface waters (Sin *et al.* 2000). Sources of NH_4^+ appeared to be from organic matter remineralization and release from the sediments (Sin *et al.* 1999) as the water column becomes stratified. With high temperatures and stratified conditions, benthic regeneration rates peak during the summer (Kemp and Boynton 1981). It has been calculated that the shoal sediments in Gloucester Point released sufficient NH_4^+ and PO_4^{3-} to supply 36 and 75% of N and P, respectively (Rizzo *et al.* 1990). Sediments may supply 16-35% of phytoplankton demand for N (Nixon *et al.* 1980; Callendar and Hammond 1982; Fisher *et al.* 1982; Hopkinson and Wetzel 1982). Tidal destratification may supply benthic-regenerated nutrients into the surface water and replenish oxygen in the deep water in the lower York River (Webb and D'Elia 1980). Similar to NO_3^- and NO_2^- , rainfall and run-off may also contribute to high NH_4^+ . A recent study of anthropogenic inputs to the York River documented concentrations of NH_4^+ in soil and urban run-off of ~ 19 and $93 \mu\text{mol NH}_4^+\text{-N L}^{-1}$, respectively (Killberg-Thoreson, Chapter 5).

Nutrient loads and nutrient limitation are known to exhibit strong temporal patterns in estuaries. Based on ratios of DIN:DIP that ranged from 0.5 to >28.9

(Table 2), the York River is increasingly N-limited during the summer/fall, but switches to P-limitation in the winter and following spring. This pattern has been previously noted in the York River (Sin *et al.* 1999) and other estuaries (Malone *et al.* 1996; Bronk *et al.* 1998). Nitrogen limitation may even remain in the mid and lower mesohaline of the York throughout the year except during periods of peak riverine discharge (Sin *et al.* 1999). In this study, apparent DIN limitation was particularly pronounced at the upriver station during summer (Clay Bank; DIN:DIP = 0.5-9.5) and even spring (DIN:DIP = 0.8), while P-limitation was the greatest farthest downriver (Goodwin Islands) in the winter (DIN:DIP > 28.9) and spring (DIN:DIP >25.9; Table 2).

Nitrogen limited systems are typically dominated by nutrient recycling (McCarthy *et al.* 1977; Stanley and Hobbie 1981; Collos *et al.* 2003). Particularly, Chesapeake Bay surface waters are controlled by heterotrophic processes in the summer months, with regeneration of reduced N forms often exceeding autotrophic uptake (Bronk *et al.* 1998; Smith and Kemp 1995). If regeneration:uptake ratios are ~1, regeneration processes are likely responsible for maintaining phytoplankton populations. Measured rates of regeneration at Gloucester Point and Clay Bank often far exceeded uptake rates (> 18-fold; Table 3), indicating that regeneration was a major N source to phytoplankton, which also allowed for accumulation of N pools and transportation of N downriver in August and September 2008. In contrast, *in situ* regeneration:uptake ratios in the winter and spring were often less than 1 and indicated additional sources of N were needed to balance uptake. In a North Carolina estuary, it was estimated that throughout the year, approximately two-thirds of the DIN utilized by phytoplankton originated from recycling processes (Stanley and Hobbie 1981). Ammonium is the primary form of N released in

the water column (Bronk and Steinberg 2008) and rates of NH_4^+ regeneration are often tightly coupled with uptake so that concentrations of NH_4^+ can be near the limit of detection, even in systems that are running largely on NH_4^+ (e.g., McCarthy and Goldman 1979). This appears to be the case for the blooms on 9/7/07 and 9/22/08 when NH_4^+ regeneration:uptake were 0.8 and 1.0, respectively and ambient nutrient concentrations $\leq 0.3 \mu\text{mol L}^{-1}$ (Table 3).

At all sites in this study the observed pattern of uptake was $\text{NH}_4^+ > \text{NO}_3^- > \text{urea} > \text{AA} > \text{NO}_2^-$ in the GF/F fraction. Ammonium uptake in the three size fractions averaged 59-61% of total absolute uptake at all stations, with individual uptake rates as high as $1.7 \mu\text{mol L}^{-1} \text{h}^{-1}$ in the GF/F fraction. These rates are comparable to those calculated for the Chesapeake Bay in previous studies (Bronk *et al.* 1998; Glibert *et al.* 1991; Bradley *et al.* 2010). This dominance of NH_4^+ uptake by phytoplankton has been documented in lagoons and estuaries (Carpenter and Dunham 1985; Collos *et al.* 2003; Twomey *et al.* 2005), particularly in Chesapeake Bay (McCarthy *et al.* 1977; Carpenter and Dunham 1985; Bradley *et al.* 2010). Ammonium has also been known to inhibit the uptake and assimilation of NO_3^- , particularly at concentrations in excess of $1 \mu\text{mol N L}^{-1}$, (Dortch 1990, Lomas and Glibert 1999, Cochlan and Bronk 2003) although this trend is not universal (e.g., McCarthy *et al.* 1975; Garside 1981). In this study the greatest diversity in N uptake, and greatest individual NO_3^- and NO_2^- uptake rates were on 8/7/08 when NH_4^+ concentrations were some of the lowest measured. Additionally, when NH_4^+ concentrations ranged from 2-10 $\mu\text{mol N L}^{-1}$, NO_3^- uptake was depressed. While there was no correlation between ambient NH_4^+ concentration and NO_3^- uptake rates at any of

the sites, given the high concentrations of NH_4^+ present, inhibition of NO_3^- was possible, particularly in late summer 2008.

Based on the results from this study and others we can summarize conditions in the York River that likely lead to harmful algal blooms. First, the decline of the annual spring bloom sequesters N in the sediments as the organic matter is broken down. In the summer the York River is more N limited with DIN:DIP ratios as low as 1.6 (Table 2). Additionally, rates of remineralization are high and nutrients are released from the sediment under low oxygen conditions. Next, a storm event mixes the water column, providing oxygen to the bottom waters as well as bringing regenerated N to the surface. Increased rainfall and freshwater flow to the river bring large fluxes of N from a variety of sources including agricultural and urban run-off as well as soil from tidal erosion. A variety of inorganic and organic species are found in the run-off including NH_4^+ , NO_3^- , and DON (Killberg-Thoreson, Chapter 5). A link between rainfall and HABs have been suggested for other species in Chesapeake Bay tributaries, including *P. minimum* (Glibert *et al.* 2001) and *C. polykrikoides* (Mulholland *et al.* 2009a). The NH_4^+ then gets oxidized to NO_2^- and NO_3^- via nitrification with the greatest rates observed upriver. High concentrations of NH_4^+ may inhibit uptake of NO_3^- and possibly NO_2^- , aiding in N accumulation the high turbidity at Clay Bank, which may create light limiting conditions for phytoplankton. If sufficient phytoplankton biomass is not present to draw down N, it may accumulate in the water column and be transported further downstream to Gloucester Point and Goodwin Islands.

A combination of physical conditions (e.g., temperature, salinity) and large N pulse stimulate *A. monilatum* growth as well as co-occurring phytoplankton including

other harmful dinoflagellate species. Juhl *et al.* (2005) suggested that *A. monilatum* has a high N requirement because of its large size and high growth rate. This is supported by the high observed K_s (7.3 ± 1.8) and V_{max} (33.7 ± 2.7) kinetic parameters as well as and uptake rates presented here. Nitrogen uptake rates in the bloom were highest for NH_4^+ , in all size fractions, followed by nearly equal rates for NO_3^- and urea, and finally AA (Figure 4); high rates of inorganic C uptake were also observed though little to no evidence for mixotrophic use of organic N (Figure 7). Regeneration processes are extremely important, as ratios of regeneration:uptake during the 9/22/08 bloom (1.0 for NH_4^+ regeneration, 2.4 for NO_3^- regeneration) indicate that all the NH_4^+ and NO_3^- taken up could easily be supplied from *in situ* regeneration processes (Table 3). Perhaps bloom decline is a function of an increased degree of uncoupling of regeneration:uptake; as biomass and N demand increases, nutrient supply from regeneration processes may not be able to keep up with demand.

Understanding triggers to bloom formation are key as both *A. monilatum* and *C. polykrikoides* form a resting cyst stage that sinks to the sediment and will “seed” a new population under favorable conditions, so future blooms are a likely occurrence. Forecasted climate change scenarios in the region include increased temperatures and periods of summer drought as well as more sporadic and intensified storm systems (Pyke *et al.* 2008), prime conditions for HAB proliferation. While there are numerous dynamics underlying HAB events, understanding nutrient concentrations, transformations, and utilization prior to, during and after HAB events is vital to understanding why blooms initiate and flourish in the York River.

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Literature Cited

- Antia, N., Harrison, P., Oliveira, L., 1991. The role of dissolved organic nitrogen in phytoplankton nutrition, cell biology, and ecology. *Phycologia* 30, 1-89.
- Berg, G., Glibert, P.M., Lomas, M.W., Burford, M.A. 1997. Organic nitrogen uptake and growth by the chrysophyte *Aureococcus anophagefferens* during a brown tide event. *Mar. Biol.* 129, 377-387.
- Bradley, P.B. 2008. Multi-system analysis of nitrogen use by phytoplankton and heterotrophic bacteria. Ph.D. Dissertation. The College of William & Mary-School of Marine Science. pp. 227.
- Bradley, P.B., Lomas, M.W., Bronk, D.A. 2010. Inorganic and organic nitrogen use by phytoplankton along Chesapeake Bay, measured using a flow cytometric sorting approach. *Estuaries and Coasts*. 33: 971-984.
- Bronk, D. A., P. M. Glibert, T. C. Malone, E. Sahlstenand and S. Banahan. 1998. Inorganic and organic nitrogen cycling in Chesapeake Bay: autotrophic versus heterotrophic processes and relationships to carbon flux. *Aquat. Microb. Ecol.* 15: 177-189.
- Bronk, D.A. 2002. Dynamics of organic nitrogen. In: *Biogeochemistry of marine dissolved organic matter*. Hansell, DA, and Carlson, CA [Eds] San Diego, Academic Press,153-247
- Bronk, D.A., Lomas, M.W., Glibert, P.M., Schukert, K.J., Sanderson, M.P. 2000. Total dissolved nitrogen analysis: Comparisons between the persulfate, UV, and high temperature oxidation methods. *Marine Chemistry*. 69, 163-178.
- Bronk, D. A. and D. Steinberg. 2008. Nitrogen regeneration. In: *Nitrogen in the Marine Environment*. Capone, D. G., Bronk, D. A., Mulholland, M. and Carpenter, E. (eds). Elsevier Press. pg. 385-468.
- Boynton, W.R. and W.M. Kemp 2008. In: *Nitrogen in the Marine Environment*. Capone, D. G., Bronk, D. A., Mulholland, M. and Carpenter, E. (eds). Elsevier Press. pg. 385-468.
- Callender, E. and Hammond, D.E.1982. Nutrient exchange across the sediment-water interface in the Potomac River estuary. *Estuarine Coastal Shelf Science* 15:395-413.
- Capone, D.G. 2000. The marine microbial nitrogen cycle. In: Kirchman, D.L. [Ed.] *Microbial Ecology of the Oceans*. Wiley-Liss, Inc., New York, pp. 455-493.

- Carpenter, E. J., Dunham, S. (1985). Nitrogenous nutrient uptake, primary production and species composition of phytoplankton in the Carmans River estuary, Long Island, N.Y. *Limnol. Oceanogr.* 30:513-526
- Cochlan, W. P. and D. A. Bronk. 2003. Effects of ammonium on nitrate utilization in the Ross Sea, Antarctica: Implications for f-ratio estimates. In: *Biogeochemistry of the Ross Sea*. DiTullio, G. R. and Dunbar, R. B. (eds). Antarctic Research Series. 78:159-178.
- Collos, Y., Vaquer, A., Bibent, B., Souchu, P., Slawyk G., Garcia, N. 2003. Response of coastal phytoplankton to ammonium and nitrate pulses: seasonal variations of nitrogen uptake and regeneration, *Aquat. Ecol.* 37: 227–236.
- Connell, C.H. and Cross, J.B. 1950. Mass mortality of the fish associated with the protozoan *Gonyaulax* in the Gulf of Mexico. *Science.* 112:359-363
- Crossett, K., T. J. Culliton, P. Wiley, and T. R. Goodspeed. 2004. *Population Trends along the Coastal United States, 1980–2008*. Silver Spring, Maryland: National Oceanic and Atmospheric Administration. 47. pp.
- Dauer, D., Marshall, H., Donat, J., Lane, M., Morton, P., Doughten, S., Hoffman, F. 2005. Status and trends in water quality and living resources in the Virginia Chesapeake Bay: York River (1985-2004). Final Report. Virginia Department of Environmental Quality, Richmond, VA. 63 pp.
- D’Elia, C.F., Sanders, F.J., Boynton, W.R. 1986. Nutrient enrichment studies in a coastal plain estuary: phytoplankton growth in large scale, continuous cultures. *Can. J. Fish. Aquat. Sci.* 43, 397-406.
- Dortch, Q. 1990. The interaction between ammonium and nitrate uptake in phytoplankton. *Mar. Ecol. Prog. Ser.* 61:183-201.
- Dudek, N., Brzezinski, M.A. & Wheeler, P.A. 1986. Recovery of ammonium nitrogen by solvent extraction for the determination of relative ¹⁵N abundance in regeneration experiments. *Mar. Chem.* 18(1), 59-69.
- Dugdale, R.C. and Goering, J.J. 1967. Uptake of new and regenerated forms of nitrogen in primary productivity. *Limnol. Oceanogr.* 12, 196-206.
- Fan, C., Glibert, P., Burkholder, J. 2003. Characterization of the affinity for nitrogen, uptake kinetics, and environmental relationships for *Prorocentrum minimum* in natural blooms and laboratory cultures. *Harmful Algae* 2, 283-299.
- Fisher, T.R., Carlson, P.R., Barber, R.T. 1982. Sediment nutrient regeneration in three North Carolina estuaries. *Estuar. Coast. Shelf. Sci.* 14(1):101-116.

- Garside, C. 1981. Nitrate and ammonia uptake in the apex of the New York Bight. *Limnol. Oceanogr.* 26:731-739.
- Gasol, J.M. and Morán, X.A.G. 1999. Effects of filtration on bacterial activity and picoplankton community structure as assessed by flow cytometry. *Aquatic Microbial Ecology* 16: 251-264.
- Glibert, P.M., F. Lipschultz, J.J. McCarthy, Altabet, M.A., 1982. Isotope dilution models of uptake and remineralization of ammonium by marine plankton. *Limnol. Oceanogr.* 27, 639-650.
- Glibert, P.M., C. Garside, J.A. Fuhrman, and M.R. Roman. 1991. Time-dependent coupling of inorganic and organic nitrogen uptake and regeneration in the plume of the Chesapeake Bay estuary and its regulation by large heterotrophs. *Limnol. Oceanogr.* 36: 895–909
- Glibert, P.M., Magnien, R., Lomas, M.W., Alexander, J., Fan, C., Haramoto, E., Trice, M., Kana, T.M. 2001. Harmful algal blooms in the Chesapeake and coastal bays of Maryland, USA: comparison of 1997, 1998, and 1999 events. *Estuaries* 24(6A): 875-883
- Glibert, P., Harrison, J., Heil, C., Seitzinger, S. 2006. Escalating worldwide use of urea – a global change contributing to coastal eutrophication. *Biogeochemistry* 77(3), 441-463.
- Glibert, P.M. and Legrand, C. 2006. The diverse nutrient strategies of harmful algae: focus on osmotrophy. In: *Ecology of Harmful Algae*, E. Granéli and J.T. Turner. (Eds.) Springer-Verlag, New York.
- Haas, L.W. 1975. Plankton dynamics in a temperate estuary with observations on a variable hydrographic conditions. Doctoral dissertation. School of Marine Science. The College of William & Mary, Gloucester Point, VA. 202 pp.
- Hagy, J.D., Boynton, W.R., Keefe, C.W., Wood, K.V. 2004. Hypoxia in Chesapeake Bay, 1950-2001: long term change in relation to nutrient loading and river flow. *Estuaries* 27, 634-658.
- Hallegraeff, G.M. 2003. Monographs on Oceanographic methodology. Harmful Algal Blooms: A Global Overview. Paris, United Nations Educational
- Hansell, D.A., Carlson, C.A., Bates, N.R., Poisson, A.P. 1997. Horizontal export of organic matter in the Equatorial Pacific Ocean. *Deep Sea Res. I.* 45, 2115-2130.

- Harding, J.M., Mann, R., Moeller, P., Hsia, M.S. 2009. Mortality of the veined rapa whelk, *Rapana venosa*, in relation to a bloom of *Alexandrium monilatum* in the York River, United States. *J. Shellfish Res.* 28(2), 363-367
- Hecky, R., Kilham P., 1988, Nutrient limitation of phytoplankton in freshwater and marine environments: a review of recent evidence of the effects of enrichment. *Limnol. Oceanogr.* 33, 796-822.
- Ho, M. and Zubkoff, P. 1979. The effects of a *Cochlodinium heterolobatum* bloom on the survival and calcium uptake by larvae of the American oyster, *Crassostrea virginica*. In: Taylor, F. and H.H. Seliger [Eds.] *Toxic Dinoflagellate Blooms*. New York, pp. 409-412.
- Hopkinson, C.S. and Wetzel, R.L. 1982. *In situ* measurements of nutrient and oxygen fluxes in a coastal marine benthic community. *Mar. Ecol. Prog. Ser.* 10:29–35.
- Hsia, M.H., Morton, S.L., Smith, L.L., Beauchense, K.R., Huncik, K.M., Moeller, P.D.R. 2006. Production of goniodomin A by the planktonic, chain-forming dinoflagellate *Alexandrium monilatum* (Howell) Balech isolated from the Gulf Coast of the United States. *Harmful Algae.* 5:290-299.
- Juhl, A.R. 2005. Growth rates and elemental composition of *Alexandrium monilatum*, a red-tide dinoflagellate. *Harmful Algae.* 4: 287-295.
- Kemp, W.M., Wetzel, R.L., Boynton, W.R., D'Elia, C.F., Stevenson, J.C. 1982. Nitrogen cycling in estuarine interfaces: Some concepts and research directions, In *Estuarine comparisons*. Academic. p. 209-220.
- Kemp, W.M. and W.R. Boynton. 1981. External and internal factors regulating metabolic rates of an estuarine benthic community. *Oecologia.* 51:19-27.
- Koroleff, F., 1983. Simultaneous oxidation of nitrogen and phosphorus compounds by persulfate. In: Grasshoff, K., Eberhardt, M., Kremling, K. (Eds.), *Methods of Seawater Analysis* 2nd ed., Verlag Chemie, Weinheimer, FRG.
- Lee, S.H., Fuhrman, J.A. 1987. Relationships between biovolume and biomass of naturally derived marine bacterioplankton. *Applied and Environmental Microbiology.* 53, 1298-1303.
- Lewitus, A.J., Willis, B.M., Hayes, K.C., Burkholder, J.M., Glasgow Jr., H.B., Glibert, P.M., Burke, M.K. 1999. Mixotrophy and nitrogen uptake by *Pfiesteria piscicida* (Dinophyceae). *J. Phycol.* 35, 1430-1437.
- Lomas, M.W., and P.M. Glibert. 1999. Interactions between NH_4^+ and NO_3^- uptake and assimilation: Comparison of diatoms and dinoflagellates at several growth temperatures. *Mar. Biol.* 133: 541–551.

- Mackiernan, G. 1968. Seasonal distribution of dinoflagellates in the lower York River, Virginia. Thesis. The College of William and Mary. 104 pp.
- Malone, T.C., Conley, D.J., Fisher, T.R., Glibert, P.M., Harding, L.W., Sellner, K.G. 1996. Scales of nutrient-limited phytoplankton productivity in Chesapeake Bay. *Estuaries*. 19(2B): 371-385.
- Marshall, H.G. 1994. Succession of dinoflagellate blooms in the Chesapeake Bay, U.S.A. In: P. Lassus *et al.* [Eds.] Harmful Marine Algal Blooms, Proceedings of the 6th International Conference on Toxic Marine Phytoplankton. Intercept Ltd., Andover, MD. 615-62
- McCarthy, J.J., Taylor, W.R., Taft, J.L. 1975. The dynamics of nitrogen and phosphorus cycling in the open waters of the Chesapeake Bay. In T.M. Church [Ed.] Marine chemistry in the coastal environment. Am. Chem. Soc. Pp. 664-681.
- McCarthy, J.J., W.R. Taylor, and J.L. Taft. 1977. Nitrogenous nutrition of the plankton in the Chesapeake Bay. 1. Nutrient availability and phytoplankton preferences. *Limnol. Oceanogr.* 22: 996-1011.
- McCarthy, J.J., and W. Kaplan, and J.L. Nevins. 1984. Chesapeake Bay nutrient and plankton dynamics. 2. Sources and sinks of nitrite. *Limnol. Oceanogr.* 29: 84-98.
- McCarthy, J.J. and J.C. Goldman 1979 Nitrogenous nutrition of marine phytoplankton in nutrient depleted waters. *Science* 23: 670-672.
- Morse, D.C. 1947. Some observations on variations in plankton populations, Patuxent River, Maryland 1943-1945. Chesapeake Biological Laboratory 65. Solomons, MD. pp. 1-31
- Mulholland, MR, Gobler, CJ, Lee, C (2002) Peptide hydrolysis, amino acid oxidation, and nitrogen uptake in communities seasonally dominated by *Aureococcus anophagefferens*. *Limnology and Oceanography* 47(4):1094-1108
- Mulholland, M.R., Boneillo, G., Minor, E. 2004. A comparison of N and C uptake during brown tide (*Aureococcus anophagefferens*) blooms from two coastal bays on the east coast of the USA. *Harmful Algae* 3, 361-376.
- Mulholland, M.R., Boneillo, G.E., Bernhardt, P.W., Minor, E.C. 2009b. Comparison of nutrient and microbial dynamics over a seasonal cycle in a Mid-Atlantic coastal lagoon prone to *Aureococcus anophagefferens* (brown tide) blooms. *Estuaries and Coasts* 32, 1176-1194.

