ABSTRACT

Title of Dissertation: RESPONSE OF PLANKTON COMMUNITIES
IN COASTAL LAGOONS TO CHANGES IN
NUTRIENT QUALITY AND QUANTITY:
CASE STUDY OF FLORIDA BAY

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The Comprehensive Everglades Restoration Plan was initiated to return Florida Bay to a more natural ecological state. The C-111 project, one phase of this plan and initiated in 2012, was designed to increase freshwater flow into northern Florida Bay. However, it also alters the nutrient regime and, potentially, phytoplankton biomass and assemblage. This dissertation investigated the combined effects of changes in discharge and nutrient on phytoplankton biomass and assemblage in several interconnected, mesohaline coastal lagoon systems (lakes) of Florida Bay using field observations, mesocosm experiments, and a statistical box model.

Field measurements on nutrients and phytoplankton were performed before and after C-111 implementation. After C-111, increased freshwater flow and phosphorus (P) input, but decreased salinity and nitrogen (N) input were observed. One set of the lagoon lakes, previously highly eutrophic (average chlorophyll *a* >20 μg L^{-1}), had a nearly 50% decline in overall phytoplankton biomass. The other set of lakes,

originally oligotrophic (average chlorophyll *a* <2 μg L^{-1}), had a doubling of phytoplankton biomass. Phytoplankton assemblage in both sets of lakes shifted to picocyanobacteria.

Mesocosm experiments (5 independent experiments, 5-10 day duration, 1000 L tanks) were conducted to test the effects of nutrient additions. Phytoplankton biomass increased 3 to 10-fold in the +P treatments (alone or +N), but did not increase substantially in the +N alone treatments. The +N+P treatments, particularly the $+NO_3^-$ +P at a +N:P molar ratio of 32 led to a 20-fold increase in diatoms, whereas N in the form of $+NH_4^+$ yielded a > 2-fold increase in picocyanobacteria.

A statistical box model based on relationships measured in the field under different salinity regimes was developed to simulate flow, nutrients, and phytoplankton changes in the eutrophic lake chain. Model output showed that higher freshwater discharge decreased phytoplankton biomass in the upper of the connected lakes, but the lower lake had a high potential to generate algal blooms, which is consistent with the field data. Also, picocyanobacteria tripled following an increase in dissolved organic nitrogen (DON). This study recommends co-management of both P and N, particularly NH_4^+ and DON in Florida Bay if picocyanobacteria blooms are to be controlled.

RESPONSE OF PLANKTON COMMUNITIES IN COASTAL LAGOONS TO CHANGES IN NUTRIENT QUALITY AND QUANTITY IN FLORIDA BAY

by

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Dedication

I dedicate this dissertation to my loving and supportive family.

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Chapter 1 : Introduction and Background

Introduction

The Comprehensive Everglades Restoration Plan (CERP) was initiated to return Florida Bay to its previous ecological state. Seagrasses in Florida Bay were the dominant primary producer for several decades during the 20th century. However, Florida Bay has sustained ecological degradation since late 1980s, ranging from episodic massive seagrass die-offs (Hall et al., 1999; Koch et al., 2007), to prolonged picocyanobacterial, *Synechococcus* sp. blooms (Phlips et al., 1999; Glibert et al., 2009; Berry et al., 2015), to mortality of higher trophic-level organisms (Robblee et al., 1991; Fourqurean & Robblee, 1999; Peterson et al., 2006). Hypersalinity has been considered to be one major contributing factor to the seagrass loss (McIvor et al., 1994; Zieman et al., 1999), but confounding effects due to nutrient changes have also been shown to play important roles in these changes (Boesch et al., 1993; Lapointe & Barile, 2004). The aim of CERP was to direct water south through the Everglades and into Florida Bay, thus lowering the salinity levels to restore ecosystem health.

The C-111 Spreader Canal Western Features (C-111 SCWF) project, a component of CERP, began operations in 2012. The C-111 project is designed to retain and increase freshwater flow within the primary drainage system into northern Florida Bay, Taylor Slough. The general goal of the restoration program is to shift the salinity regime by changing the timing and distribution of freshwater flow. The increase in water flow will also likely change the quantity and quality of nutrients discharged to Florida Bay, however, leading to unknown impacts on the composition of phytoplankton assemblages and ultimately ecosystem health. Therefore, it is crucial to understand how the changes in flow and salinity affect the nutrient regime in the system, and how they together affect the abundance and assemblage of phytoplankton.

Northern Florida Bay should be the first area to respond to changes from the C-111 project, as it lies at the interface between Florida Bay and the Everglades. This region has many quasi-Intermittent Closed Open Lakes and Lagoons (ICOLLs) (Suzuki et al., 1998; Frankovich et al., 2011; Maher et al., 2011). ICOLLs are coastal water wholly or partially separated from the adjacent sea by one or more restricted inlets. They are considered highly productive ecosystems (Suzuki et al., 1998; Menendez & Comin, 2000). They often act as sinks of land-derived nutrients because of their restricted water exchange, and thus they have the potential to become eutrophic due to increased nutrient enrichment (Kjerfve & Magill, 1989). Florida Bay itself is intrinsically sensitive to allochthonous nutrient inputs. Compared to new nutrients from outside the system, the regeneration rate of nutrients is high, due to the physical nature of this system, high surface:volume ratios and long residence time (Glibert et al., 2010). The recurring and/or sustained picocyanobacteria blooms are considered to be supported by regenerated nutrients in the system (Rudnick et al., 1999; Glibert et al., 2004; Glibert et al., 2010). The extent to which nutrients originating from the Everglades watershed would change phytoplankton biomass and assemblage is uncertain. The goal of this study was to undertake field measurements, mesocosm experiments, and modeling to strengthen understanding of changes occurring in water flow, nutrients, and phytoplankton after implementation of C-111 project in the northern Florida Bay region.

Background and Justification

A brief overview of Florida Bay and study location

Florida Bay is a wedge-shaped, subtropical coastal lagoon approximately 2,200 km^2 in area. It has an open boundary with the Gulf of Mexico in the west, but the connection with the Atlantic Ocean on the eastern and southern boundaries is restricted to narrow tidal passes through the Florida Keys. The Bay has an average annual temperature of 25°C and two distinct meteorological seasons: a November-April dry season and a May-October rainy season (Duever et al., 1994). Riverine inputs along the northern boundary are considerable during the wet season, and the major freshwater input is through slow sheet flow from the Everglades watershed (Boyer et al., 2006; Lee et al., 2007). Not only does the Bay have limited water exchange, but also hydrological circulation within the Bay itself is small as there are many shoals and shallow basins restricting water transport (Lee et al., 2002). Consequently, most of central and northeastern Florida Bay has relatively long residence times, on the order of a month or more.

Northern Florida Bay is a dynamic transition zone between Everglades and the Bay. In the southern Everglades draining into Florida Bay, there are two main chains of ICOLLs, the West Lake Chain (WLC) and the Seven Palm Lake Chain (SPLC). The WLC includes West Lake, Long Lake, and its northern Bay receiving site, Garfield Bight. The SPLC includes Seven Palm Lake, Middle Lake, and its receiving site, Terrapin Bay.

The WLC and SPLC are very different in their geological connections and ecological states (Frankovich et al., 2011). Both the WLC and the SPLC receive freshwater mainly from Taylor Slough, one of major two wetland drainage systems in the Everglades. Yet, they have distinct groundwater sources of nutrients (Price et al., 2006). Notably, there is a bird island in the upstream lakes of the WLC, where nutrients, particularly P in guano, have been deposited. Also, the lakes in the WLC are effectively separated by narrow mangrove creeks and small, shallow (<50 cm) ponds within them, whereas lakes in the SPLC are comparatively more hydrologically connected (Frankovich et al., 2011). In addition, the receiving site of the WLC, Garfield Bight is fairly open to the Bay, while Terrapin Lake, the receiving site of the SPLC, is relatively closed. Nutrients are differentially trapped during tropical storms as a result of this morphometric difference (Craighead, 1971; Castañeda-Moya et al., 2010). Consequently, these two drainage systems have different amounts and assemblages of phytoplankton and submerged vegetation (Frankovich et al., 2011).

The WLC is typically plankton-dominated and nutrient rich, with low light penetration and very high suspended algal chlorophyll *a* (chl *a*) concentrations, while the SPLC typically is dominated by benthic production, lower in nutrient content, and much lower in suspended algal chl *a*, with significantly higher light penetration to the bottom and has much greater abundance of submersed aquatic vegetation, both angiosperms (e.g., *Halodule wrightii*, the dominant seagrass group) and macroalgae (e.g., *Chara* sp., the dominant macrophyte group; Frankovich et al., 2011).

The constructed C-111 Spreader Canal Western Feature Project should increase the water flow in the Taylor Slough. Therefore, differences in responses in the two sets of ICOLLs might be triggered because: 1) they inherently represent different ecological states; and 2) they will not be affected by water management to same extent due to morphometric differences. Understanding how both the eutrophic WLC and the oligotrophic SPLC ecosystems will be affected in terms of nutrients and phytoplankton is critical to assessment of the overall ecological response to the increasing freshwater flow in Florida Bay.

Algal preferences for nutrient forms and N:P ratios

It is well acknowledged that different algal taxa have distinct requirements and capabilities to use different forms of nitrogen (N; Berg et al., 2003; Dugdale et al., 2007; Parker et al., 2012; Xu et al., 2012; Glibert 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 & Glibert, 1999a; Glibert et al., 2016 and references therein). These patterns have been 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, as illustrated in Florida Bay, Maryland-Virginia Chincoteague Bay, Long Island Sound and Laguna Madre (DeYoe & Suttle, 1994; Laroche et al., 1997; Berg et al., 2002; Glibert et al., 2010; Glibert et al., 2014a). In contrast, river-dominated estuaries typically have higher concentrations of NO_3^-, favoring proportionately larger spring diatom blooms (Chavez et al., 1991; Glibert et al., 2010; Fawcett & 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 & Ho, 1997; Sterner & Elser, 2002; Heil et al., 2007; Finkel et al., 2009; 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 by atoms (Redfield, 1958; Falkowski, 2000). The canonical Redfield ratio is not a universal optimum, however, and numerous studies have shown that the organismal N:P ratios in different phytoplankton functional groups differ, with the Redfield ratio being, at best, a general average (Rhee & Gotham, 1980; Geider & La Roche, 2002; Sterner & 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 mechanisms or "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 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 & Burkholder, 2011). Picoplankton such as *Synechococcus*, a dominant phytoplankton genus in northern Florida Bay (Phlips & Badylak, 1996; Fourqurean & Robblee, 1999; Phlips et al., 1999; Berry et al., 2015), is a comparatively good competitor under low P conditions. Due to its small size, *Synechococcus* generally has 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., 2009). Ribosomes are important growth “machinery” and particularly high in P relative to N (Sterner & Elser, 2002). *Synechococcus* may 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 P for *Synechococcus* blooms in Florida Bay (Heil et al., 2007). 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; Heil et al., 2009) 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 growth “machinery” and thus have a lower N:P requirement (Moloney & Field, 1989; Sterner & Elser, 2002; Klausmeier et al., 2004; Finkel et al., 2009; Hillebrand et al., 2013).

The nutrient regime in Florida Bay has several general features. First, it is characterized as having proportionately more chemically reduced (organic N 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 & Keller, 2007; Yarbro & Carlson, 2008). Second, this system is generally considered to be P limited, primarily because of P-adsorbing carbonate sediment. Sediment absorption of P leads to TDN:TDP (total dissolved

N:total dissolved P) 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 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 P concentrations.

The different TDN:TDP ratios of Florida Bay contribute to distinct phytoplankton assemblages spatially. The western region, which is generally more N-poor, tends to have diatom blooms (Phlips & Badylak, 1996; Jurado et al., 2007). However, central Florida Bay, the transition 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 & Robblee, 1999; Phlips et al., 1999; Glibert et al., 2004; Glibert et al., 2009; Berry et al., 2015). Historically, eastern Florida Bay had been free of algal blooms, thought to be a result of more severe P limitation, but it sustained *Synechococcus* spp. blooms from 2005 to 2008 due to nutrient inputs from hurricane-related flooding (Hurricanes Katrina, Rita and Wilma), and from nutrient and organic matter input related to construction of a causeway linking the Keys to the mainland (Glibert et al., 2009; Goleski et al., 2010; Wall et al., 2012).

How would the ecosystem respond to increased freshwater inputs?

Historically, Florida Bay has sustained catastrophic ecological degradation. Widespread seagrass loss that was most evident beginning in 1987 (Hall et al., 1999; Koch et al., 2007) was followed by a cascade of ecological effects, including frequent phytoplankton blooms (Boyer et al., 1999; Fourqurean & Robblee, 1999; Richardson

& Zimba, 2002; Glibert et al., 2004) and mortality of sponges, spiny lobster, pink shrimp, and game fish (Robblee et al., 1991; Butler IV et al., 1995; Fourqurean & Robblee, 1999; Peterson et al., 2006). Consequently, this system is relatively fragile and it has low ecological resilience (Gunderson, 2000).

Northern Florida Bay is a sensitive ecosystem also, by virtue of the inherent characteristics of the quasi-ICOLLs. Although not completely physically separated from the Bay, and thus not true ICOLLs, these quasi-ICOLLs have low nutrient loading, low flushing, and long – but variable – residence times. When the ICOLLs receive little external nutrient inputs, benthic nutrient flux and internal nutrient cycling largely exceeds the nutrient input.

Interactions between phytoplankton and seagrass include competition for nutrients and light (Burkholder et al., 2007). These ICOLLs thus have a high likelihood to shift between alternative stable states of turbid phytoplankton-dominated and clear seagrass-dominated. The state shifts of these ICOLLs can be compared to those of shallow lakes, which also have been shown to be sensitive to regime shifts (Scheffer et al., 1997; Scheffer, 2004; Peckham et al., 2006). When a perturbation is larger than the resilience of a lake, as for example when a nutrient addition generates high phytoplankton biomass, an alternative stable state results. In general, small and shallow lakes have a stronger hysteresis than bigger and deeper lakes, which means small and shallow lakes tend to shift dramatically to the alternative stable state and hard to reverse. Therefore, it is more difficult to restore the seagrass-dominated clear state in small and shallow lakes compared to larger/deeper lakes (Carpenter et al., 1999; Scheffer, 2004). Consequently, it was expected that after the C-111 project, northern Florida Bay and its ICOLLs would become more dynamic. Improved understanding of nutrient-phytoplankton relationships is needed to avoid transition to a less desirable alternate stable state, particularly in SPLC, which presently is a clear, seagrass-dominated system.

Research Hypotheses, Expectations, and Approaches

To restore Florida Bay and return its historic salinity regime, it has been estimated that a three-fold increase in freshwater flow from the Everglades to Florida Bay will be required (Herbert et al., 2011). Because freshwater flows are laden with land-based nutrients, such a freshwater increase may very likely increase total N (TN) but not necessarily total P (TP) loads (Rudnick et al., 1999; Sutula et al., 2003), due to more rapid biotic P removal (Gleason & Spackman, 1974) and abiotic P retention in Everglades wetlands (Jones & Amador, 1992; Reddy et al., 1993). Since nutrients are not easily flushed out of Florida Bay, particularly in Northern Florida Bay, the increasing TN might increase eutrophication in the system, and thus increase phytoplankton bloom frequency, duration, and intensity.

It is expected that due to the CERP C-111 project, the fraction of new nutrients, NO_3^-, to the total nutrients in northern Florida Bay may increase. It was previously shown that the NH_4^+ import from Taylor Slough/C-111 canal to Florida Bay was only 3.8% of the total TN load during 1996-1998 (Sutula et al., 2003) and DON was 23.7% of the total TN load during 1984-1995 (Rudnick et al., 1999), thus suggesting a proportionately higher load of NO_3^- from the Everglades. It is also expected that

N:P ratios (TN:TP, DIN:DIP, and DON:DOP) will likely increase (Rudnick et al., 1999; Sutula et al., 2003).

The following overarching hypothesis was thus tested in this dissertation: After implementation of the C-111 canal project, increasing freshwater discharged into Florida Bay from the Everglades will increase nutrient loading to the quasi-ICOLLs, but the increases in N and P will be disproportional. As a result, the phytoplankton assemblages will respond differentially to the altered nutrient regime. More specifically, it is expected that (A) there will be an increase in N relative to P because P is proportionately more retentive than N within sediments; (B) the ratio of oxidized to chemically-reduced forms of N will increase because new or the oxidized form of N should be enhanced, whereas N regeneration rates should be comparatively unaffected or reduced from decreasing salinity; and (C) the smaller size fraction of phytoplankton, especially picocyanobacteria, will increase because picocyanobacteria are more competitive under conditions of comparatively low P (Smith & Kalff, 1982; Agawin et al., 2000; Zubkov et al., 2007; Van Mooy et al., 2009), but this effect may be offset by the disproportionate input of oxidized relative to reduced forms of N. Where oxidized forms of N predominant, diatoms will be favored. The research included long-term field investigations in the semi-enclosed ICOLLs of northern Florida Bay and eastern Florida Bay reference stations (Chapter 2), mesocosm experiments (Chapter 3), and statistical modeling (Chapter 4). The findings are summarized in the chapter synopses below.

Chapter 2: Nutrients and phytoplankton in semi-enclosed lagoon systems in Florida Bay and their responses to changes in flow from Everglades restoration

This chapter focused on a long-term data set of nutrients (concentration and composition) and phytoplankton (biomass and assemblage) from the two chains of quasi-ICOLLs previously described, at the northern Florida Bay border, and reference stations in eastern Florida Bay (EFB) that were presumed to have been unaffected by altered flow from the C-111 canal. The differential responses of these two chains both to each other and to the stations in EFB were compared. The two chains of ICOLLs were previously characterized as poised at different stable states (sensu Scheffer & Carpenter, 2003). One ICOLL chain was considered to be eutrophic and in a plankton-dominated state, while the second ICOLL chain was oligotrophic and maintained in a benthic-dominated state (Carpenter et al., 1999; Poor, 2010; Stanley et al., 2010).

Discharge from the C-111 canal increased freshwater flow, decreased salinity, decreased total dissolved nitrogen (TDN), including all forms of N, but increased total dissolved phosphorus (TDP), including all forms of P. The West Lake Chain (WLC), previously highly eutrophic, sustained a significant, nearly 50%, decline in overall phytoplankton biomass, and a shift to smaller-sized cells, picocyanobacteria. However, in the oligotrophic Seven Palm Lake Chain (SPLC), phytoplankton biomass doubled upstream and more-than-doubled downstream after the C-111 project, also comprised of smaller-sized cells. These latter changes, while not significant, suggest a trajectory of response that may be further promoted with further changes in flow. This study suggests that the increasing freshwater flow and N and P availability from the C-111 canal are likely to continue to affect nutrients and phytoplankton in this region. The oligotrophic system has higher potential for increased phytoplankton biomass, while the highly eutrophic ICOLL may become less eutrophic, but changes in phytoplankton assemblage composition at its downstream Florida Bay receiving site are likely. The presence of these saline lakes may also serve as buffers, dampening the effects in Florida Bay itself.

Chapter 3: Phytoplankton assemblage response to changing nutrients in Florida Bay: Results of mesocosm studies

This chapter characterized changes in phytoplankton biomass and assemblage composition in response to N form and N: P ratio in five mesocosm experiments. The study aimed to answer how alteration of specific nutrients, a likely result from the ongoing Comprehensive Everglades Restoration Project, may lead to specific changes in phytoplankton.

Nutrient additions included a matrix of different forms of N (NO_3^-, NH_4^+, and DON) and of organic P, in varying N:P ratios (4, 16, 32), P alone addition, and a control. Total phytoplankton biomass as chl *a* tripled in the +P treatments and increased 10-fold in the +N+P treatments, whereas chl *a* did not increase in the +N treatments. Typically the +N+P treatments, particularly the +NO_3^-+P at an +N:P molar ratio = 32, promoted high concentrations of fucoxanthin (generally indicative of diatoms) relative to chl *a*. Although overall chl *a* did not increase substantially in the +N alone treatments, there was a change in the phytoplankton assemblage. The +N treatments, especially N 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 chl *a* increased, whereas the relative concentrations of zeaxanthin (generally indicative of photosynthetic dinoflagellates) declined. This study recommends co-management of both P and N, particularly NH_4^+ and DON in Florida Bay if picocyanobacterial blooms are to be controlled.

Chapter 4: Quantifying the effects of freshwater discharge and nutrient loading on phytoplankton biomass and assemblage in semi-enclosed lagoon system in Florida Bay using a statistic model

This chapter used a statistical model to examine relationships among freshwater discharge, nutrient concentrations and forms, and phytoplankton biomass and assemblage composition. The model was developed based on previously established relationships between nutrients and phytoplankton from field data collected during 2010 to 2014 in the WLC of the ICOLLs. Sensitivity analysis was conducted on phytoplankton versus freshwater discharge and nutrient loads from the Everglades in the three different lakes within the WLC.

Modeling simulations indicated that higher freshwater discharge from the Everglades would affect the three lakes somewhat differently. The simulation of increasing freshwater flow without changes in inflowing nutrients resulted in decreased salinity and nutrients due to dilution. Under such conditions, phytoplankton biomass, and the phytoplankton functional groups of picocyanobacteria and diatoms, all decreased in upper West Lake; phytoplankton biomass and picocyanobacteria decreased in the next lake, Long Lake, while diatoms increased; while Garfield Bight, the northern Florida Bay receiving embayment, showed high potential to develop algal blooms of both major functional groups. Alteration of nutrient loads from the Everglades in the model resulted in differing effects depending on the nutrient. Most notable was the potential impact from a change in dissolved organic nitrogen (DON), which was predicted to triple cyanobacteria biomass in West Lake, confirming that cyanobacteria would be the most likely phytoplankton group to bloom in that system. This model may be applied as a research tool to help understand the effects of the ongoing restoration of flow in the Everglades on the nutrient regime and on the biomass and assemblage of phytoplankton in this lake system and in northern Florida Bay.

Chapter 5: Summary and synthesis

The conclusions of this dissertation are relevant to the dynamics of other coastal lagoon ecosystems that share the common features of shallowness, long residence times, high nutrient regeneration rates, and intrinsic sensitivity to allochthonous nutrient inputs. These findings also provide some valuable information pertinent to water quality management. As mentioned previously, control of both N and P land-based inputs from the Everglades is suggested to avoid noxious algal blooms in northern Florida Bay.

Considering directions for future research, there is much yet to be understood in terms of phytoplankton responses to altered nutrient regimes in northern Florida Bay. The flow of C-111 freshwater directly into Taylor Slough is projected to double by as much as 2.5×10^{10} L (6.5 billion gallons) per year, ostensibly designed to promote the recovery of seagrasses based on previous conjecture that hypersalinity had caused their decline since the late 1980s. This research indicates high likelihood of a nutrient-induced regime shift from the increased freshwater flow, favoring phytoplankton blooms in both WLC and SPLC and/or their receiving waters, and thus long-term control of both N and P is highly recommended. More studies are also needed to understand how specific phytoplankton groups and certain phytoplankton species of concern will respond to the changes in nutrient conditions imposed by the C-111 canal. Such data will continue to inform resource managers and policymakers, and can contribute appropriate parameters to strengthen modeling efforts. Future meshing of the current nutrient-phytoplankton model with the Florida Bay Seagrass Ecological Assessment and Community Organization Model will refine predictions about the thresholds of nutrient loads that promote phytoplankton blooms, associated seagrass coverage, and overall habitat quality of the sensitive northern Florida Bay ecosystem.

