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# Nitrogen uptake and regeneration (ammonium regeneration, nitrification and photoproduction) in waters of the West Florida Shelf prone to blooms of *Karenia brevis*

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ABSTRACT

The West Florida Shelf (WFS) encompasses a range of environments from inshore estuarine to offshore oligotrophic waters, which are frequently the site of large and persistent blooms of the toxic dinoflagellate, *Karenia brevis*. The goals of this study were to characterize the nitrogen (N) nutrition of plankton across the range of environmental conditions on the WFS, to quantify the percentage of the plankton N demand met through *in situ* N regeneration, and to determine whether planktonic N nutrition changes when high concentrations of *Karenia* are present. In the fall of 2007, 2008, and 2009 we measured ambient nutrient concentrations and used stable isotope techniques to measure rates of primary production and uptake rates of inorganic N (ammonium, $NH_4^+$, and nitrate, $NO_3^-$), and organic N and carbon (C; urea and amino acids, AA) in estuarine, coastal, and offshore waters, as well as coastal sites with *Karenia* blooms present. In parallel, we also measured rates of *in situ* N regeneration – $NH_4^+$ regeneration, nitrification, and photoproduction of $NH_4^+$, nitrite and AA. Based on microscope observations, ancillary measurements, and previous monitoring history, *Karenia* blooms sampled represented three bloom stages – initiation in 2008, maintenance in 2007, and late maintenance/stationary phase in 2009. Nutrient concentrations were highest at estuarine sampling sites and lowest at offshore sites. Uptake of $NH_4^+$ and $NO_3^-$ provided the largest contribution to N nutrition at all sites. At the non-*Karenia* sites, *in situ* rates of $NH_4^+$ regeneration and nitrification were generally sufficient to supply these substrates equal to the rates at which they were taken up. At *Karenia* sites, $NO_3^-$ was the most important N substrate during the initiation phase, while $NH_4^+$ was the most important N form used during bloom maintenance and stationary phases. Rates of $NH_4^+$ regeneration were high but insufficient ($85 \pm 36\%$ of uptake) to support the measured $NH_4^+$ uptake at all the *Karenia* sites although nitrification rates far exceeded uptake rates of $NO_3^-$. Taken together our results support the "no smoking gun" nutrient hypothesis that there is no single nutrient source or strategy that can explain *Karenia*'s frequent dominance in the waters where it occurs. Consistent with other papers in this volume, our results indicate that *Karenia* can utilize an array of inorganic and organic N forms from a number of N sources.



## 1. Introduction

*Karenia brevis* is a toxic dinoflagellate responsible for massive fish kills in the Gulf of Mexico (Heil and Steidinger, 2009). It is considered a coastal bloom species but is found in environments ranging from nutrient depleted offshore waters, where blooms are believed to initiate (Steidinger, 1979; Steidinger et al., 1998), to nutrient replete estuarine and coastal waters. In the offshore and coastal regions, dissolved inorganic nitrogen (DIN), ammonium ($NH_4^+$) and nitrate ($NO_3^-$), comprise less than 1% of the total dissolved N (TDN) pool, making dissolved organic nitrogen (DON) the most abundant form of N present (Vargo et al., 2008).

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Offshore *Karenia* populations are thought to be transported by currents and winds and concentrated in near shore regions (Steidinger and Haddad, 1981) where toxic blooms may continue for months leading to numerous harmful impacts on finfish, shellfish, marine mammals, birds, and humans (Kirkpatrick et al., 2004; Landsberg et al., 2009; Fleming et al., 2011). Overall, standing stocks of dissolved nutrients are insufficient to support the growth requirements of *Karenia*, creating a paradox – in a seemingly nutrient-poor environment, where do cells obtain nutrients to allow for growth and subsequent accumulation of large populations of cells, often at concentrations of millions of cells per liter (Walsh and Steidinger, 2001; Vargo et al., 2008)?

### 1.1. Sources of nitrogen to the West Florida Shelf

A number of nutrient sources have been hypothesized to be available to *Karenia*. During the offshore initiation phase of *Karenia* blooms, sources such as atmospheric deposition (Vargo et al., 2008), continental shelf upwelling (Walsh et al., 2006), N release from *Trichodesmium* spp. (Lenes et al., 2001; Bronk et al., 2004; Mulholland et al., 2004, 2006, 2014), and remineralized nutrients from the decay of diatom populations (Vargo et al., 2008) have all been considered. Nutrient sources suggested to support blooms in coastal and estuarine areas include nutrient flux from rivers and estuaries such as the Caloosahatchee River, Tampa Bay, and Charlotte Harbor (Vargo et al., 2004, 2008; Brand and Compton, 2007), N regeneration from zooplankton excretion (Lester, 2005), and sediment N remineralization (Vargo et al., 2008; Dixon et al., 2014). More recently, nutrients released from the degradation of fish killed as a result of the *Karenia* blooms themselves has been suggested as a source of regenerated N to support the maintenance of *Karenia* blooms (Vargo et al., 2000; Walsh et al., 2006; Killberg-Thoreson et al., 2014a). Analysis of the $\delta^{15}N$ of particulate N (PN) samples collected during *Karenia* blooms on the WFS indicated that estuarine inputs, seagrass, and recently fixed dinitrogen supplied by *Trichodesmium* were all utilized by *Karenia* cells (Havens, 2004), thus reinforcing the idea that multiple N sources likely contribute to blooms.