- Mulholland, M.R., Morse, R.E., Boneillo, G.E., Bernhardt, P.W., Filippino, K.C., Procise, L.A., Blanco-Garcia, J.L., Marshall, H.G., Egerton, T.A., Hunley, W.S., Moore, K.A., Berry, D.L., Gobler, C.J. 2009a. Estuaries and Coasts. Understanding causes and impacts of the dinoflagellate, *Cochlodinium polykrikoides*, blooms in the Chesapeake Bay. 32(4), 734-747.
- Nixon, S.W., Kelly, J.R., Furnas, B.N., Oviatt, C.A., Hale, S.S. 1980. Phosphorus regeneration and the metabolism of coastal marine bottom communities. In K. R. Tenore and B. C. Coull [Eds.], *Marine Benthic Dynamics*. Univ. South Carolina Press, Columbia, South Carolina. pp. 219–242.
- Officer, C.B. & Ryther, J.H. 1980. The possible importance of silicon in marine eutrophication. *Mar. Ecol. Progr. Ser.* 3, 83-91.
- Parsons, T., Maita, Y., Lalli, C., 1984. *A manual of chemical and biological methods for seawater analysis* Pergamon Press, New York.
- Paerl, H.W. 1988. Nuisance phytoplankton blooms in coastal, estuarine and inland waters. *Limnology and Oceanography* 33: 823-847
- Paerl, H.W. and M.F. Piehler 2008 In: *Nitrogen in the Marine Environment*. Capone, D. G., Bronk, D. A., Mulholland, M. and Carpenter, E. (eds). Elsevier Press. pg. 385-468.
- Press, W.H., Teukolsky, S.A., Vetterling, W.T., Flannery, B.P. 1992. *Numerical recipes*. Cambridge University Press, New York, 994 pp.
- Price, N. and Harrison, P. (1987) A comparison of methods for the measurement of dissolved urea concentrations in seawater. *Mar. Biol.* 94, 307-317.
- Pyke, C. R., R. G. Najjar, M. B. Adams, D. Breitburg, M. Kemp, C. Hershner, R. Howarth, M. Mulholland, M. Paolisso, D. Secor, K. Sellner, D. Wardrop, and R. Wood. 2008. *Climate Change and the Chesapeake Bay: State-of-the-Science Review and Recommendations*. A Report from the Chesapeake Bay Program Science and Technical Advisory Committee (STAC), Annapolis, MD. 59 pp.
- Reay W. 2009. Water quality within the York River estuary. *J. Coast. Res.* 57, 23-39.
- Reece, K.S. (2009) *Monitoring for HAB species in VA Waters of Chesapeake Bay during 2008: Emerging HAB species in Chesapeake Bay*. Annual Report submitted to the VA Dept. of Health.
- Reece, K.S. (2010) *Monitoring for HAB species in VA Waters of Chesapeake Bay during 2009: Emerging HAB species in Chesapeake Bay*. Annual Report submitted to the VA Dept. of Health.

- Rizzo, W.M. 1990 Nutrient exchanges between the water column and a subtidal benthic microalgal community. *Estuaries and Coasts* 13:219-226.
- Ryther, J., Dunstan, W. 1971. Nitrogen , phosphorus, and eutrophication in the coastal marine environment. *Science* 171, 1008-1112.
- Sellner, K.G., Doucette, G.J., Kirkpatrick , G.J. 2003. Harmful algal blooms: Causes, impacts and detection. *J. Ind. Microbiol. Biotechnol.* 30, 383-406.
- Shen, J., Haas, L. 2004. Calculating age and residence time in the tidal York River using three-dimensional model experiments. *Estuar. Coast. Shelf Sci.*, 61, 449-461
- Sigman, D.M., Casciotti, K.L., Andreani, M., Barford, C., Galanter, M., and J. K. Böhlke. 2001. A bacterial method for the nitrogen isotopic analysis of nitrate in seawater and freshwater. *Anal. Chem.* 73(17):4145-4153.
- Sin, Y., Wetzel, R., Anderson, I. 1999. Spatial and temporal characteristics of nutrient and phytoplankton dynamics in the York River estuary, Virginia: analyses of long-term data. *Estuaries.* 22(2A): 260-275.
- Sin, Y., Wetzel, R.L., Anderson, I.C. 2000 Seasonal variations of size-fractionated phytoplankton along the salinity gradient in the York River estuary, Virginia (USA). *J. Plank. Res.* 22(10) 1945-1960.
- Sin, Y., and R. L. Wetzel. 2002a. Ecosystem modeling analysis of size-structured phytoplankton dynamics in the York River estuary, Virginia (USA). I. Development of a plankton ecosystem model with explicit feedback controls and hydrodynamics. *Mar. Ecol. Prog. Ser.* 228, 75-90.
- Sin, Y., and R. L. Wetzel. 2002b. Ecosystem modeling analysis of size-structured phytoplankton dynamics in the York River estuary, Virginia (USA). II. Use of a plankton ecosystem model for investigating controlling factors on phytoplankton and nutrient dynamics. *Mar. Ecol. Prog. Ser.* 228, 91-101.
- Sin, Y., Wetzel, R.L., Lee, B.G., Kang, Y.H. 2006. Integrative ecosystem analyses of phytoplankton dynamics in the York River estuary (USA). *Hydrobiologia*, 571, 93-108.
- Smayda, T.J. 1990. Novel and nuisance phytoplankton blooms in the sea: evidence for a global epidemic. E., Sundstrom, B., Edler, L., Anderson, D.M. [Eds.] *Toxic Marine Phytoplankton*. New York, Elsevier, 29-40.
- Smayda, T.J. 1997. Harmful algal blooms: Their ecophysiology and general relevance to phytoplankton blooms in the sea. *Limnol. Oceanogr.* 42, 1137-1153.

- Smayda, T.J., Reynolds, C., 2001. Community assembly in marine phytoplankton: Application of recent models to harmful dinoflagellate blooms. *J. Plankt. Res.* 23, 447-461.
- Smith, E.M., and W.M. Kemp. 1995. Seasonal and regional variations in plankton community production and respiration for Chesapeake Bay. *Mar. Ecol. Prog. Ser.* 116: 217–231.
- Stanley, D. W., and J. E. Hobbie. 1981. Nitrogen recycling in a North Carolina coastal river. *Limnol. Oceanogr.* 26:30-42.
- Tilman, D. 1977. Resource competition between planktonic algae: An experimental and theoretical approach. *Ecology* 58, 338-348.
- Twomey, L.J., M.F. Piehler, and H.W. Paerl. 2005. Phytoplankton uptake of ammonium, nitrate and urea in the Neuse River Estuary, NC, USA. *Hydrobiologia* 533: 123–134.
- Webb, K.L. and D’Elia, C.F. 1980. Nutrient and oxygen redistribution during a spring neap tidal cycle in a temperate estuary. *Science.* 207:983-985.
- Zubkoff, P., Munday, J., Rhodes, R., Warinner, J. 1979. Mesoscale features of summer (1975-1979) dinoflagellate blooms in the York River, Virginia (Chesapeake Bay estuary). In: D. Taylor and H. Seliger [Eds.] *Toxic Dinoflagellate Blooms*, Elsevier, Inc., New York, pp. 279-286.

Table1. Water quality data, biomass parameters, and species composition at monitoring sites in the York River, VA. Data in italic represent the highest biomass and most monospecific blooms. Standard deviations are given in parentheses and n.d. indicates not determined. *Pfiesteria*-like organisms are abbreviated as PLOs. The presence of a species is denoted by x and species with cell counts are marked with a letter (a-i) with concentrations provided. All are in units of cells L⁻¹, a = 20, b = 1.9 x 10⁶, c = 250, d = 250, e = 4.98 x 10⁴, g = 1.3 x 10³, h = 2.8 x 10⁶, i = 1.57 x 10⁵, j = 9.3 x 10⁵

Date	Temp (°C)	Salinity	DO (mg L ⁻¹)	Chl <i>a</i> (µg L ⁻¹)	Part N (µmol N L ⁻¹)	Part C (µmol C L ⁻¹)	PC:PN	<i>Alexandrium monilatum</i>	<i>Akashiwo sanguinea</i>	<i>Ceratium furca</i>	<i>Cochlodinium polykrikoides</i>	<i>Gyrodinium instratum</i>	<i>Heterocapsa rotunda</i>	<i>Karlodinium veneficum</i>	<i>Phaeopolykrikos hartmanni</i>	<i>PLOs</i>	<i>Prorocentrum micans</i>	<i>Prorocentrum minimum</i>	<i>Prorocentrum spp</i>	<i>Protoperedrium spp</i>	<i>Scrippsiella trochoidea</i>	<i>Chaetocerus spp</i>	<i>Chattonella spp</i>	<i>Diatom spp</i>	<i>Myrionecta rubra</i>		
Clay Bank																											
8/7/08	27.2	19.5	7.1	13.1 (1.0)	18.0 (0.8)	126.1 (7.6)	7.0		x				x														
8/29/08	26.0	20.6	7.2	3.5 (0.1)	8.1 (0.3)	69.7 (3.7)	8.6	a		x				x		x			x								
9/9/08	nd	21.4	nd	7.3 (0.2)	11.2 (0.8)	100.7 (12.9)	9.0		x										x		x						
9/22/08	23.4	19.2	7.8	15.7 (5.3)	11.4 (0.5)	96.3 (9.8)	8.5										x	x									
1/22/09	2.1	15.2	13.1	8.6 (1.5)	6.9 (0.2)	64.3 (3.3)	9.4							x					x						x		
5/26/09	23.0	15.9	8.0	14.6 (0.9)	14.1 (0.7)	133.5 (15.0)	12.9						x	x				x							x		
8/18/09	29.0	18.7	7.2	23.1 (1.1)	9.8 (1.1)	170.0 (25.5)	9.8		x												x				x		
Gloucester Pt																											
9/7/07	31.0	24.0	nd	374.3 (210)	9.4 (0.6)	nd	nd	b																			
8/7/08	26.5	21.7	7.1	10.8 (0.6)	17.5 (2.0)	110.6 (20.3)	6.3			x	x						x	x						x			
8/29/08	24.9	21.1	21.1	4.0 (0.1)	11.4 (0.6)	86.9 (9.2)	7.6	c		x				x						x							
9/9/08	26.6	21.4	6.9	5.4 (0.1)	9.4 (0.2)	79.4 (6.8)	8.5	d											x						x		
9/22/08	23.7	21.8	7.4	6.2 (0.3)	8.1 (0.4)	66.7 (18.0)	8.3										x							x			
1/22/09	2.4	20.4	12.6	4.7 (0.7)	6.9 (0.1)	78.1 (2.7)	11.3						x	x		x									x		
5/26/09	21.3	18.9	7.9	9.0 (0.2)	11.5 (0.8)	139.9 (46.2)	10.1			x	x	x		x			x								x		
8/18/09	27.9	21.4	7.2	11.7 (0.6)	5.6 (0.4)	179.9 (24.0)	9.2									e											
8/28/09	28.8	21.0	nd	119.0 (nd)	nd	nd	nd			x	x	f			x		x				x				x		
Goodwin Is.																											
8/7/08	28.3	20.4	7.1	8.4 (0.7)	16.6 (1.2)	125.1 (16.7)	7.5		x	x							x	x									
8/29/08	24.4	20.9	7.6	5.2 (0.3)	12.9 (3.6)	94.0 (5.8)	7.3	g		x							x			x							
9/9/08	26.3	21.6	7.5	17.5 (0.9)	22.8 (0.9)	200.0 (10.8)	8.8	h		x											x						
9/22/08	22.2	22.4	8.0	9.2 (0.3)	10.8 (0.4)	85.1 (9.7)	7.9														x						
1/22/09	2.7	20.5	12.3	3.7 (0.4)	6.6 (0.1)	76.7 (2.8)	11.6				x			x		x									x		
5/26/09	22.0	19.5	7.7	5.9 (0.5)	8.8 (0.4)	121.2 (44.5)	11.5						x	x			x										
8/18/09	28.1	20.0	7.1	6.6 (0.6)	12.5 (0.5)	108.2 (12.0)	8.7			x	x			x		i	x				x					x	
PWP																											
9/12/07	27.0	23.0	nd	38.3 (nd)	39.1 (1.0)	nd	nd	J									x										

Table 2. Dissolved inorganic and organic nutrients at monitoring sites in the York River, VA from 2007-2009. Data in italic represent the highest biomass/most monospecific blooms. Standard deviations are given in parentheses and n.d. indicates the parameter was not determined.

Date	NH ₄ ⁺ μmol N L ⁻¹	NO ₃ ⁻ μmol N L ⁻¹	NO ₂ ⁻ μmol N L ⁻¹	Urea μmol N L ⁻¹	AA μmol N L ⁻¹	TDN μmol N L ⁻¹	DON μmol N L ⁻¹	DOC μmol C L ⁻¹	PO ₄ ³⁻ μmol P L ⁻¹	SiO ₂ μmol Si L ⁻¹	DIN: DIP	DON: DIN	DOC: DON
Clay Bank													
8/7/08	0.25 (0.09)	0.43 (0.00)	2.18 (0.00)	0.36 (0.13)	0.38 (0.02)	23.2 (0.2)	20.3 (0.2)	312.4 (2.1)	1.82 (0.20)	36.0 (6.0)	1.6	7.1	15.4
8/29/08	9.95 (0.22)	1.64 (0.09)	3.57 (0.03)	1.90 (0.03)	0.30 (0.02)	37.8 (0.7)	23.9 (0.7)	283.7 (4.5)	2.48 (0.01)	45.6 (2.3)	6.1	1.6	11.9
9/9/08	7.38 (0.30)	3.45 (0.40)	6.19 (0.73)	1.39 (0.04)	0.25 (0.04)	38.7 (0.1)	21.7 (0.9)	322.9 (13.3)	2.34 (0.01)	52.8 (0.5)	7.3	1.3	14.9
9/22/08	7.57 (0.19)	6.89 (0.07)	7.56 (0.02)	1.45 (0.04)	0.12 (0.02)	42.0 (0.1)	20.0 (0.2)	328.7 (12.2)	2.31 (0.00)	57.6 (0.1)	9.5	0.9	16.4
1/22/09	0.34 (0.08)	0.50 (0.01)	0.01 (0.00)	0.31 (0.03)	0.17 (0.01)	22.0 (0.0)	21.1 (0.1)	292.2 (6.5)	0.05 (0.03)	19.1 (1.7)	18.8	25.0	13.8
5/26/09	0.10 (0.00)	0.11 (0.01)	0.00 (0.00)	0.15 (0.03)	0.34 (0.00)	17.7 (0.9)	17.4 (0.9)	269.0 (8.4)	0.26 (0.11)	18.1 (0.5)	0.8	80.0	15.4
8/18/09	0.42 (0.11)	0.21 (0.00)	0.11 (0.00)	0.28 (0.00)	0.38 (0.01)	23.1 (0.2)	22.4 (0.2)	292.6 (1.0)	1.51 (0.100)	29.5 (2.7)	0.5	30.1	13.1
Gloucester Point													
9/7/07	<i>0.20 (0.03)</i>	<i>0.14 (0.08)</i>	<i>0.00 (0.00)</i>	<i>0.41 (0.14)</i>	<i>0.73 (0.18)</i>	<i>23.8 (0.3)</i>	<i>23.5 (0.3)</i>	<i>258.3 (3.6)</i>	<i>0.33 (0.01)</i>	<i>19.8 (0.25)</i>	<i>1.03</i>	<i>68.2</i>	<i>11.1</i>
8/7/08	0.43 (0.17)	0.47 (0.03)	0.19 (0.02)	0.23 (0.01)	0.44 (0.01)	19.5 (0.2)	18.4 (0.3)	225.0 (5.1)	0.33 (0.02)	20.9 (9.3)	3.3	16.9	12.2
8/29/08	9.38 (0.19)	0.75 (0.00)	0.36 (0.00)	1.14 (0.04)	0.11 (0.00)	29.3 (0.1)	18.8 (0.2)	221.6 (3.1)	0.87 (0.01)	29.7 (0.2)	12.1	1.8	11.8
9/9/08	9.33 (0.17)	2.32 (0.10)	2.39 (0.00)	1.17 (0.01)	0.16 (0.00)	33.1 (0.0)	19.0 (0.2)	239.7 (n/a)	1.25 (0.04)	33.0 (0.5)	11.3	1.4	19.1
9/22/08	9.28 (0.28)	2.98 (0.00)	2.70 (0.01)	1.59 (0.04)	0.25 (0.01)	33.9 (0.4)	18.9 (0.5)	238.1 (5.8)	1.15 (0.01)	32.1 (0.3)	13.0	1.3	12.6
1/22/09	0.27 (0.06)	0.11 (0.00)	0.00 (0.00)	0.29 (0.01)	0.17 (0.01)	20.2 (0.1)	19.9 (0.1)	236.4 (3.8)	0.00 (0.00)	10.2 (0.7)	>18.6	53.4	11.9
5/26/09	0.87 (0.02)	0.35 (0.00)	0.01 (0.00)	0.25 (0.00)	0.25 (0.01)	17.3 (0.1)	16.1 (0.1)	232.0 (5.0)	0.07 (0.01)	14.6 (0.6)	17.1	13.1	14.4
8/18/09	0.32 (0.00)	0.24 (0.00)	0.00 (0.00)	0.92 (0.03)	0.85 (0.030)	21.4 (0.1)	20.8 (0.1)	224.2 (3.2)	0.46 (0.04)	20.8 (2.2)	1.2	37.4	10.8
8/28/09	<i>0.70 (0.00)</i>	<i>0.30 (0.00)</i>	<i>nd</i>	<i>0.10 (0.00)</i>	<i>nd</i>	<i>28.0 (0.0)</i>	<i>27.0 (0.0)</i>	<i>nd</i>	<i>0.02</i>	<i>29.8 (nd)</i>	<i>53.8</i>	<i>27.0</i>	<i>nd</i>
Goodwin Islands													
8/7/08	0.48 (0.14)	0.28 (0.01)	0.00 (0.00)	0.21 (0.00)	0.31 (0.01)	17.9 (0.1)	17.2 (0.2)	216.1 (0.8)	0.16 (0.03)	21.2 (3.4)	4.7	22.6	12.6
8/29/08	6.17 (0.18)	0.45 (0.03)	0.12 (0.00)	0.76 (0.01)	0.18 (0.00)	24.7 (0.1)	18.0 (0.2)	220.2 (0.9)	0.49 (0.01)	26.7 (0.2)	13.8	2.7	12.3
9/9/08	<i>0.31 (0.04)</i>	<i>1.00 (0.01)</i>	<i>0.67 (0.02)</i>	<i>0.34 (0.05)</i>	<i>0.32 (0.01)</i>	<i>20.6 (0.3)</i>	<i>18.6 (0.3)</i>	<i>227.3 (1.1)</i>	<i>0.28 (0.00)</i>	<i>22.6 (0.1)</i>	<i>7.0</i>	<i>9.4</i>	<i>12.2</i>
9/22/08	7.31 (0.48)	2.42 (0.00)	1.73 (0.00)	1.09 (0.02)	0.14 (0.02)	28.9 (0.1)	17.4 (0.5)	229.9 (13.3)	0.67 (0.00)	25.7 (0.0)	17.2	1.5	13.2
1/22/09	0.48 (0.06)	0.09 (0.02)	0.00 (0.00)	0.32 (0.07)	0.19 (0.01)	19.5 (0.3)	18.9 (0.3)	223.1 (3.5)	0.00 (0.01)	6.7 (0.3)	>28.9	32.8	11.8
5/26/09	0.25 (0.07)	0.27 (0.00)	0.00 (0.00)	0.12 (0.01)	0.26 (0.00)	16.4 (0.1)	15.9 (0.1)	250.8 (29.5)	0.00 (0.02)	10.9 (0.0)	>25.9	30.7	15.8
8/18/09	0.13 (0.02)	0.39 (0.01)	0.00 (0.00)	0.40 (0.01)	0.42 (0.02)	18.0 (0.8)	17.5 (0.8)	221.8 (28.4)	0.19 (0.02)	17.2 (0.1)	2.8	33.4	12.7
PWP													
9/12/07	<i>0.28 (nd)</i>	<i>0.24 (0.00)</i>	<i>0.47 (0.00)</i>	<i>1.00 (0.03)</i>	<i>1.01 (nd)</i>	<i>nd</i>	<i>nd</i>	<i>275.0 (1.6)</i>	<i>0.60 (0.00)</i>	<i>26.9 (0.03)</i>	<i>1.7</i>	<i>nd</i>	<i>nd</i>

Table 3. Ratios of carbon (C) to nitrogen (N) uptake and rates of N regeneration:uptake at sites in the York River, VA from 2007- 2009. Data in italic represent the highest biomass and most monospecific blooms. Standard deviations are given in parentheses and n.d. indicates a given parameter was not determined.