Chapter 2 : Nutrients and Phytoplankton in Semi-Enclosed Lagoon Systems in Florida Bay and Their Responses to Changes in Flow from Everglades Restoration

Abstract

The C-111 project, a phase of the Comprehensive Everglades Restoration Plan (CERP), was designed to retain and increase freshwater flow into northern Florida Bay and was initiated in 2012. The imposed increase in freshwater flow to the Bay will also alter the nutrient regime available to phytoplankton. Two sets of interconnected, quasi-enclosed saline lakes at the southern end of the Everglades discharge into northern Florida Bay and were compared in terms of changes in nutrients and in phytoplankton biomass and composition before and after the first few years of the project. After C-111 implementation, these saline lakes were altered by increased freshwater flow, decreased salinity, decreased total dissolved nitrogen (TDN) including all forms of dissolved N, and increased total dissolved phosphorus (TDP) including all forms of P. One set of these lakes, previously highly eutrophic, sustained a significant, nearly 50%, decline in overall phytoplankton biomass, but also a shift to smaller-sized cells, picocyanobacteria. The other set of lakes, which had been oligotrophic, experienced a doubling of phytoplankton biomass in the upstream lakes, but an even greater increase in the downstream embayments, also associated with smaller sized cells. While the magnitude of these latter changes was not significant, the trajectory of response suggests potential further changes as flow increases. This study implies that the increasing freshwater flow and N availability

are likely to affect nutrients and phytoplankton in this region, the less nutrient-rich system has the greater potential for increased phytoplankton biomass, while the highly eutrophic one might become less eutrophic, but with potential for export of algal biomass and change in phytoplankton assemblage in northern Florida Bay. The presence of these lakes may also serve as buffers, dampening the effects in Florida Bay itself.

Introduction

Florida Bay has experienced substantial ecological degradation since the late 1980s. Its ecological problems have ranged from episodic massive seagrass die-offs (Hall et al., 1999; Koch et al., 2007), to prolonged picocyanobacteria, *Synechococcus* sp. blooms (Phlips et al., 1999; Glibert et al., 2009; Berry et al., 2015), to higher trophic level organism mortality (Robblee et al., 1991; Fourqurean & Robblee, 1999; Peterson et al., 2006). Hypersalinity was earlier considered to be the contributing factor to seagrass loss (McIvor et al., 1994; Zieman et al., 1999), but nutrient over-enrichment is also considered to be a major, indirect cause of seagrass loss (Boesch et al., 1993; Lapointe & Barile, 2004). To restore Florida Bay, the Comprehensive Everglades Restoration Plan (CERP) has been initiated to redirect water south through the Everglades and into Florida Bay, thus lowering the salinity levels. As a component of the CERP, the C-111 Spreader Canal Western Features project was initiated by the South Florida Water Management District (SFWMD) in 2012. The C-111 project is designed to retain and increase freshwater flow within the primary drainage system into northern Florida Bay, Taylor Slough. The general goal of the

CERP is to shift the salinity regime by changing the timing and distribution of freshwater flow. Yet, the increase in water flow is also expected to increase the quantity and quality of nutrients discharged to Florida Bay, leading to unknown impacts on phytoplankton communities and ultimately ecosystem health, including the benthos (Sutula et al., 2003; Koch et al., 2012).

Northern Florida Bay is particularly sensitive to changes in salinity and nutrients that might occur due to the C-111 project, as it lies at the interface between Florida Bay and the Everglades; this region should be the first to respond to changes from the C-111 project. The northern Bay has many quasi-Intermittent Closed Open Lakes and Lagoons (ICOLLs) (Suzuki et al., 1998; Frankovich et al., 2011; Maher et al., 2011). ICOLLs are coastal water wholly or partially separated from the adjacent sea by one or more restricted inlets. They are not true ICOLLs in that they are not completely physically separated from the Bay, and they are connected to each other, but they function as ICOLLs, receiving limited or even reversed flow during the dry season. ICOLLs often act as sinks of land-derived nutrients because of their restricted water exchange, and they tend to be rich in organic matter and nutrients (Kjerfve & Magill, 1989). They are considered to be highly productive ecosystems and thus have the potential to become eutrophic with increased nutrient enrichment (Suzuki et al., 1998; Menendez & Comin, 2000). The quasi-ICOLLs in northern Florida Bay are relatively isolated from each other, and each exhibits distinctive geological and ecological characteristics.

As a shallow, subtropical coastal lagoon, Florida Bay itself is also intrinsically very sensitive to allochthonous nutrient inputs. Nutrient regeneration rates are high, not only in the water column but also from the benthos (Yarbro & Carlson, 2008; Gardner & McCarthy, 2009; Glibert et al., 2010). This is due to the morphometry of this system, especially its high benthic surface:volume ratios and slow flushing (long water residence time (Madden, 2010). In the central region of Florida Bay, frequent blooms of picocyanobacteria were observed in the past several decades, a result of both new nutrient inputs and sustained nutrient regeneration (Phlips & Badylak, 1996; Hitchcock et al., 1998; Phlips et al., 1999; Glibert et al., 2004; 2009). The relationships between such blooms and nutrients originating from the Everglades watershed have been a topic of much debate (e.g., Lapointe & Clark, 1992; Boesch et al., 1993; NRC, 2002; Lapointe & Barile, 2004). Nutrients entering the Bay from the watershed are rapidly recycled, and recurring and sustained picocyanobacteria blooms can be supported by regenerated nutrients within the system (Rudnick et al., 1999; Glibert et al., 2004; 2009; 2010).

Nutrients in northern Florida Bay have three general features. First, the nutrient composition is characterized as having proportionately more chemically-reduced or regenerated nitrogen (N; organic N and ammonium, NH_4^+) compared to oxidized or new N (nitrate, NO_3^-; Glibert et al., 2004, 2010a). Second, organic N is the major N form and contributes substantially to primary productivity, particularly of picocyanobacteria (Fourqurean et al., 1993; Glibert et al., 2004; Boyer & Keller, 2007). Third, this system has limited and variable phosphorus (P) concentrations, and the dissolved inorganic N:P ratio generally is much higher than Redfield ratio, primarily because much of the P is immobilized by the P-adsorbing carbonate sediment (Boyer et al., 2006; Price et al., 2006). Thus, northern and eastern Florida

Bay have classically been viewed as P-limited (Powell et al., 1989; Fourqurean et al., 1992).

Here, the changes in hydrology, nutrient concentrations and ratios, and phytoplankton biomass and taxa composition that are due to the C-111 project were addressed. It was specifically hypothesized that the quasi-ICOLLS would have the following effects following C-111 implementation (1) water flow would increase, (2) nutrient loading would increase, but the increases in N and P would be disproportional, and (3) changes in phytoplankton biomass and assemblage would result. More specifically, it was hypothesized that (A) there would be an increase in N relative to P because P is proportionately more retentive than N within sediments, (B) the ratio of oxidized to chemically-reduced forms of N would increase because new or oxidized N should be enhanced, whereas the regeneration rate of N should be comparatively unaffected or reduced by decreasing salinity, and (C) the smaller size fraction of phytoplankton, especially picocyanobacteria, would increase because picocyanobacteria are more competitive under conditions of comparatively low P (Smith & Kalff, 1982; Owens et al., in review; Agawin et al., 2000; Zubkov et al., 2007; Van Mooy et al., 2009), but such an effect might be offset by a disproportionate input of oxidized N relative to reduced N. Under conditions of increased oxidized N, diatoms would be expected (e.g., Glibert et al. 2016).

The differential responses of two chains of quasi-ICOLLs, located in the southern Everglades, at the northern Florida Bay border were compared both to each other and to reference stations in eastern Florida Bay (EFB), presumed to have been unaffected by changes in flow imposed by the C-111 project. These two chains of ICOLLs were previously characterized as poised at different stable states (*sensu* Scheffer & Carpenter, 2003). Prior to the CERP, one ICOLL chain was considered to be eutrophic and in a planktonic-dominated state, while the second ICOLL chain was oligotrophic and in a benthic-dominated state (Carpenter et al., 1999; Poor, 2010; Stanley et al., 2010) based on the overall assessment of chlorophyll *a* (chl *a*) and submerged aquatic vegetation (SAV) (USEPA, 2005; Bricker et al., 2008; Frankovich et al., 2011). It was thus further hypothesized that the response of each chain of ICOLLs to the change in nutrient loading as a result of C-111 implementation would differ, with a greater potential for the benthic-dominated chain to become more nutrient-enriched and to shift into a planktonic-dominated state, but less of a likelihood for the planktonic-dominated ICOLL~~s~~ to shift to a benthic state. This paper is part of a broader effort to characterize both the pelagic and benthic responses of these quasi-ICOLLs (Owens et al., in review). As issues of changes in freshwater flow and their effects on ecology are of increasing concern in estuaries, e.g., San Francisco Estuary (e.g., Glibert, 2010; Thomson et al., 2010; Dugdale et al., 2012) and Apalachicola Bay, FL (e.g., Chanton & Lewis, 2002; Putland et al., 2014), this study has broader implications than just Florida Bay.

Methods

Site description and sampling times

Florida Bay is a coastal lagoon approximately 2,200 km^2 in area connected to the Everglades wetland system at its northern boundary (Fig. 2.2). Water exchange with the Atlantic Ocean is inhibited by the Florida Keys and reef tract along the eastern and southern boundaries (Boyer et al., 2006; Madden et al., 2009). The Bay exchanges with the Gulf of Mexico at the open western border, via diurnal tides and currents. Hydrological circulation within the Bay itself is limited, as there are many shoals and shallow basins that restrict water transport (Lee et al., 2002). Florida Bay is fairly shallow with an average depth of approximately 1.5 m. The Bay has an average annual temperature of 25°C and two distinct meteorological seasons: a November-April dry season and a May-October rainy season, during which 75% of annual precipitation occurs (Duever et al., 1994). The freshwater input is considerable in the wet season through slowly flowing surface freshwater from the Everglades (Lee et al., 2007).

This study focused on two highly contrasting chains of quasi-ICOLLs in northern Florida Bay, and sites in eastern Florida Bay (EFB) in both the eastern Bay and along the Florida Keys (Fig. 2.1; Table 2.1). The West Lake Chain (WLC) of these ICOLLs includes West Lake, Long Lake, and its northern Florida Bay receiving site, Garfield Bight. The Seven Palm Lake Chain (SPLC) includes Seven Palm Lake, Middle Lake, and its receiving site, Terrapin Bay.

These two lake chains have been shown to be very different in their ecological states and geological connections (Frankovich et al., 2011). The WLC is typically eutrophic, with low light penetration and very high suspended algal chl *a* concentrations, while the SPLC typically is dominated by benthic production, lower in nutrient content, and much lower in suspended algal chl *a*, with significantly higher light penetration to the bottom and has much greater abundance of submersed aquatic vegetation, both angiosperms (e.g., *Halodule wrightii*, the dominant seagrass group) and macroalgae (e.g., *Chara* sp., the dominant macrophyte group; Frankovich et al., 2011). Both the WLC and the SPLC receive freshwater mainly from Taylor Slough, one of two wetland drainage systems in the Everglades. Flow from Taylor Slough gradually widens as it extends south, ultimately merging with wetlands near these saline lakes. Within each chain system, flow is connected and occurs from the upper lakes to the lower (more downstream) lakes and then to northern Florida Bay. The residence time of these lakes are in the magnitude of a month (Lee et al., 2007), although it differs between the individual lakes. The lakes in the WLC are effectively separated by narrow mangrove creeks and small, shallow (<50 cm) ponds within them; lakes in the SPLC are more hydrologically connected than those in the WLC (Frankovich et al., 2011). The difference in residence time in each lake of the same chain is attributed to the different lake size. The lake sizes are, respectively, 1.25×10^7, 2.3×10^6, 9×10^6, 8.7×10^6, 1.4×10^6, 6.2×10^6 m^3 for West Lake, Long Lake, Garfield Bight, Seven Palm Lake, Middle Lake and Terrapin Bay. In addition, the receiving embayment of WLC, Garfield Bight is better connected to northern Florida Bay compared to the receiving embayment of SPLC, Terrapin Bay. Reference sites sampled in eastern Florida Bay (EFB) included Duck Key, Blackwater Sound and Sunset Cove (Fig. 2.1).

Sampling of these saline lakes and EFB stations occurred on single or multiple occasions in each of five years: April 2010, November 2010, April 2011, June 2012, August 2012, May 2013, November 2013, and April 2014. The intent was to study a wet and a dry season each year. Each of the lake and EFB sites were visited on each sampling trip (1-2 sites/day) to the extent that weather and other conditions permitted.

Hydrological data

Due to the dispersed nature of Taylor Slough, direct flow data are not available. Therefore, alternate metrics of changes in flow, including precipitation, water level and salinity, were used to assess long-term trends in hydrology. Data on water level and precipitation at the mouth of Taylor River, part of the Taylor Slough system located in Little Madeira (Fig. 2.1), were obtained from the Everglades Depth Estimation Network (EDEN) and United States Geological Survey (USGS; http://sofia.usgs.gov/eden/stationlist.php). Daily salinity data of Seven Palm Lake were downloaded from USGS website (http://sofia.usgs.gov/exchange/sfl_hydro_data/).

Sample collection

At each site, temperature and salinity were measured by a YSI-85 meter. Water samples from all sites were collected from about 10 cm below the surface in 20L carboys, filtered through 153 µm mesh plankton net to remove large zooplankton, and returned for processing to the National Park Service Florida Bay Interagency Science Center on Sunset Cove (eastern Florida Bay off Florida Keys) within ~1 - 4 hr of collection.

Chemical analyses

Samples from each station were analyzed for algal biomass and nutrient concentrations. Samples for chl *a* analysis were collected in duplicate by sequential size fractionation. Samples were first filtered through a 3-µm Nuclepore filter to yield the > 3 µm fraction, and then through a GF/F filter (nominally 0.7-µm pore size), to yield the 0.7-3.0 µm fraction. Filters were extracted overnight in 100% methanol at 4°C and subsequently read on a Turner 10AU fluorometer (Holm-Hansen et al., 1965), calibrated using a chl *a* standard from *Anacystis nidulans* (Sigma). A separate set of filtrations through GF/F filters was done for phytoplankton pigment analysis by high-performance liquid chromatography (HPLC; Van Heukelem & Thomas, 2001). These samples were flash-frozen in liquid N_2 and stored at -80 °C for subsequent analysis on an Agilent 1100 HPLC.

The filtrates from these samples were collected and frozen for subsequent nutrient analysis. Concentrations of NO_x^- (NO_3^- + NO_2^-), NH_4^+, and dissolved inorganic phosphorus (DIP) were measured using autoanalysis techniques (Whitledge et al., 1981; D'Elia et al., 1997). Total dissolved nitrogen (TDN) and total dissolved phosphorus (TDP) concentrations were determined by persulfate oxidation followed by autoanalysis (Valderrama, 1981). Dissolved organic nitrogen (DON) and dissolved organic phosphorus (DOP) were determined by the difference between dissolved total and inorganic fractions.

Data analysis

Hydrological long-term trends were analyzed using two different approaches. First, monthly-averaged precipitation and water level data at Taylor River, and salinity data at Seven Palm Lake, were examined from May 2004 to April 2014. A time series of a decade prior to CERP is reported here so that the effect of seasonal and annual variations could be differentiated from changes due to the CERP. Second, cumulative sums of variability (CUSUM; Page, 1954; Manly & Mackenzie, 2003; Mesnil & Petitgas, 2009) were calculated using available hydrological data. CUSUM reveals a relatively simple and visually more apparent pattern than that of the deviations plotted individually. The z-score CUSUM approach was applied (Briceño & Boyer, 2010), and the z-scores of each datum were summed through time. In the z-score CUSUM chart, a positive slope represents a period where the values are above the average in the time series, while the negative slopes indicate below-average values; they should not be interpreted as upward or downward trends. Breaks in slope in the CUSUM time series indicate differences in averages at each side of the break.

In order to assess the effects of C-111 implementation, regional, temporal and flow differences in temperature, salinity, nutrients and phytoplankton biomass (as chl *a*) and composition were examined by comparison of the averages and standard deviation of each region before and after C-111 project initiation. Comparisons were also made of the differences in the upstream lakes of each lake chain compared to its respective downstream embayment. There were 3 sampling efforts before C-111 project implementation began, and 5 after. One-way ANOVAs at significance levels of both $p = 0.05$ and $p = 0.1$ were performed with SAS software. In the regional comparison, Fisher's LSD test was followed for the pair comparison.

To characterize the extent to which flow-related nutrient concentrations may have resulted in changes in phytoplankton, changes in nutrient concentration and form were compared to changes in phytoplankton biomass (as chl *a*) and assemblage. Given the broad range in nutrient concentrations and other physical parameters across sites and seasons, nutrient and biomass data were compared to salinity with the assumption that changes in salinity were a reasonable proxy for flow changes. All data from all sites and sampling dates were used for this phase of the analysis. These comparisons yielded two distinct response groups, herein referred to as the low-salinity (LS) and high-salinity (HS) groups. The LS stations were those that had salinity $\leq$ 20, and the HS stations were those where salinity >20. These groups did not strictly follow the geographic differentiation of the two lake chains due to the differing seasonal effects of freshwater flow. While the data from the upper sites in the lake chains, specifically West Lake and Seven Palm Lake, were consistently categorized as LS, and those of the receiving embayments were generally characterized as HS, intermediate sites Long Lake and Middle Lake were variable depending on whether sampling occurred during a high-flow or low-flow period (Table 2.1). All stations in the EFB were categorized as the HS group.

These station groupings were subsequently compared to quantify the response in phytoplankton biomass (as chl *a*) and assemblage to different nutrient forms. Trends in concentrations of chl *a* and major pigments (relative to chl *a*) as a function of different nutrients and nutrient forms were examined by Pearson correlation analysis to gain insights about the potential for different algal functional groups to respond to changes in nutrients. Zeaxanthin was considered as an indicator of cyanobacteria, alloxanthin as an indicator of cryptophytes, peridinin as an indicator of peridinin-containing dinoflagellates, and fucoxanthin as an indicator of diatoms (Jeffrey & Wright, 1994; Jeffrey & Vesk, 1997; Glibert et al., 2004). The strength of the correlation (Pearson correlation coefficient, r) and the slopes for all pigments and -nutrients were calculated. Statistical significance of each correlation is reported for both $p = 0.05$ and $p = 0.1$ levels.

Results

Hydrological trends

Monthly averaged hydrological data displayed a strong seasonal pattern. Generally, comparatively high precipitation and a corresponding elevation in water level occurred in the wet (high-flow) season from May to October, while low precipitation and a decrease in water level was observed in the dry (low-flow) season from November to April. Precipitation and water level parameters were not completely consistent, however, because precipitation is one of many water input sources and there is the accumulative effect of precipitation on the water level. In the seven sampling efforts, during the 15 days prior to sampling, precipitation amounts were 32, 55, 8, 103, 62, 1, 20, and 6 mm, respectively (Fig. 2.2a), and water levels were -0.17, -0.26, -0.15, -0.13, -0.14, -0.18, 0.03, -0.24 m, respectively (Fig. 2.2b). Salinity in Seven Palm Lake averaged 15.37 and, as expected, had the opposite seasonal trend compared to the precipitation and water level at Taylor River (Fig. 2.2c).

The CUSUM calculations for water level and salinity illustrated the overarching hydrological trend of the long-term change in freshwater delivery. The z-CUSUM of water level in Taylor River showed a general downward trend before June 2009, variable flow from June 2009-2011, and an abrupt upward trend after June 2011, indicating values below the long-term mean shifting to values above the long-term mean (Fig. 2.3b). The overall salinity z-CUSUM values for Seven Palm Lake showed a general upward trend to October 2009, followed by a period through April 2011 during which CUSUM values trended downward. After the high-flow season in 2011, the trend declined again after October 2011 (Fig. 2.3c). Thus, the salinity trend was the opposite of the water level trend, although it was delayed (lagged) by up to several months.

Changes in water quality: abiotic parameters

To assess the effects of C-111 implementation, three sets of comparisons were made. Average water quality parameters are compared first for each Bay region (the two ICOLL chains WLC and SPLC, and EFB), then before and after effects of C-111 were examined by Bay region, and finally the before-and-after comparison was made by sub-region, comparing the upstream lakes with the downstream embayments.

Salinity in the two ICOLL chains was significantly lower than in EFB (Fisher's LSD test: $p < 0.05$, $n = 64$ for both pair comparisons; Table 2.2). Salinity averaged 21 in the WLC and 17 in the SPLC, while it was 28 on average in EFB. After C-111 implementation, salinity decreased in all three regions. The decrease in salinity in the WLC was only 5% following C-111 implementation, but 30% in the SPLC, and 12% in EFB (difference not statistically significant). By sub-regional basis, following C-111 implementation, salinity decreased in WLC upstream lakes, increased in WLC downstream embayment and decreased in both upstream and downstream of SPLC, but these sub-regional differences were not statistically significant (Table 2.2). Water temperatures in the WLC and the SPLC increased significantly post C-111 implementation (one-way ANOVA: $p < 0.05$, $n = 24$ for WLC; $p < 0.05$, $n = 22$ for SPLC) and it increased significantly in both upstream and downstream of WLC (one-way ANOVA: $p < 0.05$, $n = 16$ for WLC upstream; $p < 0.1$, $n = 8$ for WLC downstream; Table 2.2).

There were substantial differences in total nutrient availability, forms, and ratios between the two ICOLL chains and between them and EFB, both before and after C-111 implementation (Fig. 2.3, Table 2.2). Concentrations of TDN, DON, TDP and DOP in the WLC were significantly higher than in the SPLC and EFB (Fisher's LSD test: $p < 0.05$, $n = 64$ for TDN, DON, TDP; $p < 0.05$, $n = 64$ for DOP), and these nutrient concentrations were also significantly higher in the SPLC than in EFB (Fisher's LSD test: $p < 0.05$, $n = 64$ for TDN; $p < 0.05$, $n = 64$ for DON; Fig. 2.3a, Table 2.2). DON was the major N form in WLC, SPLC, and EFB, comprising >85% of TDN. DIN concentrations for all sites considered collectively averaged 5-7 μM-N, and NH_4^+ was the main inorganic N form (Table 2.2). In addition, DOP was the major P form in this region, composing > 60% of TDP (Fig. 2.3b, Table 2.2). The average DIP concentration for all sites considered collectively was < 0.3 μM-P. DIP in the WLC was significantly higher than in the SPLC and the EFB (Fisher's LSD test: $p < 0.05$, $n = 64$ for WLC and SPLC comparison; $p < 0.1$, $n = 64$ for WLC and EFB comparison; Fig. 2.3b). TDN:TDP ratios were > 150 in all regions, even > 225 in SPLC (Fig. 2.3c). Due to the dominant role of DON in TDN, the DIN:DIP ratios were comparatively lower than the TDN:TDP ratios, averaging 27, 48, 41 in the WLC , the SPLC, and EFB, respectively (Fig. 2.3c). They were significantly lower in the WLC than in the SPLC (Fisher's LSD test: $p < 0.1$, $n = 64$ for WLC and SPLC comparison).

Following C-111 implementation, TDN showed a downward trend while TDP generally trended upward (Fig. 2.3). Reductions in N species were only statistically significant, however, in the SPLC and the EFB (one-way ANOVA: $p < 0.01$, $n = 24$

for TDN, DON, $NH_4^+$and NO_x^- in the SPLC; $p < 0.05$, $n = 22$ for $NH_4^+$and NO_x^- in the EFB). Changes in P were more variable, but pre- vs. post-C-111 data were not statistically significant. The TDN:TDP ratio decreased in both WLC and SPLC, but it was only significant in SPLC (one-way ANOVA: $p < 0.1$, $n = 22$ in the SPLC). The DIN:DIP ratio decreased by >75% in both WLC and SPLC, and > 50% in EFB, but remained above Redfield proportions (Fig. 2.3c; one-way ANOVA: $p < 0.1$, $n = 24$ in the WLC; $p < 0.05$, $n = 22$ in the SPLC; $p < 0.05$, $n = 18$ in the EFB).

