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Phytoplankton assemblage response to changing nutrients in Florida Bay: Results of mesocosm studies

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ABSTRACT

The ongoing restoration of the Florida Everglades has changed the hydrology in south Florida and increasing freshwater discharge has contributed to a shift in nutrients and phytoplankton in northern Florida Bay. To understand the effect of the changing nitrogen (N) forms and nitrogen:phosphorus (N:P) ratios on phytoplankton biomass and assemblage composition, five mesocosm experiments were conducted. Nutrient additions included a matrix of different forms of N (NO_3^-, NH_4^+, and DON) and P (as sodium β-glycerophosphate pentahydrate) in differing N:P molar ratios (4, 16, 32), a P alone addition, and a control. In general, chlorophyll *a* increased three-fold in the +P treatments and ten-fold in the +N+P treatments, while it did not increase in the +N treatments. Typically the +N+P treatments, particularly the $+NO_3^-+P$ at the N:P molar ratio of 32, promoted high concentrations of fucoxanthin (generally indicative of diatoms) relative to chlorophyll *a*. While chlorophyll *a* did not increase significantly in the +N alone treatments, there was a change in the phytoplankton assemblage. The +N treatments, when N was in the form of $+NH_4^+$ yielded proportionally higher zeaxanthin (generally indicative of picocyanobacteria). When the +N:P ratio increased, the relative concentrations of fucoxanthin and alloxanthin (generally indicative of cryptophytes) to chlorophyll *a* increased, whereas the relative concentrations of zeaxanthin and dinoflagellates (generally indicative of photosynthetic dinoflagellates) declined. This study highlights the importance of dual P and N control, particularly N in the forms of NH_4^+ and DON if picocyanobacterial blooms are to be controlled.

1. Introduction

The Comprehensive Everglades Restoration Plan (CERP) was initiated to restore the water resources of southern Florida in the early 2000s and current plans call for considerable expansion of flow to southern Florida. The Plan has redirected water originating in the Kissimmee River and Lake Okeechobee, Florida, and now flowing to the Atlantic Ocean and Gulf of Mexico, south through the Everglades wetlands and into Florida Bay. The general goal of CERP is to increase freshwater flow and relieve hypersalinity which has been considered to be a contributing factor to seagrass loss and the subsequent ecological degradation of Florida Bay since the late 1980s (Robblee et al., 1991; McIvor et al., 1994; Hall et al., 1999; Zieman et al., 1999; Lapointe and Barile, 2004; Koch et al., 2007). As a component of CERP, the C-111 Spreader Canal Western Features project was designed to retain and increase freshwater flow within the major drainage system into northern Florida Bay, Taylor Slough. However, this ongoing restoration of freshwater flow has altered the concentrations and composition of nutrients, contributing to a shift in phytoplankton biomass and assemblage composition in northern Florida Bay (Shangguan et al., 2017). In order to better understand how specific nutrient changes may lead to specific changes in phytoplankton, mesocosm experiments were conducted in which nutrient concentration, form and ratio were altered and the responses of Florida Bay phytoplankton communities were followed.

It is well acknowledged that different algal taxa have distinct requirements and capabilities to use different forms of nitrogen (N; e.g., Lomas and Glibert, 1999a; Lomas and Glibert, 1999b; Berg et al., 2003; Dugdale et al., 2007; Parker et al., 2012; Xu et al., 2012; Glibert

Abbreviations: CERP, Comprehensive Everglades Restoration Plan; N, nitrogen; P, phosphorus; chl *a*, chlorophyll *a*; TDN, total dissolved nitrogen; TDP, total dissolved phosphorus; DON, dissolved organic nitrogen; DOP, dissolved organic phosphorus; HPLC, high performance liquid chromatography; SD, standard deviation; SE, standard error