Date	C up: N up GF/F	C up: N up > 5 μ m	C up: N up < 5 – 0.2 μ m	NH ₄ ⁺ regen (μ mol N L ⁻¹ h ⁻¹)	NO ₃ ⁻ regen (μ mol N L ⁻¹ h ⁻¹)	NH ₄ ⁺ regen : uptake	NO ₃ ⁻ regen : uptake
Clay Bank							
8/7/08	0.5	0.7	0.2	1.4 (0.4)	0.5 (0.0)	1.9	0.9
8/29/08	4.7	2.7	1.2	5.2 (0.1)	1.7 (0.0)	24.4	116.6
9/9/08	9.9	9.7	3.2	2.2 (0.4)	3.4 (0.0)	7.3	94.3
9/22/08	5.2	4.5	1.9	2.8 (0.5)	4.7 (0.1)	7.3	50.9
1/22/09	2.3	n.d.	n.d.	0.1 (0.0)	0.4 (0.0)	0.6	2.3
5/26/09	0.9	n.d.	n.d.	0.2 (0.0)	0.2 (0.0)	0.5	1.8
8/18/09	0.2	0.1	0.1	0.3 (0.0)	0.2 (0.1)	0.5	2.7
Gloucester Point							
9/7/07	<i>nd</i>	<i>nd</i>	<i>nd</i>	<i>0.33 (0.1)</i>	<i>0.9 (nd)</i>	<i>0.8</i>	<i>0.5</i>
8/7/08	0.2	0.3	0.1	1.1 (0.2)	0.2 (0.1)	22.0	67.0
8/29/08	5.3	6.0	4.6	5.7 (3.2)	0.4 (0.1)	20.6	95.3
9/9/08	4.6	6.5	1.5	7.5 (1.1)	2.3 (0.1)	18.0	59.5
9/22/08	6.7	8.7	1.5	3.5 (0.3)	1.7 (0.0)	0.9	1.3
1/22/09	1.4	n.d.	n.d.	0.5 (0.0)	0.2 (0.0)	1.0	11.1
5/26/09	1.8	n.d.	n.d.	0.2 (0.2)	0.2 (0.0)	0.7	4.6
8/18/09	0.2	0.2	0.1	0.2 (0.0)	0.2 (0.0)	0.8	0.5
Goodwin Islands							
8/7/08	0.1	0.2	0.1	1.1 (0.0)	1.3 (0.3)	0.6	0.9
8/29/08	3.3	4.8	2.4	1.2 (0.0)	0.4 (0.0)	2.7	61.4
9/9/08	2.7	7.8	0.9	<i>1.5 (0.3)</i>	<i>0.8 (0.0)</i>	<i>1.0</i>	<i>2.4</i>
9/22/08	3.6	4.2	0.5	1.6 (0.2)	1.5 (0.1)	3.5	14.0
1/22/09	1.1	n.d.	n.d.	0.6 (0.2)	0.2 (0.0)	1.0	1.6
5/26/09	0.2	n.d.	n.d.	0.4 (0.0)	0.3 (0.0)	0.5	0.8
8/18/09	0.21	0.2	0.1	0.2 (0.0)	0.1 (0.0)	0.4	1.8

Figure Legend

Figure 1. Map of York River, VA. Study sites are Clay Bank, Gloucester Point, Goodwin Islands.

Figure 2. Rainfall in the York River basin measured at the Taskinas Creek continuous monitoring station TSK000.23.

Figure 3. Pamunkey River discharge from Hanover, VA, reported by the USGS.

Figure 4. Total absolute N uptake ($\mu\text{mol N L}^{-1} \text{ h}^{-1}$) of ammonium (NH_4^+), nitrate (NO_3^-), nitrite (NO_2^-), amino acids (AA) and urea at Clay Bank (A, B, C), Gloucester Point (D, E, F), and Goodwin Islands (G, H, I) for the GF/F, $> 5\mu\text{m}$, and $5\mu\text{m} - 0.2\mu\text{m}$ size fractions. No size fractionation was conducted on 1/22/09 or 5/26/09, as noted by the asterisk (*) for the $> 5\mu\text{m}$ and $5\mu\text{m} - 0.2\mu\text{m}$. Urea uptake rates could not be determined for the $> 5\mu\text{m}$ and $5\mu\text{m} - 0.2\mu\text{m}$ size fractions for 8/18/09 due to mass spectrometer error. Data is included for the 9/7/07 bloom at Gloucester Point; only the $> 5\mu\text{m}$ size fraction was examined. Note the differences in scales of the y-axes.

Figure 5. Percentage of total absolute uptake for ammonium (NH_4^+), nitrate (NO_3^-), nitrite (NO_2^-) amino acids (AA) and urea at Clay Bank (A, B, C), Gloucester Point (D, E, F), and Goodwin Islands (G, H, I) for the GF/F, $> 5\mu\text{m}$, and $5\mu\text{m} - 0.2\mu\text{m}$ size fractions. No size fractionation was conducted on 1/22/09 or 5/26/09 as noted by the asterisk (*) for the $> 5\mu\text{m}$ and $5\mu\text{m} - 0.2\mu\text{m}$ size fractions. Urea uptake rates could not be determined for the $> 5\mu\text{m}$ and $5\mu\text{m} - 0.2\mu\text{m}$ size fractions for 8/18/09 due to mass spectrometer error, therefore urea data is not presented here; the percentages of total absolute uptake that were calculated without urea may be overestimated. Data is included for the 9/7/07 bloom at Gloucester Point; only the $> 5\mu\text{m}$ size fraction was examined.

Figure 6. Kinetics curve for NH_4^+ from an *A. monilatum* bloom at station PWP in the York River, VA., sampled on 9/12/07.

Figure 7. Total absolute carbon (C) uptake ($\mu\text{mol C L}^{-1} \text{ h}^{-1}$) of bicarbonate (HCO_3^-) and amino acids (AA) at Clay Bank (A, B, C), Gloucester Point (D, E, F), and Goodwin Islands (G, H, I) for the GF/F, $> 5\mu\text{m}$, and $5\mu\text{m} - 0.2\mu\text{m}$ size fractions. No size fractionation was conducted on 1/22/09 or 5/26/09, as noted by the asterisk (*) for the $> 5\mu\text{m}$ and $5\mu\text{m} - 0.2\mu\text{m}$ size fractions. Note the differences in scales of the y-axes. Urea uptake was also measured, however, no uptake occurred.

Figure 1.

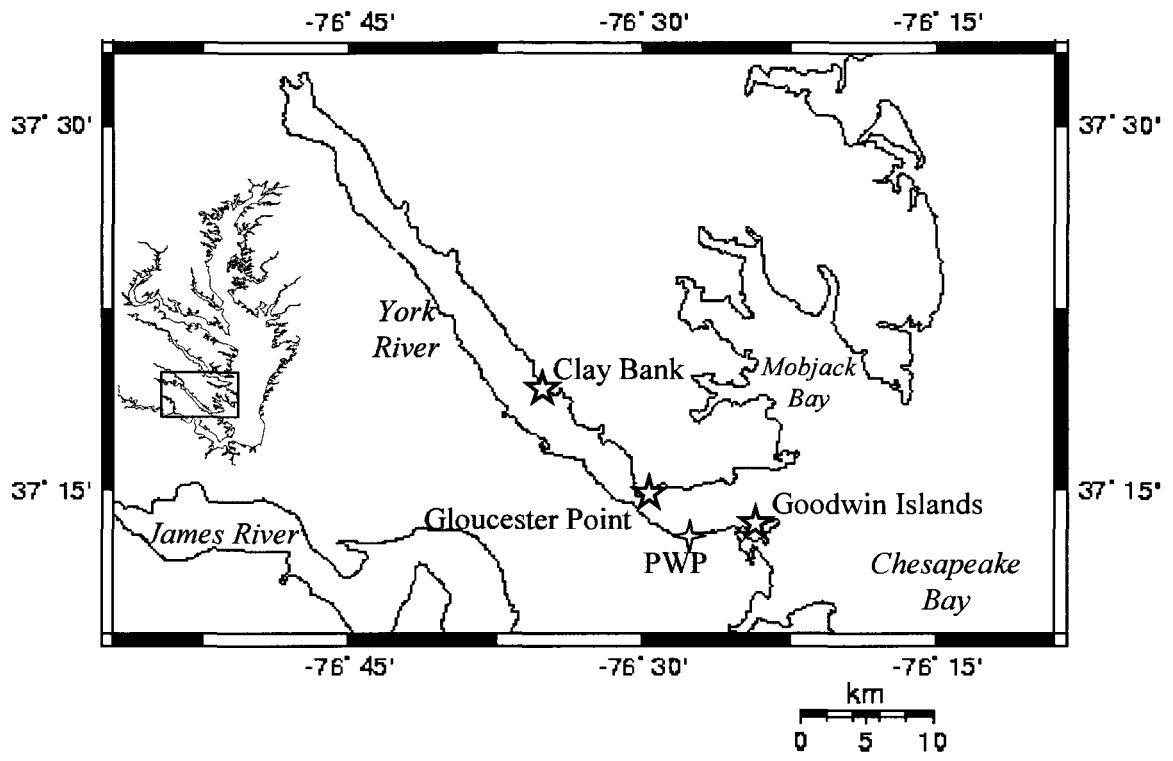


Figure 2.

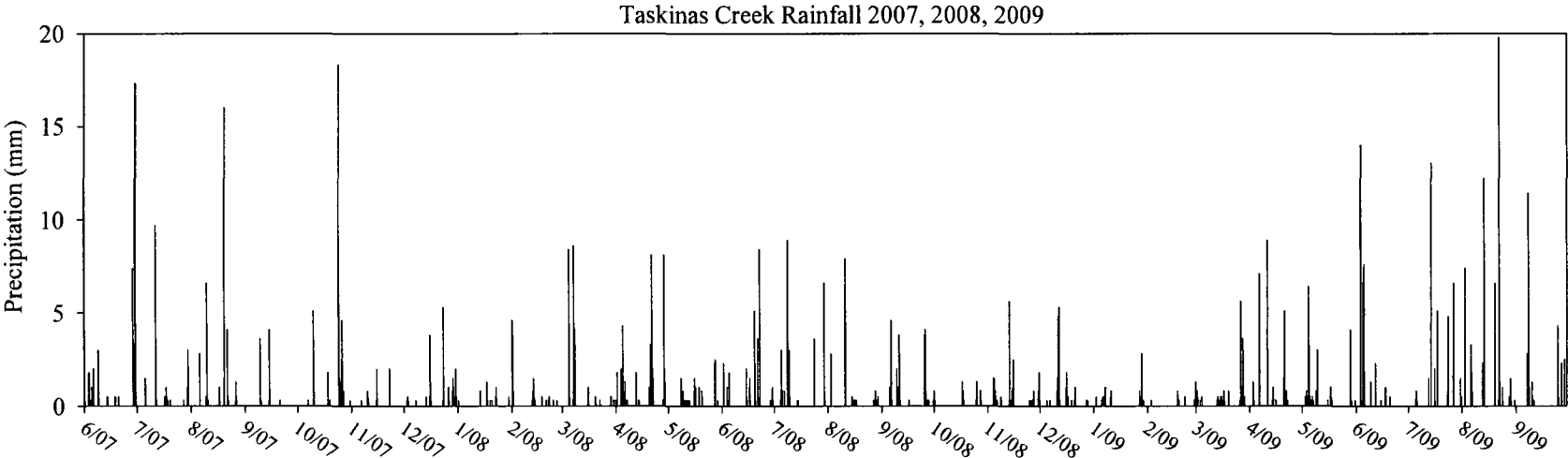


Figure 3.

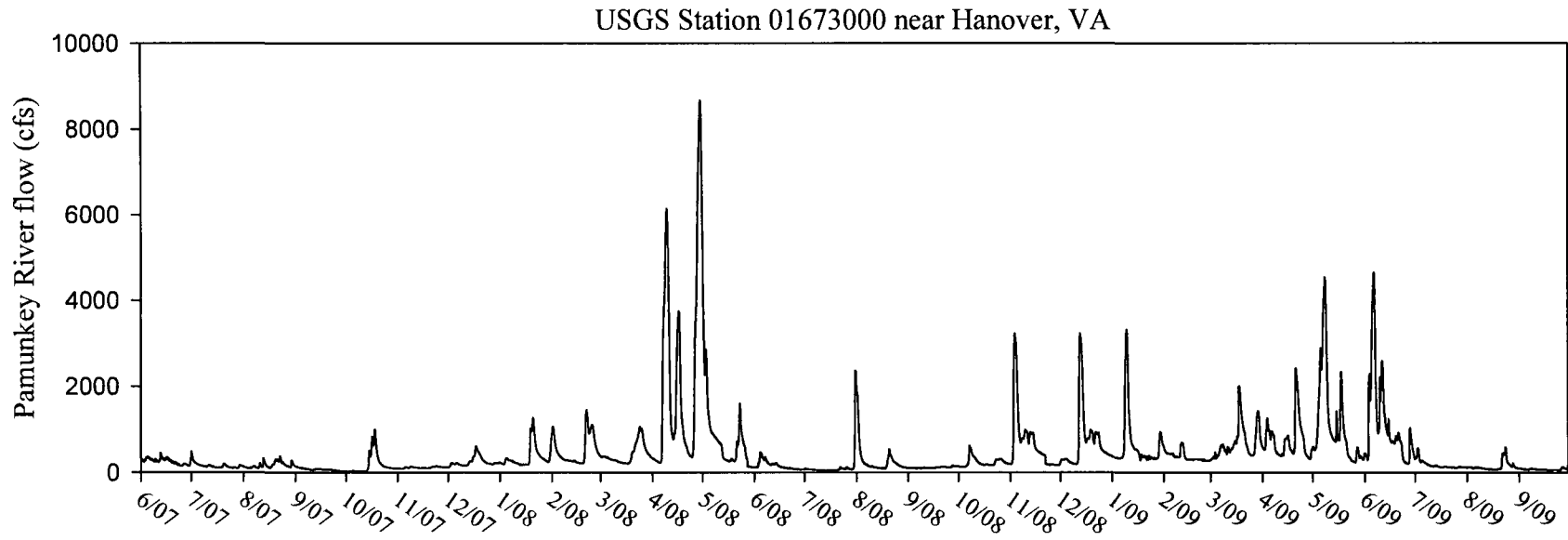


Figure 4.

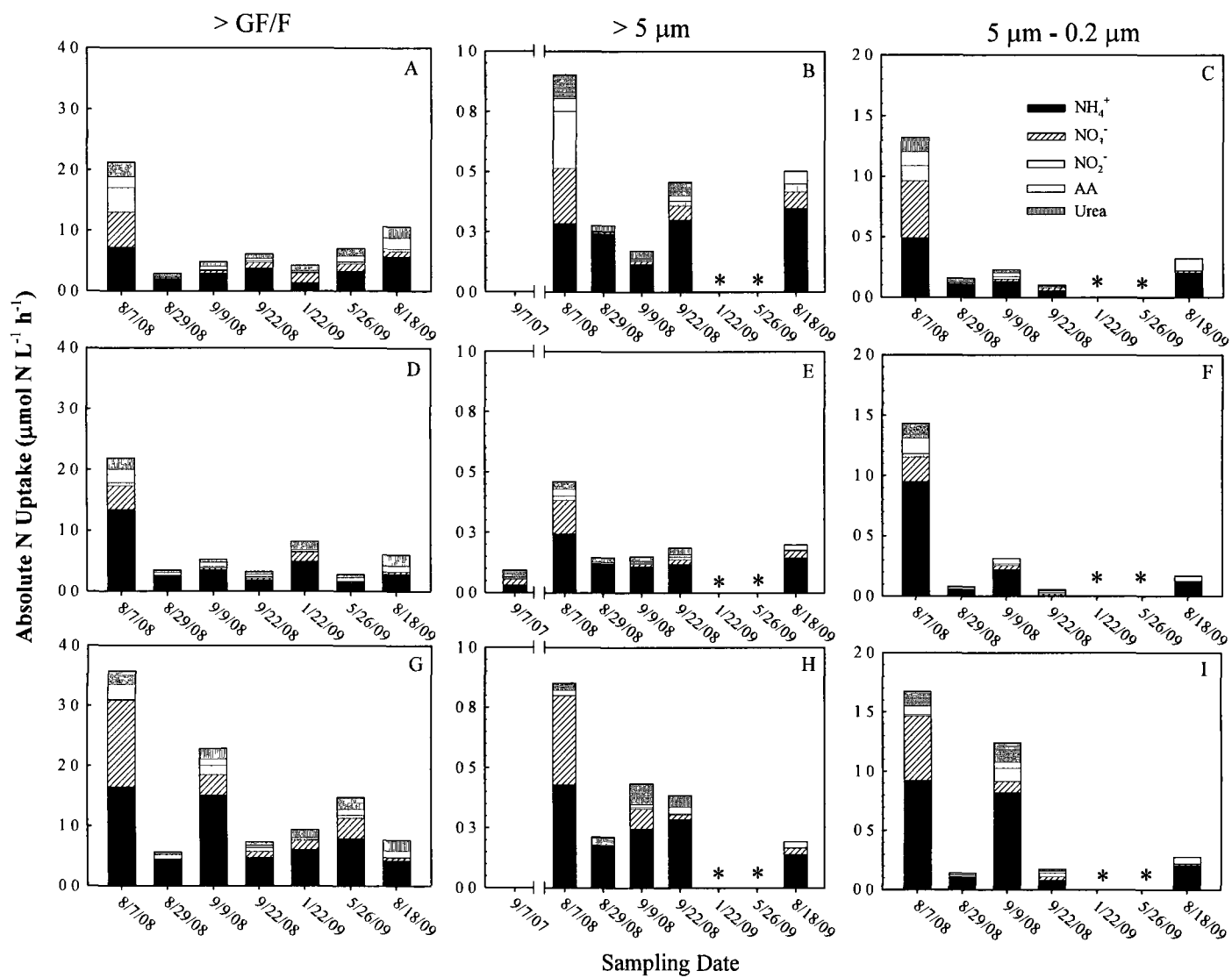


Figure 5.

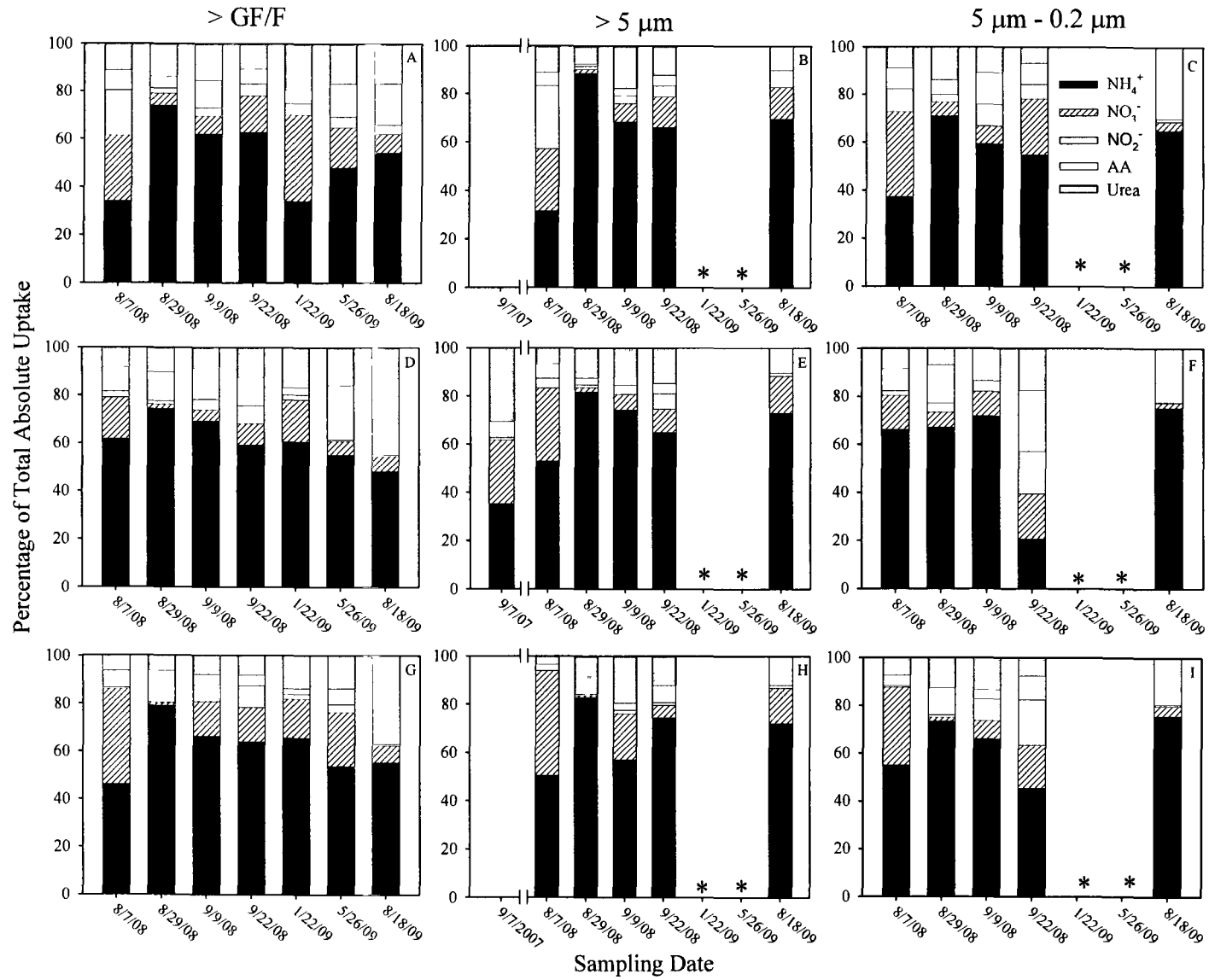


Figure 6.