Changes in sub-regions WLC and SPLC differed following C-111 implementation (Table 2.2). In the WLC, TDN and all forms of N increased and both forms of P decreased in the upstream, whereas all forms of N other than DON decreased and P increased in the downstream. The only significant changes were found in the upstream, NO_x^- (one-way ANOVA: $p < 0.05$, $n = 16$ for NO_x^-). In the SPLC upstream, TDN, DON, NH_4^+, and NO_x^- significantly decreased (one-way ANOVA: $p < 0.05$, $n = 16$ for TDN, DON, NH_4^+, and NO_x^-). Consequently, although not statistically significant, the TDN:TDP ratio decreased (by 31-52%) in all sub-regions other than the downstream WLC, and the DIN:DIP ratio decreased (by 53-78%) in all sub-regions except the SPLC downstream embayment. The TDN:TDP ratio significantly decreased in the upstream WLC (one-way ANOVA: $p < 0.1$, $n = 16$) and the DIN:DIP ratio significantly decreased in the SPLC upstream lakes (one-way ANOVA: $p < 0.05$, $n = 15$; Table 2.2).

Phytoplankton biomass and assemblage

Phytoplankton chl *a* concentrations and structure of the assemblages differed by size (as chl *a* fractions) and taxa between the lake chains and between them and EFB (Fig.

2.4). Concentrations of chl *a* were > 10-fold and 35-fold higher in the WLC than in the SPLC and EFB, respectively (Fisher's LSD test: $p < 0.01$, $n = 64$ for both the WLC vs. SPLC, and the WLC vs. EFB pair comparisons; Fig. 2.4a). The 0.7-3.0 µm size fraction of chl *a* samples accounted for 50% of total chl *a* in the WLC, while it was ~40% of total chl *a* in the SPLC and EFB. Phytoplankton abundance as both size fractions of chl *a* in the WLC were significantly higher than in those in either the SPLC or EFB (one-way ANOVA: $p < 0.01$, $n = 64$ for comparisons of both the WLC and the SPLC, and the WLC and EFB).

In terms of the composition of the phytoplankton assemblage pre-C-111 implementation, the WLC was dominated by picocyanobacteria, while the SPLC and EFB had a comparatively more diverse assemblage. EFB had more phytoplankton within the larger size fraction, such as diatoms and dinoflagellates (Fig. 2.4b-e). The relative concentration of zeaxanthin:chl *a* in the WLC was about double that in the SPLC, and more than 4 times that in EFB (Fisher's LSD test: $p < 0.05$, $n = 62$ for comparisons of the WLC vs. SPLC, and the WLC vs. EFB; $p < 0.1$, $n = 62$ for the comparison of the SPLC vs. EFB; Fig. 2.4b). The relative concentration of alloxanthin:chl *a*, indicative of the proportion of cryptophytes in the total phytoplankton biomass, was low in the WLC compared to the other regions (Fisher's LSD test: $p < 0.1$, $n = 62$ for comparisons of the WLC vs. EFB; Fig. 2.4c). In the WLC, the relative concentrations of fucoxanthin:chl *a* and peridinin:chl *a*, indicating the diatom and peridinin-containing dinoflagellate proportions, respectively, were about half of those in SPLC, which were also about half of that in EFB (Fisher's LSD test: $p < 0.01$, $n = 62$ for comparisons in fucoxanthin:chl *a* of the WLC vs. the SPLC, the WLC vs. EFB, and the SPLC vs. the EFB; $p < 0.01$, $n = 62$ for comparisons in peridinin:chl *a* of the WLC vs. EFB, and the SPLC vs. EFB; $p < 0.05$, $n = 62$ for comparison in peridinin:chl *a* of the WLC vs. the SPLC).

After the C-111 project was initiated, chl *a* concentrations, the small-size and large-size fractions both decreased ~60% on average in the WLC lakes (one-way ANOVA: $p < 0.1$, $n = 22$ for the small-size chl *a*; $p < 0.05$, $n = 22$ for the large-size chl *a*; Fig. 2.4a). There were no statistically significant differences in the zeaxanthin:chl *a*, alloxanthin: chl *a*, fucoxanthin:chl *a*, and peridinin:chl *a* ratios in the WLC before and after C-111 implementation (Fig. 2.4b - e). In contrast, in the SPLC after implementation of the C-111 canal, the fucoxanthin:chl *a* ratio decreased by ~40% (one-way ANOVA: $p < 0.05$, $n = 22$; Fig. 2.4d). In EFB, there were no significant differences in in phytoplankton assemblages after C-111 initiation.

The trends in phytoplankton assemblage in the sub-regions of each lake chain were typically the same, but the extent of change was generally larger in the downstream embayments compared to the upstream lakes (Table 2.2). In the WLC after C-111 initiation in the upstream lakes, the smaller size fraction (29 to 16 $\mu m\ g^{-1}$) and the larger size fraction (22 to 11 $\mu m\ g^{-1}$) of chl *a* decreased ~50%, (one-way ANOVA: $p < 0.1$, $n = 16$ for chl *a*; $p < 0.05$, $n = 16$ for 0.7-3 µm chl *a*; Table 2.2). In addition, alloxanthin concentrations were also reduced significantly, declining by 80% (one-way ANOVA: $p < 0.1$, $n = 16$) in the upstream WLC. Fucoxanthin:chl *a* decreased significantly by 40% in the SPLC upstream lakes post C-111 initiation (one-way ANOVA: $p < 0.05$, $n = 15$).

Nutrients and phytoplankton in relation to salinity

There were distinctly different relationships between salinity and nutrients and between phytoplankton and nutrients for the LS and HS station groupings. For TDN and DON, correlations with salinity were significant and positive for the LS stations (Pearson correlation: $p < 0.05$, $n = 30$) but not for the HS stations (Fig. 2.5a, c, Table 2.3). Both TDP and DOP showed significant, positive relationships with salinity, but with differing slopes for the two salinity groupings (Pearson correlation: $p < 0.05$, $n = 30$ for TDP and DOP in LS and HS; Fig. 2.5b, d, Table 2.3). For the inorganic nutrients, NH_4^+ and DIP were significantly and positively related with salinity in the LS stations (Pearson correlation: $p < 0.05$, $n = 30$ for NH_4^+ and DIP), but not in the HS stations, and NO_x^- was not correlated in either (Table 2.3). Interestingly, the ratios of TDN:TDP showed a downward trend with the increase of salinity in both salinity groupings despite both N and P increasing, suggesting that the N increase was proportionately less than the P increase. The TDN:TDP ratios were significantly correlated with salinity in both salinity categories (Pearson correlation: $p < 0.05$, $n = 30$ for both LS and HS; Table 2.3). However, DIN:DIP ratios were not significantly correlated with salinity in either the LS or HS groups (Table 2.3).

Trends in phytoplankton biomass and composition relative to salinity were similar to those in the nutrient-salinity relationships. Concentrations of total chl *a,* as well as the different size fractions of chl *a*, were positively correlated with salinity at the LS stations, but were negatively correlated at the HS sites (Fig. 2.6). In the LS stations, the correlations were significant for chl *a* and 0.7-3.0 µm chl *a* fraction (Pearson correlation: $p < 0.05$, $n = 30$ for chl *a* and 0.7-3 chl *a* fraction) and the slope for the

0.7-3.0 µm chl *a* was higher than that for 3 µm chl *a*, which was not significantly correlated with salinity (Table 2.3). The relative concentrations of pigments all increased with salinity in the LS stations, but none of these relationships were statistically significant. In the HS stations, the proportions of zeaxanthin:chl *a* was negatively correlated with salinity, while alloxanthin:chl *a* was positively correlated (Pearson correlation: $p < 0.05$, $n = 30$; Table 2.3).

Phytoplankton biomass and assemblage in relation to different forms of nutrients

The biomass and composition of the phytoplankton assemblage varied as nutrient quantity and quality changed. Total chl *a* and its different size fractions generally increased with increasing nutrients in both the LS and HS station groupings, but with different slopes (Pearson correlation, $p < 0.05$ for TDN and DON in all the LS and HS, $n = 30$; Fig. 2.7, Table 2.4). The relationships between chl *a* and TDP and DOP in the LS, and between all chl *a* categories and DIP in the HS were positive and statistically significant (Pearson correlation: $p < 0.05$, $n = 30$ for correlations between chl *a* and DOP in LS; $p < 0.05$, $n = 30$ for correlations between all fractions of chl *a* and DIP in HS; $p < 0.1$, $n = 30$ for correlations between chl *a* and TDP in LS; Fig. 2.7, Table 2.4).

Zeaxanthin:chl *a* was significantly and positively related to both TDN and DON in both the LS and the HS station groups (Pearson correlation: $p < 0.05$, $n = 30$ for both TDN and DON in LS and HS), whereas the alloxanthin:chl *a* was significantly, but negatively related to DON in the LS stations (Pearson correlation: $p < 0.05$, $n = 30$) and fucoxanthin:chl *a* and peridinin:chl *a* were significantly and negatively related with TDN and DON in the HS stations (Pearson correlation: $p < 0.05$, $n = 30$ for all correlations; Fig. 2.8, Table 2.4). The proportions of zeaxanthin, fucoxanthin and peridinin to chl *a* were nonlinearly related to the DIN:DIP ratio. Increasing at first, zeaxanthin:chl *a* and peridinin:chl *a* declined when NH_4^+ exceeded 5 μM, or when DIN: DIP increased >100, suggestive of P limitation. Fucoxanthin:chl *a* was significantly, positively correlated with NO_x^- in the LS stations (Pearson correlation: $p < 0.05$, $n = 30$). In these LS stations, alloxanthin:chl *a* was significantly, positively correlated with DOP and zeaxanthin:chl *a* was significantly, positively correlated with DIP (Pearson correlation: $p < 0.05$, $n = 30$ for alloxanthin:chl *a* in relation to DOP; $p < 0.1$, $n = 30$ for zeaxanthin:chl *a* in relation to DIP; Fig. 2.8).

Discussion

Changes in freshwater flow to estuaries are an increasingly important – yet controversial – issue, as ecological function in estuaries is linked to freshwater supply. Increased flow typically decreases the salinity and increases the nutrient loadings. Variable freshwater inflow, which is a function of natural meteorological and climatic changes (high-flow, low-flow seasons, cycles of drought, flooding), and water diversion projects have led to debates about effects on ecology and economies, as for example in the San Francisco Estuary (e.g., Thomson et al., 2010; Dugdale et al., 2012) and Apalachicola Bay, Florida (e.g., Chanton & Lewis, 2002; Putland et al., 2014), among many other estuaries. Here, the direct effects of a flow restoration effort in Florida Bay were examined in terms of changes in salinity, nutrients and the amount and assemblage structure of the phytoplankton. The major objective of the ongoing restoration of flow for Florida Bay is the potential for reduction in phytoplankton blooms in northern Florida Bay and the restoration of beneficial SAV. The unique nature of the quasi-ICOLLs in the southern Everglades, northern Florida Bay border, provided an opportunity to compare responses of highly contrasting systems and to interpret responses in terms of the differing roles of these semi-enclosed systems in modulating flow effects on the Bay.

Overarching trends

The initiation of water diversion operations in the C-111 Spreader Canal Western Project was evident from the increased water levels and reduced salinity in the ICOLLs (Fig. 2.2b-c). It is of note that an extremely high rainfall was observed in the latter half of the 2012 dry season, around the same time the C-111 project was initiated (Fig. 2.2a). Therefore, the data reflected the net effects of both C-111 implementation and an earlier-than-normal shift from the dry season to the wet season.

It was hypothesized that the higher freshwater discharge would not only increase the nutrient input but also alter the nutrient composition due to an increase in N and a comparatively smaller change in P, leading to an increase in the N:P ratio and in the proportion of the N pool in chemically-oxidized forms. As a result, one expected outcome was an increase of the picocyanobacteria proportion, but the interacting effect of a hypothesized simultaneous increase in the proportion of NO_3^-, and its likely promotion of diatoms, was uncertain. Our results differed from these expectations in terms of nutrient loads, but substantiated our prediction of increased picocyanobacterial dominance. After C-111 project implementation, TDN and all forms of N decreased in both ICOLLs and EFB, whereas TDP and both forms of P generally increased in all regions except TDP and DOP in EFB. This resulted in decrease in TDN:TDP in both ICOLLs and decrease in DIN:DIP in all regions (Fig. 2.3). In addition, higher proportions of NH_4^+ to NO_3^-, and generally higher levels of DON were observed. Thus, the N pool became increasingly dominated by reduced forms of N rather than oxidized forms.

In response, phytoplankton parameters differed in each of the ICOLLs and in EFB. In the WLC, which was the historically more eutrophic ICOLLs chain, total phytoplankton biomass as chl *a*, and the 0.7-3.0 μm size fraction, declined significantly, by ca. 50%. The > 3 μm sized fraction of chl *a* significantly decreased by about two thirds (Fig. 2.4a). Even though total phytoplankton biomass decreased in the WLC, the relative proportions of picocyanobacteria and cryptophytes (based on pigment markers) to chl *a* increased, as originally hypothesized, and, in the case of cryptophytes, increased more than 4-fold in Garfield Bight, the downstream embayment (Fig. 2.4b-c). In the less nutrient-rich SPLC, phytoplankton biomass, as chl *a*, more than doubled compared to the pre-C-111 condition, although the change was not significant. And, as originally hypothesized, the picocyanobacteria and cryptophytes increased, and the large phytoplankton, diatoms and peridinin-containing dinoflagellates, both decreased. The diatoms in the upstream lakes declined significantly by ~50% (Fig. 2.4d). In EFB, the region that had the lowest nutrient and chl *a* levels pre-C-111, had a slight increase in phytoplankton biomass, and an increase in the relative concentrations of picocyanobacteria and diatoms, and a reduction in the cryptophytes and peridinin-containing dinoflagellates proportions

(Fig. 2.4). Thus, while the community composition generally responded as expected, the overall biomass changes were more variable. The benthic-dominated ICOLLs, SPLC, shifted to a more planktonic state as hypothesized, but the large decline in plankton biomass in the previously eutrophic WLC was not expected. A conceptual model is proposed for why this occurred.

Conceptual model

The responses by phytoplankton in the differing ICOLLs to C-111 implementation are analogous to the behavior of phytoplankton in chemostats, the steady-state culture systems often used in microalgal culture (Novick & Szilard, 1950). In a chemostat, fresh medium is continuously added while the culture is continuously removed to keep the volume constant. The growth rate of the microalgae equals the dilution rate and the biomass value is determined by the limiting nutrient in the system. In steady state, all environmental parameters remain constant and growth occurs at a constant rate. However, once the dilution rate is greater than the maximum growth rate of phytoplankton, they are washed out. The ICOLLs systems of northern Florida Bay appear to act as interconnected chemostats, with water flowing from the Everglades to the upper lakes, then from them to the lower embayments and Florida Bay (Figure 2.9). This basic functioning is similar to the conceptual/biogeochemical model developed for northern San Francisco Estuary to predict the initiation of phytoplankton blooms, and which highlights the combined effect of freshwater flow and NH_4^+ concentration (Dugdale et al., 2012; Dugdale et al., 2013; Glibert et al., 2014b).

In the upper lakes of the WLC, as flow increased, the total chl *a* and its >3 µm size fraction decreased, and this included the cryptophytes (based on alloxanthin). The phytoplankton biomass reduction is likely because the residence time of the lakes decreased, increasing the likelihood of washout of the more slower-growing phytoplankton from the lakes. The rate of change due to washout would be expected to differentially impact phytoplankton groups because they have different growth rates. Cryptophytes were washed out from the upstream lakes, whereas picocyanobacteria increased. In contrast, in the upper lakes of the SPLC, as well as its downstream embayment, there was an increase in phytoplankton biomass. This suggests that the SPLC lakes have a longer residence time than those of the WLC, or more likely that the phytoplankton in the SPLC were capable of reaching a higher maximum growth rate due to potentially higher light available in the SPLC. The latter is the more likely possibility since it has previously been shown that the SPLC lakes have shorter residence times and more rapid flushing rates (Søballe & Kimmel, 1987; Frankovich et al., 2012). As in a chemostat, the increase in the limiting resource promoted an increase in biomass. Increases in TDP and DOP, but not DIP, occurred in the upper SPLC post-C-111 while increases in TDP and DIP, but not DOP, were observed in the upper WLC. These trends were in contrast to N (all forms) that all declined. Also, changes in picocyanobacteria were coincident with DOP and TDP in all subregions post C-111 (Table 2.2). Picocyanobacteria were the first functional group to respond to the increase in DOP.

Unique ICOLL responses

Compared to the SPLC, the WLC, which is more isolated and more slowly flushed, was expected to retain more of the new N and P and to generate persistent algal blooms. These conditions historically have maintained the SPLC in a less nutrient-rich state, with relatively good water quality and abundant *Halodule* and *Chara* (Frankovich et al., 2011), and the WLC in a more eutrophic condition. In this study, increasing plankton abundance may lead to loss of submersed aquatic vegetation in the SPLC if the trajectory of increased phytoplankton biomass continues with further increased freshwater flow. In contrast, in the eutrophic WLC, the increased flow and reduced residence time may have tipped the ecosystem toward a lower biomass state and possibly dilution/washout of phytoplankton, a condition where phytoplankton growth could not keep pace, resulting in lower chl *a* after increased flow. With substantial further increases in flow, lower salinity, and higher light availability resulting from the reduced phytoplankton biomass, SAV may eventually become established in the WLC (Fig. 2.10).

The different responses in the two ICOLL chains lead to the question of whether the changes in nutrients are a response to changes in flow or changes in internal nutrient cycling with altered flow regimes. In a related study on benthic nutrient fluxes in the SPLC, Owens et al. (in review) showed that DIP and NO_3^- fluxes were mostly low under both light and dark incubation conditions while NH_4^+ effluxes were occasionally high under dark incubations, and suggested that *Chara* loss might result in increases in the NH_4^+ effluxes, thus further fueling algal blooms. They did not find a corresponding benthic DIP source for the algae. Thus, their results support the conclusion that the changes in nutrients resulted from changes in external loading, not internal processing, although the uncertain effect of groundwater remains (Price et al., 2006). The benthic nutrient flux results are consistent with our study, and show elevated rates characterized by low DIP, low NO_3^-, and a relatively high proportion of NH_4^+. If flows further increase in the CERP, with a trajectory characterized by higher phytoplankton productivity, further losses in SAV (and decay of dead SAV), and lower salinity, further increases in benthic NH_4^+ efflux could result.

Finally, the different changes in chl *a*, nutrients, and phytoplankton with respect to salinity further support the role of altered flow in these ICOLLs. The LS stations located more adjacent to the Everglades, with their lower salinity/higher nutrient and phytoplankton levels, showed significant correlations between chl *a* and DON, and chl *a* and DOP, while the HS stations which were closer in proximity to the Bay with their higher salinity, had lower nutrient and phytoplankton levels, and displayed significant relationships between chl *a* and both DON and DIP. The emergence of distinct patterns with respect to nutrients and salinity gives some predictive power to understanding how future phytoplankton responses may occur as salinity changes.

Implications

The increasing freshwater discharge from the C-111 canal project within the broader ongoing CERP program is altering nutrient concentrations and composition, and the biomass and assemblage of phytoplankton in both the upstream receiving and downstream waters. Whereas the upper ICOLLs, especially the eutrophic WLC, sustained a decrease in total chl *a* and a change in the relative contribution of different functional groups, increases in total chl *a* were detected in the relatively nutrient-poor SPLC, in particular in Terrapin Bay. Changes in the phytoplankton assemblage were also seen in EFB. To date, these changes in EFB, a reference site that was assumed to have been unaffected by implementation of the C-111 canal, have been small. Overall, these findings on early changes from the C-111 project suggest that the potential exists for nutrient composition and concentration, as well as phytoplankton biomass and composition, to change with increased freshwater flow. The ICOLLs could be acting as reactors, buffering these changes. However, changes in residence time due to changes in flow also affect the ability of these systems to buffer changes, as was the case in WLC where washout of the phytoplankton apparently occurred. Also, the highly contrasting character of the ICOLL chains suggests that with increasing flow and N availability, the SPLC has the greater potential for increased phytoplankton biomass, while the WLC has the greater potential to become less eutrophic, moving phytoplankton production from the upstream ICOLLS to the receiving water body. Without the ICOLLS, it is likely that the potential for phytoplankton blooms in Florida Bay with increased flow and its associated nutrient changes would be greater.

Historically, P has been considered the limiting nutrient in Florida Bay, and therefore its control has been thought to be critically important in controlling eutrophication (Fourqurean et al., 1993; Moustafa et al., 1999; Knight et al., 2003; Kadlec & Wallace, 2008; Frankovich et al., 2011). Based on previous studies in these ICOLLs, it was estimated that to maintain the clear state in SPLC, a target annual mean water-column TP concentration of 2.2 ± 1.1 μM was suggested (Frankovich et al., 2011). A much lower concentration, as yet unquantified, was suggested as necessary to restore the turbid WLC system (Frankovich et al., 2011). Here, all the TP concentrations measured were well below 2.2 µM, but most of the ICOLLs sustained an increase in TP, rather than a decrease following C-111 implementation. Such a trend will continue to present challenges in meeting water quality goals. In addition to P management, management of N is needed. While N concentrations in the upper ICOLLs did decline with increasing flow rate, an important trend was the reduction in both TDN:TDP and DIN:DIP due to the combination of lower N and increased P. While still far in excess of Redfield stoichiometry, there is potential for increased phytoplankton biomass and changes in phytoplankton community composition as this ratio begins to converge on Redfield values. With DON as a major contributor to total N, the picocyanobacterial fraction of the chl *a*, 0.7-3 µm, may disproportionately increase with increases in DON regardless of P limitation (Fig. 2.8, 2.10). Although in freshwater the concern is most often that cyanobacteria will increase under N limitation, some picocyanobacteria outcompete other groups in low DIP environments, attributed to their ability to substitute non-phosphorus membrane lipids for phospholipids (Van Mooy et al., 2009), and their capacity to use organic P (Wetzel, 1992; Li et al., 1998; Glibert et al., 2004). In all, these quasi-ICOLLs have presented interesting new insights into changes in nutrients and phytoplankton as flow changes. They are worthy of continued study as large-scale "mesocosms", and as potential sentinels of broader change in EFB. They also help to highlight the variable nature of estuarine responses to changes in flow, reflecting the importance of hydrology and prior ecological conditions in addition to changes in flow and the chemical character carried with that flow.

Table 2.1. Sites sampled, date and the regional, temporal and salinity category designation of all samples. LS indicates low salinity, and HS indicates high-salinity differentiation.