### 1.2. Nitrogen Nutrition of Karenia brevis

*Karenia* appears to possess a flexible N metabolism and is able to take up and utilize a variety of DIN and DON sources. In fact, *Karenia* has been shown to grow on both chemically simple molecules, such as $NH_4^+$ (Wilson, 1966) and amino acids (Wilson, 1966; Baden and Mende, 1979; Shimizu et al., 1995), as well as more complex organic compounds such as humic substances (Heil unpublished data), DON released from *Trichodesmium* (Bronk et al., 2004; Mulholland et al., 2006; Sipler, 2009; Sipler et al., 2013) and decaying fish (Killberg-Thoreson et al., 2014b). *Karenia brevis* also appears to be mixotrophic (Shanley and Vargo, 1993; Vargo et al., 1987; Heil et al., 2004), and has been shown to incorporate carbon from leucine (Shimizu and Wrensford, 1993; Shimizu et al., 1995) and to take up glucose in the light (Baden and Mende, 1979). It can also utilize N from amino acids (AA) by enzymatically cleaving the amino group (Baden and Mende, 1979). In addition, *Karenia* can graze on co-occurring picoplankton (Glibert et al., 2009).

Uptake rates for several DON and DIN compounds have been quantified in both cultured (Sinclair et al., 2006a,b; Glibert et al., 2009; Sinclair et al., 2009; Killberg-Thoreson et al., 2014b) and natural populations (Bronk et al., 2004; Killberg-Thoreson et al., 2014b) of *Karenia* using $^{15}N$ isotopic techniques. Nitrogen uptake rates for the closely related species *Karenia mikimotoi* were also measured in the East China Sea (Li et al., 2012). Many of these studies have been conducted to determine the kinetic parameters for *Karenia* (Bronk et al., 2004; Sinclair et al., 2006a, 2009; Glibert et al., 2009; Killberg-Thoreson et al., 2014b). Kinetic studies aimed at determining the maximum uptake velocity ($V_{max}$) and half-saturation constants ($K_s$) for different N compounds can be used to assess an organism's affinity for a particular N species and maximum uptake rates. While this information can be useful for predicting competitive outcomes between species, it does not necessarily reflect N uptake in the environment, especially along the WFS where regeneration and uptake rates are often tightly coupled thus keeping ambient nutrient concentrations very low (Heil et al., 2001; Vargo et al., 2008).

In this study, we had three objectives: first, to characterize the N nutrition of plankton across the range of environmental conditions on the WFS; second, to determine what percentage of the planktonic N demand could be met with *in situ* N regeneration; and third, to determine whether N uptake and regeneration varied significantly when high concentrations of *Karenia* were present during blooms *versus* when there is a more diverse microbial assemblage. At the outset of this project, we hypothesized that there was no single nutrient factor or "smoking gun" that triggered bloom initiation or that sustained *Karenia* blooms on the WFS. We encountered *Karenia* populations on the three cruises presented here although not always in high concentrations. Taken together the data presented below supports the "no smoking gun" hypothesis in that we could not identify a single nutrient source that caused or sustained *Karenia* blooms.

## 2. Methods

### 2.1. Field sampling

Research cruises were conducted in the eastern Gulf of Mexico aboard the *R/V Pelican* October 15–27, 2007, October 1–13, 2008, and October 1–13, 2009. Each cruise was divided into two legs. The first leg was devoted to characterizing ambient nutrient and biomass concentrations and microbial processes at seven estuarine, coastal and offshore sites. The second leg was devoted to more detailed sampling within waters with high *Karenia* abundance (Table 1 and Fig. 1).

### 2.2. Ambient nitrogen and pigment concentrations

Water was collected at two or three depths each day within an hour of sunrise with a CTD with Niskin bottle rosette. Here we report results from surface water samples (1 m). Surface water samples were filtered through pre-combusted (450 °C for 2 h) Whatman® GF/F filters; the filter was used to measure the concentration of chlorophyll *a* (Chl *a*), and the filtrate was frozen in low-density polyethylene (LDPE) centrifuge tubes (Corning®) for later determination of TDN, $NH_4^+$, $NO_3^-$, nitrite ($NO_2^-$), urea, total dissolved phosphorus (TDP), phosphate ($PO_4^{3-}$), and silica (Si) in the laboratory. Samples for AA were stored in high-density

**Table 1**
Approximate location of the sample sites for this study and characterization as estuarine (E), coastal (C), or offshore (O) stations. Sites with *Karenia* were selected while following a drogue and so varied from cruise to cruise.

| Station | Type | Location | Latitude | Longitude |
|---|---|---|---|---|
| 1 | O | Offshore | 27.1050° N | 83.0580° W |
| 2 | C | Outside Charlotte Harbor | 26.7157° N | 82.2860° W |
| 3 | C | Caloosahatchee River[a] | 26.2595° N | 82.0092° W |
| 4 | E | Inside Charlotte Harbor | 26.7268° N | 82.1905° W |
| 5 | C | Outside Sarasota Bay | 27.2675° N | 82.5900° W |
| 6 | E | Inside Tampa Bay | 27.6835° N | 82.5925° W |
| 7 | C | Outside Tampa Bay | 27.5680° N | 82.8050° W |

[a] May also be referred to as Mudhole in some publications.