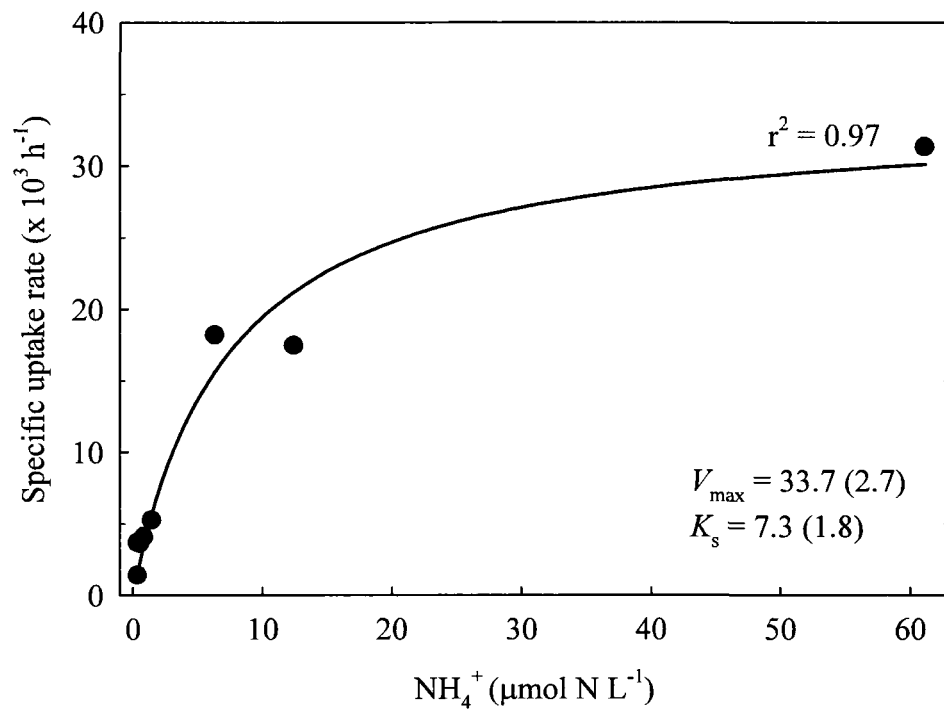
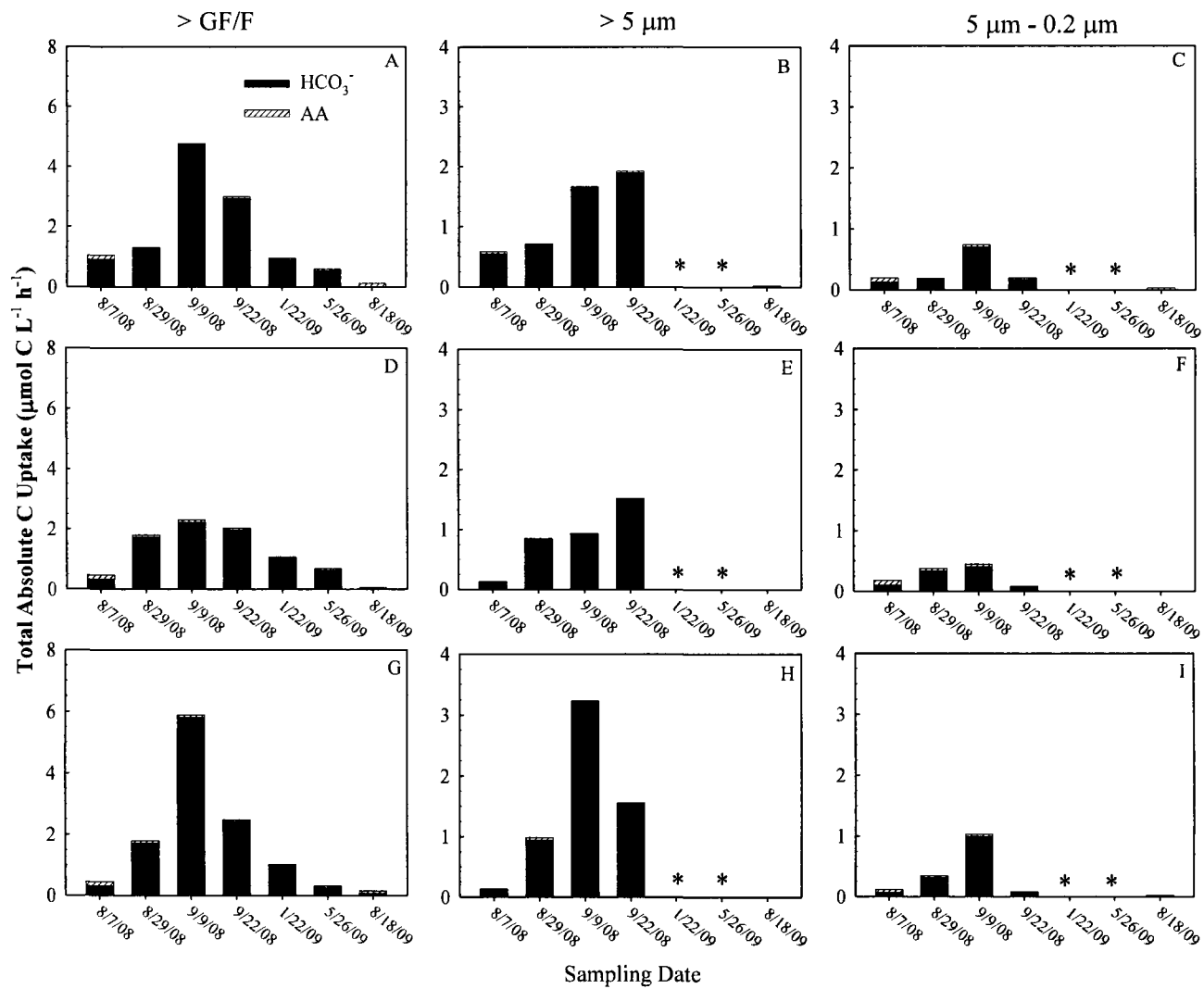


Figure 7.



CHAPTER 5

**Anthropogenic nitrogen inputs to the York River, Virginia, stimulate
growth of harmful algae**

Abstract

Three different anthropogenic nitrogen (N) sources were used to assess their role in exacerbating harmful algal blooms (HABs) in the York River, VA. Urban run-off (+ Urban), soil extracts from a construction site (+ Soil) and run-off from a paper mill (+ Industrial) were added to a natural bloom assemblage as a nutrient source during a bioassay experiment conducted over a period of seven days. Additional treatments of ammonium (NH_4^+) and urea represented comparative inorganic and organic N sources, respectively. Results indicate that anthropogenic sources had unique N compositions at the time of collection with DIN comprising approximately 60%, 91% and 20% of + Urban, + Soil, and + Industrial, respectively. The N sources stimulated the growth of harmful algae, particularly the dinoflagellate *Cochlodinium polykrikoides*. The + Urban and + Soil treatments elicited the greatest response with a doubling in Chl *a* biomass and/or cell concentrations along with concomitant nutrient drawdown of both organic and inorganic sources within two days; division rates of *C. polykrikoides* during the first day in those treatments were 0.6 ± 0.1 and 0.7 ± 0.4 divisions d^{-1} , respectively. The + Industrial treatment did not exhibit significant changes in HAB biomass, indicating either the organic dominated pool was relatively refractory or the phytoplankton were influenced by the inhibitory nature of tannins that were likely present. Shifts in community composition from dinoflagellate- to diatom-dominated assemblages were observed over time, particularly in the + Soil and + Industrial treatments; by day 7 when >95% of total cell abundance were diatoms. With drawdown of added nutrients early in the

bioassay, regenerated nutrients were likely the N source responsible for maintaining phytoplankton populations. Results of this study indicate the anthropogenic sources tested could exacerbate harmful algal blooms in the York River.

Introduction

Over enrichment of coastal waterways by increased nutrient inputs is considered a major pollution problem worldwide (e.g. Vitousek *et al.* 1997; Howarth *et al.* 2002; reviewed in Glibert and Burkholder 2006). With approximately 70 and 90% of the populations of Virginia and Maryland living within coastal counties, respectively (Crossett *et al.* 2004), Chesapeake Bay and its tributaries have experienced eutrophication attributed to human influence. An increasing population and the shifts in land use that accompany it have significantly impacted the delivery of nitrogen (N) and phosphorus (P) to adjacent water bodies (Vitousek and Mooney 1997). For example, anthropogenic activities are estimated to have increased N inputs to the coastal waters of Chesapeake Bay six to eight-fold since the pre-colonial period (Boynton *et al.* 1995).

Eutrophication has also been implicated as an important factor in the global expansion of harmful algal blooms (HABs) both geographically and temporally (e.g., Smayda 1990; Riegman 1995; Anderson *et al.* 2002; Glibert *et al.* 2005a; Glibert *et al.* 2005b). Increased nutrient concentrations are able to support higher biomass blooms of phytoplankton and have been correlated to increased HAB species abundance (reviewed in Anderson *et al.* 2002; Glibert and Burkholder 2006). In

addition to increases in total phytoplankton biomass, eutrophication may cause selective enrichment of a single nutrient, which can favor domination of one (often harmful) species over others (Ryther and Dunstan 1971; Tilman 1977, Officer and Ryther 1980; Smayda 1990; Hallegraeff 2003; Smayda 1997). For instance, blooms of *Pfiesteria* spp. occur along the eastern U.S. coast when N:P ratios are low, due to high P concentrations from highly concentrated animal feeding operations (Mallin 2000). Increases in the occurrences of HABs in the lower Chesapeake Bay have been attributed to changes in water quality, specifically, the enrichment of estuarine waters with dissolved organic matter (DOM) relative to inorganic nutrients (Paerl 1988, Glibert *et al.* 2001).

Approximately half of the N transported to the coastal ocean from rivers is in the form of dissolved organic nitrogen (DON; Meybeck 1982; reviewed in Glibert *et al.* 2005a or b). Once considered refractory, DON is a dynamic pool that many phytoplankton, particularly HAB species, have been found to utilize (reviewed in Berman and Bronk 2003). Many HABs use DON to meet a portion of their N nutrition and may prefer it to inorganic N (e.g. Berg *et al.* 1997, Mulholland *et al.* 2002). Mixotrophic phytoplankton species, those that can utilize both inorganic and organic nutrients, may possess a variety of mechanisms to access organics giving them a competitive advantage when inorganic nutrients are low or when organic nutrients are present in high concentrations (reviewed in Glibert and Legrand 2006). For instance proliferation of *Prorocentrum minimum* has been linked to eutrophication (Heil *et al.* 2005), particularly with inputs of anthropogenic DON in the form of humic substances (Carlsson *et al.* 1999; Heil 2005). Brown tide blooms

of *Aureococcus anophagefferens* have been correlated with periods of low rainfall (LaRoche *et al.* 1997), which correspond to higher DON:DIN ratios.

Anthropogenic N may come from a variety of point and non-point sources and is comprised of complex mixtures of compounds. These sources include but are not limited to atmospheric, agricultural, industrial and sewage effluents, groundwater inputs, and aquaculture run-off. The main source of N and P to major rivers in the U.S. is from non-point inputs (Newman 1995), which are more difficult to identify and monitor than point sources. Of particular concern is nutrient delivery via atmospheric deposition. Often comprised of a wide variety of compounds, atmospheric nutrient delivery may occur through dry or wet deposition. Studies have shown that rainwater can enhance productivity more than the addition of a single N source (Paerl 1997). Urban run-off is another significant non-point source and includes construction sites, lawn fertilizer run-off, and pet wastes. Although occupying a relatively small percentage of land area, construction sites are of particular concern due to the high erosion rates associated with them (Carpenter *et al.* 1998).

The York River, VA is an estuarine tributary of Chesapeake Bay that experiences both eutrophication and harmful algal blooms. Dinoflagellate blooms have been documented in the York River for decades (Ho and Zubkoff 1979, Marshall 1994) and are prevalent in the late summer/early fall months, although conditions leading to HABs in the York River are not well understood. These blooms are often multi-species assemblages and can last for weeks. The athecate, chain-forming, coastal dinoflagellate, *Cochlodinium polykrikoides* has produced the most

extensive blooms annually (Mackiernan 1968; Zubkoff *et al.* 1979; Marshall 1994). Blooms of *C. polykrikoides* have been documented worldwide (e.g. Margalef 1961; Gárate-Lizárranga *et al.* 2004; Gobler *et al.* 2008), particularly in Asian countries (e.g. Kim 1998; Iwataki *et al.* 2008; Kim *et al.* 1999), however, it appears that these strains are genetically dissimilar to those found in North American waters (Gobler *et al.* 2008; Mulholland *et al.* 2009). Additionally, *C. polykrikoides* has been shown to possess ichthyotoxic properties (Kim *et al.* 1999; Kim *et al.* 2002; Tang and Gobler 2009) leading to massive fish kills. *Cochlodinium polykrikoides* is mixotrophic (Jeong *et al.* 2004; 2005) and can utilize a variety of inorganic and organic N species (Mulholland *et al.* 2009), however, little research has been conducted on N nutrition of blooms along the eastern U.S. coast, particularly within Chesapeake Bay tributaries. Recent studies suggest that blooms of *C. polykrikoides* have coincided with periods of intense rainfall and storm water runoff in the nearby James River (Mulholland *et al.* 2009) as well as in Korean waters (Lee and Lee 2006). *Cochlodinium polykrikoides* form benthic resting cysts that initiate future bloom events (Seaborn and Marshall 2008), so continued blooms in the lower Chesapeake Bay are likely.

Identifying anthropogenic nutrient sources that stimulate blooms and understanding their N composition is critical to understanding where, when and why HABs occur. With this in mind, the goals of this study were to: (1) determine the N composition of three anthropogenic sources to the York River, and (2) determine if those sources would stimulate the growth of a harmful algal bloom in the York River.

Materials and Methods

Three anthropogenic N sources were collected: run-off from an urban parking lot, soil from a construction site, and Industrial run-off from an outflow adjacent to a paper mill. Seven day bioassays were conducted to determine the bioavailability of the anthropogenic N sources to a bloom of harmful phytoplankton in the York River.

Study Area

The York River, VA is the 5th largest estuary of Chesapeake Bay at ~6900 km² (2662 mi²). It is considered a microtidal, partially-mixed estuary with a salinity range from polyhaline to freshwater. It receives freshwater from two tributaries, the Mattaponi and Pamunkey Rivers, which intersect at West Point, ~48 km from the mouth of the river (Figure 1). While the York River watershed is quite rural compared to many other Chesapeake Bay rivers, it is influenced by the urban areas of Hampton Roads and Richmond. Gloucester Point is one of the population centers of the region. Land use in the York River basin is primarily forest (61%) and agricultural (21%); wetlands make up 7% of the area, followed by developed lands (2%), barren lands (1%) and water (8%). Of the developed lands, the percentage of impervious surfaces is ~1% (Chesapeake Bay program watershed profiles, <http://chesapeake bay.net>).

Preparation of anthropogenic nutrient sources

Anthropogenic N sources were collected within one week of bioassay initiation. Urban run-off was collected from a street-level storm sewer grate that received runoff from adjacent streets, parking lots and shopping centers, in Newport News, VA (37 ° 6' 18.0" N, 76 ° 29' 39.6" W) on August 22, 2009. The water was collected in an acid washed (10% HCl) carboy, within 1 h of the start of a rain storm to ensure collection of a majority of compounds being washed from the asphalt. Soil that had been exposed during construction was collected in an acid-cleaned and combusted (500°C for 4 h) glass beaker from a site adjacent to the Virginia Institute of Marine Science located in Gloucester Point, VA (37 ° 15' 1.9"N, 76 ° 29' 55.8"W) on August 22, 2009. The soil was then suspended in 1.5 L of distilled water (DIW) and allowed to settle over a 24 h period. The soil-water mixture was then decanted. Tea-colored industrial run-off was collected at a lot filled with hewn logs that was adjacent to a paper mill located in West Point, VA (37 ° 32' 9.8"N, 76 ° 48' 8.0"W) on August 23, 2009. The water was collected from a small drainage ditch that flowed directly into the York River. Water was collected in a 20 L carboy and returned to the lab. All nutrient source waters were passed through a GF/F filter (nominal pore size 0.7 µm) to remove debris and stored in a temperature controlled room at 5 °C prior to use.

Field sample collection and bioassay

A multi-species algal bloom was collected from a pier adjacent to the Virginia Institute of Marine Science in Gloucester Point, Virginia on August 28, 2009. York River surface water was collected using buckets and gently filtered through a 100 μm Nitex mesh to remove any larger zooplankton. Bloom water was evenly divided into 18 acid washed (10% HCl) 2.5 L polycarbonate bottles. The bioassay consisted of six treatments run in triplicate: (1) control, (2) + ammonium (NH_4^+), (3) + Urea, (4) + urban run-off (+ Urban), (5) + Soil, (6) + industrial run-off (+ Industrial). The salinity of the bloom water was 22. All N sources were modified with a salt mixture (NaCl , MgSO_4 and NaHCO_3) to obtain a uniform salinity. The control treatment contained no added N although artificial seawater (ASW) was added to make the total volume in each treatment the same. Other than the control, all treatments received an addition of $\sim 18 \mu\text{mol N L}^{-1}$ in whatever form N was added. Previous studies of N uptake in the York River have shown both NH_4^+ and urea to be bioavailable during bloom events with the highest uptake rates for NH_4^+ (Killberg-Thoreson, Chapter 4), and were used for comparison with the other N sources.

The bottles were placed in an incubator under *in situ* light and temperature (28 °C) conditions. The incubator was set on a 14.5: 9.5 light dark cycle with the start of the light period beginning at natural daybreak at 0600. An aliquot (150 mL) was sub-sampled from each bottle at time points 0, 0.5, 1, 2, 3, 5 and 7 days. Samples were collected for chlorophyll *a* (Chl *a*) and phytoplankton cells counts (preserved in 1% glutaraldehyde, final concentration). Nutrient samples were

collected for NH_4^+ , urea, total dissolved nitrogen (TDN), total dissolved phosphorus (TDP), nitrate + nitrite (NO_x^-), dissolved organic carbon (DOC), phosphate (PO_4^{3-}) and silica (SiO_2).

Sample analyses

Water samples to be analyzed for TDN, TDP, NH_4^+ , NO_x^- , urea, PO_4^{3-} , SiO_2 were stored in low density polyethylene (LDPE) centrifuge tubes (Corning®). All analyses were performed in duplicate (urea, NO_x^-) or triplicate (all others). Ammonium concentrations were analyzed using the colorimetric phenol-hypochlorite method of Koroleff (1983). A Lachat QuikChem 8500 autoanalyzer were used to measure concentrations of NO_3^- and NO_2^- , PO_4^{3-} , and SiO_2 (Parsons *et al.* 1984). Concentrations of TDN were analyzed according to the persulfate oxidation technique (Bronk *et al.* 2000). Urea was analyzed using the diacetyl monoxmine thiosemicarbazide method adapted from Price and Harrison (1987). Concentrations of DON were determined as the difference between the TDN and dissolved inorganic nitrogen (DIN); the errors from the TDN, NH_4^+ , NO_x^- measurements were propagated to provide a standard error for DON estimates. A Shimadzu TOC-V was used to measure dissolved organic C (DOC) concentrations (Hansell *et al.* 1997). Concentrations of TDP were measured using an Element2 ICPMS (ThermoFisher; Bremen, Germany) in medium resolution mode. Indium was used as an internal standard to correct for instrument drift and variability in sample introduction. The instrument was calibrated by the standard addition technique using

NIST-traceable standards obtained from Inorganic Ventures (Christiansburg, VA). Concentrations of DOP were determined as the difference between the TDP and dissolved inorganic P (PO_4^{3-}) and the errors from those measurements were propagated to provide a standard error for DOP estimates.

Concentrations of Chl *a* were measured fluorometrically on a Turner Design Model 10-AU fluorometer; each sample had been previously filtered onto a pre-combusted GF/F filter, extracted in 90% acetone overnight at 4°C, and analyzed according to Parsons *et al.* (1984). Phytoplankton cell counts were enumerated using an inverted microscope. Growth rates were calculated based on cell counts for *C. polykrikoides* using the following equation:

$$\text{Growth rate } (\mu): K' = \ln(N_2 / N_1) / (t_2 - t_1)$$

Where N_1 and N_2 = biomass at time 1 (t_1) and time 2 (t_2), respectively (Levasseur *et al.* 1993). Divisions per day were calculated from the specific growth rate:

$$\text{Divisions per day: Div. d}^{-1} = K' / \ln(2)$$

Results

Bloom site and anthropogenic N source characteristics

Cell counts from the bloom sample revealed a mixture of dinoflagellates dominated by *C. polykrikoides* (62 % of total abundance). *Gyrodinium instriatum*, *K. veneficum*, *Phaeopolykrikos hartmanni*, *Prorocentrum micans*, *P. minimum*, and *S. trochoidea* were also present in addition to diatoms. Total cell abundance was

1,079 cells mL⁻¹ and Chl *a* was 22.5 µg L⁻¹ (data not shown). Concentrations of TDN were 22 ± µmol N L⁻¹ and of that, ~95% was comprised of DON. Concentrations of NH₄⁺, NO_x⁻ and urea were all less than 1 µmol N L⁻¹ and PO₄³⁻ and SiO₂ were 1.1 and 26, respectively (Table 1). The bloom water had a N:P ratio of 20. The salinity and temperature were 22 and 28 °C, respectively (data not shown).

Each of the anthropogenic N sources had a distinct N composition. The Soil had the highest TDN concentration (159 µmol N L⁻¹) and Urban and Industrial sources had nearly equal amounts of TDN (63 and 64 µmol N L⁻¹; Table 1). Of that TDN, there were varying proportions of DIN and DON among the sources. The Soil source was composed primarily of DIN (91 %), Urban was a more equal mixture (59 % DIN) while the Industrial source was dominated by DON (79 %; Table 1). Over half of the Urban DON pool was comprised of urea, while urea contributed only 5% of the DON in the Industrial source, leaving the majority of the organic pool uncharacterized. Total dissolved P ranged from 1 to 4 and was highest in the Industrial water (Table 1). Ratios of N:P were highest in the Soil (76) followed by Urban (58) and then Industrial (16).

Changes in nutrient concentrations

A decline in nutrient concentrations was observed in all treatments with added N (Figure 2). Except for the Control, there was a 5 to 15 µM N drawdown of TDN within 0.5 to 1 days. Concentrations of DON experienced a 1-3 µM N initial decrease by 0.5 days, and the + Urea treatment had complete drawdown by day 2

(Figure 2 B). Ammonium concentrations in the + NH₄⁺, + Urban and + Soil treatments were negligible after 1 day, representing drawdown of 17, 5, and 11 μmol N L⁻¹, respectively (Figure 2 C). Drawdown of NH₄⁺ in the +Urban treatment occurred faster than NO_x⁻ drawdown, although initial concentrations of both substrates were equal, indicating preferential utilization of NH₄⁺. The majority of the DON pool was comprised of urea (64 %) which was also rapidly consumed by day 1 (Figure 2). The + Urea treatment exhausted its ~19 μmol urea-N L⁻¹ addition by day 2 and the +Urban treatment depleted ~ 3 μmol urea-N L⁻¹ after 1 day. Concentrations of SiO₂ gradually declined over the course of the experiment but were never limiting (Figure 3).

Concentrations of TDP, DOP and PO₄³⁻ declined primarily during the first day of the bioassay, as much as ~ 1 μmol P L⁻¹. Concentrations of PO₄³⁻ became negligible in the + NH₄⁺ treatment, conversely, concentrations in the + Urea treatment increased. Concentrations of DOP decreased in all treatments except for the Control, with the greatest decreases observed in the + NH₄⁺, + Urea, and + Soil treatments.