Regional Station Category	Station	Date	Pre or Post the C-111 Project	Salinity Category
WLC	West Lake	Apr. 2010	Pre	LS
		Nov. 2010	Pre	LS
		Apr. 2011	Pre	LS
		Jun. 2012	Post	LS
		Aug. 2012	Post	LS
		May 2013	Post	LS
		Nov. 2013	Post	LS
		Apr. 2014	Post	LS
	Long Lake	Apr. 2010	Pre	LS
		Nov. 2010	Pre	LS
		Apr. 2011	Pre	HS
		Jun. 2012	Post	LS
		Aug. 2012	Post	LS
		May 2013	Post	HS
		Nov. 2013	Post	LS
		Apr. 2014	Post	LS
	Garfield Bight	Apr. 2010	Pre	HS
		Nov. 2010	Pre	LS
		Apr. 2011	Pre	HS
		Jun. 2012	Post	HS
		Aug. 2012	Post	HS
		May 2013	Post	HS
		Nov. 2013	Post	HS
		Apr. 2014	Post	HS
SPLC	Seven Palm Lake	Apr. 2010	Pre	LS
		Nov. 2010	Pre	LS
		Apr. 2011	Pre	LS
		Jun. 2012	Post	LS
		Aug. 2012	Post	LS
		May 2013	Post	LS
		Nov. 2013	Post	LS
		Apr. 2014	Post	LS
	Middle Lake	Apr. 2010	Pre	HS
		Apr. 2011	Pre	HS
		Jun. 2012	Post	LS
		Aug. 2012	Post	LS
		May 2013	Post	LS
		Nov. 2013	Post	LS
		Apr. 2014	Post	LS
	Terrapin Bay	Apr. 2010	Pre	HS
		Nov. 2010	Pre	HS
		Apr. 2011	Pre	HS
		Jun. 2012	Post	LS
		Aug. 2012	Post	LS
		May 2013	Post	HS
		Nov. 2013	Post	HS
		Apr. 2014	Post	HS
EFB	Blackwater Sound	Apr. 2011	Pre	HS
	Duck Key	Apr. 2011	Pre	HS

	Aug. 2012	Post	HS
	May 2013	Post	HS
	Nov. 2013	Post	HS
	Apr. 2014	Post	HS
Sunset Cove	Jun. 2012	Post	HS

Table 2.2. Mean and standard deviation of various water quality characteristics, including temperature, salinity, nutrients, chlorophyll *a*, and the proportion of signature pigments to chlorophyll *a* in different sub-regions of the West Lake Chain (WLC) and the Seven Palm Lake Chain (SPLC) before and after C-111 implementation. One-way ANOVA was used to statistically compare the pre- and post-conditions within each sub-region. Asterisks * and ** indicate statistically significant differences at $p < 0.1$ and $p < 0.05$, respectively.

	WLC			
Parameter	Upstream Lakes		Downstream Embayment	
	Pre	Post	Pre	Post
Temperature (°C)	24±3	28±2**	23±4	28±1*
Salinity	17±9	14±6	30±15	32±9
TDN (µM)	133±21	115±31	92±13	103±17
DON (µM)	125±17	103±21	85±12	106±4
NH_4^+ (µM)	4.9±4.7	3.1±3.0	6.1±5.2	2.9±2.9
NO_x^- (µM)	2.8±4.5	1.0±0.9**	0.8±0.2	0.4±0.3
TDP (µM)	0.65±0.21	0.93±0.53	0.66±0.28	0.55±0.29
DOP (µM)	0.49±0.16	0.54±0.22	0.43±0.28	0.36±0.27
DIP (µM)	0.17±0.06	0.40±0.39	0.23±0.05	0.20±0.04
TDN:TDP	219±55	152±60*	159±52	226±84
DIN:DIP	53±71	15±9	32±21	15±12
Chl *a* (µg L^{-1})	51±28	29±9*	8.0±10	2.8±0.06
0.7-3 µm chl *a* (µg L^{-1})	29±20	16±9	5.8±5.7	1.4±1.2
>3 µm chl *a* (µg L^{-1})	22±9.7	10±8.7**	5.6±5.4	1.4±1.2
Zeaxanthin:chl *a*	0.24±0.91	0.32±0.12	0.15±0.19	0.11±0.11
Alloxanthin:chl *a*	0.008±0.010	0.002±0.003*	0.011±0.010	0.046±0.036
Fucoxanthin:chl *a*	0.026±0.019	0.026±0.013	0.060±0.034	0.070±0.026
Peridinin:chl *a*	0.018±0.020	0.016±0.021	0.050±0.031	0.047±0.034

	SPLC			
	Upstream Lakes		Downstream Embayment	
Parameter	Pre	Post	Pre	Post
Temperature (°C)	26±3	28±2	24±3	28±2
Salinity	14±7	10±4	31±6	25±9
TDN (µM)	76±9	55±8**	76±6	66±7
DON (µM)	65±10	56±8**	71±6	66±3*
NH_4^+ (µM)	6.3±3.7	2.2±1.6**	3.7±2.0	3.8±2.7
NO_x^- (µM)	4.3±2.9	0.5±0.3**	0.5±0.1	0.5±0.4
TDP (µM)	0.30±0.13	0.41±0.23	0.40±0.26	0.63±0.22
DOP (µM)	0.19±0.15	0.31±0.22	0.23±0.25	0.48±0.21
DIP (µM)	0.11±0.05	0.10±0.05	0.17±0.03	0.15±0.05
TDN:TDP	315±136	207±155	263±115	162±61
DIN:DIP	122±46**	27 ±11**	24±8	27±11
Chl *a* (µg L^{-1})	1.8±0.6	3.5±0.6	0.97±0.26	2.3±1.4
0.7-3 µm chl *a* (µg L^{-1})	0.71±0.30	2.1±2.5	0.26±0.03	1.1±1.0
>3 µm chl *a* (µg L^{-1})	1.0±0.62	1.4±0.69	0.71±0.23	1.3±0.49
Zeaxanthin:chl *a*	0.09±0.09	0.16±0.11	0.06±0.08	0.10±0.06
Alloxanthin:chl *a*	0.017±0.007	0.020±0.017	0.019±0.0042	0.018±0.0064
Fucoxanthin:chl *a*	0.086±0.031	0.047±0.024**	0.130±0.037	0.091±0.036
Peridinin:chl *a*	0.092±0.055	0.050±0.055	0.045±0.0076	0.051±0.030

Table 2.3. Regression statistics for various parameters and salinity for data differentiated by low salinity (LS) and high salinity (HS) station groupings. Asterisks * and ** indicate correlation coefficients that were significant at $p < 0.1$ and $p < 0.05$, respectively ($n = 30$). All significant values are also shown in bold font.

Parameter	Salinity category	Regression coefficient (slope)	Correlation coefficient (r)	Significance (P) of r
TDN	LS	5.58	**0.62****	<0.01
	HS	0.66	0.16	0.41
DON	LS	5.14	**0.61****	<0.01
	HS	0.63	0.15	0.44
NH_4^+	LS	0.37	**0.51****	<0.01
	HS	0.02	0.04	0.83
NO_x^-	LS	0.07	0.10	0.60
	HS	0.003	0.01	0.97
TDP	LS	0.06	**0.60****	<0.01
	HS	0.02	**0.45****	0.01
DOP	LS	0.04	**0.58****	<0.01
	HS	0.02	**0.48****	<0.01
DIP	LS	0.03	**0.45****	0.01
	HS	0.001	0.08	0.68
TDN:TDP	LS	-15.17	**-0.50****	<0.01
	HS	-5.07	**-0.38****	0.04
DIN:DIP	LS	-0.68	-0.06	0.77
	HS	-0.37	-0.09	0.65
Chl *a*	LS	2.30	**0.42****	0.02
	HS	-0.09	-0.06	0.77
0.7-3 µm chl *a*	LS	1.36	**0.37****	0.05
	HS	-0.05	-0.04	0.85
>3 µm chl *a*	LS	0.86	0.31	0.11
	HS	-0.03	-0.06	0.74
Zeaxanthin:chl *a*	LS	0.002	0.06	0.74
	HS	-0.005	**-0.46****	0.01
Alloxanthin:chl *a*	LS	0.0002	0.06	0.74
	HS	0.002	**0.52****	<0.01
Fucoxanthin:chl *a*	LS	0.0001	0.02	0.94
	HS	0.001	0.15	0.42
Peridinin:chl *a*	LS	0.003	0.29	0.12
	HS	-0.001	-0.11	0.55

Table 2.4. Correlation coefficients for the regressions of nutrients or nutrient ratios and phytoplankton pigments or pigment ratios for data differentiated by low salinity (LS) and high salinity (HS) station groupings. Asterisks * and ** indicate correlation coefficients that were significant at $p < 0.1$ and $p < 0.05$, respectively ($n = 30$). All significant values are also shown in bold font.

Parameter		LS	HS
Chl *a*	TDN	__0.69**__	__0.67**__
	DON	__0.70**__	__0.66**__
	NH_4^+	0.24	-0.01
	NO_x^-	0.08	-0.01
	TDP	__0.33*__	__0.32*__
	DOP	__0.36**__	0.13
	DIP	0.21	__0.57**__
	TDN:TDP	-0.15	0.03
	DIN:DIP	-0.08	-0.14
0.7-3.0 µm chl *a* size fraction	TDN	__0.60**__	__0.65**__
	DON	__0.62**__	__0.64**__
	NH_4^+	0.17	-0.01
	NO_x^-	0.07	<0.01
	TDP	0.30	__0.39**__
	DOP	0.31	0.16
	DIP	0.20	__0.62**__
	TDN:TDP	-0.17	-0.01
	DIN:DIP	-0.07	-0.16
>3 µm chl *a* size fraction	TDN	__0.72**__	__0.72**__
	DON	__0.74**__	__0.71**__
	NH_4^+	0.22	0.07
	NO_x^-	0.10	-0.05
	TDP	0.21	0.29
	DOP	0.29	0.15
	DIP	0.08	__0.39**__
	TDN:TDP	-0.06	0.11
	DIN:DIP	-0.03	-0.07

Zeaxanthin:chl *a*	TDN	**0.42****	**0.42****
	DON	**0.48****	**0.43****
	NH_4^+	-0.13	-0.10
	NO_x^-	-0.24	-0.06
	TDP	0.18	0.04
	DOP	0.11	-0.0
	DIP	0.18	**0.32***
	TDN:TDP	-0.14	0.16
	DIN:DIP	**-0.32***	-0.22
Alloxanthin:chl *a*	TDN	-0.30	-0.08
	DON	**-0.35****	-0.05
	NH_4^+	0.19	-0.08
	NO_x^-	0.24	-0.11
	TDP	-0.21	0.33
	DOP	-0.07	**0.44****
	DIP	-0.28	-0.16
	TDN:TDP	-0.04	**-0.36****
	DIN:DIP	**0.35***	-0.09
Fucoxanthin:chl *a*	TDN	-0.19	**-0.58****
	DON	-0.25	**-0.58****
	NH_4^+	0.25	0.03
	NO_x^-	**0.38****	0.05
	TDP	-0.14	-0.16
	DOP	-0.04	-0.11
	DIP	-0.20	-0.17
	TDN:TDP	0.02	-0.19
	DIN:DIP	**0.57****	0.12
Peridinin:chl *a*	TDN	-0.21	**-0.47****
	DON	-0.22	**-0.49****
	NH_4^+	0.01	0.17
	NO_x^-	-0.01	0.14
	TDP	-0.10	-0.21
	DOP	-0.03	-0.11
	DIP	-0.14	-0.30
	TDN:TDP	-0.02	-0.14
	DIN:DIP	0.12	**0.32***

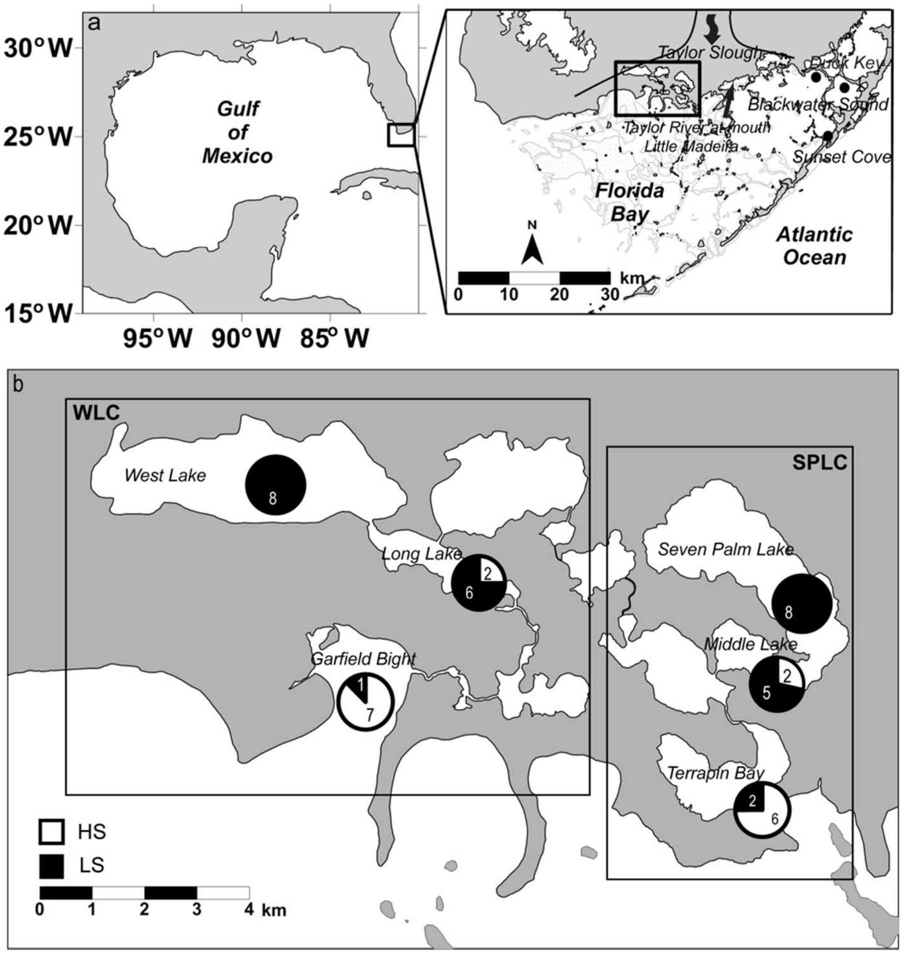


Figure 2.1 Map of Florida Bay and the study stations. (a) The general location of Florida Bay (insert map), Taylor Slough, Taylor River at the mouth station, and the sampling sites in the northern Florida Bay ICOLLs (in the box) and in eastern Florida Bay (black dots). (b) Enlargement showing the northern Florida Bay ICOLLs. The boxes differentiate the two "lake chains", the West Lake Chain (WLC) and the Seven Palm Lake Chain (SPLC). The pie charts represent the number of sampling periods in each site that fell in the high-nutrient/low-salinity (LS) versus the low-nutrient/high-salinity (HS) category. The positioning of the black dots and pie charts indicate sampling sites. The base map was modified from Frankovich et al. (2011) and used with permission of the publisher.

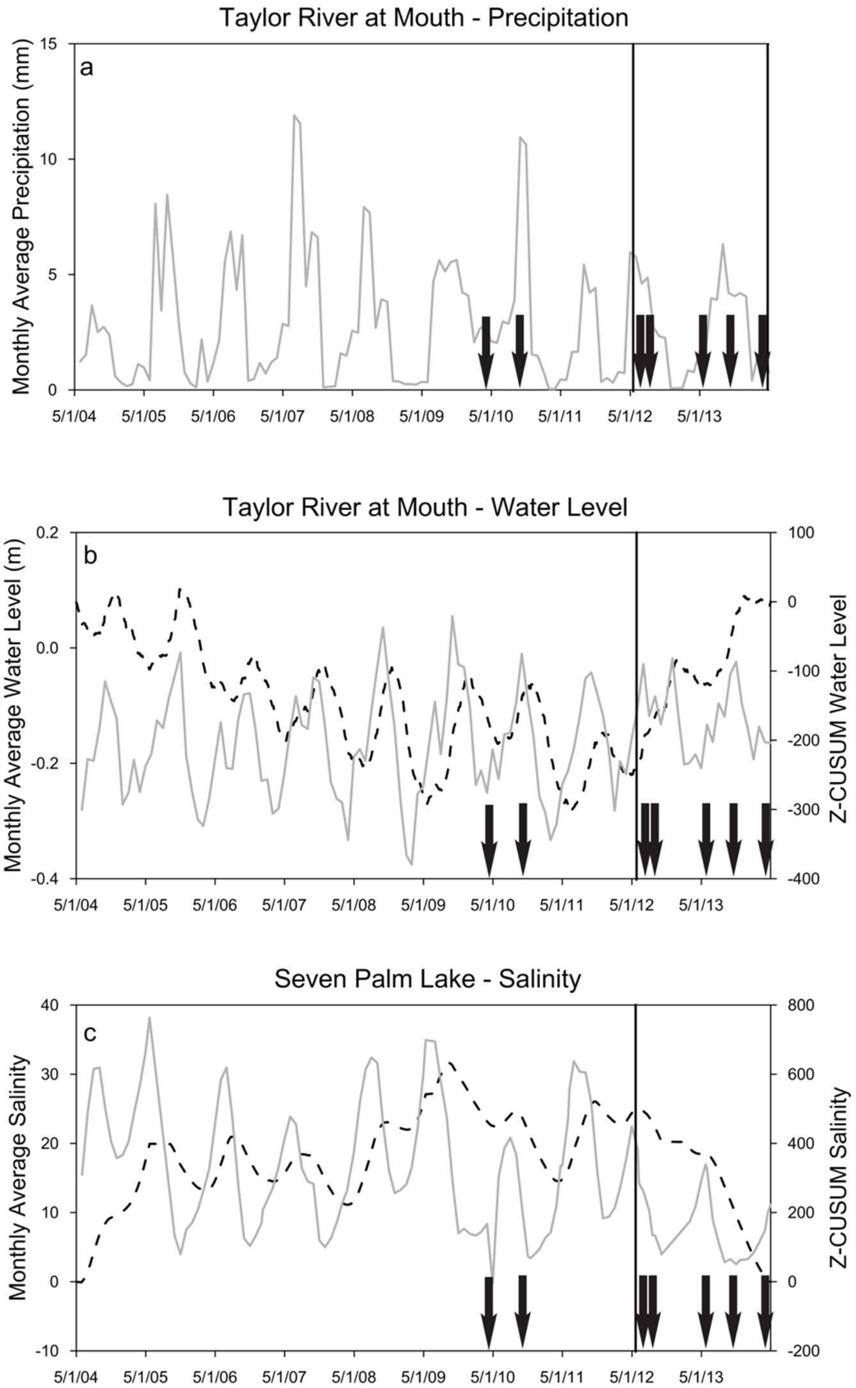


Figure 2.2 Hydrological data from May 2004 to April 2014. (a) Monthly precipitation in Taylor River. (b) Monthly water level in Taylor River (grey solid line) and the z-CUSUM of the same data (black dotted line). (c) Monthly average salinity at Seven Palm Lake (black solid line) and the z-CUSUM of the same data (black dotted line). Vertical black line represents the initiation of the C-111 Spreader Canal Western Project water diversion operation on May 17, 2012. Arrows indicate sampling in this study.

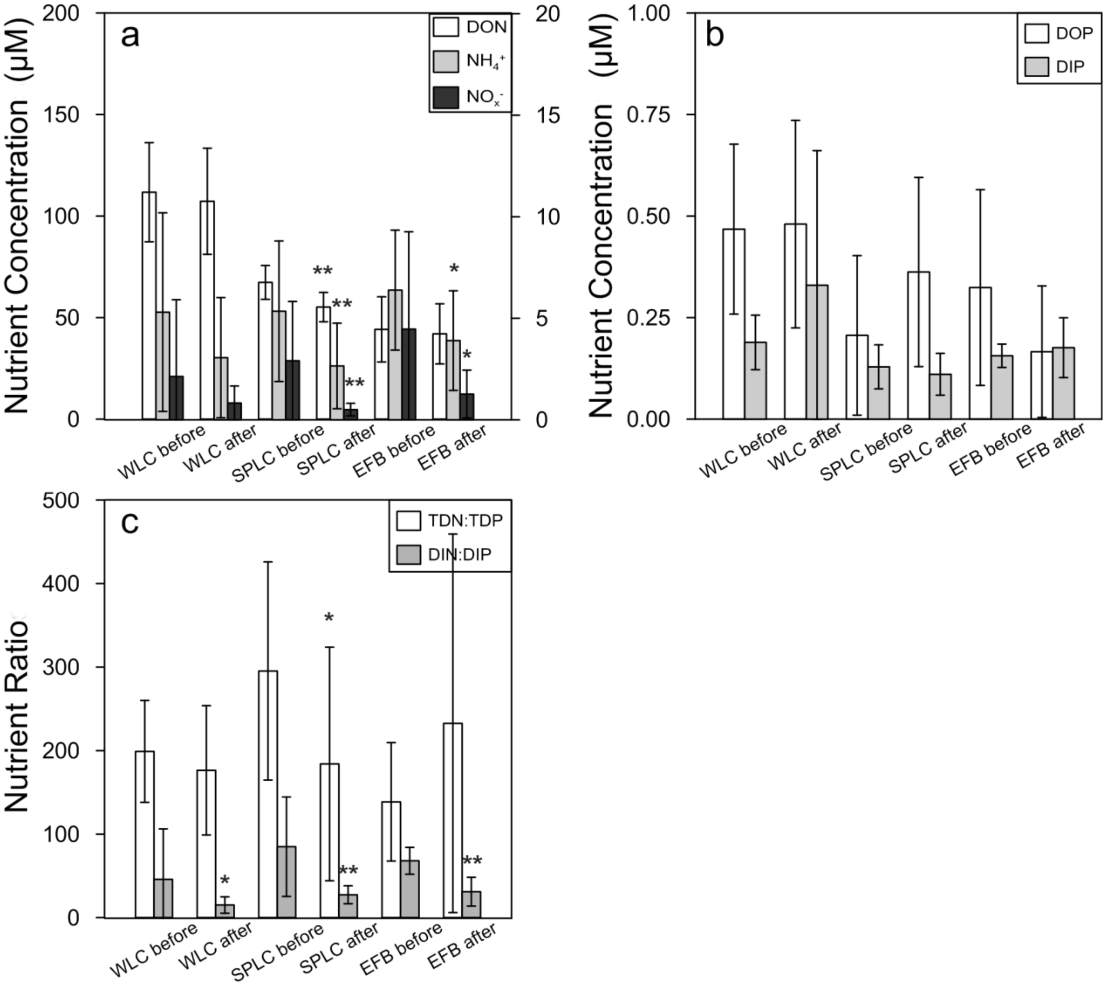


Figure 2.3 Average concentrations (± 1 SD) of (a) DON, NH$_4^+$, and NO$_x^-$, (b) DOP and DIP, and (c) TDN:TDP and DIN:DIP among samples in different regions (West Lake Chain, WLC; Seven Palm Lake Chain, SPLC; and East Florida Bay, EFB) and time periods before and after C-111 implementation. Significant changes are noted by * (p < 0. 1) and ** (p < 0.05).