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et al., 2014b; Glibert et al., 2016). Diatoms are typically NO_3^- opportunists, while picocyanobacteria and many dinoflagellates and cryptophytes generally favor chemically-reduced forms of N, mainly NH_4^+ and organic N (Lomas and Glibert, 1999b; Glibert et al., 2016 and references therein). These patterns have been well demonstrated in coastal lagoons and river-dominated estuaries, two estuarine typologies differing in the forms of N typically received (Glibert et al., 2010). Coastal lagoons generally have comparatively high proportions of organic and chemically-reduced forms of N, often leading to picoplankton blooms that can be sustained for long periods of time, as illustrated in Florida Bay, Maryland-Virginia Chincoteague Bay, Long Island Sound and Laguna Madre (DeYoe and Suttle, 1994; LaRoche et al., 1997; Berg et al., 2002; Glibert et al., 2010; Glibert et al., 2014a). On the contrary, river-dominated estuaries, such as Chesapeake Bay, more typically have higher concentrations of NO_3^-, favoring proportionately larger spring diatom blooms (Chavez et al., 1991; Glibert et al., 2010; Fawcett and Ward, 2011; Carstensen et al., 2015).

The stoichiometry of N and phosphorus (P) has been extensively studied as another important factor shaping phytoplankton biomass and assemblage composition (Smayda, 1990; Hodgkiss and Ho, 1997; Sterner and Elser, 2002; Heil et al., 2007; Finkel et al., 2010; Glibert et al., 2011; Glibert, 2012; Hillebrand et al., 2013; De Senerpont Domis et al., 2014; Li et al., 2015). The most widely applied stoichiometric relationship is the Redfield ratio, phytoplankton N:P = 16:1 on a molar basis (Redfield, 1958; Falkowski, 2000). However, the canonical Redfield ratio is not a universal optimum, and numerous studies have shown that the organismal N:P ratios in different phytoplankton functional groups differ, with the Redfield ratio being a general average (Rhee and Gotham, 1980; Geider and La Roche, 2002; Sterner and Elser, 2002; Ho et al., 2003; Klausmeier et al., 2004; Arrigo, 2005; Hillebrand et al., 2013; Martiny et al., 2013). Different types of phytoplankton have physiological strategies that allow them to thrive under N:P ratios that deviate from Redfield stoichiometry (e.g., Glibert and Burkholder, 2011). For example, in a high N:P ratio environment, organisms that benefit are those that have use of P sources that are not available to competitors (e.g., organic forms of P), or they may have a lower cellular P requirement overall (Glibert and Burkholder, 2011). Picoplankton such as *Synechococcus*, a dominant species in northern Florida Bay (Phlips and Badylak, 1996; Fourqurean and Robblee, 1999; Phlips et al., 1999; Glibert et al., 2004; Berry et al., 2015), are comparatively good competitors under low P conditions. Due to their small size, *Synechococcus* generally have less allocation of P-rich growth "machinery" and more of N-rich resource-acquisition "machinery" (Klausmeier et al., 2004; Irwin et al., 2006; Finkel et al., 2010). These cyanobacteria are also able to substitute P-containing lipids with non-P-containing lipids under conditions of P limitation (Van Mooy et al., 2009; Popendorf et al., 2011). Moreover, organic P is likely an important source of nutrients of *Synechococcus* blooms in Florida Bay (Glibert et al., 2004; Heil et al., 2009). Thus *Synechococcus* appear to thrive under conditions of comparatively low inorganic P. Strong correlations between the abundance of *Synechococcus* and alkaline phosphatase activity have been found in Florida Bay (Glibert et al., 2004) as well as in northern Gulf of Aqaba (Li et al., 1998), among many other P-limited systems. Diatoms and dinoflagellates, on the other hand, generally require higher allocation of P-rich growth "machinery" and thus have a lower N:P requirement (Moloney and Field, 1989; Sterner and Elser, 2002; Klausmeier et al., 2004; Finkel et al., 2010; Hillebrand et al., 2013).