Changes in species composition and Chlorophyll a

Initial Chl *a* concentrations in the bioassay ranged between 10 and 13 μg L⁻¹ for all treatments. There was approximately a 2-fold increase in Chl *a* in the inorganic and organic controls (+ NH₄⁺, + Urea) as well as the + Soil and + Urban treatments within the first day (Figure 4) while the Control treatment declined steadily during the experiment. Chlorophyll *a* concentrations in the + NH₄⁺ and

+ Soil treatment remained steadiest and highest throughout the experiment while concentrations in the + Urea and + Urban treatments began to decline earlier (Figure 4). In the + Industrial treatment, there was no significant difference in Chl *a* concentrations from the Control treatment until day 7 ($t = 11.0$, $p < 0.001$) when Chl *a* doubled (Figure 4).

All treatments began with a composition of ~ 250 cells mL^{-1} (Figure 5). The Control treatment exhibited no significant change in total cell abundance or composition (t-test, $p > 0.05$) until days 5 and 7 when dinoflagellate abundance became negligible and only diatoms remained (84% of total abundance). The + NH_4^+ and + Urea treatments exhibited a 2.5-fold increase in cell abundance within 2 days, ~ 650 cells mL^{-1} (Figure 5). Increases in *C. polykrikoides* concentrations in those treatments within the first day corresponded to division rates of 0.57 ± 0.31 divisions d^{-1} ($\mu = 0.39 \pm 0.22$) for + NH_4^+ and 0.92 ± 0.38 divisions d^{-1} ($\mu = 0.64 \pm 0.35$) for + Urea. The + NH_4^+ treatment sustained the highest cell abundance, with the greatest proportion of *C. polykrikoides* remaining at the end of the experiment on day 7 ($\sim 47\%$).

From the anthropogenic N sources, + Urban elicited the greatest response, similar to that of the + NH_4^+ treatment, with a doubling of mean total cell concentrations and *C. polykrikoides* concentrations. This corresponded to a division rate for *C. polykrikoides* of 0.56 ± 0.11 divisions d^{-1} ($\mu = 0.39 \pm 0.07$) within the first day. In the + Soil treatment, the mean total cell abundance and individual cell abundances increased, however these increases were not significant (t-test, $p > 0.05$), due to high bottle variability, unlike the Chl *a* values which exhibited large and

significant increases ($t = 8.4, p = 0.001$) within the first day. Division rates for *C. polykrikoides* in the + Soil treatment during the first day correspond to 0.71 ± 0.39 divisions d^{-1} ($\mu = 0.49 \pm 0.27$), however, by days 5 and 7 dinoflagellates were negligible and diatoms were the dominant species present ($> 81\%$). The + Industrial treatment exhibited the least change in total cell abundance compared to the other treatments with no significant increase in *C. polykrikoides* abundance (t-test, $p > 0.05$) during the first 2 days of the experiments, however, between days 5 and 7 there was a significant increase in cell concentrations ($t = 5.2, p = 0.01$) due to diatom biomass, which comprised 97% of total cell abundance (Figure 5).

Discussion

Harmful algal blooms, particularly of the dinoflagellate *C. polykrikoides*, are a common feature in the tributaries of the lower Chesapeake Bay in the late summer and early fall. Summer months are typically associated with N limited conditions in the York River and blooms have been associated with low N:P ratios (Mulholland *et al.* 2009; Killberg-Thoreson, Chapter 4). Cells have been shown to utilize a variety of N substrates including both inorganic and organic N sources (Mulholland *et al.* 2009; Killberg-Thoreson, Chapter 4) and low N concentrations measured during blooms are likely a function of that N utilization. An extensive *C. polykrikoides* bloom coincided with intense rainfall and storm water runoff after a period of summer drought in the James River, a neighboring Chesapeake Bay tributary (Mulholland *et al.* 2009), and elevated cell densities have also been associated with

heavy rainfall and N inputs in Korea (Lee and Lee 2006), however, there have been no direct studies of allochthonous N sources to blooms, particularly in the York River.

This bioassay study examined the N composition and bioavailability of three potential anthropogenic sources to harmful algae in the York River. The sources represented different land uses within the watershed and provided a spectrum of N compositions, ranging from DON dominated (Industrial, 80%) to DIN dominated (Soil, 91%) as well as a nearly equal mixture of both (Urban: 59% DIN, 41% DON, Table 1), and allowed for a unique assessment of how these inputs may shape the phytoplankton community of the river. Results indicate that rainfall and subsequent run-off have the potential to add large amounts of N to the York River with TDN concentrations ranging from 63 to 159 $\mu\text{mol N L}^{-1}$ (Table 1).

Within this study we demonstrated that a bloom of harmful algae, dominated by *C. polykrikoides* utilized anthropogenic nutrient sources of varying N composition. The largest responses to the anthropogenic sources tested were in the + Urban and + Soil treatments, which had large proportions of DIN. Additionally, the DON portion of the + Urban treatment contained a substantial proportion (~65%) of the labile substrate urea. Rapid utilization of N in the + Urban and + Soil treatments allowed the bloom to double in biomass and allow *C. polykrikoides* to achieve division rates of 0.56 ± 0.11 divisions d^{-1} and 0.71 ± 0.39 divisions d^{-1} . The growth rates and corresponding divisions d^{-1} measured here fall within values measured in previous lab studies, ranging from 0.13 to 0.91 divisions d^{-1} (Kim *et al.* 2004) and estimates from the field based on carbon turnover (Tomas *et al.* 2008; ≤ 1.0 divisions

d⁻¹) and nitrogen uptake in dense blooms (Mulholland *et al.* 2009; 0.48-1.30 divisions d⁻¹).

Maximum drawdown in N concentrations for all treatments was compared to the largest change in *C. polykrikoides* and total cell concentrations to determine the quantity of N needed to support a 10⁵ increase in total community abundance (Table 2). It was determined that on average 2.4 ± 0.5 μM N from the Urban source and 7.3 ± 6.7 μmol N L⁻¹ would be required to increase bloom abundance to 10⁵ cells. Values for the inorganic (+ NH₄⁺) and organic (+ Urea) control treatments indicate similar N requirements of 3.6 ± 0.3 μmol N L⁻¹ and 5.8 ± 1.5 μmol N L⁻¹, respectively (Table 2). The molar N:P drawdown of nutrients required per 10⁵ cells ranged from 13.9 to 23.5. Interestingly, the increasing PO₄³⁻ and decreasing DOP concentrations in the + Urea treatment indicate DOP was the primary source of P to the bloom.

As shown in this study, NH₄⁺, NO₃⁻ and urea derived from Urban run-off and Soil were particularly utilized by *C. polykrikoides* and co-occurring phytoplankton in the York River. These results are consistent with findings of both organic and inorganic utilization by *C. polykrikoides* in the lower Chesapeake Bay (Mulholland *et al.* 2009) as well as by a closely related species *C. fulvescens* (Kudela *et al.* 2008). Utilization of urea is important as it is the primary N source used in agricultural fertilizer (Glibert *et al.* 2006) and increased urea usage has been correlated with an increase in HABs (Anderson *et al.* 2002). In addition to the nutrients measured here, soil may have additional stimulatory effects on phytoplankton because of high humic content as well as trace metals. Changed patterns in land, use such as deforestation and subsequent erosion, have been shown to alter phytoplankton species composition

by increasing the concentrations of humic substances in land run-off (Hallegraeef 2003). River water draining from forested areas (rich in humic and fulvic acids) can stimulate dinoflagellate blooms of *P. minimum* (Graneli and Moreira 1990). Tidal erosion is the dominant sediment source in the York River (Reay 2009). Typically, the lowest sediment loads in the York River have been associated with the historic dry years, and peak sediment loads associated with historic wet years and are dominated by nonpoint agricultural inputs (52%; reviewed in Reay 2009). Human impacts, such as population increases and greater construction near waterways can lead to destabilization of the coast line and higher erosion rates which can cause greater sediment delivery to the river. Urban run-off and soil from erosion are non-point sources and, as often observed, non-point sources are often more important contributors to eutrophication and more difficult to manage than point sources.

In contrast, the + Industrial treatment was a comparatively localized N source at the head of the river and results indicate the mixture, dominated by DON did not contribute substantially to cellular growth. There was an initial drawdown of inorganic N ($\sim 3 \mu\text{mol N L}^{-1}$ of NO_x^- ; Figure 2), and P ($\sim 0.2 \mu\text{mol P L}^{-1}$ of PO_4^{3-} ; Figure 3) however, little change in the bulk organic pools over time. While it is possible that microbial processing of the organic pool may alter its composition without measureable changes in bulk DON measurements, this did not translate into large changes in phytoplankton concentrations or Chl *a*, indicating that the DON pool present was relatively refractory. While GF/F filters have been shown to retain > 50% of bacterial biomass (Gasol and Moran 1999; Lee and Fuhrman 1987), specifically 53-71% in the York River (Bradley 2008), it is also possible that bacteria

present in the Industrial source water may have utilized the labile organic fractions prior to the start of the bioassay.

Additionally, the reddish coloration of the water at the time of collection implied that natural phenolic materials, tannins, released from degradation of the logs adjacent to the paper mill were likely present. While not specifically measured in this study, tannic acid has been shown to inhibit growth of microalgae at high concentrations $\sim 18 \text{ mg L}^{-1}$, with growth similar to the control at lower concentrations (3 mg L^{-1} ; Herrera-Silveira and Ramírez- Ramírez 1996). It is likely that a combination of the refractory nature of the DON pool as well as the inhibitory nature of the tannins in the + Industrial treatment were responsible for the low phytoplankton response as compared to the other treatments. The tannins, however, did not seem to inhibit diatom growth as observed at the end of the bioassay (days 5 and 7). Either tannins negatively impact dinoflagellate growth more than diatoms or the inhibitory properties of the tannins were degraded by microbial processing and no longer retained their inhibitory function over time.

In both the + Soil and + Industrial treatments there was an increase in diatom biomass as *C. polykrikoides* cells declined. With the major decrease in added N concentrations during the first 2 days of the bioassay, regenerated nutrients were likely fueling the remaining cells after the initial drawdown, particularly the increases in diatoms between days 5 and 7. In fact, NH_4^+ and NO_3^- regeneration rates measured in the same bloom that was used for the bioassay, a few days prior, averaged $0.2 \mu\text{mol N L}^{-1} \text{ h}^{-1}$ (Killberg-Thoreson, Chapter 4).

A likely explanation for dominance of *C. polykrikoides* early in the bloom is that *C. polykrikoides* simply out-competed the diatoms for the available nutrients. Alternatively, *C. polykrikoides* has been shown to possess allelopathic properties on co-occurring phytoplankton and may facilitate blooms by eliminating competing species (Tang and Gobler 2010). The large increase in diatom biomass only after the *C. polykrikoides* populations were negligible suggests allelopathy may have been imposed upon the diatoms and only alleviated once *C. polykrikoides* declined. This scenario matches well with the suggestion that allelopathic agents are short-lived and dependent on cell viability (Tang and Gobler 2010).

Currently the York River watershed is relatively rural, however, human populations continue to increase with three of the surrounding counties (Gloucester, James City, and New Kent) experiencing some of the highest population increases in the state. Nutrient water quality status reports indicate currently degraded conditions in the Chesapeake Bay and York River estuaries (Reay 2009). Increasing populations and associated shifts in land use that accompany it may significantly impact the delivery of nitrogen (N) and phosphorus (P) to the York River and enhance eutrophication. Additionally, climate change models have predicted increases in temperature and precipitation for the Chesapeake Bay watershed, with summer months experiencing more sporadic intense storms and associated high discharge rates (Pyke *et al.* 2008) adding even greater N inputs to the watershed. As shown in this study Urban run-off and Soil extract elicited the greatest responses in HAB biomass, particularly the dinoflagellate *C. polykrikoides*, therefore it can be suggested that increased urbanization, including construction and associated soil disturbance

will not only contribute to eutrophication of the York River but may also support nuisance and harmful species. Considering the ability of *C. polykrikoides* to form benthic resting cysts that seed future populations, and the history of blooms in the York River, future bloom events are likely to continue. Not investigated here, but also important, is how the toxicity of *C. polykrikoides* and other HAB species may vary depending on nutrient status and availability. It is unclear whether *C. polykrikoides* toxicity may vary dependent on the N or P species present but this may have important implications for the health of fish and shellfish in the York River. HAB-nutrient relationships are often complex and understanding the connection between human impacts and bloom proliferation is an important for future forecasting of bloom events and mitigation of their deleterious consequences.

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Literature Cited

- Anderson, DM, Glibert, PM, Burkholder, JM. 2002. Harmful Algal Blooms and Eutrophication: Nutrient Sources, Composition, and Consequences. *Estuaries* 25(4b):704-726
- Berg, G, Glibert, PM, Lomas, MW, Burford, MA (1997) Organic nitrogen uptake and growth by the chrysophyte *Aureococcus anophagefferens* during a brown tide event. *Marine Biology* 129:377-387
- Berman, T., Bronk, D.A., 2003. Dissolved organic nitrogen: a dynamic participant in aquatic ecosystems. *Aquat. Microb. Ecol.* 31, 279-305.
- Bradley, P.B. 2008. Multi-system analysis of nitrogen use by phytoplankton and heterotrophic bacteria. Ph.D. Dissertation. The College of William & Mary-School of Marine Science. pp. 227.
- Bronk, D.A., Lomas, M.W., Glibert, P.M., Schukert, K.J., Sanderson, M.P. 2000. Total dissolved nitrogen analysis: Comparisons between the persulfate, UV, and high temperature oxidation methods. *Marine Chemistry*. 69: 163-178.
- Boynton, W.R., Garber, J.H., Summers, R., Kemp, W.M. 1995. Inputs, transformations, and transport of N and P in Chesapeake Bay and selected tributaries. *Estuaries* 18: 285-314
- Carlsson, P, Granéli, E, Segatto, AZ (1999) Cycling of biologically available nitrogen in riverine humic substances between marine bacteria, a heterotrophic nanoflagellate and a photosynthetic dinoflagellate. *Aquatic Microbial Ecology* 18:23-36
- Carpenter, S. R., N. F. Caraco, D. L. Correll, R. W. Howarth, A. N. Sharpley, and V. H. Smith. 1998. Nonpoint pollution of surface waters with phosphorus and nitrogen. *Ecological Applications* 8:559-568.
- Crossett, K., Culliton, T.J., Wiley, P., Goodspeed, T.R. 2004. Population trends along the coastal United States, 1980-2008. Silver Spring, Maryland: National Oceanic and Atmospheric Administration, 47 p.
- Gárrate-Lizárraga, I., D.J. Lopez-Cortes, J.J. Bustillis-Guzrnan, and R.Hernandez-Sandoval. 2004. Blooms of *Cochlodinium polykrikoides* (Gymnodiniaceae) in the Gulf of California, Mexico. *Revista de Biología Tropical* 52: 51–58.

- Glibert, P.M., Magnien, R., Lomas, M.W., Alexander, J., Fan, C., Haramoto, E., Trice, M., Kana, T.M. 2001. Harmful algal blooms in the Chesapeake and coastal bays of Maryland, USA: comparison of 1997, 1998, and 1999 events. *Estuaries* 24(6A): 875-883
- Glibert, P, Anderson, D, Gentien, P, Granéli, E, Sellner, K (2005a) The global, complex phenomena of harmful algal blooms. *Oceanography* 18(2): 130-141
- Glibert, P, Seitzinger, S, Heil, C, Burkholder, J, Parrow, M, Codispoti, L, Kelly, V (2005b) The role of eutrophication in the global proliferation of harmful algal blooms: new perspectives and new approaches. *Oceanography* 18(2):198-209
- Glibert, P.M and Burkholder, J.M. 2006 The complex relationships between increases in fertilization of the earth, coastal eutrophication and proliferation of harmful algal blooms. In: *Ecology of Harmful Algae*, E. Granéli and J.T. Turner. (Eds.) Springer-Verlag, New York.
- Glibert, P.M. and Legrand, C. 2006. The diverse nutrient strategies of harmful algae: focus on osmotrophy. In: *Ecology of Harmful Algae*, E. Granéli and J.T. Turner. (Eds.) Springer-Verlag, New York.
- Glibert, P.M., J. Harrison, C. Heil, and S. Seitzinger. 2006. Escalating worldwide use of urea – a global change contributing to coastal eutrophication. *Biogeochemistry* 77: 441-463.
- Granéli, E. and Moreira, M.O. 1990. Effects of river water of different origin on the growth of marine dinoflagellates and diatoms in laboratory cultures. *J. Exp. Mar. Biol. Ecol.* 136(2): 89-106
- Gobler, C.J., D.L. Berry, O.R. Anderson, A. Burson, F. Koch, B.S., Rodgers, L.K. Moore, J.A. Goleski, B. Allam, P. Bowser, Y. Tang, and R. Nuzzi. 2008. Characterization, dynamics, and ecological impacts of harmful *Cochlodinium polykrikoides* blooms on eastern Long Island, NY, USA. *Harmful Algae* 7: 293–307.
- Hallegraeff, G.M. 2003. Monographs on Oceanographic methodology. Harmful Algal Blooms: A Global Overview. Paris, United Nations Educational
- Hansell, D.A., Carlson, C.A., Bates, N.R., Poisson, A.P. 1997. Horizontal export of organic matter in the Equatorial Pacific Ocean. *Deep Sea Res. I.* 45, 2115-2130.
- Heil, CA (2005) Effects of humic acids on growth, photosynthesis, and respiration of the dinoflagellate *Prorocentrum minimum*. *Harmful Algae* 4:603-613

- Heil, C.A., Glibert, P.M., Fan, C. 2005. *Prorocentrum minimum* (Pavillard) Schiller: A review of a harmful algal bloom species of growing worldwide importance. *Harmful Algae* 4(3): 449-470
- Herrera-Silveira, J.A., Ramírez-Ramírez, J. 1996. Effects of natural phenolic material (tannin) on phytoplankton growth. *Limnol. Oceanogr.* 41(5): 1018-1023.
- Ho, M.-S. and P.L. Zubkoff 1979. The effects of a *Cochlodinium heterolobatum* bloom on the survival and calcium uptake by larvae of the American oyster, *Crassostrea virginica*. In: D.L. Taylor & H.H. Selinger (eds), *Toxic Dinoflagellate Blooms*, Elsevier / North-Holland, New York: 409-412.
- Howarth, R.W., Boyer, E.W., Pabich, W.J., Galloway, J.N. 2002. Nitrogen use in the United States from 1961 – 2000 and potential future trends. *Ambio* 31(2): 88-96.
- Iwataki, M., H. Kawami, K. Mizushima, C.M. Mikulski, G.J. Doucette, J.R. Relox Jr., A. Antone, Y. Fukuyo, and K. Matsuoka. 2008. Phylogenetic relationships in the harmful dinoflagellate *Cochlodinium polykrikoides* (Gymnodiniales, Dinophyceae) inferred from LSU rDNA sequences. *Harmful Algae* 73: 271–277.
- Jeong, H.J., Y.D. Yoo, J.S. Kim, T.H. Kim, J.H. Kim, N.S. Kang, and W. Yih. 2004. Mixotrophy in the phototrophic harmful alga *Cochlodinium polykrikoides* (Dinophyceae): Prey species, the effects of prey concentration, and grazing impact. *Journal of Eukaryotic Microbiology* 51: 563–569
- Jeong, H.J., J.Y. Park, J.H. Nho, M.O. Park, J.H. Ha, K.A. Seong, C. Jeng, C.N. Seong, K.Y. Lee, and W.H. Yih. 2005. Feeding by red tide dinoflagellates on the cyanobacterium *Synechococcus*. *Aquatic Microbial Ecology* 41: 131–143.
- Kim, H.G. 1998. *Cochlodinium polykrikoides* blooms in Korean coastal waters and their mitigation. In *Harmful algae*, ed. B. Reguera, J. Blanco, M.L. Fernandez, and T. Wyatt, 227–228. Galicia: Xunta de Galicia and Intergovernmental Oceanographic Commission of UNESCO.
- Kim, C.S., Lee, S.G., Kim, H.G., Jung, J. 1999. Reactive oxygen species as causative agents in the ichthyotoxicity of the red tide dinoflagellate *Cochlodinium polykrikoides*. *J. Plankton. Res.* 21: 2105-2115.
- Kim, D., Oda, T., Muramatsu, T., Kim, D., Matsuyama, Y., Honjo, T. 2002. Possible factors responsible for the toxicity of *Cochlodinium polykrikoides*, a red tide phytoplankton. *Comp. Biochem. Physiol. C. Toxicol. Pharmacol.* 132: 415-423.

- Koroleff, F., 1983. Simultaneous oxidation of nitrogen and phosphorus compounds by persulfate. In: Grasshoff, K., Eberhardt, M., Kremling, K. (Eds.), *Methods of Seawater Analysis*. 2nd ed., Verlag Chemie, Weinheimer, FRG.
- Kudela, R.M., J.P. Ryan, M.D. Blakely, J.Q. Lane, and T.D. Peterson. 2008. Linking the physiology and ecology of *Cochlodinium* to better understand harmful algal bloom events: A comparative approach. *Harmful Algae* 7: 278–292.
- LaRoche, J, Nuzzi, R, Wyman, K, Falkowski, P, Wallace, DWR, Wallance, D. 1997. Brown tide blooms in Long island's coastal waters linked to interannual variability in groundwater flow. *Global Change Biology* 3(5): 397-410(14)
- Lee, Y.S. and Lee, S.Y. 2006. Factors affecting outbreaks of *Cochlodinium polykrioides* in coastal areas of Korea. *Mar. Pollut. Bull.* 52, 624-634.
- Levasseur, M., Thompson, P. A. and Harrison, P. J. 1993. Physiological acclimation of marine phytoplankton to different nitrogen sources. *J. Phycol.*, 29 (5):587–595.
- Mackiernan, G.B. 1968. Seasonal distribution of dinoflagellates in the lower York River, Virginia. M.A. Thesis. The College of William & Mary. Williamsburg, VA. pp. 104
- Mallin, M.A. 2000. Impacts of industrial-scale swine and poultry production on rivers and estuaries. *American Scientist* 88:26-37.
- Margalef, R., 1961. Hidrografia y fitoplancton de un area marina de la costa meridional de Puerto Rico. *Invest. Pesq.* 18, 76–78.
- Marshall, H. G. 1994. Succession of dinoflagellate blooms in the Chesapeake Bay, U.S.A. In Lassus, P., et al (eds.). *Harmful Marine Algal Blooms*. Intercept Ltd. Andover, Md. pp. 615–620.
- Meybeck, M. 1982. Carbon, nitrogen, phosphorus transport by world rivers. *Am. J. Sci.* 282: 401-450
- Mulholland, MR, Gobler, CJ, Lee, C (2002) Peptide hydrolysis, amino acid oxidation, and nitrogen uptake in communities seasonally dominated by *Aureococcus anophagefferens*. *Limnology and Oceanography* 47(4):1094-1108
- Mulholland, M.R., Morse, R.E., Boneillo, G.E., Bernhardt, P.W., Filippino, K.C., Procise, L.A., Blanco-Garcia, J.L., Marshall, H.G., Egerton, T.A., Hunley, W.S., Moore, K.A., Berry, D.L., Gobler, C.J. 2009a. Estuaries and Coasts. Understanding causes and impacts of the dinoflagellate, *Cochlodinium polykrikoides*, blooms in the Chesapeake Bay. 32(4), 734-747.