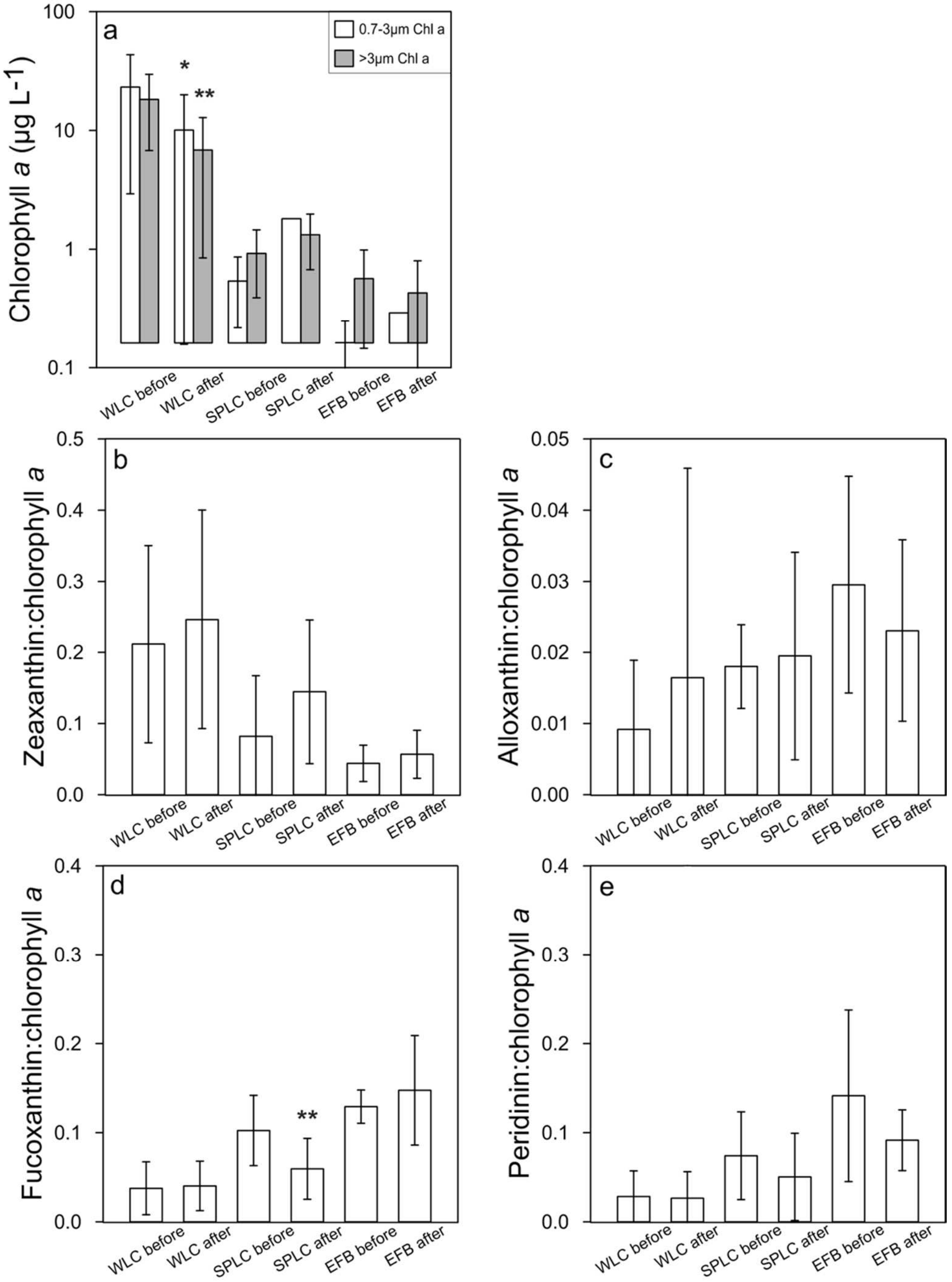


Figure 2.4 Average concentrations (± 1 SD) of (a) different size fractions of chlorophyll *a*, 0.7-3.0 µm and >3 µm (log scale), and (b-e) different pigments in relation to chlorophyll *a* for samples collected in different regions (West Lake Chain, WLC; Seven Palm Lake Chain, SPLC; and East Florida Bay, EFB) and time periods before and after C-111 implementation. Significant changes are noted by * ($p < 0.\ 1$) and ** ($p < 0.05$).

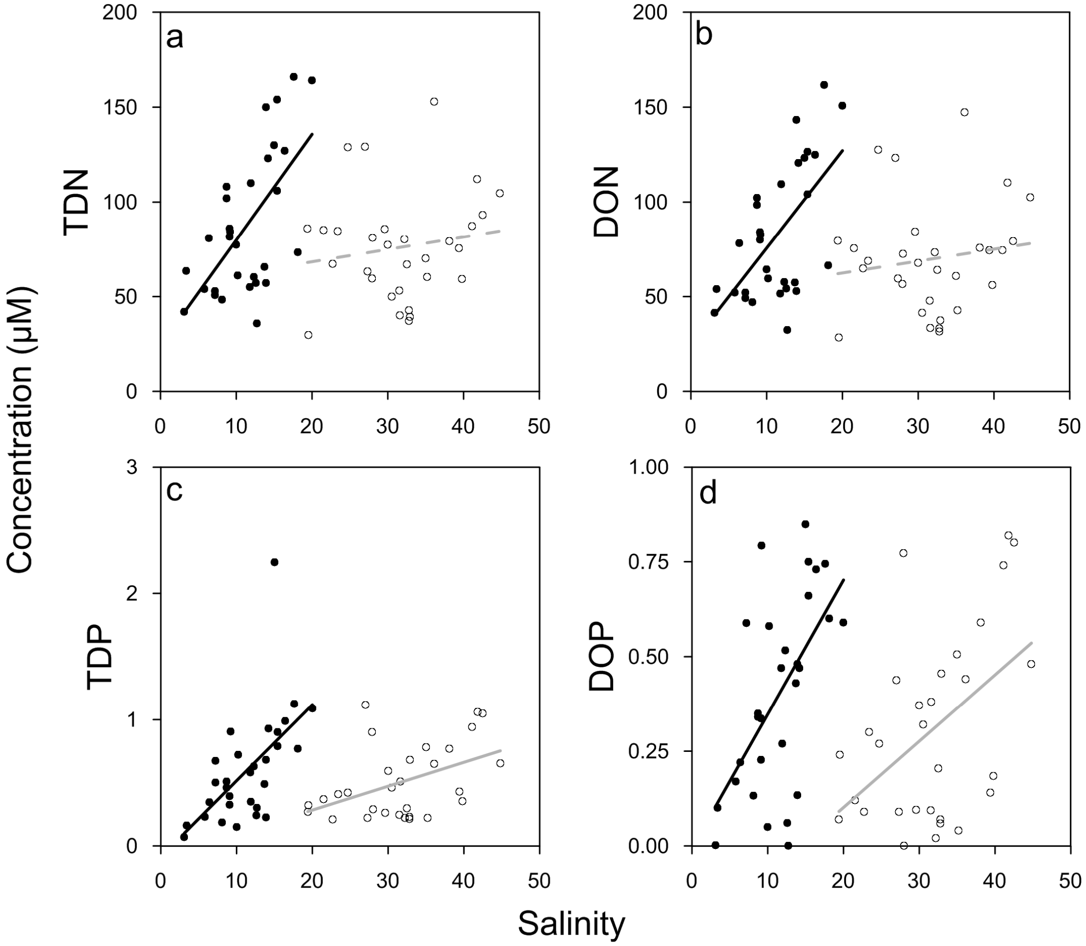


Figure 2.5 Relationship of (a) TDN, (b) DON, (c) TDP, and (d) DOP to salinity for all samples collected in all Florida Bay regions and all time periods (April 2010 – April 2014). Data points are individual sampling events and are separated into two different categories, low salinity (LS, salinity ≤ 20, filled circles, black lines) and high salinity (HS, salinity > 20, open circles, grey lines). Significant correlations are shown by the solid line ($p < 0.1$); non-significant relationships are shown in dashed lines.

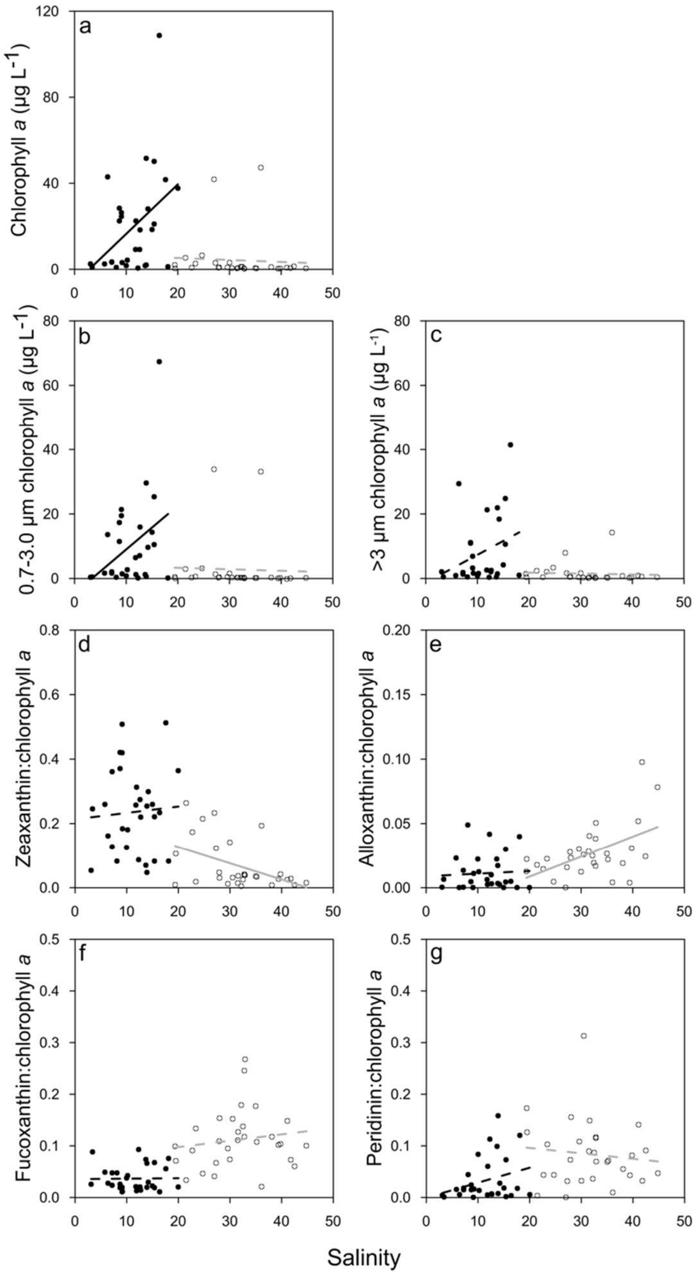


Figure 2.6 Relationship between (a) chlorophyll *a*, (b) 0.7-3.0 µm chlorophyll *a* size fraction, (c) > 3 µm chlorophyll *a* size fraction; (d) zeaxanthin:chlorophyll *a* ratio; (e) alloxanthin:chlorophyll *a* ratio; (f) fucoxanthin:chlorophyll *a* ratio; and (g) peridinin: chlorophyll *a* ratio and salinity for all samples collected in all regions and all time periods. Data points are individual sampling events (2010 April – 2014 April) and are separated into two different categories, low salinity (LS, salinity ≤ 20, filled circles, black lines) and high Salinity (HS, salinity > 20, open circles, grey lines). Significant correlations are shown in solid line ($p < 0.1$); non-significant relationships are shown in dashed lines.

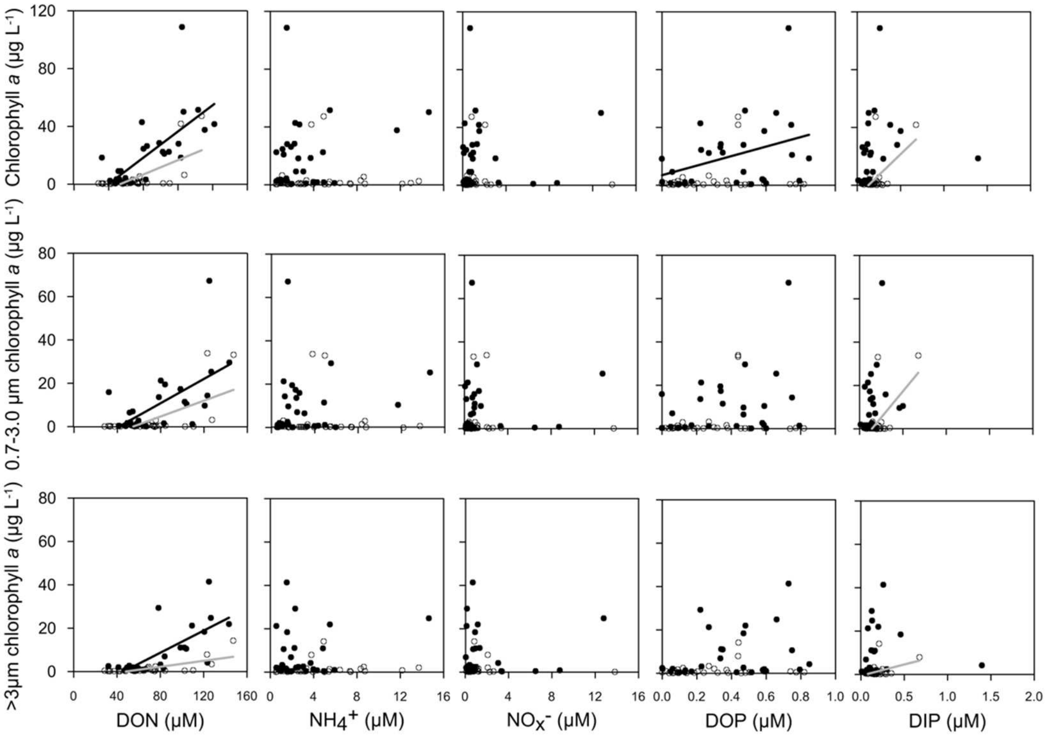


Figure 2.7 Relationship of total chlorophyll *a* and two size fractions, 0.7-3.0 µm and > 3 µm, to different nutrients and nutrient forms, DON, NH_4^+, NO_x^-, DOP, and DIP, in the low-salinity (salinity < 20, filled circles, black lines) and high-salinity (salinity > 20, open circle, grey lines) sites, considering all samples. Only significant correlations are shown ($p < 0.1$).

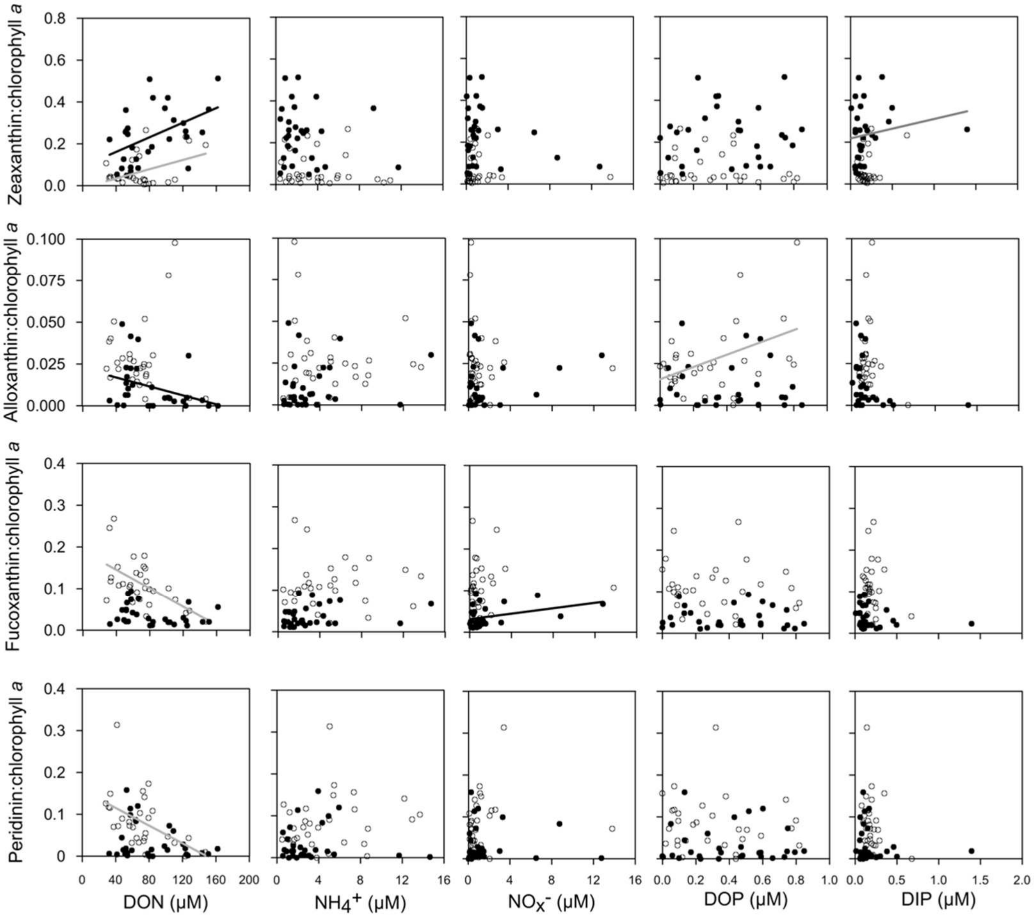


Figure 2.8 Relationships between zeaxanthin:chlorophyll *a*, alloxanthin:chlorophyll *a*, fucoxanthin:chlorophyll *a*, and peridinin:chlorophyll *a* versus different forms of nutrients, DON, NH_4^+, NO_x^-, DOP, and DIP in the low-salinity (salinity lower than 20, filled circles, black lines) and high-salinity (salinity higher than 20, open circles, grey lines) sites of all samples. Only significant correlations are shown ($p < 0.1$).

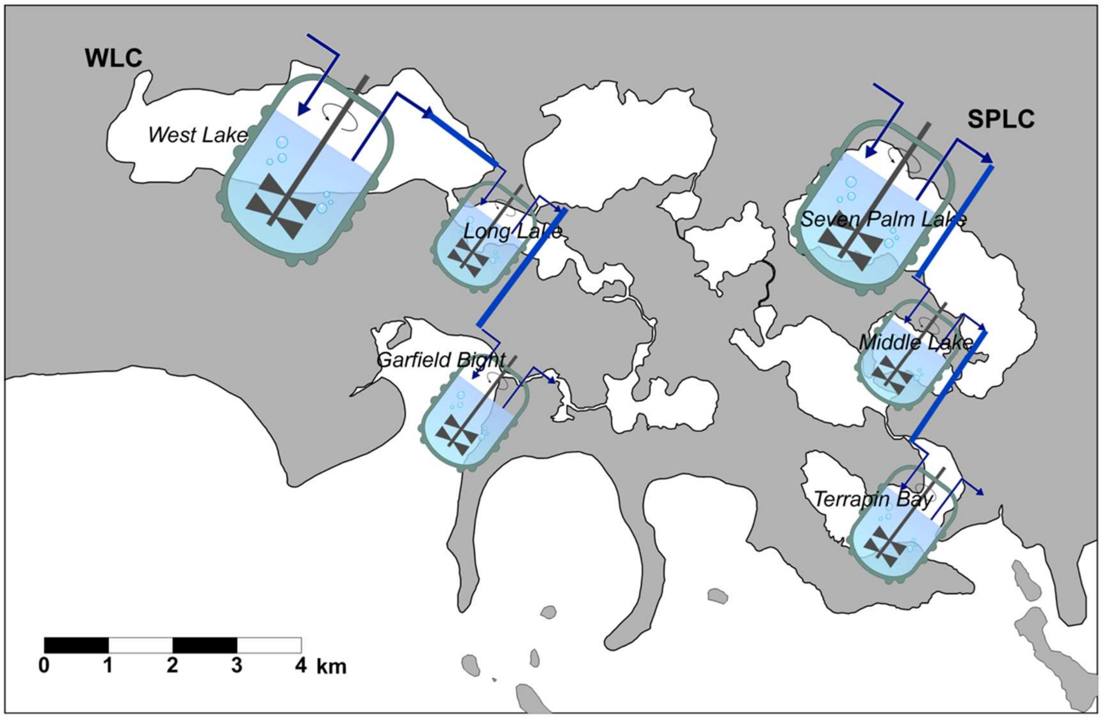
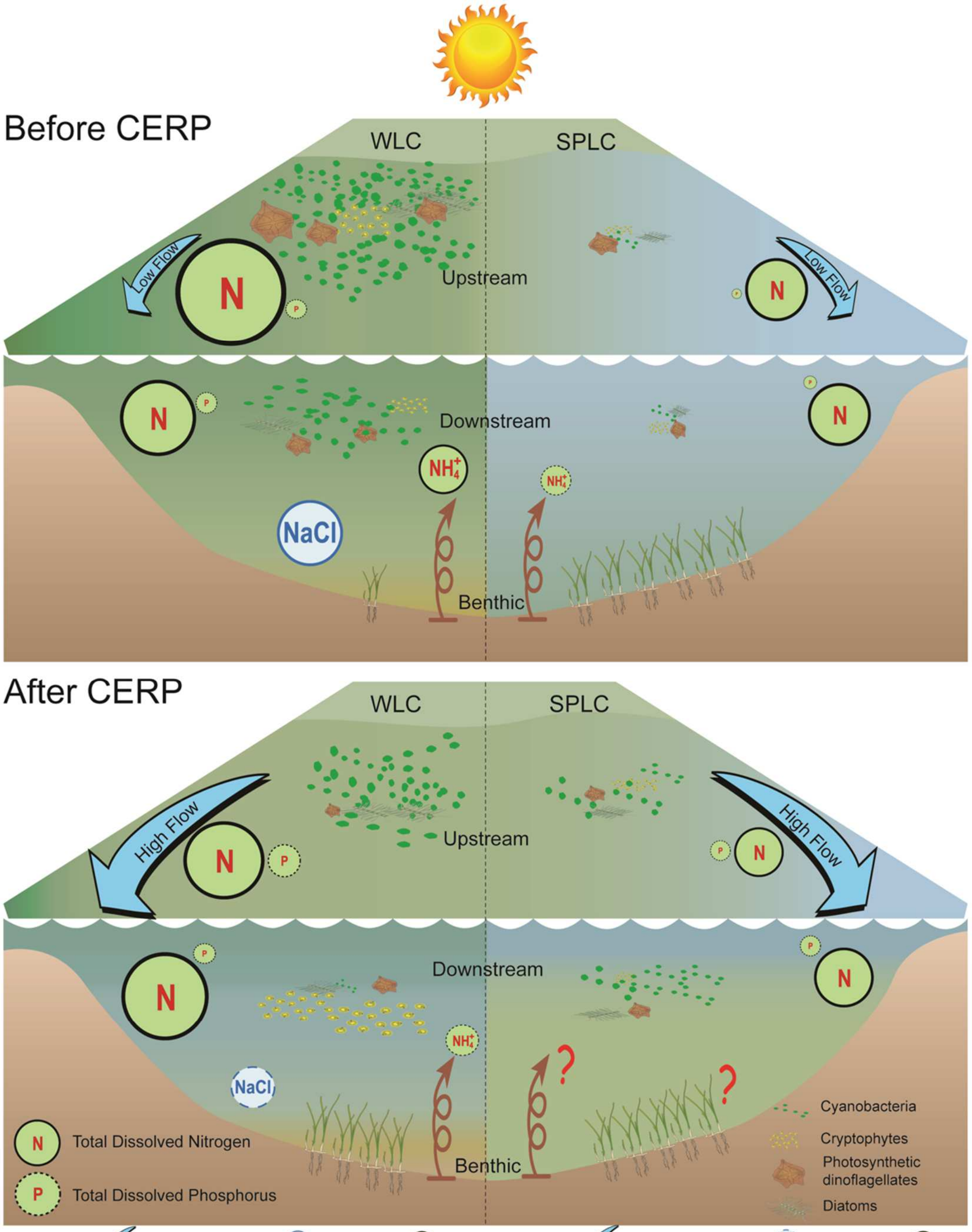


Figure 2.9 Conceptual diagram of lakes connected in the ICOLL chains analogous to the chemostat model.



Before CERP, low freshwater flow, NaCl high salinity, N high TDN input, P low TDP input. High phytoplankton abundance, low benthic vegetation coverage, NH$_4^+$ high ammonium efflux in WLC and low phytoplankton abundance, high benthic vegetation coverage, NH$_4^+$ low ammonium efflux in SPLC.

After CERP, high freshwater flow, NaCl lower salinity, N lower TDN input, P higher TDP input. Decreased phytoplankton abundance, increased benthic vegetation coverage, NH$_4^+$ lower ammonium efflux in WLC and increased phytoplankton abundance, uncertain changes in benthic vegetation coverage and ammonium efflux in SPLC.

