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Nitrogen, phosphorus and silica on the West Florida Shelf: Patterns and relationships with *Karenia* spp. occurrence

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ABSTRACT

A large scale investigation was conducted in major estuaries and the oligotrophic coastal waters of the West Florida Shelf during the fall of 2007 through 2010 to identify statistically significant and persistent associations of the nearly annual blooms of the harmful algae, *Karenia brevis*, with dissolved and particulate nutrients, and with other taxonomic groups of the coastal phytoplankton community. Conflicting interannual patterns of *Karenia* densities and riverine flows indicated that links between *Karenia* blooms and terrestrial contribution of nutrients were neither direct nor certain. There was no evidence in these data that *Karenia* blooms were enhanced by estuarine outflow. There was no characteristic nutrient ensemble with which *Karenia* presence was associated, which implied a wide range of nutrient strategies for the organism. Drawdowns of urea and DIN were not a universal feature of the blooms sampled. The only persistent relationship with individual parameters were a correlation of *Karenia* cell density with DON and an inverse correlation with DIN:$Si(OH)_4$. Temporal and spatial patterns of DON and $\delta^{15}N$ provided evidence of regional processes controlling nitrogen supply. Correlation of $\delta^{15}N$ values with cyanophyte percentage of biomass demonstrated the significance of N_2-fixation to the region. *Karenia* was correlated with haptophyte and cyanophyte biomass and inversely correlated with diatoms. The phytoplankton community was not significantly different with *Karenia* present and indicated that blooms replaced differing components of the phytoplankton community at different times. Principal component analysis of phytoplankton community composition identified a modest association of *Karenia* with nutrient-poor conditions. The results emphasized that strong interannual variability existed in nutrient regimes, phytoplankton community, and nutrient-mediated *Karenia* responses.

1. Introduction and rationale

The southwest coast of Florida is characterized by nearly annual blooms of the toxic dinoflagellate, *Karenia brevis* (Davis) G. Hansen & Moestrup (Steidinger et al., 1998; Daugbjerg et al., 2000), with highest incidence in the late summer and early fall months (Tester and Steidinger, 1997). Blooms initiate offshore in oligotrophic waters (Steidinger and Haddad, 1981; Steidinger et al., 1998; Heil et al., 2001, 2007; Vargo et al., 2004; Bissett et al., 2005), are physically transported by winds and tidal currents, can accumulate at thermal or salinity density fronts (Vargo et al., 2001; Stumpf et al., 2008), and can persist in higher nutrient environments nearshore for many months (Heil et al., 2007). While motility and aggregation behavior (Heil, 1986; Kamykowski et al., 1998; Schofield et al., 2006; Sinclair and Kamykowski, 2008; Heil et al., 2014b) undoubtedly account for some portion of the rapid increase in cell densities observed at the onset of a bloom, the rate of apparent increase in biomass of this relatively slow-growing member of the coastal phytoplankton community remains puzzling. Additionally, the water column standing stocks of dissolved inorganic nutrients are inadequate to support the biomass of *K. brevis* achieved except in nearshore environments with modest cell densities (Steidinger et al., 1998; Vargo et al., 2001, 2004). The source and magnitude of additional likely nutrient sources has been the subject of a number of studies (Lenes et al., 2001; Walsh and Steidinger, 2001; Heil et al., 2001; Lester et al., 2001; Walsh et al., 2003; Vargo et al., 2004, 2008; Heil et al., 2007; Yentsch et al., 2008) but some potential sources remain poorly constrained or unquantified.

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The identification of the key nutrient sources supporting *Karenia brevis* blooms is made more difficult by the demonstrated variety of nutrient strategies; uptake of both inorganic and organic nitrogenous compounds (Bronk et al., 2004; Mulholland et al., 2004, 2006), uptake of organic phosphorus (Steidinger et al., 1998; Vargo and Shanley, 1985), heterotrophic abilities (Baden and Mende, 1978, 1979; Shimizu and Wrensford, 1993; Shimizu et al., 1995; Mulholland et al., 2002), as well as phagocytosis (Jeong et al., 2005; Glibert et al., 2009). Potential nutrient sources include: estuarine outflow, coastal upwelling, atmospheric deposition, *in situ* bacterial remineralization and photolysis, zooplankton excretion, flux from sediments, decomposition from fish kills, and releases from nitrogen-fixing cyanobacteria. The absolute magnitude and relative proportions of contributions from the various sources also vary both spatially and temporally.

In October 2006, a regional NOAA EcoHAB multidisciplinary effort began, targeting the simultaneous examination of many previously under-evaluated N and P nutrient sources in the West Florida Shelf (WFS) region. Multiple investigators (O'Neil and Heil, Eds. this volume) addressed a number of the more poorly characterized sources and associated *Karenia brevis* response, with a desired end result of providing better estimates of rates, processes, and pools to support ecological modeling efforts predicting the onset and progress of *K. brevis* blooms (Lenes et al., 2012, 2013). Spatially organized to evaluate relative nutrient contributions from multiple sources over multiple years and the resulting nutrient regimes associated with *K. brevis*, the overall project targeted specific habitats (both inside and outside of major estuaries and a smaller lagoonal estuary, as well as an offshore environment) for process investigations by other investigators. The project succeeded in capturing a range of bloom conditions and stages in a variety of environments.