- Newman, A. 1995. Water pollution point sources still significant in urban areas. *Environmental Science and Technology* 29:114
- Officer, C.B. & Ryther, J.H. 1980. The possible importance of silicon in marine eutrophication. *Mar. Ecol. Progr. Ser.* 3, 83-91.
- Paerl, H.W. 1988. Nuisance phytoplankton blooms in coastal, estuarine and inland waters. *Limnology and Oceanography* 33: 823-847
- Paerl, HW (1997) Coastal eutrophication and harmful algal blooms: importance of atmospheric deposition and groundwater as "new" nitrogen and other nutrient sources. *Limnology and oceanography* 42:1154-1165
- Parsons, T., Maita, Y., Lalli, C., 1984. A manual of chemical and biological methods for seawater analysis. Pergamon Press, New York.
- Price, N. and Harrison, P. (1987) A comparison of methods for the measurement of dissolved urea concentrations in seawater. *Mar. Biol.* 94, 307-317.
- Pyke, C. R., R. G. Najjar, M. B. Adams, D. Breitburg, M. Kemp, C. Hershner, R. Howarth, M. Mulholland, M. Paolisso, D. Secor, K. Sellner, D. Wardrop, and R. Wood. 2008. Climate Change and the Chesapeake Bay: State-of-the-Science Review and Recommendations. A Report from the Chesapeake Bay Program Science and Technical Advisory Committee (STAC), Annapolis, MD. 59 pp.
- Reay W. 2009. Water quality within the York River estuary. *J. Coast. Res.* 57, 23-39.
- Riegman, R. 1995. Nutrient-related selection mechanisms in marine phytoplankton communities and the impact of eutrophication on the planktonic food web. *Water Science and Technology* 32 (4): 63-75
- Ryther, J.H. and Dunstan, W.M. 1971. Nitrogen, Phosphorus, and Eutrophication in the Coastal Marine Environment. *Science.* 171 (3975):1008-1013
- Seaborn, D.W., and H.G. Marshall. 2008. Dinoflagellate cysts within sediment collections from the southern Chesapeake Bay and tidal regions of the James, York, and Rappahannock rivers. *Virginia Journal of Science* 59: 135–141.
- Smayda, T.J. 1990. Novel and nuisance phytoplankton blooms in the sea: evidence for a global epidemic. *Toxic Marine Phytoplankton*. E, Sundstrom, B, Edler, L, Anderson, DM (eds) New York, Elsevier: 29-40
- Smayda, T.J. 1997. Harmful algal blooms: Their ecophysiology and general relevance to phytoplankton blooms in the sea. *Limnol. Oceanogr.* 42, 1137-1153.

- Tang, Y.Z., Gobler, C.J. 2009. Characterization of the toxicity of *Cochlodinium polykrikoides* isolates from Northeast US estuaries to finfish and shellfish. *Harmful Algae* 8: 454-462.
- Tang, Y.Z., Gobler, C.J. 2010. Allelopathic effects of *Cochlodinium polykrikoides* isolates and blooms from the estuaries of Long Island, New York, on co-occurring phytoplankton. *Mar. Ecol. Prog. Ser.* 406: 19-31.
- Tilman, D. 1977. Resource competition between planktonic algae: An experimental and theoretical approach. *Ecology* 58, 338-348.
- Vitousek, Peter M., John D. Aber, Robert W. Howarth, Gene E. Likens, Pamela A. Matson, David W. Schindler, William H. Schlesinger, and David G. Tilman. 1997. Human alteration of the global nitrogen cycle: sources and consequences. *Ecological Applications* 7:737-750.
- Vitousek, P.M. and Mooney, H.A. 1997. Estimates of coastal populations. *Science* 278: 1211-1212
- Zubkoff, P.L., Munday, J.C., Rhodes, R.G., Warriner, J.E., 1979. Mesoscale features of summer (1975 to 1977) dinoflagellate blooms in the York River, Virginia (Chesapeake Bay Estuary). In: *Toxic Dinoflagellate Blooms*. F.J.R. Taylor and H.H. Seliger (Eds.) Elsevier North Holland, Inc. New York, New York

Table 1. Nutrient concentrations present in the initial York River bloom water and the +Urban, +Soil, + Industrial nutrient sources and percent composition of the N present upon collection. Concentrations are in units of $\mu\text{mol N, P, C or Si L}^{-1}$ and ± 1 standard deviations are provided in parentheses. NO_x is defined as nitrate + nitrite.

Source type	TDN	NH_4^+	NO_x	DON	Urea	TDP	PO_4^{3-}	SiO_2	% NH_4^+	% NO_x	% DON	N:P
York River Bloom	21.6 (0.1)	0.8 (0.4)	0.3 (0.0)	20.5 (0.1)	0.1 (0.1)	n.d.	1.1 (0.0)	25.5 (0.0)	3.7	1.4	94.9	19.6*
Urban	64.0 (0.0)	18.5 (0.1)	19.0 (0.0)	26.5 (0.1)	17.1 (0.8)	1.1 (0.1)	n.d.	n.d.	28.9	29.7	41.4	58.2
Soil	159.0 (0.0)	92.5 (0.6)	51.5 (0.2)	15.0 (0.2)	n.d.	2.1 (0.1)	n.d.	n.d.	58.2	32.4	9.4	75.7
Industrial	63.0 (0.0)	2.2 (0.8)	10.5 (0.0)	50.3 (0.1)	2.5 (0.1)	3.9 (0.1)	n.d.	n.d.	3.5	16.7	79.8	16.2

* indicates the ratio was calculated from the PO_4^{3-} concentration instead of the TDP concentration.

Table 2. The maximum cellular response of the natural bloom population to anthropogenic nitrogen additions. Maximum responses were observed on day 1 for + NH₄⁺, + Urea, and + Urban treatments and on day 2 for + Soil and + Industrial treatments. Standard deviations are given in parentheses. The amount of N added to each treatment at the start of the experiment was ~18 μmol N L⁻¹.

Treatment	N drawdown (μmol N L ⁻¹)	P drawdown (μmol P L ⁻¹)	N:P drawdown molar ratio	Change in <i>C. polykrikoides</i> (cells ml ⁻¹)	Change in total community abundance (cells ml ⁻¹)	μmol N per 10 ⁵ cells (total community)	μmol P per 10 ⁵ cells (total community)
Control	-0.5 (0.1)	0.34 (0.10)	-1.2	9 (41)	29 (99)	-	-
+ NH ₄ ⁺	13.9 (0.1)	0.71 (0.05)	19.6	254 (21)	384 (35)	3.6 (0.3)	0.18 (0.02)
+ Urea	13.0 (0.2)	0.59 (0.03)	22.0	226 (117)	468 (119)	5.8 (1.5)	0.13 (0.03)
+ Urban	8.1 (0.5)	0.58 (0.03)	13.9	211 (52)	343 (66)	2.4 (0.5)	0.17 (0.03)
+ Soil	16.0 (0.1)	0.68 (0.03)	23.5	105 (140)	218 (198)	7.3 (6.7)	0.31 (0.28)
+ Industrial	3.21 (0.7)	0.45 (0.06)	7.1	63 (152)	187 (131)	1.7 (1.3)	0.24 (0.17)

Figure Legend:

Figure 1. Map of York River, VA. Locations where nutrient sources were collected are shown.

Figure 2. Nutrient concentrations are presented for each treatment: Control (solid circles), + Ammonium (NH_4^+ ; solid triangles), + Urea (solid square), + Urban run-off (+ Urban; open diamond), + Industrial run-off (+Industrial; open triangles), + Soil extract (+ Soil; open hexagon). Concentrations of total dissolved nitrogen (TDN; A), dissolved organic nitrogen (DON; B), NH_4^+ (C), urea (D), and nitrate + nitrite (NO_x^- ; E) are given in concentrations of $\mu\text{mol N L}^{-1}$. Dissolved organic carbon (DOC; F) values are in units of $\mu\text{mol C L}^{-1}$. Values are the means ± 1 standard deviation and are frequently smaller than the symbols. Note the differences in the scales of the y-axes.

Figure 3. Nutrient concentrations are presented for each treatment: Control (solid circles), + Ammonium (NH_4^+ ; solid triangles), + Urea (solid square), + Urban run-off (+ Urban; open diamond), + Industrial run-off (+Industrial; open triangles), + Soil extract (+ Soil; open hexagon). Concentrations of total dissolved phosphorus (TDP; A), phosphate (PO_4^{3-} ; C) and dissolved organic phosphorus (DOP; D) are given in concentrations of $\mu\text{mol P L}^{-1}$. Concentrations of silica are presented in units of $\mu\text{mol Si L}^{-1}$ (SiO_2 ; B). Values are the means ± 1 standard deviation and are frequently smaller than the symbols. Note the differences in the scales of the y-axes.

Figure 4. Chlorophyll *a* concentrations ($\mu\text{g L}^{-1}$) for each nutrient treatment: Control (solid circles), + Ammonium (NH_4^+ ; solid triangles), + Urea (solid square), + Urban run-off (+ Urban; open diamond), +Industrial run-off (+ Industrial; open triangles), + Soil extract (+ Soil; open hexagon). Values are the means ± 1 standard deviation, which are frequently smaller than the symbols.

Figure 5. Total average cell concentration (cells ml^{-1}) in each treatment during the 7 day bioassay including concentrations of *Cochlodinium polykrikoides* (black), other dinoflagellates (cross-hatch), and diatoms (grey).

Figure 1.

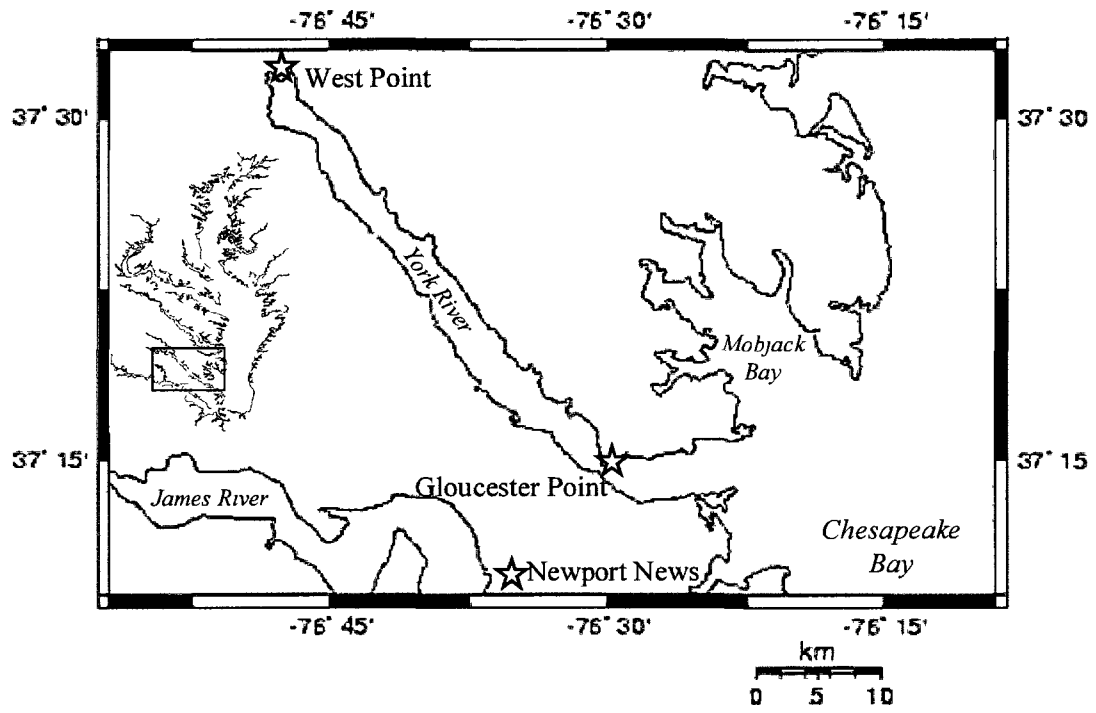


Figure 2.

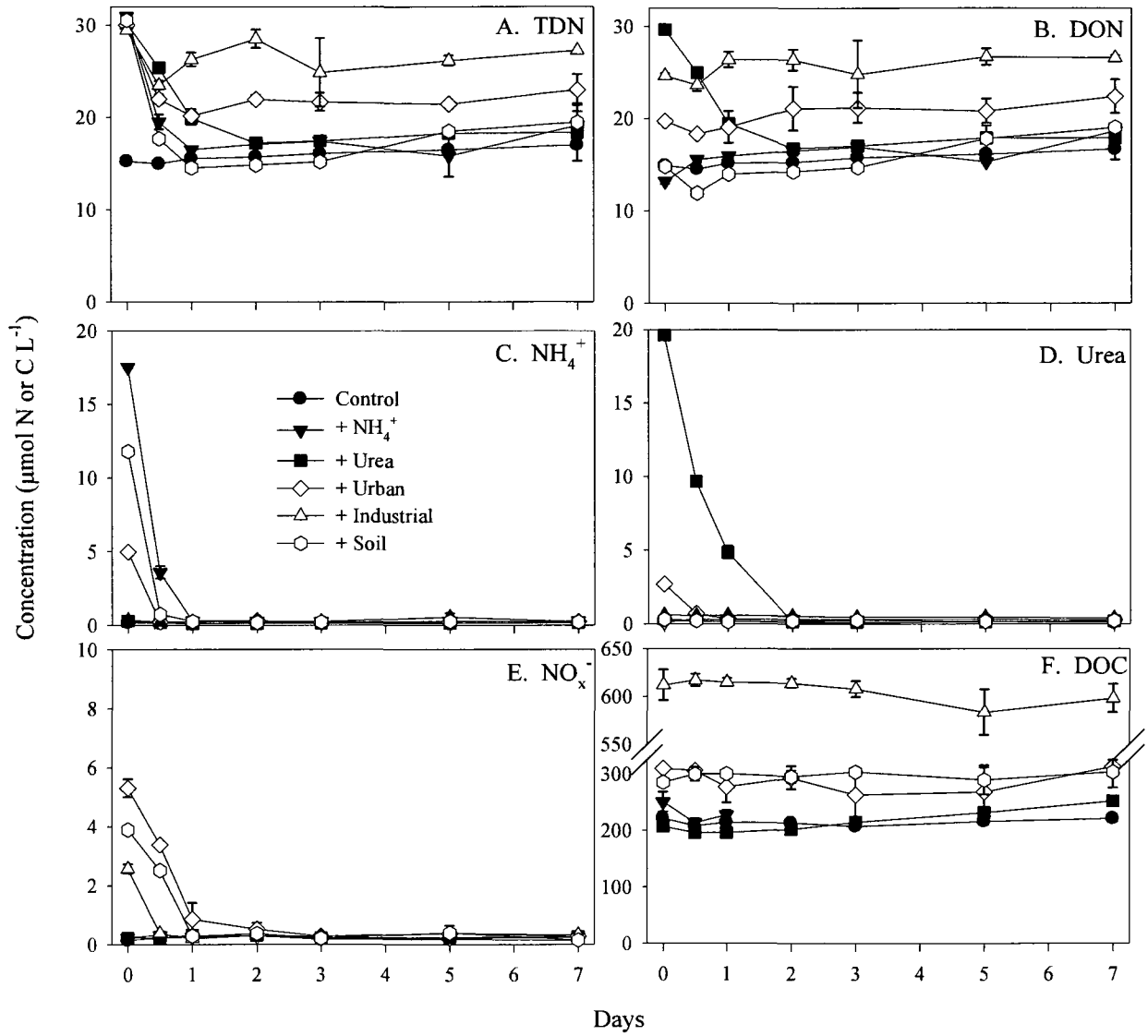


Figure 3.

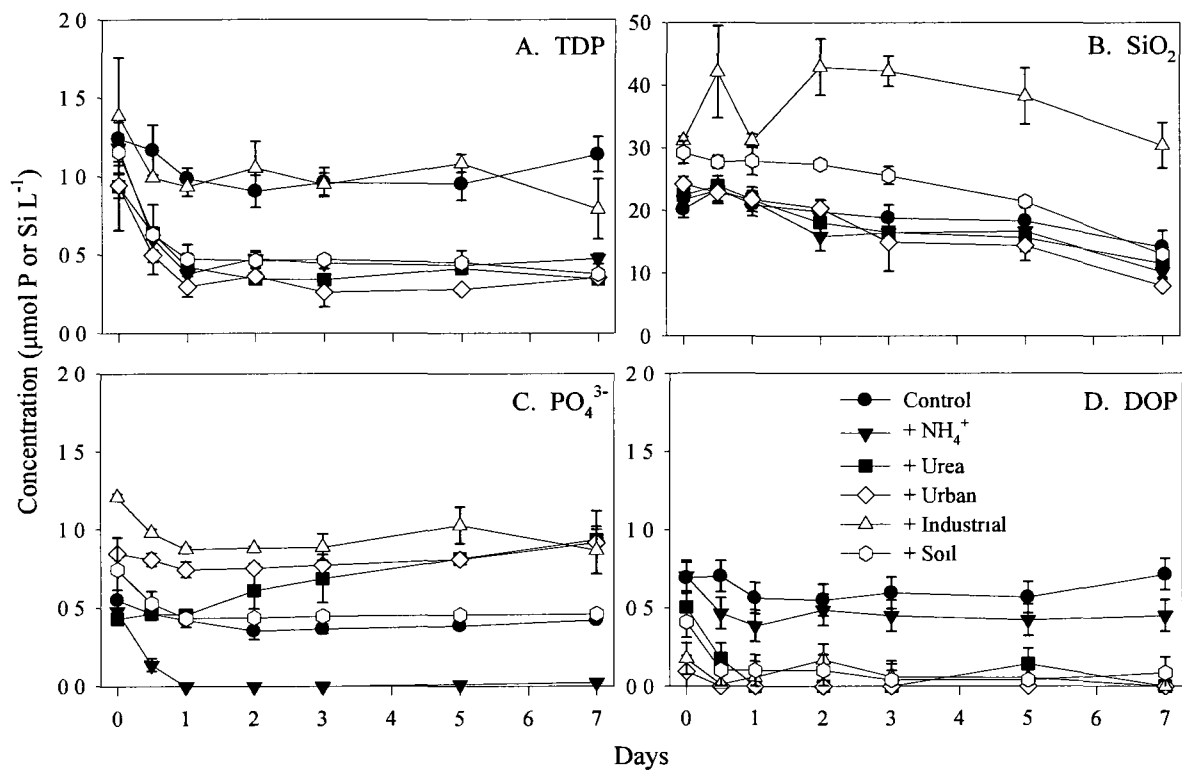


Figure 4.