Figure 2.10 Conceptual diagram of several main factors alteration, including freshwater flow, TDN, TDP, different phytoplankton communities, benthic vegetation, and sediment nutrient efflux in upstream and and downstream of the two lake chains, the WLC and the SPLC, after CERP.

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Chapter 3 : Phytoplankton Assemblage Response to Changing Nutrients in Florida Bay: Results of Mesocosm Studies

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 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 assemblage composition. The +N treatments, especially N 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.

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 & 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., submitted). 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 & Glibert, 1999b; Lomas & Glibert, 1999a; Berg et al., 2003; Dugdale et al., 2007; Parker et al., 2012; Xu et al., 2012; Glibert 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 & Glibert, 1999a; 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 & 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 & 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 & Ho, 1997; Sterner & Elser, 2002; Heil et al., 2007; Finkel et al., 2009; 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 & Gotham, 1980; Geider & La Roche, 2002; Sterner & 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 & Burkholder, 2011). Picoplankton such as *Synechococcus*, a dominant species in northern Florida Bay (Phlips & Badylak, 1996; Fourqurean & Robblee, 1999; Phlips et al., 1999; Berry et al., 2015), is a comparatively good competitor under low P conditions. Due to its small size, *Synechococcus* generally has 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., 2009). It is 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 growth "machinery" and thus have a lower N:P requirement (Moloney & Field, 1989; Sterner & Elser, 2002; Klausmeier et al., 2004; Finkel et al., 2009; 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 & Keller, 2007; Yarbro & Carlson, 2008). Second, this system is generally considered to be P limited, primarily because of P-adsorbing carbonate sediment. Sediment absorption 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 & 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* since mid-1990s (Fourqurean & Robblee, 1999; Phlips et al., 1999; Glibert et al., 2004; Glibert et al., 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).

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 & Clark, 1992; Boesch et al., 1993; Lapointe & Barile, 2004). Therefore, Florida Bay serves as a case study, particularly with the C-111 project, of changing hydrology, and of effects of changing nutrients on phytoplankton (Shangguan et al. submitted a).

As part of a broader study on phytoplankton blooms after the C-111 project implementation, the response in phytoplankton biomass and assemblage composition to N form and N:P ratio alterations in Florida Bay were investigated in a series of mesocosm experiments. 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 optima for growth 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-dominated system, and other nutrient regimes would lead to a relatively mixed algal assemblage structure. The objectives of this study were thus to demonstrate how phytoplankton abundance and the major phytoplankton taxa may be altered over time as nutrient composition is changed.

Methods

Site description

Florida Bay is wedge-shaped subtropical coastal lagoon of approximately 2,200 km^2 in area (Fig. 3.1). It has an open boundary with the Gulf of Mexico in the west, but the connection with the Atlantic Ocean on the eastern and southern boundaries is restricted to narrow tidal passes in the Florida Keys. The Bay has an average annual temperature of 25°C and two distinct meteorological seasons: a November-April dry season and a May-October rainy season (Duever et al., 1994). Riverine input in the northern boundary is considerable in the wet season, and the major freshwater input is through slow sheet flow from the Everglades watershed (Boyer et al., 2006; Lee et al., 2007). Not only does the Bay have limited water exchange, but hydrological circulation within the Bay itself is also small because many shoals and shallow basins restrict water transport (Lee et al., 2002). Consequently, most of central and northeastern Florida Bay has relatively long residence times, on the order of a month or more.

Experimental treatments

Mesocosm experiments were performed five times, in June 2012, August 2012, May 2013, November 2013, and April 2014, at the National Park Service Florida Bay Interagency Science Center on Sunset Cove (eastern Florida Bay off the Florida Keys). Water was transferred into a head tank from the pump located in the dock of the Science Center. The outdoor mesocosm tanks (length × width × height; 222 × 80 × 50 cm; total volume 1000 L; surface area 1.78 m^2) were washed and scrubbed before the experiment and then were simultaneously filled by pumping 1 m^3 of seawater from the holding tank into each. Small aquarium pumps were placed in each mesocosm to supply airflow to create water circulation. The mesocosms were covered by a neutral density screen and maintained under experimental condition for either 10 days (June 2012) or five days (Aug. 2012, May and Nov. 2013, and Apr. 2014).

The overall objective of the five experiments was to determine the effects of changes in nutrient concentration, form and ratio on phytoplankton abundance and assemblage composition. In all experiments, controls did not receive nutrient additions. In June, treated mesocosms received nutrients in a daily dose of 4 μM-N (in different forms) and/or 1 μM-P (as sodium β-glycerophosphate pentahydrate) for 5 days, and were sampled daily for 10 days that included 5 days with enrichment and 5 days post- nutrient enrichment. The nutrient treatments for the mesocosms were, respectively: +P, +NO_3^-+P, +NH_4^++P, +DON+P, +NO_3^-, +NH_4^+, +DON. In August, the mesocosms received the same suite of nutrient treatments as in June, but all nutrients were delivered in a single pulse, 20 μM-N and/or 5 μM-P at day 0 (D_0), and responses were monitored for 5 days. In May, 5 mesocosms received nutrients as a daily dose of 4 μM-N and/or 1 μM-P for 5 days, and the other 5 each received a single pulse of 20 μM-N and/or 5 μM-P at D_0. All mesocosms in the May experiment were sampled daily for 5 days. Other than the different nutrient delivery frequencies, the nutrient treatments were the same for the 2 sets of nutrient delivery frequencies in the May experiment: +NH_4^+, +NO_3^-+P, +NH_4^++P, and +DON+P, and also a control and a +P in a single pulse. In November, 5 mesocosms all received nutrients in a single pulse of 20 μM-N and/or 5 μM-P at D_0 and were monitored for 5 days. The nutrient treatments were +P, +NO_3^-+P, +NH_4^++P, and +DON+P. In April, the mesocosms received nutrients in a single pulse at D_0 and again monitored for 5 days. The nutrient treatments included a 3×3 matrix, with N forms NO_3^-, NH_4^+, and DON, and P varied to yield additions that were below, equivalent to, and above the Redfield ratio (N:P ratio of 4, 16, 32) using a constant 20 μM-N addition but varied P additions at 5, 1.25, and 0.625 μM-P, respectively.

All +DON treatments were a mixture of 50%, 25%, and 25% from urea, arginine and glutamine, respectively (Glibert et al., 2004), and the P additions were prepared as dissolved organic P (DOP) with sodium β-glycerophosphate pentahydrate. All +N+P additions were added as a molar ratio of 4 except for the April experiments (explained above).

On a daily basis at 0800 hr, nutrients were added to treatment mesocosms, water was stirred by paddles (using a unique paddle for each mesocosm to avoid cross-contamination), and samples were immediately collected for analysis of algal chlorophyll *a* (chl *a*) and nutrient concentrations. Samples for analysis of signature pigments were collected at the same time on day 0, day 2 and day 4 (D_0, D_2, and D_4) as described below. At noon, temperature and salinity were measured in each mesocosm using a YSI-85.

Chemical analyses

Samples for chl *a* analysis were collected in duplicate after filtration through a combusted GF/F filter (nominally 0.7 μm). Filters were extracted overnight in 90% acetone at 4°C and subsequently read on a Turner 10AU fluorometer. Values were corrected for phaeophytin (Arar & Collins, 1992). The fluorometer was calibrated with commercially available chl *a* (Turner Designs). A separate set of filtrations through GF/F filters was completed for phytoplankton pigment analysis by high-performance liquid chromatography (HPLC; Van Heukelem & Thomas, 2001). These samples were flash-frozen in liquid nitrogen and stored at -80 °C for subsequent analysis on an Agilent 1100 HPLC. Four algal functional groups were chosen as the focus in this study: zeaxanthin was considered as an indicator of picocyanobacteria, alloxanthin as an indicator of cryptophytes, fucoxanthin as an indicator of diatoms, and peridinin as an indicator of peridinin-containing dinoflagellates (Jeffrey & Wright, 1994; Jeffrey & Vesk, 1997; Glibert et al., 2004).

The filtrates of these biomass collections were collected and frozen for subsequent nutrient analysis. Concentrations of NO_x^- (NO_3^- + NO_2^-), NH_4^+, and dissolved inorganic phosphorus (DIP) were measured using autoanalysis techniques, following D'Elia et al. (1997) and Whitledge et al. (1981). Concentrations of TDN and TDP were determined by persulfate oxidation (Solorzano & Sharp, 1980 for TDP; Valderrama, 1981). Concentrations of DON and DOP were determined by the difference between dissolved total and inorganic fractions.

Data analyses

Effects of N form and N:P ratio on total biomass (as chl *a*) and phytoplankton taxa were evaluated in several ways. To assess the general phytoplankton growth trend in all 5 mesocosm experiments, the chl *a* and signature pigment data from days 1 to 4 (D_1 to D_4) were tested for statistically significant correlations, considering controls and all consistent treatments across all experiments (+P alone, +NO_3^-+P, +NH_4^++P, +DON+P, +NO_3^-, +NH_4^+, and +DON). Only treatments receiving a single pulse of nutrients (rather than daily additions) were included in this aspect of data analysis. All experiments in which the ratio of N:P was manipulated were similarly compared.

To assess growth in relation to nutrient changes, daily changes in phytoplankton abundance (as chl *a*), phytoplankton functional group (as pigments), and nutrients were calculated. Both daily-pulse and single-pulse treatments were included in this analysis. Because the focus was on growth, rather than biomass decline, only periods when phytoplankton showed positive trends in growth were considered. Linear regression analysis was used to compare the daily changes in chl *a* in relation to the daily changes in signature pigments for each N form and N:P ratio used in treatments. The rate of change (slope) and the coefficient of determination, R^2, were calculated using SAS software, and were reported at significance levels of $p = 0.01$ and $p = 0.05$. A high slope suggested a high contribution from one signature pigment to the total biomass (as chl *a*). A similar calculation was made to compare daily changes in chl *a* and signature pigments with daily changes in nutrients, to estimate the chl *a* concentration per nutrient concentration consumed. The more negative the slope, the more rapidly the phytoplankton taxa were assumed to have taken up the nutrient. In this daily-change comparison, the interval was one day for phytoplankton abundance and nutrients, and the interval for calculation of taxa changes was 2 days since signature pigment data were not collected daily. Over this short time interval the change in nutrients was assumed to reflect use of the nutrients, both added and ambient. Therefore, for the N-form analysis, there were 6, 17, 14, 15, 3, 9, and 3 paired daily-change comparisons for controls and $+NO_3^-$+P, $+NH_4^+$+P, +DON+P, $+NO_3^-$, $+NH_4^+$, and +DON treatments, respectively; and for the N:P ratio analysis, there were 6, 4, 30, 7, 9, and 15 paired comparisons for controls and +P alone, +N:P = 4, +N:P = 16, and +N:P = 32, and +N alone treatments, respectively. Only treatments receiving a single pulse of nutrients (rather than daily additions) were included in this aspect of data analysis because changes were related to the consumption of nutrients from day to day, and the addition of the daily nutrients precluded such calculations. Therefore, there were 6, 17, 14, 15, and 5 paired daily change comparisons for controls and $+NO_3^-+P$, $+NH_4^++P$, +DON+P, and $+NH_4^+$ treatments for N form analysis, respectively; and for the N:P ratio analysis there were 6, 3, 22, 7, 9, and 9 paired comparisons for controls and +P alone, +N:P = 4, +N:P = 16, and +N:P = 32, and +N alone treatments, respectively.

Results

Ambient environmental conditions

Mesocosm experiments were initiated during periods when the temperatures ranged from 28.6 to 32°C and salinities were above 28 (Table 3.1). Ambient nutrient concentrations for dissolved inorganic nitrogen (DIN, sum of NO_x^- and NH_4^+) were quite variable. Concentrations of NO_x^- and NH_4^+ were as low as 1.36 and 1.15 µM-N, respectively, in experiment 2 (August), but in experiment 4 (November) they were both several fold higher, with maxima at 4.17 and 7.94 µM-N, respectively (Table 3.1). Concentrations of NH_4^+ were generally 1.5 times higher than those of NO_x^-. Across all experiments, DON ranged from 27.65 to 38.91 µM-N and TDN ranged from 32.90 to 47.17 µM-N. Inorganic and organic forms of P were very low in all experiments, < 0.5 µM-P, and TDP ranged from 0.23 to 0.73 µM-P. Molar ratios of DIN:DIP in the initial samples ranged from 11 – 74, and TDN:TDP ratios were substantially higher than the Redfield ratio in all experiments (Table 3.1).

Ambient chl *a* concentrations differed somewhat in each experiment, but all were below 3.1 µg L^{-1}, when the experiments were initiated (Table 3.1). Zeaxanthin concentrations did not vary by more than a factor of ~3 at the beginning of all experiments (range, 0.02 - 0.06 µg L^{-1}), while alloxanthin and fucoxanthin concentrations were highly variable (range, 0.03 to 0.18 µg L^{-1}, and 0.06 to 0.62 µg

L^{-1} respectively) (Table 3.1). Highest concentrations of these three pigments were all found in experiment 4 (November), when the maximum value of chl *a* was also observed. The highest initial peridinin concentration occurred in experiment 1 (0.17 μg L^{-1}) and the lowest in experiment 5 (0.04 μg L^{-1}).

Trends in phytoplankton biomass and assemblage composition

Concentrations of chl *a* increased in the +P alone treatments, and particularly in the +N+P treatments of all 5 experiments (Fig. 3.2a). The grand mean chl *a* at D_0 (all experiments considered collectively) was 1.35 ± 0.88 μg L^{-1} (mean ± one standard deviation (SD). Concentrations of chl *a* ranged from 0.7-3.3, 0.6-4.2, 0.6-13.8, 0.6-8.6, 0.6-7.3, and 0.6-4.0 μg L^{-1} in the +P, +NO_3^-+P, +NH_4^++P, +DON+P, and +NH_4^+ treatments, respectively. By D_1 in the +P alone treatments, mean chl *a* concentrations had increased by ~60%; by D_2 they had more than doubled; and subsequently chl *a* decreased. In all of the +N+P treatments, mean chl *a* concentrations showed strong upward trends in first 3 days. Of these treatments, increases in mean chl *a* in +NO_3^-+P were highest (20.79 ± 8.78 μg L^{-1}, *n* = 4), ~16 times the initial chl *a*, by D_3. In contrast to the substantial change observed in the +P alone treatments, +N alone did not significantly stimulate phytoplankton growth. Nevertheless, in the +NH_4^+ treatments, chl *a* increased throughout the experiment, and averaged more than 3-fold higher than the initial chl *a* by the end of the experiments (Fig. 3.2a).

In the daily-pulse experiments, phytoplankton (as chl *a*) responded similarly as in the single-pulse enrichments, but with lower maximal chl *a*. In the May experiment, chl *a* in the controls ranged from 0.7-3.3 μg L^{-1}. Chl *a* in the daily pulse treatments ranged from 0.6-7.3, 0.6-7.0, 0.6-5.2, and 0.6-3.2 in the $+NO_3^-$+P, $+NH_4^+$+P, +DON+P, and $+NH_4^+$, respectively.

Different algal groups displayed distinct patterns through time but, as for chl *a*, maxima generally were higher in +N+P treatments than in the +P alone or +N alone treatments. Zeaxanthin concentrations generally increased in all nutrient treatments (Fig. 3.2b). By D_4, zeaxanthin had increased ~5-fold, reaching an average of 0.20, 0.24, and 0.15 µg L^{-1} in the $+NO_3^-$+P, $+NH_4^+$+P, and +DON+P treatments, respectively. Zeaxanthin in the $+NH_4^+$ alone treatment also increased, and by D_4 averaged 0.16 µg L^{-1}, roughly the same level as the +N+P treatments. In the +P alone and +N+P treatments, alloxanthin concentrations more than tripled in the first few days and then declined by D_4, while in control and +N alone treatments, they declined after D_0 (Fig. 3.2c). Concentrations of fucoxanthin increased more than 10-fold on average in the +N+P treatments, but in other nutrient treatments fucoxanthin did not significantly increase (Fig. 3.2d). These concentrations increased on average from 0.24 µg L^{-1} at D_0 to 8.9, 6.3, 13.1 µg L^{-1} in the $+NO_3^-$+P, $+NH_4^+$+P, and +DON+P treatments, respectively, by D_2, and then declined. Similarly as for alloxanthin, peridinin concentrations increased ~3-fold on average by D_2 and then declined in the +P alone and +N+P treatments. Peridinin levels decreased in the controls and remained at similar levels in the +N alone treatments (Fig. 3.2e). While the signature pigments changed similarly as chl *a*, the more limited pigment sampling may not have captured the maxima, particularly the pigment concentrations at D_3 for the +N+P treatments, where the maxima was observed in the chl *a* concentration.

N form

Considering all experiments collectively, several relationships emerged when daily changes in signature pigments were compared to daily changes in chl *a* (Fig. 3.3-3.5). All zeaxanthin responses were positive when compared to the changes in chl *a*, indicating proportionately more picocyanobacteria in the phytoplankton assemblage as the phytoplankton grew (Fig. 3.3). The slopes of these changes in the +N alone treatments were substantially higher than those in the +N+P treatments (Fig. 3.5a). In addition, the relationship was significant and the slope was highest in the $+NH_4^+$ treatments, suggesting that picocyanobacteria largely and significantly contributed to the total phytoplankton biomass in $+NH_4^+$-enriched mesocosms ($R^2 = 0.71$, $p < 0.01$, $n = 9$; Fig. 3.3f) regardless of the +P concentrations. The slope for the $+NH_4^+$ treatments was twice as high as the same comparison for the $+NO_3^-$ treatments (not significant), while the slope for the +DON treatments (not significant) was 1.5 times that of the $+NO_3^-$ treatments. No consistent, significant relationships between chl *a* daily changes and daily changes of zeaxanthin were observed in the $+NO_3^-$+P, $+NH_4^+$+P, and +DON+P treatments (Fig. 3.5a).

The rate of change in alloxanthin in relation to the rate of change in chl *a* was positive in the controls and the +N+P, and $+NO_3^-$ treatments, but negative in the $+NH_4^+$ and +DON treatments (Fig. 3.3). These relationships were significant in the $+NO_3^-$+P ($R^2 = 0.44$, $p < 0.01$, $n = 17$) and $+NH_4^+$ treatments ($R^2 = 0.47$, $p < 0.05$, $n = 9$; Fig. 3.5b). Additionally, the slopes in the $+NO_3^-$+P and $+NO_3^-$ treatments were both higher than in the $+NH_4^+$+P, +DON+P and the $+NH_4^+$, +DON treatments (Fig. 3.5b).

The rate of change in fucoxanthin relative to that of chl *a* was positive under all nutrient treatments (Fig. 3.4). The slopes of the +N+P treatments were more than twice those of controls, and 2-10 times higher than those of the +N alone treatments, and only the relationships in the control and the +N+P treatments were significant ($R^2 = 0.99$, $p < 0.01$, $n = 6$ for control; $R^2 = 0.77$, $p < 0.01$, $n = 17$ for $+NO_3^-+P$ treatments; $R^2 = 0.40$, $p < 0.05$, $n = 14$ for $+NH_4^++P$ treatments; $R^2 = 0.78$, $p < 0.01$, $n = 15$ for +DON+P treatments; Fig. 3.5c). Similarly as for alloxanthin, the slope for fucoxanthin in the $+NO_3^-+P$ treatments was higher than that in the $+NH_4^++P$ and the +DON+P treatments, and the slope in the $+NO_3^-$ treatments was higher than that in the $+NH_4^+$ and the +DON treatments (Fig. 3.5c). These data suggest that the diatom contribution to the increase in phytoplankton biomass increased when enriched with +N+P, particularly with N as NO_3^-. The apparent diatom contribution in the $+NO_3^-+P$ treatments was more than 5-fold higher than that in the +N alone treatments, and more than 50% and 20% higher than that in $+NH_4^++P$ and +DON+P respectively.

In contrast to the above changes, the rate of change in peridinin compared to that of chl *a* varied with nutrient treatment. The relationships were positive in controls and in $+NH_4^++P$, $+NO_3^-$, and +DON treatments, but negative in the other treatments (Fig. 3.4). Relationships were only significant under the $+NO_3^-+P$ treatments ($R^2 = 0.27$, $p < 0.05$, $n = 17$) (Fig. 3.5d).

When the daily changes in chl *a* and individual signature pigments were compared to the daily changes in nutrients for the combined data for TDN, the responses were also varied (Table 3.2). The yield in chl *a* per µM N consumed was highest in the +DON+P treatments ($R^2 = 0.55$, $p < 0.01$, $n = 15$), and ~30% higher than that in the $+NO_3^-$+P treatments ($R^2 = 0.62$, $p < 0.01$, $n = 17$). Interestingly, although not significant, the zeaxanthin yield per N in the $+NH_4^+$ treatments was more than 5 times higher than that in the +DON+P treatments, implying that zeaxanthin utilized N fairly efficiently when the mesocosms were enriched with $+NH_4^+$. The fucoxanthin yield per N consumed was relatively high in all +N+P treatments ($R^2 =$ 0.57, $p < 0.01$, $n = 17$ for $+NO_3^-$+P treatments; $R^2 = 0.60$, $p < 0.01$, $n = 14$ for $+NH_4^+$+P treatments; $R^2 = 0.56$, $p < 0.01$, $n = 15$ for +DON+P treatments; Table 3.2), much higher than that in all +N alone treatments.

N:P ratio

All data from all experiments were compiled and grouped into differing N:P ratio treatments, including control, +P alone, and +N:P = 4, +N:P = 16, +N:P = 32, and +N alone based on the N and P additions. However, due to the highly skewed ambient TDN:TDP ratio, the final nutrient ratios in the mesocosms were very different from the ratios of the nutrients added. The final N:P ratios averaged 93, 6, 10, 32, 70, and 108 for the treatments control, +P alone, +N:P = 4, +N:P = 16, +N:P = 32, and +N alone, respectively. Only the +P alone and +N:P = 4 treatments resulted in TDN:TDP initial ratios below the Redfield ratio (Fig. 3.6).

The daily rates of change in signature pigments were compared to those of chl *a* for each +N:P ratio treatment (Figs. 3.7-3.9). Increases in zeaxanthin were generally positive compared to increases in chl *a* in all treatments except +P alone and molar N:P additions = 32 (Fig. 3.7). These increases in zeaxanthin relative to those of chl *a* were significant when the molar N:P additions were 16 ($R^2 = 0.59$, $p < 0.05$, $n = 7$) and 32 ($R^2 = 0.64$, $p < 0.05$, $n = 9$). The slopes of daily rates of change of zeaxanthin relative to those of chl *a* in the +N+P treatments were low compared to those slopes in both the controls and the +N alone treatments, however, and the increase in zeaxanthin relative to chl *a* was only significant when +N alone was the experimental addition ($R^2 = 0.74$, $p < 0.01$, $n = 15$; Fig. 3.9a).

The slopes of the daily rates of change in alloxanthin (cryptophytes marker) and those of chl *a* were mostly positive, suggesting that cryptophytes were significantly contributing to total algal biomass as chl *a*, with exception of the +N alone treatment (Fig. 3.7). Also for alloxanthin, the slope for the +P treatments was the highest, almost twice as high as that for controls, but the relationship between alloxanthin and chl *a* was not significant in either the +P or control treatments (Fig. 3.9b). The contributions of alloxanthin to total algal biomass as chl *a* when the N:P additions were 16 ($R^2 = 0.76$, $p < 0.05$, $n = 7$) and 32 ($R^2 = 0.47$, $p < 0.05$, $n = 9$) were roughly half those of the control treatment, and the contributions from alloxanthin to the increase in algal biomass was significant. The slopes for the daily rates of change in alloxanthin and those of chl *a* exhibited an increasing trend when N:P additions increased from 4 to 32 (Fig. 3.9b).