The nutrient regime in Florida Bay has several general features. First, it is characterized as having proportionately more chemically-reduced (dissolved organic N, DON and ammonium, NH_4^+) compared to oxidized forms (nitrate, NO_3^-) of N (Glibert et al., 2004, 2010). Organic N, particularly in the northern part of the Bay, is the major N form contributing to primary productivity (Rudnick et al., 1999; Glibert et al., 2004; Boyer et al., 2006; Boyer and Keller, 2007; Yarbro and Carlson, 2008). Second, this system is generally considered to be P limited, primarily because of P-adsorbing carbonate sediment. Sediment adsorption of P thus leads to total dissolved N:total dissolved P (TDN:TDP) ratios much higher than the Redfield ratio (Lapointe, 1989; Fourqurean et al., 1992; Fourqurean et al., 1993; Glibert et al., 2004; Boyer et al., 2006; Price et al., 2006). Limitation by P in Florida Bay shows a west-east gradient as the major P source is considered from the west, Gulf of Mexico, while eastern Florida Bay has proportionately more P-adsorbing carbonate sediment (Fourqurean et al., 1992; Noe et al., 2001) and therefore very low concentrations of P.

The different TDN:TDP ratios of Florida Bay contribute to distinct phytoplankton communities spatially. The western region, which is generally more N poor, tends to have diatom blooms (Phlips and Badylak, 1996; Jurado et al., 2007). However, central Florida Bay, the transitioning zone from P limitation to N limitation from west to east, has been the site of several intense and prolonged *Synechococcus* blooms since mid-1990s (Fourqurean and Robblee, 1999; Phlips et al., 1999; Glibert et al., 2004, 2009; Berry et al., 2015). Eastern Florida Bay had been historically free of algal blooms, thought to be a result of more severe limitation by P, but it experienced sustained *Synechococcus* spp. blooms from 2005 to 2008 due to nutrient inputs from hurricane flooding (Hurricanes Katrina, Rita and Wilma) and from nutrient and organic matter input due to construction of a causeway linking the Keys to the mainland (Glibert et al., 2009; Goleski et al., 2010; Wall et al., 2012). Additional *Synechococcus* blooms occurred in northeastern Florida Bay in late 2016 (Madden, pers. comm.).

As a shallow, subtropical coastal lagoon, Florida Bay is intrinsically sensitive to allochthonous nutrient input. Compared to new nutrients from outside of the system, the regeneration rate of nutrients is high because of its high benthic surface:volume and long residence time (Glibert et al., 2010). The recurring and/or sustained picocyanobacteria blooms are considered to be supported by autochthonous regenerated nutrients in the system (Rudnick et al., 1999; Glibert et al., 2004, 2010). The extent to which nutrients originating from the Everglades watershed contribute to these blooms has been subject to controversy (Lapointe and Clark, 1992; Boesch et al., 1993; Lapointe and Barile, 2004).

Florida Bay serves as a case study, particularly with the C-111 project, of changing hydrology, changing nutrients, and of effects of changing nutrients on phytoplankton. Due to the combination of the C-111 project operation and 2012–2014 heavy rainfall (http://sofia.usgs.gov/exchange/sfl_hydro_data/), TDN and all forms of N have had a recent decreasing trend, whereas TDP and all forms of P have an increasing trend in northern Florida Bay (Shangguan et al., 2017). Also, the chemically reduced forms of N (NH_4^+, DON) have increased. In several sub-estuaries of northern Florida Bay, at the southern edge of the Everglades, these changes in nutrient quantities, forms, and proportions have been shown to influence phytoplankton biomass and assemblage composition (Shangguan et al., 2017).

As part of a broader study on phytoplankton blooms after the C-111 project implementation, a series of mesocosm experiments were designed to help understand the response in phytoplankton biomass and assemblage composition to N form and N:P ratio alterations in Florida Bay. We hypothesized that (1) NO_3^- additions would contribute to increased production by diatoms while additions of organic and reduced forms of N (NH_4^+ and DON) would contribute to production of higher proportions of picocyanobacteria, cryptophytes and dinoflagellates because NO_3^- favors diatoms and an environment with more reduced forms of N is more suitable for the others (Glibert et al., 2016), and (2) high N:P additions would yield increased production of picocyanobacteria or cryptophytes, while additions with a low N:P would favor diatoms or a dinoflagellate-dominated system as the N:P preferences and optimal nutrients of these different algal functional groups differ (e.g., Hillebrand et al., 2013). Therefore, it was expected that low N:P ratios with N in the form of NO_3^- would shape the system to be diatom-dominated, high N:P ratios with N in the form of NH_4^+ and DON would instead generate a picocyanobacteria-