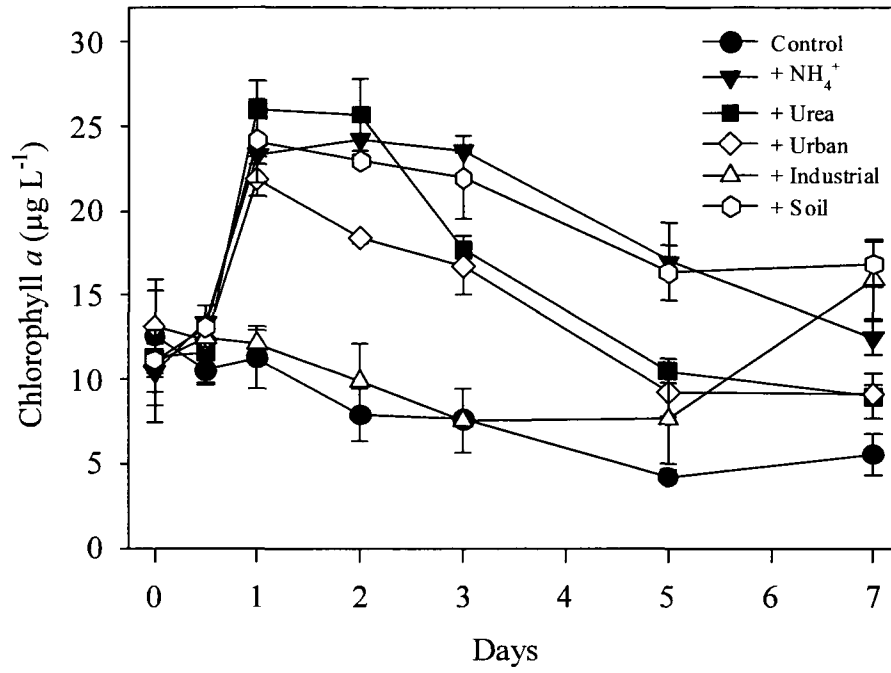
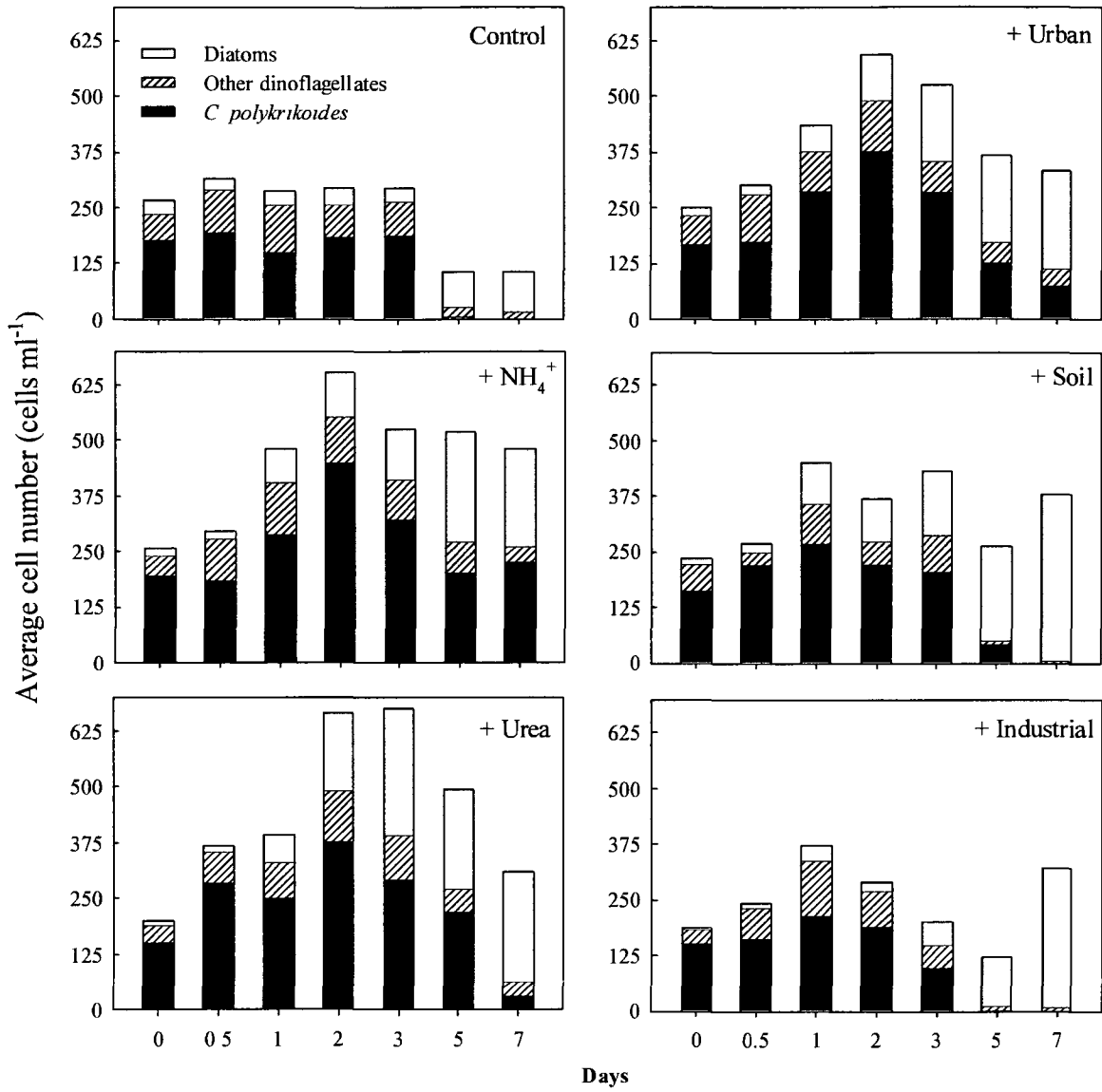


Figure 5.



CHAPTER 6

CONCLUSIONS

The intensity and frequency of harmful algal blooms (HABs) has risen dramatically over the past two decades (Hallegraeff 2003). This increase brings with it a suite of detrimental ecological, economic, and human health impacts. Anthropogenic nitrogen (N) input to coastal waterways and subsequent eutrophication has been identified as a likely trigger for bloom initiation for many HABs (Tilman 1977; Officer and Ryther 1980; Lam and Ho 1989; Smayda 1997; Glibert *et al.* 2005).

In the past, most studies of N utilization by phytoplankton have examined the dissolved inorganic species, nitrate (NO_3^-) and ammonium (NH_4^+), because dissolved organic nitrogen (DON) was thought to be refractory or utilized primarily by bacteria (reviewed in Berman and Bronk 2003). However, DON may play an important role in phytoplankton nutrition (reviewed in Berman and Bronk 2003; Bronk *et al.* 2007) and DON uptake has been observed in a number of species, including many harmful species (e.g. Lomas *et al.* 1996; Fan *et al.* 2003; Mulholland *et al.* 2004; Glibert *et al.* 2006; Herndon and Cochlan 2007). The goal of this work was to study the significance of DIN and DON to blooms of harmful phytoplankton in two distinct regions that experience frequent and persistent blooms, using a combination of stable isotopic (^{15}N) tracer techniques and nutrient bioassays. Chapters 2 and 3 focus on the Eastern Gulf of Mexico region, which experiences severe blooms of the red tide, *Karenia brevis*. Chapters 4 and 5 report on studies within the York River estuary, which experiences blooms of numerous harmful dinoflagellates, including *Cochlodinium polykrikoides* and *Alexandrium monilatum*. Finally, data from the sites and species are synthesized into an overall description of the major N sources to and utilized by HABs.

Karenia brevis in the Gulf of Mexico

Although typically considered a coastal bloom species (Smayda and Reynolds 2001), *K. brevis* can survive a variety of environments and nutrient regimes. The Eastern Gulf of Mexico, in which *K. brevis* blooms thrive, is characterized by oligotrophic conditions. Blooms of *K. brevis* are believed to initiate offshore (Steidinger 1975; Tester and Steidinger 1997) where concentrations of dissolved inorganic nitrogen (DIN) are relatively low (0.1 - 0.2 μM) (Heil *et al.* 2001) compared to concentrations of dissolved organic nitrogen (DON), which are one to two orders of magnitude higher (5-10 μM) (Heil *et al.* 2001). *Karenia brevis* may be transported by winds and tidal currents and entrained near shore by thermal and salinity fronts resulting in blooms that form due to concentration processes.

In Chapter 2, I investigated N uptake kinetics of *K. brevis* in field populations as well as with cultured strains using stable ^{15}N techniques. This study documented uptake of a variety of substrates, including ammonium (NH_4^+), nitrate (NO_3^-), urea, an amino acid (AA) mixture, the individual amino acids alanine and glutamate, and humic-N. Field experiments were conducted using populations collected from high biomass accumulations during cruises along the West Florida Shelf (WFS) in October 2001, 2002, and 2007 and the strains Jacksonville C4 (CCFWC 251) and Texas B4 (CCFWC 267), were used for the culture study. Parameters for maximum uptake velocity (V_{max}), half-saturation constant (K_s), and affinity constant (α) were determined and indicated the greatest preference for NH_4^+ followed by NO_3^- , urea, and humics.

In Chapter 3, I describe the variability in N uptake rates by *K. brevis* during a diel cycle using a variety of inorganic and organic substrates. The study included uptake rates in a field population and three cultured strains. In the field, N uptake experiments were conducted using water collected at the surface (< 1 m) and bottom (ranging from 6.5 to 9 m) of a drogue-tracked water mass containing a *K. brevis* bloom at 4 h intervals during a 25 h period. For comparison, additional experiments were performed in the laboratory using three cultured strains of *K. brevis*: Sarasota B3 (CCFWC 254), Jacksonville C4 (CCFWC 251), and Charlotte C2 (CCFWC 257). In both the field and laboratory, short-term (0.5 to 1 h) incubations were performed with ¹⁵N-labeled inorganic (NH₄⁺ and NO₃⁻) and organic substrates (urea and amino acid mixture) added at either trace (0.1 μmol N L⁻¹) or saturating (10 μmol N L⁻¹) final concentrations. There was pronounced periodicity in diel uptake rates with the maximum and minimum rates measured early in the light and dark periods, respectively. The three strains of *K. brevis* exhibited significant differences in N uptake rates as well as patterns of uptake over the diel cycle. Additionally, there was uptake variability between N substrates with the highest rates observed for NH₄⁺.

While NH₄⁺ uptake rates were the highest observed, ambient concentrations of NH₄⁺ are generally very low. This apparent paradox is a function of tight coupling between NH₄⁺ uptake and regeneration (e.g. McCarthy and Goldman 1979). When regeneration:uptake ratios are ~1, regeneration processes are likely responsible for maintaining phytoplankton populations. Rates of NH₄⁺ regeneration:uptake were 1.2 in the Chapter 3 field study, highlighting this tight coupling between the two processes. Based on the high uptake rates measured in these studies, ammonium uptake and

regeneration could be important to *K. brevis* bloom initiation as well as bloom maintenance through a variety of possible sources of regenerated NH_4^+ .

Nitrogen sources, particularly in the form of NH_4^+ , include recent release from *Trichodesmium* spp. (Mulholland *et al.* 2006; Vargo *et al.* 2008), benthic remineralization (Vargo *et al.* 2008), as well as release from decaying fish (Vargo *et al.* 2001; Walsh *et al.* 2006; Walsh *et al.* 2009). *Trichodesmium* spp. along the West Florida Shelf releases an average of 52% of recently fixed N (Mulholland *et al.* 2006), with NH_4^+ and DON being the products released in the highest concentrations (Mulholland *et al.* 2004, 2006). *Trichodesmium* spp. undergo DVM by adjusting their carbohydrate ballast (Walsby 1992) and it has been hypothesized that *K. brevis* may concurrently migrate with the *Trichodesmium* colonies and could intercept the excreted NH_4^+ and AAs (Walsh *et al.* 2006). Utilization of these concentrated transient NH_4^+ pools by *K. brevis* would be beneficial during the bloom initiation phase offshore.

Another potentially important source of N may be regenerated from populations of centric diatoms that develop at near-bottom waters during summer stratification (Heil *et al.* 2001). Their decay and subsequent remineralization combined with breakdown of thermal stratification by vertical mixing in the fall may be one source of N for bloom initiation (Vargo *et al.* 2008). Only a few benthic flux rates of NH_4^+ have been measured in the region where blooms occur with rates of $0.002 \mu\text{mol N L}^{-1} \text{d}^{-1}$ (Marinelli *et al.* 1998) and $0.1 \mu\text{mol N L}^{-1} \text{d}^{-1}$ (Darrow *et al.* 2003). In more recent work, it was determined that at the 10 m isobath, sediment flux could supply of 3-7% of the N requirement of *K. brevis* (K. Dixon, personal communication)

On-going work with *K. brevis* includes quantifying utilization of potentially important regenerated N sources, including nutrients released from decaying fish. With millions of fish often perishing during *K. brevis*-induced fish kills, this is potentially a very important N and P source for maintaining high bloom densities, particularly when blooms and fish are trapped within coastal bays and inlets (Vargo *et al.* 2001; Walsh *et al.* 2006; Walsh *et al.* 2009). Some of my preliminary experiments have demonstrated high (mM) nutrient concentrations released from fish (Appendix 2, 3), and making the connection between those high release rates and direct uptake by *K. brevis* is the next step in understanding the complex nutrient sources that keep *Karenia* blooming.

Harmful algal blooms in the York River

In contrast to the Eastern Gulf of Mexico, the York River system is considerably more eutrophic. Poor water clarity is a persistent problem due to high suspended sediments and phytoplankton biomass (Dauer *et al.* 2005). Harmful algal blooms are frequent occurrences in the late summer and early fall. The dominant bloom-forming species in the York River, *C. polykrikoides* and *A. monilatum*, are considered near-shore/estuarine species, although, both *C. polykrikoides* and *A. monilatum* may also be in the Gulf of Mexico in the same nearshore habitats in which *K. brevis* may be located. Land use in the York River watershed is predominantly rural, with developed lands only covering ~5% of the watershed (Chesapeake Bay watershed profiles: <http://www.chesapeakebay.et>). However, with population increases projected in the

coming years, the York River system may become even more eutrophic due to anthropogenic nutrient input and land use conversion.

In Chapter 4, I examined seasonal variations in ambient conditions and N uptake rates at three stations from 2007-2009 in the York River, Virginia. Uptake of inorganic and organic N sources was examined within a variety of phytoplankton assemblages, including blooms dominated by *A. monilatum*. The goals of this study were to measure a suite of parameters spatially and temporally in the York River including: 1) ambient nutrient concentrations, 2) uptake rates of NH_4^+ , NO_3^- , NO_2^- , urea, an AA mixture, and ^{13}C -bicarbonate in three size fractions (GF/F, $> 5 \mu\text{m}$, and $5 \mu\text{m} - 0.2 \mu\text{m}$), and 3) NH_4^+ and NO_3^- regeneration rates. Spatially, results exhibited a trend of decreasing nutrient concentrations from the upriver (Clay Bank) to downriver (Gloucester Point and Goodwin Islands) sites. High nutrient concentrations of DIN were measured in late August and early September 2008, with NH_4^+ and NO_2^- concentrations reaching 9.9 and 7.6 $\mu\text{mol N L}^{-1}$, respectively. Ratios of DIN:DIP indicated the York River was relatively N-limited during the summer and fall and P-limited in the winter and spring. High rates of NH_4^+ and NO_3^- regeneration were measured via isotope dilution and often exceeded uptake rates, which likely contribute to the high N concentrations observed. Overall, the pattern of uptake at each station was $\text{NH}_4^+ > \text{NO}_3^- > \text{urea} > \text{AA} > \text{NO}_2^-$ in the GF/F fraction, however, urea uptake was often utilized at equal or greater rates than NO_3^- in the $> 5 \mu\text{m}$ and $5 \mu\text{m} - 0.2 \mu\text{m}$ size fractions. Ammonium had the highest uptake rates at all stations, for all size fractions and for all seasons, ranging from 34 to 80% of total absolute uptake. Rates of N uptake in *A. monilatum* blooms are the first reported for this species in the York River estuary, and demonstrate uptake of a diverse suite of inorganic and

organic substrates, dominated by NH_4^+ . A preference for NH_4^+ has been documented in other Chesapeake Bay studies (McCarthy *et al.* 1977; Carpenter and Dunham 1985; Bradley *et al.* 2010). McCarthy *et al.* (1977) documented that NH_4^+ remained the preferred N form used, throughout Chesapeake Bay multiple experiments along a Chesapeake bay transect, even when NO_3^- accounted for >90% of N nutrient. Again, the dominance of NH_4^+ uptake is similar to what was observed for *K. brevis* in the Eastern Gulf of Mexico.

Based on my results from this study I presented a series of events that likely occur spatially and temporally in the river to initiate and maintain HABs. In the summer, high concentrations of nutrients, particularly NH_4^+ and NO_2^- were observed, particularly at Clay Bank. One source of this N is likely benthic regeneration of organic matter from the previous spring bloom. In fact, Sin *et al.* (1999) found that summer NH_4^+ concentrations at a station located slightly downriver of Gloucester Point was correlated with spring Chl *a*, from the previous year, located in the middle mesohaline region of the river. Ammonium and phosphorus are regenerated and released from the sediment in the bottom waters as the water column becomes stratified. Then tidal destratification may supply benthic-regenerated nutrients into the surface water and replenish oxygen in the deep water in the lower York River (Webb and D'Elia 1980). Additionally, storm events, which are common during the summer, may aid in stirring up nutrients from the bottom (Bradley *et al.* 2010). It has been calculated that the shoal sediments in Gloucester Point released sufficient NH_4^+ and PO_4^{3-} to supply 36 and 75% of N and P, respectively (Rizzo *et al.* 1990). High concentrations of NH_4^+ then get oxidized to NO_2^- and NO_3^- via nitrification. Particularly upriver at Clay Bank, a combination of high turbidity coupled

with light limitation due to suspended sediment may inhibit phytoplankton growth and accumulation of biomass. If sufficient phytoplankton biomass is not present to take up the N, it may accumulate in the water column and be transported further downstream. A combination of physical conditions as well as the high N concentrations may stimulate *A. monilatum* and co-occurring phytoplankton, including other harmful species.

Understanding triggers to bloom formation in the York River is important as future blooms will likely occur, due to *A. monilatum*'s ability to form benthic resting cysts, which can seed future populations.

Because conditions in estuaries can change so quickly, future work would include much more frequent sampling of the York River, particularly during the summer months to get better resolution of the spatial and temporal variability of nutrients as well as uptake rates. Most bloom studies examine nutrient dynamics once a visible bloom is already apparent and miss that critical phase of bloom initiation, therefore more work is necessary to examine nutrient and physical conditions that lead up to bloom events. Additional work would also involve examining the nutrient conditions under which *A. monilatum* cells undergo encystment and excystment processes and quantifying cyst concentrations within York River sediments, as that information is key to understanding bloom initiation and decline. Finally, in addition to nutrient enrichment, physical forcings, such as riverine flow, circulation and vertical mixing play an important role in the development, extent and persistence of HABS (Sellner *et al.* 2003; GEOHAB 2006). Further investigation of the physical conditions in the York River would aid in the understanding of overall bloom dynamics

In Chapter 5, I investigated the role anthropogenic N inputs and eutrophication play in stimulating HABs in the York River, dominated by the dinoflagellate, *C. polykrikoides*. Three different anthropogenic N sources were used to assess their role in exacerbation of a HAB including: Urban parking lot run-off (+ Urban), soil from a construction site (+ Soil) and run-off from a local paper mill (+ Industrial). These anthropogenic N sources were added to a natural bloom assemblage during a 7 day bioassay and additional treatments of NH_4^+ and urea represented comparative inorganic and organic N sources, respectively. Results indicated that each anthropogenic source had a unique N composition; DIN comprised ~9%, 91% and 20% of + Urban, + Soil, and + Industrial, respectively. All N sources stimulated the growth of phytoplankton with the + Urban and + Soil treatments eliciting the greatest response, a doubling in Chl *a* and/or cell concentrations along with nutrient drawdown of both DIN and DON within two days. The + Industrial treatment did not exhibit significant changes in HAB biomass, indicating either the organic dominated pool was relatively refractory or the phytoplankton were influenced by the inhibitory nature of tannins that were likely present. Shifts in community composition were observed over time, switching from dinoflagellate to diatom dominated assemblages, particularly in the + Soil and + Industrial treatments. By day 7, >95% of total cell abundance were diatoms in those treatments. Results of this study indicate that phytoplankton in the York River may be exacerbated by anthropogenic N inputs

The York River watershed is currently relatively rural compared to other Chesapeake Bay tributaries, however, human populations continue to increase in the region. Increasing populations and the associated land use shifts that accompany them

may significantly impact the delivery of N and P to the York River and exacerbate its already degraded conditions. Considering the ability of *C. polykrikoides* to form benthic resting cysts that seed future populations, as well as the history of blooms in the York River, future bloom events are likely to occur. Not investigated here, but important work for the future is how the toxicity of *C. polykrikoides* and other York River HAB species may vary depending on nutrient status and availability. It is unclear whether *C. polykrikoides* toxicity may vary dependent on the inorganic or organic N or P species present but this may have important ramifications for the health of finfish and shellfish in the York River.

Determining the variable composition of DOM and the extent to which it is contributing to eutrophication within coastal environments is another challenging and important issue. While in our study there were few changes in the measured bulk DON and DOP pools over times, it is highly likely that the constituents of the pools were being utilized, but on an individual compound level. One relatively new and useful tool aiding this quest is the application of electrospray ionization mass spectrometry (ESI-MS). ESI-MS allows natural mixtures of DOM to be examined at the compound level and makes it possible to determine if individual compounds are used or modified by organisms (Seitzinger *et al.* 2005; Sipler and Seitzinger 2008). Future analysis of the complex chemical compositions of anthropogenic DOM sources and their utilization by microbes using ESI-MS will be useful to understanding organic matter cycling within estuaries and HABs and this work is ongoing in our lab.

Phytoplankton blooms are a function of a decoupling between bottom-up (i.e. nutrient supply) and top-down processes (i.e. grazing). The goal of this work was to focus on the bottom-up or nutrient controls on phytoplankton growth and bloom dynamics in two regions that experience frequent and often severe HABs. The significance of DIN, as well as DON, to phytoplankton physiology was examined. From this research, I can draw three primary conclusions between the HAB species and systems studied.

First, the harmful algae examined have flexible metabolisms. The research demonstrated that the HAB species were able to utilize all inorganic and organic species offered during N uptake experiments (Chapters 2, 3, 4) and draw down a suite of N sources in the bioassay (Chapter 5). This flexible metabolism is likely one of the reasons for the success of the HAB species examined over co-occurring phytoplankton. Frequently, the N uptake affinity of dinoflagellates is lower than the affinity of co-occurring phytoplankton, particularly diatoms, and utilization of DON and particulate N may be a strategy to offset that ecological disadvantage (Smayda 1997). Opportunistic phytoplankton such as the harmful algae examined here may be favored over species that are more adapted to utilize a particular substrate. Harmful algae from both the Gulf of Mexico and York River were able to utilize every substrate offered, including more complex molecules (i.e. humics) in the case of *K. brevis*. The humic uptake results presented in Chapter 2 are the first measured for *K. brevis* using the ^{15}N tracer approach. Additionally, N uptake rates had not previously been examined for *A. monilatum*, particularly in the York River and this research provides the first measured rates of N uptake.

Secondly, NH_4^+ uptake dominated in both systems and for all species examined. While all inorganic and organic substrates offered were taken up, rates of NH_4^+ far surpassed uptake of other substrates. Both kinetic (Steidinger 1998; Bronk *et al.* 2004; Killberg-Thoreson Chapter 2) and tracer uptake studies of *K. brevis* in the light and dark (Killberg-Thoreson Chapter 3) have found a high affinity for NH_4^+ as compared to other substrates. For *A. monilatum*, a high V_{max} value for NH_4^+ as well as tracer uptake rates were presented in Chapter 4. The dominance of NH_4^+ as a favored N source is not surprising given that the molecule is already in a reduced form, is energetically efficient for cells to use, and requires little additional energy to be assimilated (reviewed in Mulholland and Lomas 2008). What is an interesting, and unexpected, observation from this research is the similarity in nutrient uptake given the distinct regions examined, unique species characteristics, and bloom dynamics of each species. The experiments demonstrate that the species investigated may not have as unique nutrient preferences as previously hypothesized, rather, NH_4^+ is preferred in all cases.