When compared to the daily changes in chl *a*, the daily changes in fucoxanthin were positive for all treatments, and the relationships were significant in the control ($R^2 = 0.99$, $p < 0.01$, $n = 6$), and when the N:P additions were 4 ($R^2 = 0.70$, $p < 0.01$; $n = 30$), +16 ($R^2 = 0.75$, $p < 0.05$, $n = 7$), and 32 ($R^2 = 0.94$, $p < 0.01$, $n = 9$; Fig. 3.8), but the relationships were not significant in the +P or +N. The rate of changes in fucoxanthin in relation to that in chl *a* increased when the N:P of the additions increased from 4 to 32 (Fig. 3.9c). The slope for the daily rate of changes in fucoxanthin and those of chl *a* in the +N:P = 32 addition treatments was approximately 6 times that of control. This trend indicates that diatom contributed disproportionately in phytoplankton assemblage as the +N:P ratio increased.

Moreover, the daily changes in peridinin in relation to the daily changes in chl *a* were positive in the control and +P alone treatments while negative in the 3 +N+P and +N alone treatments (Fig. 3.8). This relationship was negative and significant only in the +N:P = 32 treatments ($R^2 = 0.66$, $p < 0.01$, $n = 9$), consistent with the observation that other taxa contributed proportionately more to the algal growth while suppressing the growth of peridinin-containing dinoflagellates (Fig. 3.9d).

When the yields in chl *a* and signature pigments were compared to the daily changes in nutrients for each N:P ratio addition treatment, the significant relationships were typically observed in the three +N+P treatments but not the other treatments (Fig. 3.10). The relationships were significant between the daily changes in chl *a* and the daily changes in NO_x^- in the controls ($R^2 = 0.77$, $p < 0.05$, $n = 6$) and between the daily changes in zeaxanthin and daily changes in NH_4^+ in the +N alone treatments ($R^2=0.60$, $p<0.05$, $n=9$). Other than these relationships, there were no significant relationships between signature pigments and nutrients, and signature pigments vs. TDN. Consumption of TDN significantly contributed to the increases in daily changes in chl *a* and fucoxanthin in the +N+P treatments (TDN in relationship with chl *a*, N:P = 4, $R^2 = 0.34$, $p < 0.01$, $n = 22$; N:P = 16, $R^2 = 0.92$, $p < 0.01$, $n = 7$; N:P = 32, $R^2 = 0.66$, $p < 0.01$, $n = 9$; TDN in relationship with fucoxanthin, N:P = 4, $R^2 = 0.34$, $p < 0.01$, $n = 22$; N:P = 16, $R^2 = 0.91$, $p < 0.01$, $n = 7$; N:P = 32, $R^2 = 0.57$, $p < 0.05$, $n = 9$) and the highest yield per TDN consumed in both chl *a* and fucoxanthin were observed in the treatments of +N:P = 16 addition. It is suggested that when enriched with +N+P, particularly in the +N:P ratio = 16, diatoms were good competitors using N. Also, the yield of alloxanthin per μM TDN consumed was significant when enriched with +N:P = 16 ($R^2 = 0.74$, $p < 0.05$, $n = 7$). The relationships between the daily changes in nutrients and peridinin were typically positive, indicating that other phytoplankton taxa assimilated the N to a proportionately greater extent.

The individual pigment yield per unit P consumed was also generally higher in the +N+P treatments than the others (Fig. 3.10). In the +N:P = 16 treatments, yields of chl *a* ($R^2 = 0.88$, $p < 0.01$, $n = 7$), zeaxanthin, alloxanthin ($R^2 = 0.73$, $p < 0.01$, $n = 7$), and fucoxanthin ($R^2 = 0.76$, $p < 0.01$, $n = 7$) per μM DIP consumed were the highest, 56, 0.14, 1, and 80 μg: μM-P, respectively. The response to DOP differed somewhat, and the changes in signature pigments relative to the changes in DOP were not statistically significant.

Discussion

The overall hypotheses tested here were that (1) NO_3^- additions would promote growth of the diatom community while the organic and reduced forms of N (NH_4^+ and DON) additions would promote growth of picocyanobacteria, cryptophytes and dinoflagellates, and (2) high N:P additions (above Redfield) would yield a cyanobacterium- or cryptophyte-dominated system, and low N:P additions (less than Redfield) would favor a diatom- or dinoflagellate-dominated system. The results largely agree with these hypotheses; however, the responses from cryptophytes and peridinin-containing dinoflagellates to the nutrient additions differed from expectations. Here, the data are considered from the perspective of algal physiology. In addition, these mesocosm results are compared to changes of nutrients and phytoplankton abundance and composition that were reported from companion field studies conducted in Florida Bay (Chapter 2, Shangguan et al. submitted a). Management implications of changes in the nutrient quantity and quality with respect to changes due to the CERP are also considered, from the perspective of algal bloom control.

It is generally accepted that P is the limiting nutrient for primary production in Florida Bay, whereas N and silicon are not (Fourqurean et al., 1993; Phlips & Badylak, 1996; Hitchcock et al., 1998; Lavrentyev et al., 1998; Price et al., 2006). These experiments demonstrated that P additions substantially elevated the overall phytoplankton biomass (Fig. 3.2a) and all studied phytoplankton groups, picocyanobacteria, cryptophytes, diatoms, and peridinin-containing dinoflagellates (Fig. 3.2b-e). Similar increases in phytoplankton biomass have been observed in P-limited lakes and coastal regions worldwide, e.g. the East China Sea (Harrison et al., 1990) and the eastern Mediterranean Sea (Zohary & Robarts, 1998) among other regions. Moreover, in comparison to the +P additions, the +N+P treatments increased the phytoplankton biomass even more substantially, by more than 10-fold. In general, diatom production increased first, followed by a picocyanobacteria bloom generally by D_4 (Fig. 3.2b). As the "bloomers", diatoms doubled rapidly and cell numbers were maximal when P limitation was relieved and the N:P ratio was low (Klausmeier et al., 2004; Arrigo, 2005). Subsequently, when water-column P was mostly consumed, diatoms were outcompeted by the relatively slow-growing “survivalists”, the picocyanobacteria. This plankton succession has been observed in many estuaries (Moore et al., 2002; Yusoff et al., 2002; Berg et al., 2003; Miller & Wheeler, 2012). In contrast to the P additions (with or without N) which yielded the highest biomass responses, the +N alone additions did not elicit large biomass increases, but in the $+NH_4^+$ treatments, picocyanobacteria biomass (indicated by signature pigment, zeaxanthin) increased, in keeping with expectations (Fig. 3.2b, Fig. 3.5a; Glibert et al., 2016).

It is now well-documented that the form of N substrates can shape phytoplankton assemblage structure (Glibert et al., 2016 and references therein). As expected, the highest contribution of diatoms to total phytoplankton biomass (as chl *a*) was observed in the treatments where $+NO_3^-$ was added either alone (+N only) or with P (Fig. 3.5c). The diatom genus *Chaetoceros* was most abundant in the ambient samples (data not shown) and has been reported as an abundant diatom genus in this region (Steidinger et al., 2001; Hitchcock et al., 2007; Richardson, 2009). Species of *Chaetoceros* are also generally observed in NO_3^--rich waters, likely because they have high affinity for NO_3^- and often display non-saturating uptake kinetics for this N form (Lomas & Glibert, 1999b; Glibert et al., 2016).

Although the $+NH_4^+$+P treatments yielded mixed phytoplankton taxa assemblages, the $+NH_4^+$ treatments (without P) triggered dominance by picocyanobacteria (Fig. 3.2b, Fig. 3.5a). These assemblages also showed high estimated consumption of TDN (Table 3.2). The data add to the substantial body of literature that picocyanobacteria are often the dominant phytoplankton when the system is NH_4^+-rich but P-limited (Berg et al., 1997; Glibert et al., 2010; Domingues et al., 2011; Donald et al., 2011; Donald et al., 2013; Glibert et al., 2016). The +DON enrichment did not trigger as much picocyanobacteria growth as the $+NH_4^+$ additions in our study (Fig. 3.2b, 3.5a). Species of S*ynechococcus*, the bloom-forming picocyanobacteria in Florida Bay, are capable of utilizing organic nutrients such as urea (Glibert et al., 2004; Boyer et al., 2006; Heil et al., 2007; Wawrik et al., 2009), but under the +DON conditions herein they evidently were outcompeted by peridinin-containing dinoflagellates (Fig. 3.5d). Previous studies also have shown that *Synechococcus* responds to DON additions in Florida Bay (Glibert et al., 2004; 2009).

Cryptophytes and dinoflagellates generally bloom in regions rich in chemically-reduced forms of N, including NH_4^+ and DON (Lewitus & Kana, 1994; Berg et al., 2001; Berg et al., 2003; Glibert et al., 2016), but the trends in cryptophytes and peridinin-containing dinoflagellates in this study were not consistent with expectations. The growth patterns of cryptophytes were comparatively unaffected by N forms, but they typically co-existed with diatoms responding to +N+P treatments (Fig. 3.5b). Cryptophytes have been observed to form blooms with other taxonomic groups or to follow the dominance of others in coastal regions (Wetz & Paerl, 2008) perhaps because they are competitive in using the NH_4^+ and DON accumulated from the diatom blooms through grazing, viral lysis, or phytoplankton excretion (Wetz & Wheeler, 2003). Peridinin-containing dinoflagellates contributed minimally to the algal biomass growth (Fig. 3.5d).

The phytoplankton assemblage alterations with +N:P ratio enrichment showed several patterns. First, with +P alone additions, cryptophytes and peridinin-containing dinoflagellates grew rapidly, but the diatom response was relatively weak (Fig. 3.9b, c, d). Considering that total phytoplankton biomass in the +P alone treatments did not increase dramatically, production from cryptophytes and dinoflagellates was comparatively small. Nevertheless, there was a tendency for peridinin-containing dinoflagellates to co-exist with the opportunistic group, i.e. cryptophytes (Reynolds, 2006; Wetz & Paerl, 2008), when nutrient conditions were below the Redfield ratio. Relationships between dinoflagellates and N:P ratios have been studied (Riegman, 1995; Hodgkiss & Ho, 1997; Anderson et al., 2002; Li et al., 2009; Glibert et al., 2012; Accoroni et al., 2015). The onset of dinoflagellate blooms is likely associated with N:P ratios lower than Redfield, sometimes from an injection of P-rich water (Fang, 2004; Glibert et al., 2012). This initiation mechanism of dinoflagellates is consistent with the data from this study. While some peridinin-containing dinoflagellates, such as *Prorocentrum minimum*, may bloom under low N:P conditions, they can, however, be sustained for long periods in N:P ratios that are substantially elevated above Redfield proportions (Li et al., 2009; Glibert et al., 2012). In addition, the data suggest that peridinin-containing dinoflagellates used NH_4^+ to a high extent when only enriched with +P and when final N:P ratios approximated 6 (Fig. 3.10). This agrees with the conceptual model for dinoflagellate bloom formation in that they prefer reduced forms of N as opposed to oxidized forms (Glibert et al., 2012; Glibert, 2016) and that reduced N forms, especially NH_4^+, are preferentially taken up during blooms (Li et al., 2009). Subsequently, dinoflagellate blooms can be sustained over a longer period following P depletion due to internal P storage and to allelopathic and mixotrophic interaction with other species, possibly cryptophytes in this case (Glibert & Burkholder, 2011).

Second, the +N alone additions generated a picocyanobacteria-dominated condition, accompanied to a lesser extent by diatoms and peridinin-containing dinoflagellates (Fig. 3.9a, c, d). The picocyanobacteria biomass with +N alone additions was less than in +P alone or +N+P treatments, however (Fig. 3.2b). Due to the low P requirement of some picocyanobacteria (Van Mooy et al., 2009; Popendorf et al., 2011) and capacity to use organic P (Glibert et al., 2004; 2009), they can be prevalent in P-limited environments.

Third, when the final N:P ratio increased from 10 - 70, the contributions from diatoms and cryptophytes both increased (Fig. 3.9b, c). Diatoms and cryptophytes in all +N+P treatments appeared to efficiently use all inorganic and organic forms of N and P, based on the regressions of their nutrient depletion as a function of biomass (signature pigments) changes. But among them, it was typically when the treatment additions were 16, corresponding to an ambient N:P of 32, the highest nutrient uptake occurred based on nutrient depletion trends (Fig. 3.10). This response of diatoms to increasing +N:P ratio countered the expectation that the lowest N:P treatment would yield the highest proportion of diatoms. While +P enrichment increased the proportion of diatoms to overall phytoplankton biomass, additional enrichment with P decreased their contribution. The data indicated that diatoms did not use DOP substantially even under P stress (Fig. 3.10). This observation is consistent with previous reports that diatoms are less able to utilize DOP than other taxa (Dyhrman & Ruttenberg, 2006; Wang et al., 2011), although phytoplankton generally are known to use DOP via enzymatic hydrolysis in response to P deficiency (Dyhrman et al., 2006; Huang et al., 2007; Lomas et al., 2010; Dyhrman et al., 2012). Once P limitation was apparently relieved with an addition of 0.6 μM-P, reaching TDP 0.8 μM and a N:P ratio of 70, a diatom-dominated bloom likely would be stimulated in this system, whereas more P or lower N:P would be expected to yield a more diverse assemblage.

An important finding of this study was the potential for different groups of phytoplankton to respond to pulsed additions of nutrients, even when there were residual or even saturating nutrients in the water column at the beginning of the experiment. This finding counters the conventional premise that nutrients cannot be regulating at saturating concentrations (sensu Reynolds, 1999), which has also been previously challenged based on physiological data (Glibert et al., 2013; 2016). Here, the phytoplankton responded to the changing ratios of +N:P additions as opposed to total ambient N:P ratios (Fig. 3.7-3.9). Whereas the natural assemblages were physiologically poised to maximize the uptake of N and P under ambient conditions, when provided new substrates, algal populations were able to respond differentially to the pulse. Here, the starting condition was not uniformly limiting for growth, and the responses to nutrient pulses underscore the dynamic ability of phytoplankton cells to respond, at nutrient levels that range from limiting to supersaturating (Glibert et al., 2013).

Phytoplankton assemblage composition alteration in Florida Bay

Changes in nutrients and phytoplankton composition have taken place in Florida Bay since implementation of the C-111 project (Chapter 2; Shangguan et al. submitted a; Owens et al. in review ms). 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 generally have decreased whereas TDP and all forms of P have increased in northern Florida Bay (Chapter 2; Shangguan et al. submitted a). Also, the reduced forms of N 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 (Chapter 2, Shangguan et al. submitted a). In one eutrophic sub-estuary at the southern Everglades, northern Florida Bay border, which had elevated nutrients (mean TDN 120 μM, mean TDP ~0.7 μM) and phytoplankton (chl *a* ~40 μg L^{-1}) levels prior to CERP, phytoplankton biomass was reduced to about half, but the concentrations of picocyanobacteria and cryptophytes in relation to chl *a* increased after CERP was implemented. Reductions in chl *a* were likely because they were washed out of the sub-estuary into northern Florida Bay as dilution rate from the increased flow exceeded maximum growth rates of the algal assemblage. In contrast, phytoplankton biomass as chl *a* in a second, adjacent sub-estuary, which was oligotrophic prior to CERP, doubled to ~3 μg L^{-1} following CERP implementation, and the shifts in phytoplankton assemblages were similar. The proportions of picocyanobacteria and cryptophytes increased, while the proportions of diatoms and peridinin-containing dinoflagellates, both decreased.

These mesocosm results provide insights about the observed field responses following nutrient changes due to CERP. Picocyanobacteria were preferred in the reduced N treatments (Fig. 3.2b, Fig. 3.5a). Cryptophytes and peridinin-containing dinoflagellates were the algal groups favored by +P alone enrichments, although growth was not significantly higher than pre-CERP (Fig. 3.2c, e, Fig. 3.9b, d). Peridinin-containing dinoflagellates generally have lower maximum growth rates compared to other phytoplankton groups such as cyanobacteria, diatoms, and cryptophytes (Banse, 1976; Geider et al., 1986; Raven et al., 2005; Irwin et al., 2006). Slow growth rates apparently also contributed to the peridinin-containing dinoflagellates decline that was observed both in the eutrophic and oligotrophic sub-embayments in northern Florida Bay under increasing water flow and nutrient loads (Chapter 2, Shangguan et al. submitted a). In addition, diatoms were not favored by +P alone enrichments (Fig. 3.9c), consistent with a decrease in diatom biomass that has been observed in these two sub-estuaries in northern Florida Bay (Chapter 2; Shangguan et al. submitted a). Picocyanobacteria increased in both sub-estuaries, likely resulted from the increasing reduced N proportion, even though the total phytoplankton biomass change in the sub-estuaries differed. These changes mirrored the combined effect of potential picocyanobacteria blooming in this nutrient regime and their high growth rate, although their response to +P alone treatments was variable in the mesocosms (Fig. 3.9a).

Implications for south Florida water management

In all, the data here indicate that +P enrichment with +N in the form of NO_3^- could potentially stimulate diatom blooms and $+NH_4^+$ nutrient additions could lead to a picocyanobacteria-dominant community, the major bloom type in this region, consistent with other studies (Berg et al., 2003; Glibert et al., 2004; Donald et al., 2011; Fawcett et al., 2011; Glibert, 2016 and references therein).

Nutrient management in the region has focused on P since P historically has been considered the primary limiting nutrient, and thus it has been generally accepted that any increase in P would enhance the likelihood for algal blooms (Fourqurean et al., 1993; Moustafa et al., 1999; Knight et al., 2003; Kadlec & Wallace, 2008; Frankovich et al., 2011). These data indicate that P enrichment alone has the potential to substantially increase algal biomass, but combined enrichment with N and P could enhance phytoplankton biomass much more (Fig. 3.2a). Thus, a diatom-dominated bloom could be generated in eastern Florida Bay in response to both N and P, even at a relatively low level of ~0.8 μM-P. This value is derived from the mean ambient P concentrations and the results of the mesocosm experiments with +N+P additions in a molar ratio of +32, the condition when the diatom contribution to the phytoplankton abundance (based on the accessory pigment, fucoxanthin) was highest. By comparison, TDP at 2.2±1.1 μM-P was previously suggested as the minimum required to maintain a P-limiting condition for phytoplankton in northern Florida Bay (Frankovich et al., 2011). Frankovich et al. (2011) calculated this value using regression equations relating light availability to phytoplankton abundance, and phytoplankton abundance to water-column TDP concentrations, based on a light availability target of 5% of surface light at the bottom to maintain the dominant SAV, *Chara*. This study suggests, however, that even lower levels of P may be required to maintain a P-limited condition.

This research also highlights the importance of N co-management with P, particularly chemically-reduced N (Conley et al., 2009; Xu et al., 2010; Glibert & Burkholder, 2011). While substantial increases in biomass were not observed following N enrichment, the results support the premise that there can be a substantial change in biodiversity and an increase in the relative proportion of picocyanobacteria, in response to N additions. The experiments reveal that the $+NH_4^+$ alone enrichment can trigger and/or sustain a picocyanobacteria bloom (Fig. 3.5a), which can use N efficiently in Florida Bay (Table 3.2). The data clearly underscore the need for greater understanding of the influences of N forms in controlling phytoplankton blooms and taxa composition, and in this region, NH_4^+ and DON are of particular concern. In all, the data indicate that while P management may be effective in controlling overall biomass, control of N - especially NH_4^+ - will be important to mitigate and reduce picocyanobacterial blooms.

Table 3.1. Ambient environmental conditions in the mesocosms at Day 0 after which they were treated with varying N forms and N:P ratios.

Parameter	Experiment 1	Experiment 2	Experiment 3	Experiment 4	Experiment 5
Date of Mesocosm D_0	6/5/2012	8/13/2012	5/20/2013	11/18/2013	4/14/2014
Temperature (°C)	30.4	32.0	29.0	28.6	29.9
Salinity	28.5	28.5	32.3	33.2	33.9
NO_x^- (µM)	1.64	1.36	3.03	4.17	2.51
NH_4^+ (µM)	3.61	1.15	5.23	7.94	7.25
DON (µM)	27.65	34.99	38.91	34.65	32.62
TDN (µM)	32.90	37.50	47.17	46.76	42.38
DIP (µM)	0.21	0.23	0.30	0.16	0.24
DOP (µM)	0.20	0.40	0.42	0.07	0.20
TDP (µM)	0.41	0.73	0.72	0.23	0.44
NH_4^+: NO_x^-	2.20	0.85	1.73	1.90	2.89
DIN:DIP	25	11	28	76	41
TDN:TDP	80	51	66	203	96
Chlorophyll *a* (µg L^{-1})	1.26	1.06	0.64	3.09	0.91
Zeaxanthin (µg L^{-1})	0.03	0.04	0.02	0.06	0.03
Alloxanthin (µg L^{-1})	0.11	0.06	0.06	0.18	0.03
Fucoxanthin (µg L^{-1})	0.16	0.15	0.06	0.62	0.13
Peridinin (µg L^{-1})	0.17	0.15	0.07	0.13	0.04

Table 3.2. Comparison of slopes for regressions of the daily rate of change in chlorophyll *a* (μg L^{-1} d^{-1}), and major signature pigments (μg L^{-1} d^{-1}) as a function of the daily rate of change in TDN and P parameters (μM L^{-1} d^{-1}) for mesocosm controls and treatments as +NO_3^-+P, +NH_4^++P, +DON+P, +NO_3^-, +NH_4^+, and +DON. To exclude the nutrient addition effect in this dataset, mesocosms that received daily nutrient additions were not included in this aspect of analysis. Sample sizes for each treatment were listed. Based on Student's t-tests, significant differences at $p < 0.01$ (**) and $p < 0.05$ (*) are indicated; all significant values are also shown in bold and italics.