The work reported here was a simultaneously conducted, larger scale investigation and mapping of nutrient conditions and phytoplankton community, at both the process investigation locations and latitudinally and longitudinally across the WFS. Within logistic constraints, we collected a large number of samples to identify statistically significant and persistent associations of *Karenia brevis* with specific nutrient regimes, to identify limiting conditions through the analysis of ambient data, and to elucidate the dominant sources of nutrients with respect to *K. brevis*. We also examined the interrelationships of multiple taxonomic elements of the entire phytoplankton community with *K. brevis*.

2. Methods

2.1. Sampling methods

Four two-week cruises were conducted during the October months of 2007 through 2010. Cruises in 2007, 2008, and 2009 were segregated into a mapping effort (Week 1), followed by process sampling and experiments in regions where *Karenia brevis* was present at above background concentrations (Week 2). The region sampled in 2007, 2008, and 2009 encompassed ~225 km from north of Tampa Bay to south of the mouth of the Caloosahatchee River, and within approximately 70 km of shore. In 2010, advance notice of low *K. brevis* counts throughout the region forced researchers to suspend extensive mapping in an attempt to find a *K. brevis* bloom, resulting in a more restricted area being sampled in 2010 and generally limiting inter-annual comparisons to the 2007–2009 data. The freshwater inflows from selected gauged rivers (http://waterdata.usgs.gov/usa/nwis/sw) from the major contributing watersheds (Tampa Bay, Charlotte Harbor, and Caloosahatchee River) were examined as indices to place the four study periods in a hydrologic context and to provide a relative comparison of the associated terrestrial nutrient loads.

At each station, physical data were obtained from casts of a Sea-Bird Electronics 911 plus conductivity-temperature-depth (CTD) instrument. Parameters for water column structure included salinity, dissolved oxygen (DO), fluorescence, and calculated density as σ_t. Discrete samples were collected concurrently with profiles. At a minimum, samples were collected at surface (1 m) and bottom (within 1 m of bottom). Physical and chemical data resulted from 86, 122, 170, and 23 samples in 2007, 2008, 2009, and 2010, respectively.

2.2. Analytical methods

Karenia was enumerated to the species level wherever possible. Samples (1 ml) were preserved with unacidified Lugol's solution, settled for 15 min, and all *Karenia* cells in the well identified using an Olympus CK30 inverted light microscope at 200× magnification. Concentrations of all *Karenia* species (*Karenia brevis*, *Karenia mikimotoi*, and *Karenia* sp. cells which could not be identified to species level due to orientation and staining) were summed as *Karenia* spp. and referred to as *Karenia* for the current analysis.

Phytoplankton community analysis was conducted using high performance liquid chromatography (HPLC). Samples were filtered onto Whatman 0.7 μm GF/F filters and held at −20 °C until analysis. Filters were analyzed by sonication and extraction in cold 98:2 methanol:ammonium acetate and photopigments were quantified with HPLC (Wright et al., 1991) using an Agilent Hypersil ODS Column (250 mm × 4.6 mm, 5.0 μm) at 30 °C and a photodiode array UV–VIS detector (SPD-M10AVP, Shimadzu, Inc.). Chlorophyll *a* biomass (Chl *a*) attributed to each taxonomic group was derived from accessory photopigments using ChemTax® software, incorporating a factor analysis of pigments and the steepest descent algorithm. The phylogenetic groups of interest included *Karenia*, other dinoflagellates, diatoms, chlorophytes, cryptophytes, cyanophytes, haptophytes, prasinophytes, and prochlorophytes.

Samples for dissolved ammonium, nitrate + nitrite, reactive phosphate, and silica species [NH_4^+, NO_{2+3}^-, PO_4^{3-}, and $Si(OH)_4$), respectively] were filtered through Pall Supor 450 0.45 μm membrane filters. Urea (Urea-N), dissolved total N and P (DTN, DTP), and particulate carbon, nitrogen, and phosphorus (PC, PN, and PP) samples were filtered through pre-combusted (450° C, 3 h) 0.7 μm Whatman GF/F filters. Samples for PC and PN were rinsed with acidified (10% HCl), filtered seawater to remove inorganic carbon. Samples for $Si(OH)_4$ were held darkened at room temperature; samples for the remaining parameters were frozen at −20 °C until analysis.

Analyses for dissolved nutrients followed colorimetric, segmented flow, autoanalyzer techniques of Bran+Luebbe/Seal (B+L/S) on an AA3 with method reference and method detection limits (MDL) as follows; NH_4^+ (B+L/S, 2005a; 0.07 μM), NO_{2+3}^- (B+L/S, 2010; 0.07 μM), Urea-N (B+L/S, 2007; 0.2 μM), PO_4^{3-} (B+L/S, 2005b, 0.03 μM), and $Si(OH)_4$ (B+L/S, 2004; 0.1 μM). Salinity corrections were applied for urea and $Si(OH)_4$ and were demonstrated as unnecessary for the remaining parameters due to colorimeter design.

Samples for DTN, DTP, and PP were analyzed according to Solorzano and Sharp (1980a,b) with in-house modifications for analysis on a segmented flow analyzer and MDLs of 0.3 μM, 0.1 μM, and 0.03 μM, respectively. For PC and PN, samples were analyzed on a Thermo FlashEA® 1112 Elemental Analyzer with MDLs of 0.2 and 0.1 μM, respectively. Analyses were conducted according to National Environmental Laboratory Accreditation Conference protocols (EPA, 2003) with assessments of instrument response, precision, accuracy, and field and laboratory blank acceptability. Dissolved inorganic nitrogen (DIN), dissolved organic nitrogen (DON) and phosphorus (DOP) were computed