Third, NH_4^+ regeneration rates documented in the studies were high, often meeting or exceeding rates of uptake, making regeneration an important process for maintaining large biomass accumulations. Nitrogen limited systems, are typically dominated by nutrient recycling (McCarthy *et al.* 1977; Stanley and Hobbie 1981; Collos *et al.* 2003), with regeneration of reduced N forms often exceeding autotrophic uptake (Bronk *et al.* 1998; Smith and Kemp 1995). Prior to bloom formation in the York River, regeneration greatly exceeded uptake, allowing N pools to form, and provided a large source of N for phytoplankton growth and bloom development. Ratios of regeneration:uptake in both the Eastern Gulf of Mexico and York River neared one

during blooms and demonstrated the tight coupling of the two processes and that regeneration processes contribute to the maintenance of phytoplankton populations. While regeneration appears to be a key process in both regions, the source of regenerated N varies. In the Gulf of Mexico, regenerated N likely results from benthic sources, release from recent fixation by *Trichodesmium* or from dead fish decay, however, in the York River regeneration is likely dominated by benthic remineralization. Overall *in situ* processes appear to be key to sustaining blooms.

In conclusion, HAB-nutrient relationships are often complex and understanding the connection between human impacts, nutrient dynamics, and the physiology of harmful phytoplankton is important to understanding overall bloom proliferation. This study provided insight into the N uptake dynamics by HABs in two regions. It documented N uptake rates for inorganic and organic N species, some of which had not been previously investigated. Overwhelmingly the results indicate that HABs have flexible metabolisms, with uptake dominated by the inorganic substrate NH_4^+ , which at times was produced by high rates of *in situ* regeneration.

Literature Cited

- Berman, T. and Bronk, D.A. 2003. Dissolved organic nitrogen: a dynamic participant in aquatic ecosystems. *Aquat. Microb. Ecol.* 31, 279-305.
- Bradley, P.B., Lomas, M.W., Bronk, D.A. 2010. Inorganic and organic nitrogen use by phytoplankton along Chesapeake Bay, measured using a flow cytometric sorting approach. *Estuaries and Coasts.* 33: 971-984.
- Bronk, D. A., Sanderson, M.P., Mulholland, M.R., Heil, C.A., O'Neil, J.M. 2004. Organic and inorganic nitrogen uptake kinetics in field populations dominated by *Karenia brevis*. In: Steidinger, K. A., J. H. Landsberg, C. R. Tomas and G. A. Vargo [Eds.], *Harmful Algae 2002*. Florida Fish and Wildlife Conservation Commission, Florida Institute of Oceanography and Intergovernmental Oceanographic Commission of UNESCO, St. Petersburg, Florida, USA. pp. 80-82.
- Bronk, D.A., See, J.H., Bradley, P., Killberg, L. 2007. DON as a source of bioavailable nitrogen for phytoplankton. *Biogeosciences* 4, 238-296.
- Carpenter, E. J., Dunham, S. (1985). Nitrogenous nutrient uptake, primary production and species composition of phytoplankton in the Carmans River estuary, Long Island, N.Y. *Limnol. Oceanogr.* 30:513-526
- Collos, Y., Vaquer, A., Bibent, B., Souchu, P., Slawyk G., Garcia, N. 2003. Response of coastal phytoplankton to ammonium and nitrate pulses: seasonal variations of nitrogen uptake and regeneration, *Aquat. Ecol.* 37: 227-236.
- Darrow, B.P., Walsh, J.J., Vargo, G.A., Masserini, R.T., Fanning, K.A., Zhang, J.-Z, (2003). A simulation study of the growth of benthic microalgae following the decline of a surface phytoplankton bloom. *Cont. Shelf. Res.* 23:1265-1283.
- Dauer, D., Marshall, H., Donat, J., Lane, M., Morton, P., Doughten, S., Hoffman, F. 2005. Status and trends in water quality and living resources in the Virginia Chesapeake Bay: York River (1985-2004). Final Report. Virginia Department of Environmental Quality, Richmond, VA. 63 pp
- D'Elia, C.F., Sanders, F.J., Boynton, W.R. 1986. Nutrient enrichment studies in a coastal plain estuary: phytoplankton growth in large scale, continuous cultures. *Can. J. Fish. Aquat. Sci.* 43, 397-406.
- Fan, C., Glibert, P., Burkholder, J. 2003. Characterization of the affinity for nitrogen, uptake kinetics, and environmental relationships for *Prorocentrum minimum* in natural blooms and laboratory cultures. *Harmful Algae.* 2, 283-299.

- GEOHAB, 2006. Global ecology and oceanography of harmful algal blooms. In: Glibert, P. (Ed.), *Global Ecology and Oceanography of Harmful Algal Blooms: Eutrophication and HABs*. IOC and SCOR, Paris and Baltimore. 74 pp.
- Glibert, P., Seitzinger, S., Heil, C., Burkholder, J., Parrow, M., Codispoti, L., Kelly, V. 2005. The role of eutrophication in the global proliferation of harmful algal blooms: new perspectives and new approaches. *Oceanography* 18(2), 198-209.
- Glibert, P.M., Burkholder, J.M., Parrow, M.W. Lewitus, A.J., Gustafson, D.E. 2006. Direct uptake of nitrogen by *Pfiesteria piscicida* and *Pfiesteria shumwayae*, and nitrogen nutritional preferences. *Harmful Algae* 5(4), 380-394
- Hallegraeff, G.M. 2003. *Monographs on Oceanographic methodology. Harmful Algal Blooms: A Global Overview*. Paris, United Nations Educational
- Heil, C., Vargo, G., Spence, D., Neely, M., Merkt, R., Lester, K., Walsh, J. 2001 Nutrient stoichiometry of a *Gymnodinium breve* Davis (Gymnodiniales: Dinophyceae) bloom: What limits blooms in oligotrophic environments? In: Hallegraeff, G.M., Blackburn, S.I., Bolch, C.J., Lewis, R.J. (Eds.) *Harmful Algal Blooms 2000*, Intergovernmental Oceanographic Commission of UNESCO 2001, Paris, pp.165-168.
- Herndon, J., and W.P. Cochlan. 2007. Nitrogen utilization by the raphidophyte *Heterosigma akashiwo*: growth and uptake kinetics in laboratory cultures. *Harmful Algae* 6: 260-270.
- Juhl, A.R. 2005. Growth rates and elemental composition of *Alexandrium monilatum*, a red-tide dinoflagellate. *Harmful Algae*. 4: 287-295.
- Lam, C.W.Y. & Ho, K.C. 1989. Red Tides in Tolo Harbour, Hong Kong. *Red Tides: Biology, Environmental Science*. Okaichi, T., Anderson, D.M., Nemoto, T. [Eds.] Elsevier Science, 49-52
- Lomas, M, Glibert, P, Berg, G. 1996. Characterization of nitrogen uptake by natural populations of *Aureococcus anophagefferens* (Chrysophyceae) as a function of incubation duration, substrate concentration, light, and temperature. *Journal of Phycology* 32:907-916
- Marinelli, R.L. Jahnke, R.A., Cravens, D.B., Nelson, J.B., Eckman, J.E. 1998. Sediment nutrient dynamics on the South Atlantic Bight continental shelf. *Limnol. Oceanogr.* 43:1305-1320.
- McCarthy, J.J., W.R. Taylor, and J.L. Taft. 1977. Nitrogenous nutrition of the plankton in the Chesapeake Bay. 1. Nutrient availability and phytoplankton preferences. *Limnol. Oceanogr.* 22: 996–1011.

- McCarthy, J.J. & Goldman, J.C. 1979. Nitrogenous nutrition of marine phytoplankton in nutrient-depleted waters. *Science* 23:670-672.
- Mulholland, M.R., Boneillo, G., Minor, E. 2004. A comparison of N and C uptake during brown tide (*Aureococcus anophagefferens*) blooms from two coastal bays on the east coast of the USA. *Harmful Algae* 3, 361-376.
- Mulholland, M.R. & Lomas M.W. 2008. Nitrogen uptake and assimilation. In Capone, D. Bronk, D., Mulholland, M., Carpenter, E. [Eds.] *Nitrogen in the marine environment*. Academic Press. New York, USA. pp. 303-384.
- Mulholland, M.R., Bernhardt, P.W., Heil, C.A., Bronk, D.A., O'Neil, J.M. 2006. Nitrogen fixation and release of fixed nitrogen by *Trichodesmium* spp. in the Gulf of Mexico. *Limnol. Oceanogr.* 51(4), 1762-1776.
- Officer, C.B. & Ryther, J.H. 1980. The possible importance of silicon in marine eutrophication. *Mar. Ecol. Progr. Ser.* 3, 83-91.
- Rizzo, W.M. 1990 Nutrient exchanges between the water column and a subtidal benthic microalgal community. *Estuaries and Coasts* 13:219-226.
- Sellner, K.G, Doucette, G.J., Kirkpatrick, G.J. 2003. Harmful algal blooms: causes, impacts and detection. *J. Indust. Microb. Biotech.* 30(7):383-406
- Seitzinger, S.P., Hartnett, H., Lauck, R., Mazurek, M., Minegishi, T., Spyres, G., Styles, R. 2005. Molecules-level chemical characterization and bioavailability of dissolved organic matter in stream water using electrospray-ionization mass spectrometry. *Limnology and Oceanography* 50: 1-12
- Sin, Y., Wetzel, R., Anderson, I. 1999. Spatial and temporal characteristics of nutrient and phytoplankton dynamics in the York River estuary, Virginia: analyses of long-term data. *Estuaries.* 22(2A): 260-275.
- Sipler, R.E. and Seitzinger, S. 2008. Use of electrospray ionization (ESI) mass spectrometry to investigate complex dissolved organic matter (DOM) and its potential applications in phytoplankton research. *Harmful Algae* 8: 182-187
- Smayda, T.J. 1997. Harmful algal blooms: Their ecophysiology and general relevance to phytoplankton blooms in the sea. *Limnol. Oceanogr.* 42, 1137-1153.
- Smayda, T.J. and Reynolds, C. 2001. Community assembly in marine phytoplankton: Application of recent models to harmful dinoflagellate blooms. *J. Plankt. Res.* 23, 447-461.

- Smith, E.M., and W.M. Kemp. 1995. Seasonal and regional variations in plankton community production and respiration for Chesapeake Bay. *Mar. Ecol. Prog. Ser.* 116: 217–231.
- Stanley, D. W., and J. E. Hobbie. 1981. Nitrogen recycling in a North Carolina coastal river. *Limnol. Oceanogr.* 26:30-42.
- Steidinger, K.A. 1975. Basic factors influencing red tides. In: V.R. LoCiero (Ed.), *Proceedings of the First International Conference on Toxic Dinoflagellate Blooms*, Massachusetts Science and Technology Foundation, Wakefield, Massachusetts. pp. 153-162.
- Steidinger, K.A., Vargo, G.A., Tester, P.A., Tomas, C.R., 1998. Bloom dynamics and physiology of *Gymnodinium breve* with emphasis on the Gulf of Mexico. In: Anderson, D.M., Cembella, A.D., Hallegraef, G.M. (Eds.). *Physiological ecology of harmful algal blooms*. Springer-Verlag, Berlin, pp. 133-153.
- Tester, P.A., Steidinger, K.A. 1997. *Gymnodinium breve* red tide blooms: Initiation, transport, and consequences of surface circulation. *Limnol. Oceanogr.* 42, 1039-1051.
- Tilman, D. 1977. Resource competition between planktonic algae: An experimental and theoretical approach. *Ecology* 58, 338-348.
- Vargo, G.A., Heil, C.A., Fanning, K.A., Dixon, L.K., Neely, M.B., Lester, K., Ault, D., Murasko, S., Havens, J., Walsh, J., Bell, S. 2008. Nutrient availability in support of *Karenia brevis* blooms on the West Florida Shelf: What keeps *Karenia* blooming? *Cont. Shelf Res.* 28, 73-98.
- Walsby, A.E. 1992. The gas vesicles and buoyancy of *Trichodesmium*. In Carpenter, E.J., Capone, D.G., Rueter, J.G. [Eds.] *Marine Pelagic Cyanobacteria: Trichodesmium and Other Diazotrophs*. Kluwer, Dordrecht, pp. 141–161
- Walsh, J.J., Joliff, J.K., Darrow, B.P., Lenes, J.M., Milroy, S.P., Dieterle, D.A., Cerder, K.L., Chen, F.R., Vargo, G.A., Weisberg, R.H., Fanning, K.A., Muller-Karger, F.E., Steidinger, K.A., Heil, C.A., Prospero, J.S., Lee, T.N., Kirkpatrick, G.J., Whittedge, T.E., Stockwell, D.A., Tomas, C.R., Villareal, T.A., Jochens, A.E., Bontempi, P.S. 2006. Red tides in the Gulf of Mexico: where, when, and why? *J. Geophys. Res.* 111, C11003.

- Walsh, J.J., Weisberg, J.M., Lenos, J.M., Chen, F.R., Dieterle, D.A., Zheng, L., Carder, K.L., Vargo, G.A., Havens, J.A., Peebles, E., Hollander, D.J., He, R., Heil, C.A., Mahmoudi, B., Landsberg, J.H. (2009) Isotopic evidence for dead fish maintenance of Florida red tides, with implications for coastal fisheries over both source regions of the West Florida shelf and within downstream waters of the South Atlantic Bight. *Prog. Oceanogr.* 80, 51-73
- Webb, K.L. and D'Elia, C.F. 1980. Nutrient and oxygen redistribution during a spring neap tidal cycle in a temperate estuary. *Science.* 207:983-985.

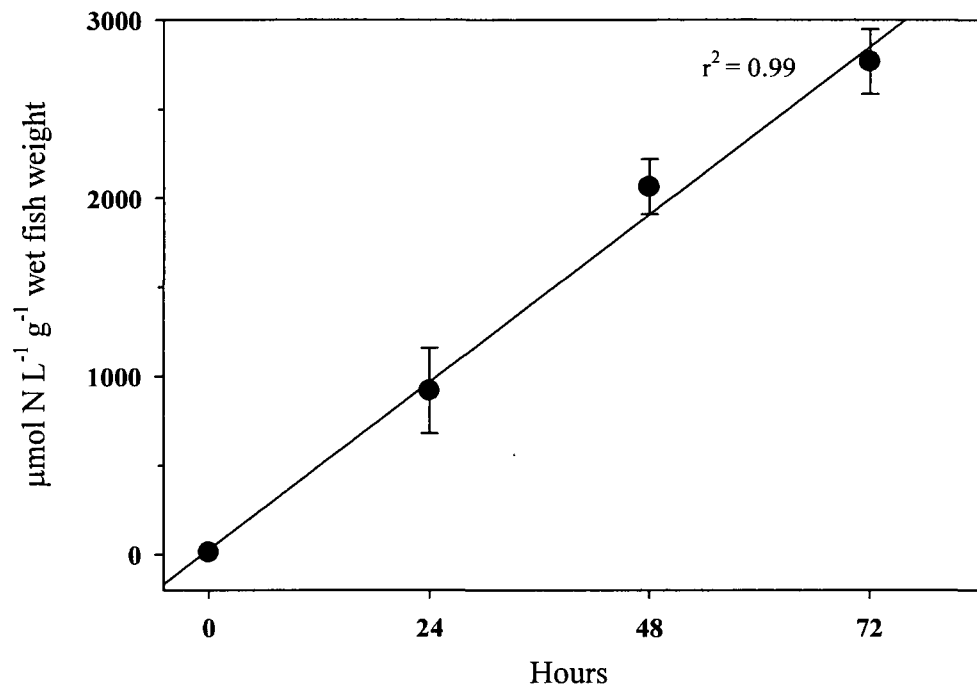
APPENDIX 1.

Calculated absolute uptake kinetics parameters of ammonium (NH_4^+), nitrate (NO_3^-), urea, an amino acid (AA) mixture, individual amino acids (alanine and glutamate), and humics by field populations and cultures of *Karenia brevis*. Ambient concentrations are in units of $\mu\text{mol N L}^{-1}$. Maximum specific rates of uptake (V_{max}) are reported in units of $\mu\text{mol N L}^{-1} \text{ h}^{-1}$ and half-saturation constants (K_s) are reported in $\mu\text{mol N L}^{-1}$. Standard error (SE) for estimates of both V_{max} and K_s parameters are given in parentheses. Substrate affinity ($\alpha = V_{max} / K_s$) is reported in units of $(\mu\text{mol N L}^{-1} \text{ h}^{-1}) / (\mu\text{mol N L}^{-1})$. n = the number of data points used in each curve. Concentrations below the limit of detection are labeled b.d. while concentrations not determined are labeled n.d. For uptake kinetics that were linear functions of concentration the equation of the line is provided. The symbol ^a denotes curves that were originally non-saturating but were re-plotted to a maximum concentration of $50 \mu\text{mol N L}^{-1}$, from which Michaelis-Menten parameters were calculable. Germanium (Ge) was added to the October 28, 2002 experiment to inhibit growth of co-occurring diatoms.

Substrate	Experiment	Ambient conc.	V_{max}	K_s	α	r^2	n
NH ₄ ⁺	2 October, 2001	0.042	0.33 (0.01)	0.30 (0.04)	1.10	0.97	10
	4 October, 2001	0.149	0.48 (0.01)	0.41 (0.02)	1.17	1.00	10
	28 October, 2002 (Ge added)	0.051	0.36 (0.01)	0.41 (0.04)	0.87	0.98	10
	18 October, 2007	0.202	1.61 (0.22)	0.63 (0.30)	2.55	0.70	9
	22 October, 2007	0.351	4.16 (0.13)	1.78 (0.16)	2.33	0.99	9
	Jacksonville C4 (CCFWC 251)	0.064	5.03 (0.34)	0.32 (0.11)	15.7	0.79	9
	Texas B4 (CCFWC 267)	0.168	7.63 (0.06)	0.76 (0.31)	19.0	0.73	8
	Culture incubated in field, 2002	0.042	0.03 (0.00)	0.50 (0.17)	0.06	0.64	8
	Average for this study		2.45 (0.43)	0.64 (0.51)	5.35		
NO ₃ ⁻	2 October, 2001	0.050	0.15 (0.01) ^a	0.18 (0.06) ^a	0.83	0.77	8
	4 October, 2001	0.001	0.20 (0.01) ^a	0.50 (0.13) ^a	0.40	0.94	7
	18 October, 2007	0.106	0.36 (0.02)	0.37 (0.09)	0.97	0.97	8
	22 October, 2007	0.091	0.60 (0.04)	0.50 (0.12)	1.2	0.92	9
		Average for this study		0.33 (0.05)	0.38 (0.21)	0.85	
Urea	28 October, 2002 (Ge added)	0.182	3.78 (0.51)	1.58 (0.97)	2.39	0.77	6
	18 October, 2007	0.326	0.62 (0.05)	0.63 (0.22)	0.96	0.62	9
		Average for this study	2.20 (0.51)	1.10 (0.99)	1.68		
AA mix	18 October, 2007	0.077	y = 0.0033x + 0.0187				7
Alanine	28 October, 2002 (Ge added)	0.011	y = 0.3467x + 4.9934				6
Glutamate	2 October, 2001	b.d.	y = 0.1434x + 3.145				7
Humic	18 October, 2007	n.d.	9.15 (1.94)	33.3 (11.4)	0.27	0.98	9
	22 October, 2007	n.d.	2.32 (0.85)	2.85 (2.84)	0.81	0.81	8
		Average for this study	5.74 (2.12)	18.1 (11.7)	0.54		

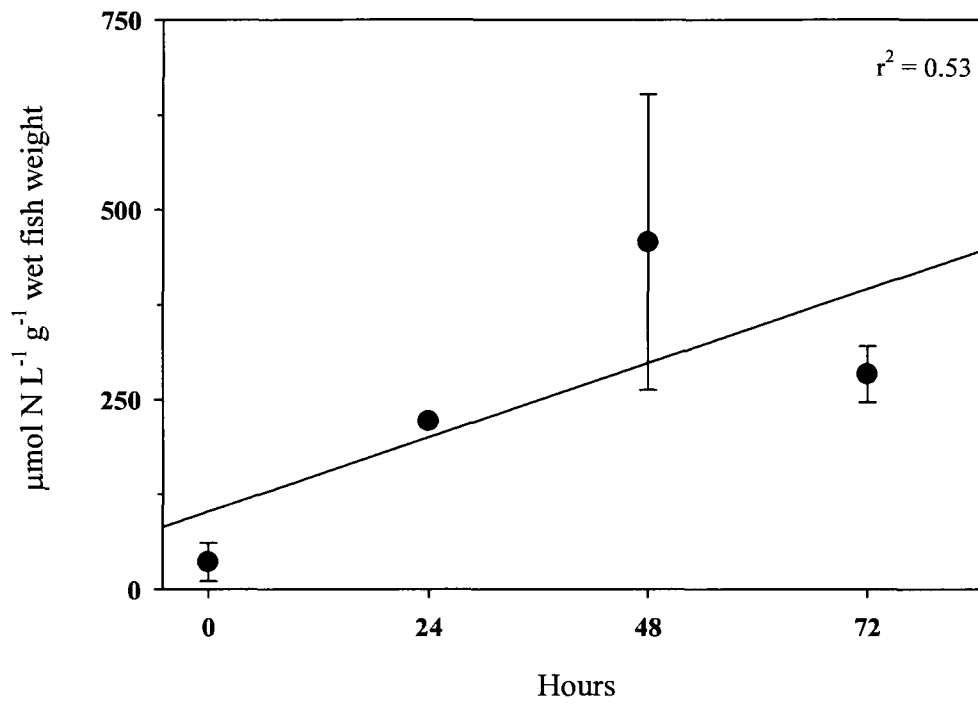
APPENDIX 2.

Concentration of ammonium (NH_4^+) released per gram wet fish weight over time (hours).



APPENDIX 3.

Concentration of amino acids (AA), measured as dissolved primary amines (DPA) released per gram wet fish weight over time (hours).



VITA

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Born in Racine, Wisconsin on October 15, 1981. Lynn graduated from J.I. Case High School in 2000. She earned a B.S. in Chemistry with an emphasis in biochemistry from Northern Illinois University in DeKalb, Illinois in May 2004 and entered the Virginia Institute of Marine Science- School of Marine Science at The College of William & Mary in August 2004. She bypassed into the Ph.D. program in 2007 and completed her dissertation work in April 2011 under the guidance of Dr. Deborah Bronk. Lynn enjoys gardening, running, yoga, cooking, and spending time with her husband, friends, and five cats.