Pigment variables	Dissolved Nutrient and Nutrient Form	Mesocosm controls and treatments				
		Control	+NO_3^-+P	+NH_4^++P	+DON+P	+NH_4^+
Sample number		6	17	14	15	5
Chlorophyll *a*	TDN	0.018	***-0.46*****	-0.22	***-0.60*****	-0.092
	TDP	1.5	-2.4	-1.2	-1.58	1.2
Zeaxanthin	TDN	-0.0002	-0.0006	-0.0013	-0.0008	-0.0069
	TDP	**0.091****	0.0056	-0.012	-0.0062	0.047
Alloxanthin	TDN	-0.016	***-0.007****	-0.0007	-0.0032	0.00004
	TDP	-0.14	-0.031	-0.0002	-0.0059	***-0.20*****
Fucoxanthin	TDN	0.016	***-0.65*****	***-0.76*****	***-0.69*****	0.040
	TDP	0.55	-3.5	-1.4	-1.5	0.15
Peridinin	TDN	-0.0010	0.0016	0.012	0.002	0.0016
	TDP	-0.015	-0.010	0.0026	0.012	-0.0042

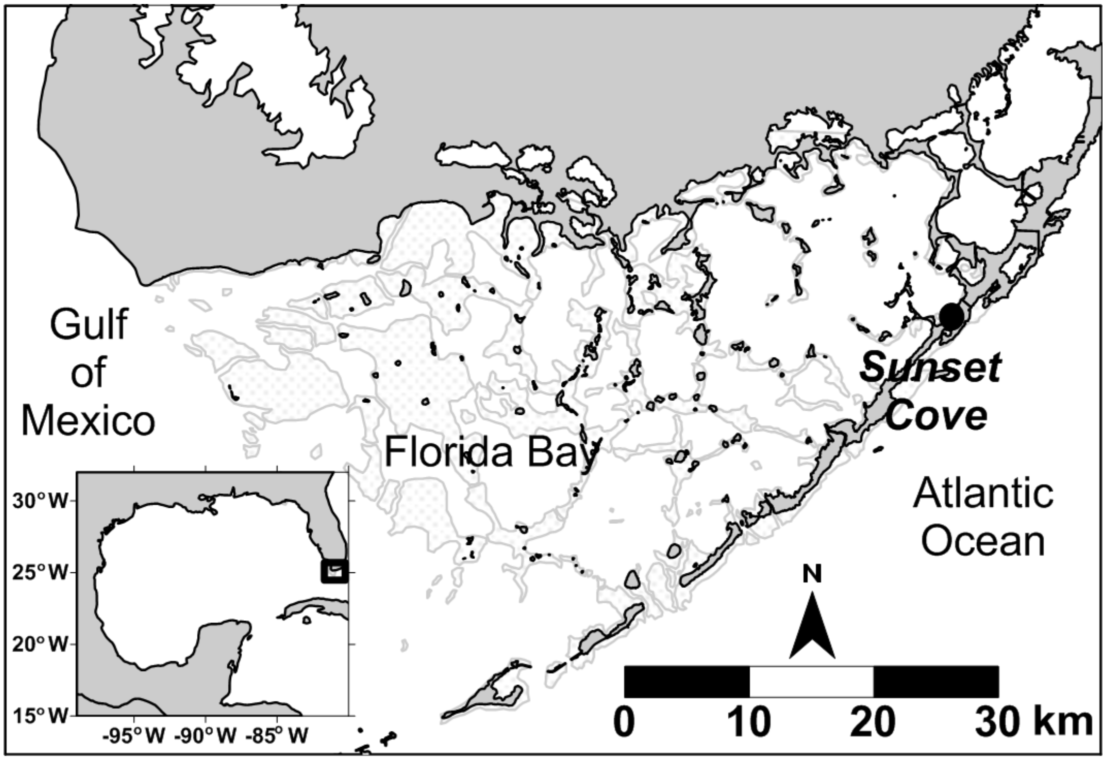

Figure 3.1 Map showing Florida Bay and the location of Sunset Cove, where the mesocosm experiments were conducted.

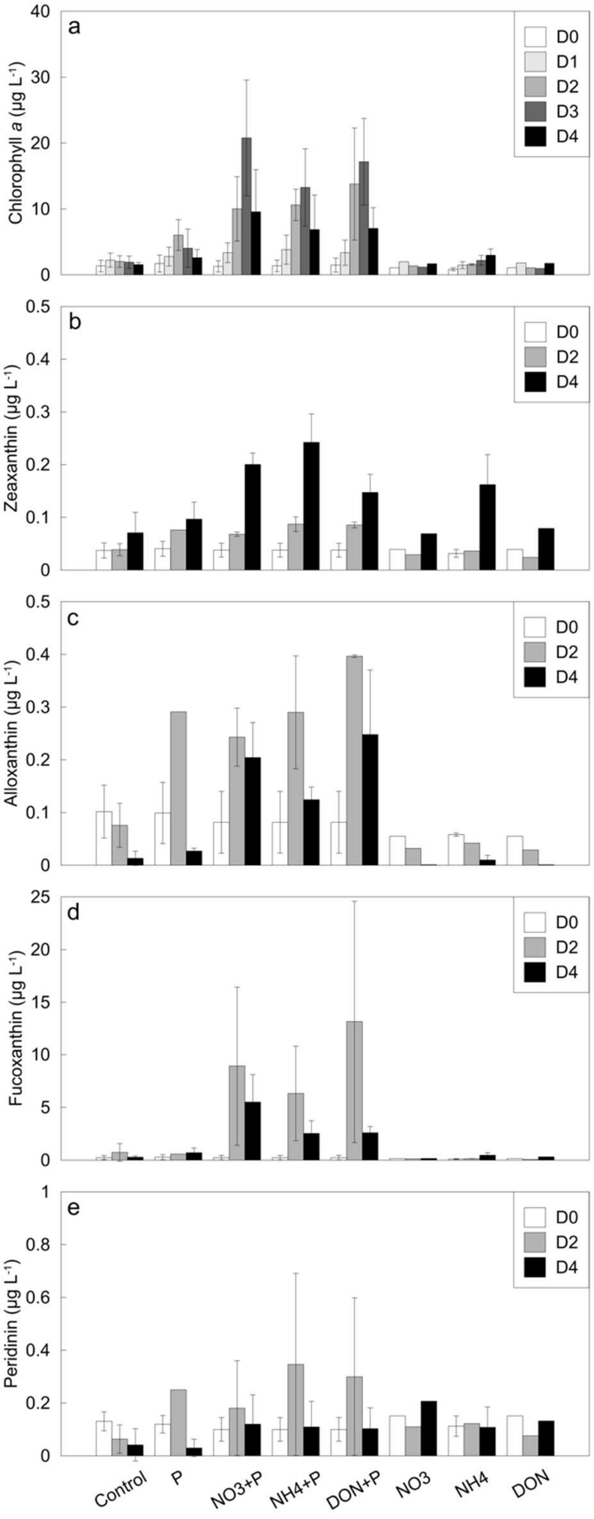

Figure 3.2 The mean concentration ($\underline{+}$ 1 SD) of chlorophyll *a* from D_0-D_4 (a) and signature pigments at D_0, D_2, and D_4 (b-e) (µg L^{-1}). These comparisons include all experiments described in text in which single nutrient additions were made.

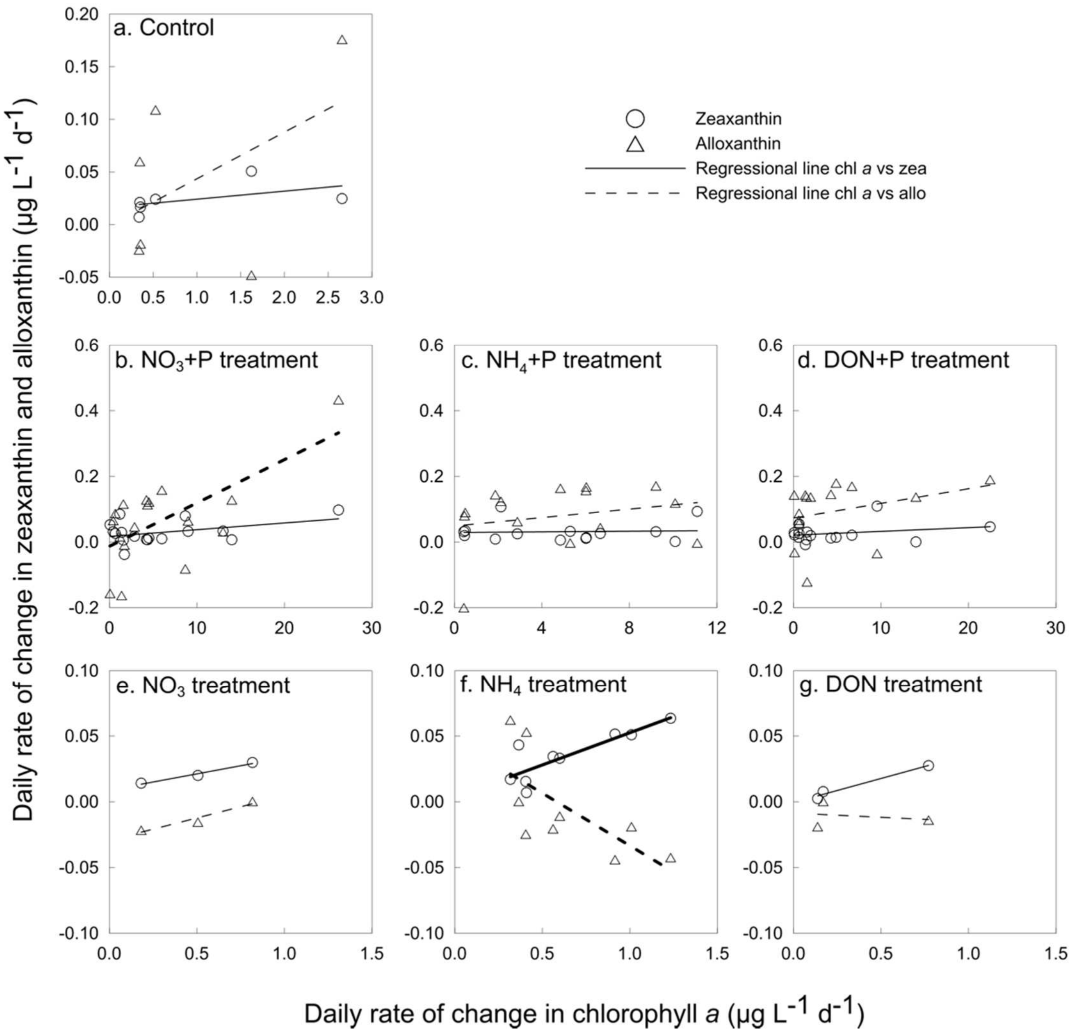

Figure 3.3 Comparisons of regressions of the daily rates of change in zeaxanthin (open circles, solid line) and alloxanthin (open triangles, dashed line) (µg L^{-1} d^{-1}) and the daily rates of change in chlorophyll *a* (µg L^{-1} d^{-1}) for data compiled from all mesocosms. Both daily-pulse and single-pulse treatments were included. Regressions that were significant ($p < 0.05$) are indicated with a bold line.

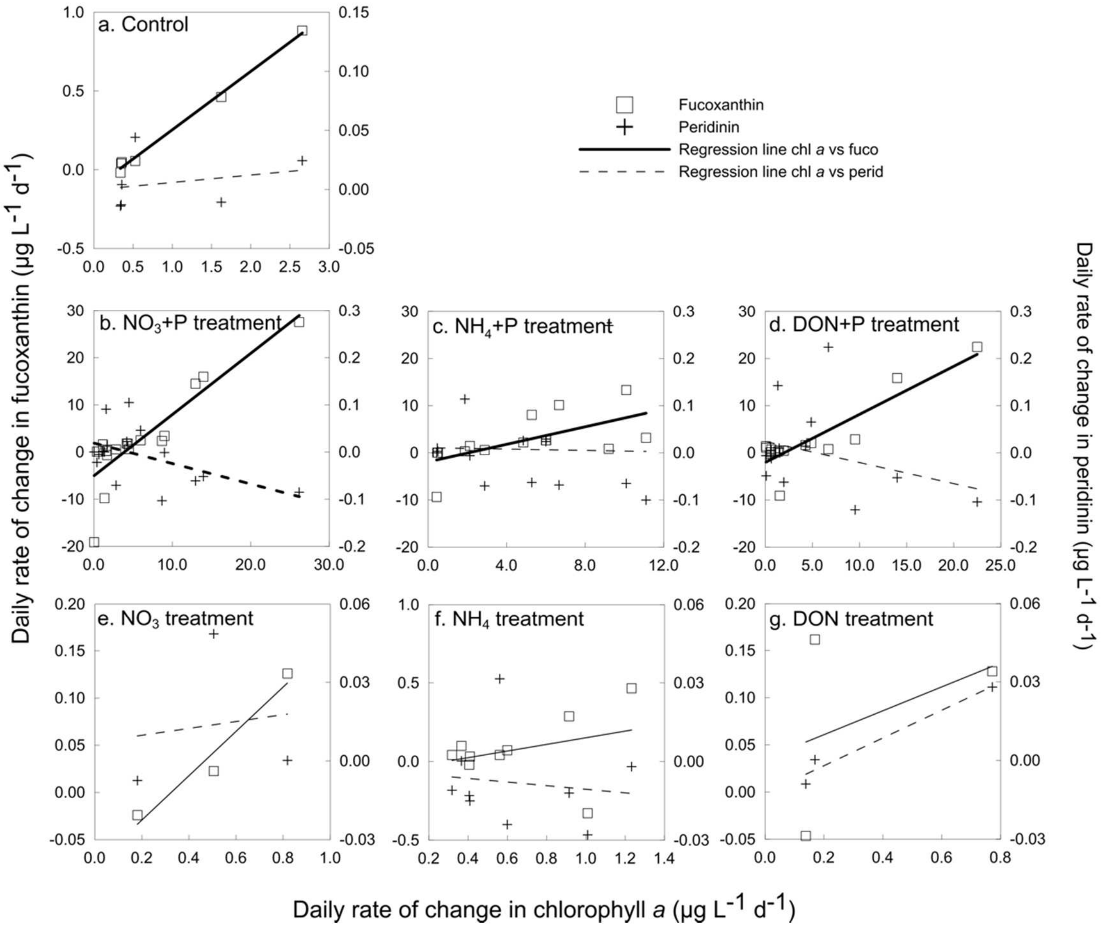

Figure 3.4 Comparisons of regressions of the daily rates of change in fucoxanthin (open squares, solid line; Y-axis is on the left) and peridinin (crosses, dashed line; Y-axis is on the right) (µg L^{-1} d^{-1}) and the daily rates of change in chlorophyll *a* (µg L^{-1} d^{-1}) for data compiled from all mesocosm samples. Both daily-pulse and single-pulse treatments were included. Regressions that were significant ($p<0.05$) are shown with a bold line.

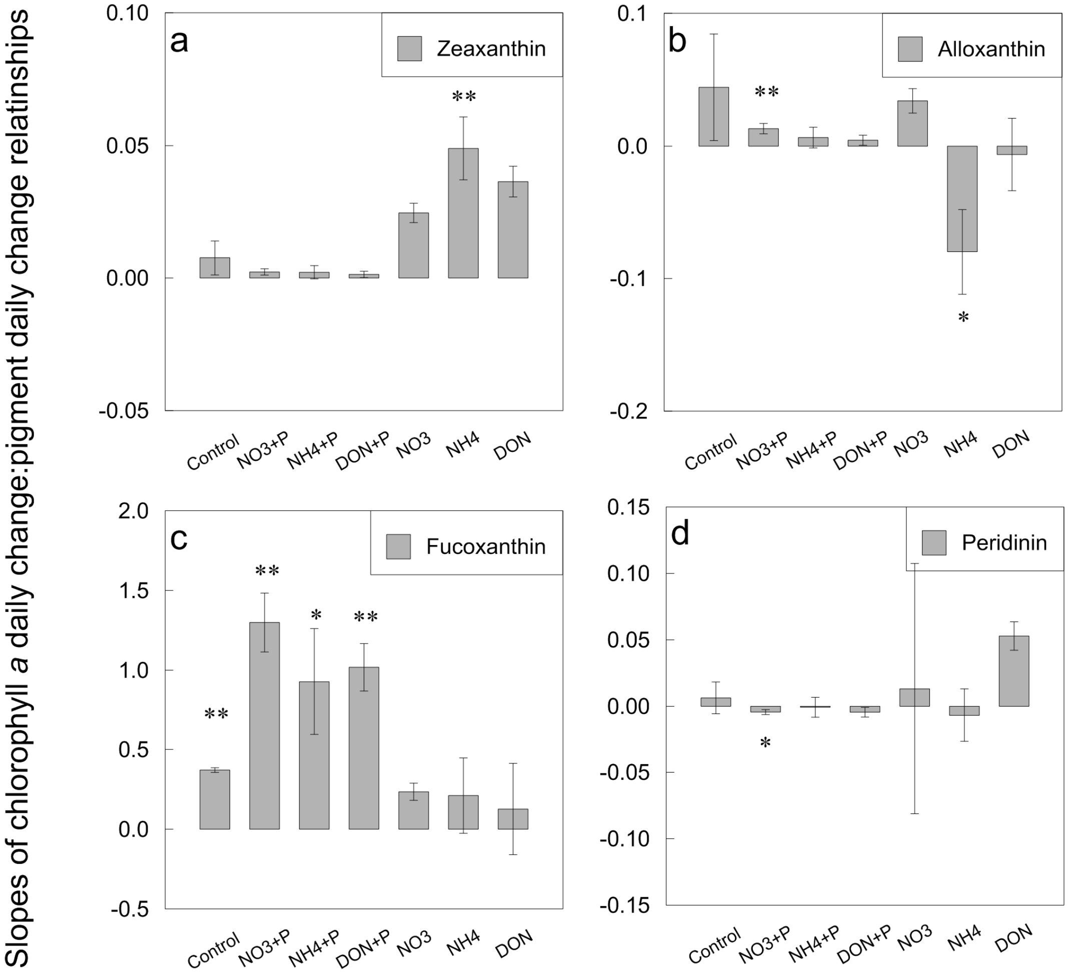

Figure 3.5 Comparisons of the slopes for the regressions of the daily rates of change in signature pigments and the rates of change in chlorophyll *a* for mesocosm treatments indicated. Both daily-pulse and single-pulse treatments were included. Data are given as means ± 1 SE. Values of R^2 that were significant at $p < 0.01$ (**) and $p < 0.05$ (*) are indicated.

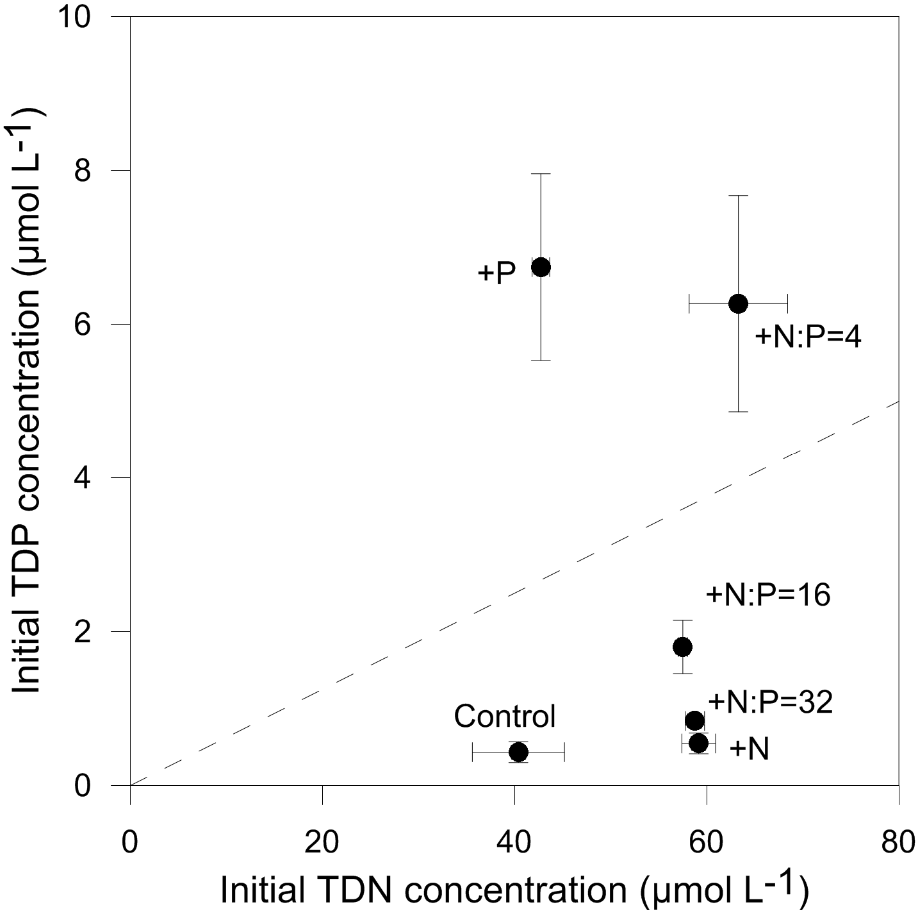

Figure 3.6 Relationship between the initial TDP and TDN concentrations in the N:P addition experiments. The data are given as means ± 1 SD.

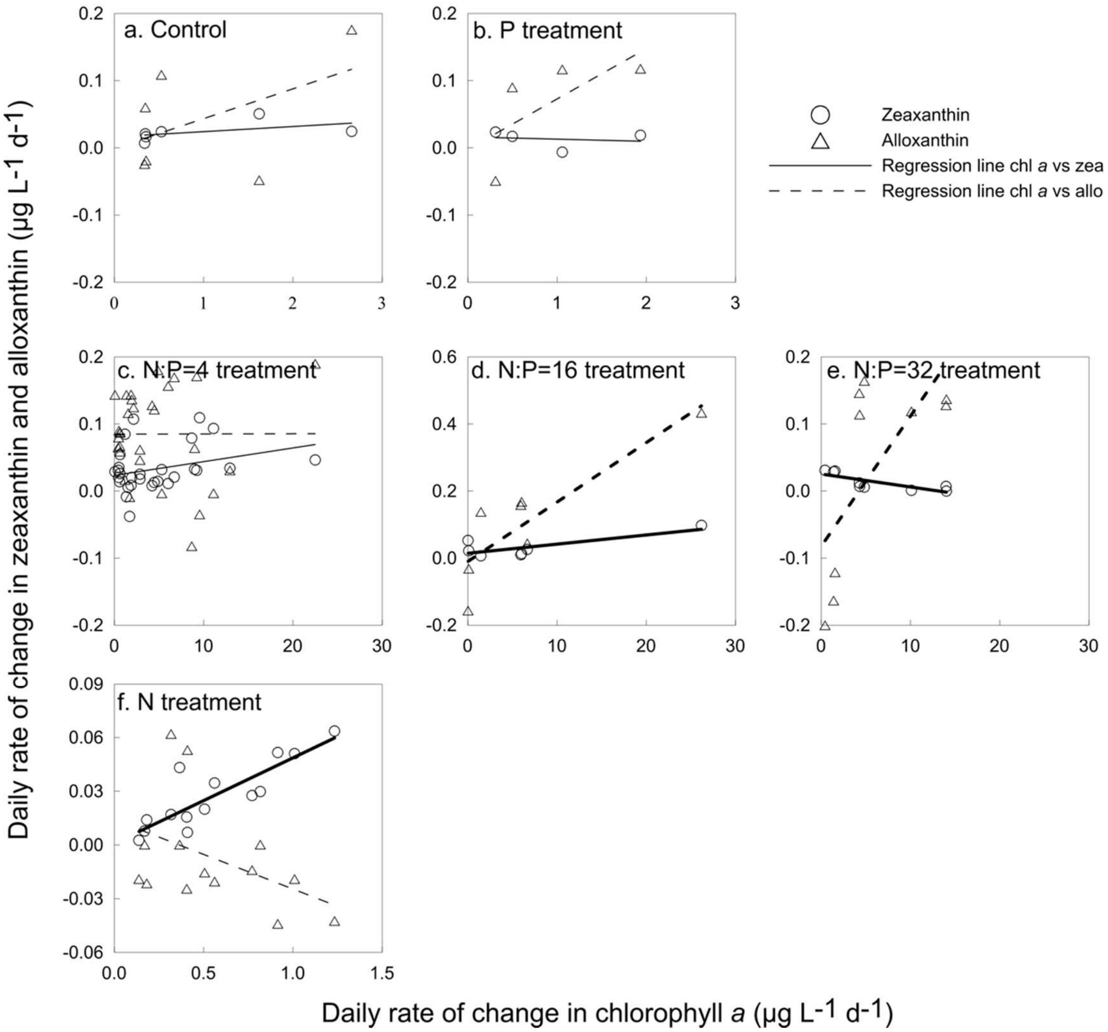

Figure 3.7 Comparisons of regressions of the daily rates of change in zeaxanthin (open triangles, solid line) and alloxanthin (open circles, dashed line) (µg L^{-1} d^{-1}) as a function of the daily rates of change in chlorophyll *a* (µg L^{-1} d^{-1}) for complied data from all mesocosm samples. Both daily-pulse and single-pulse treatments were included. Regressions that were significant (p<0.05) are shown with a bold line.

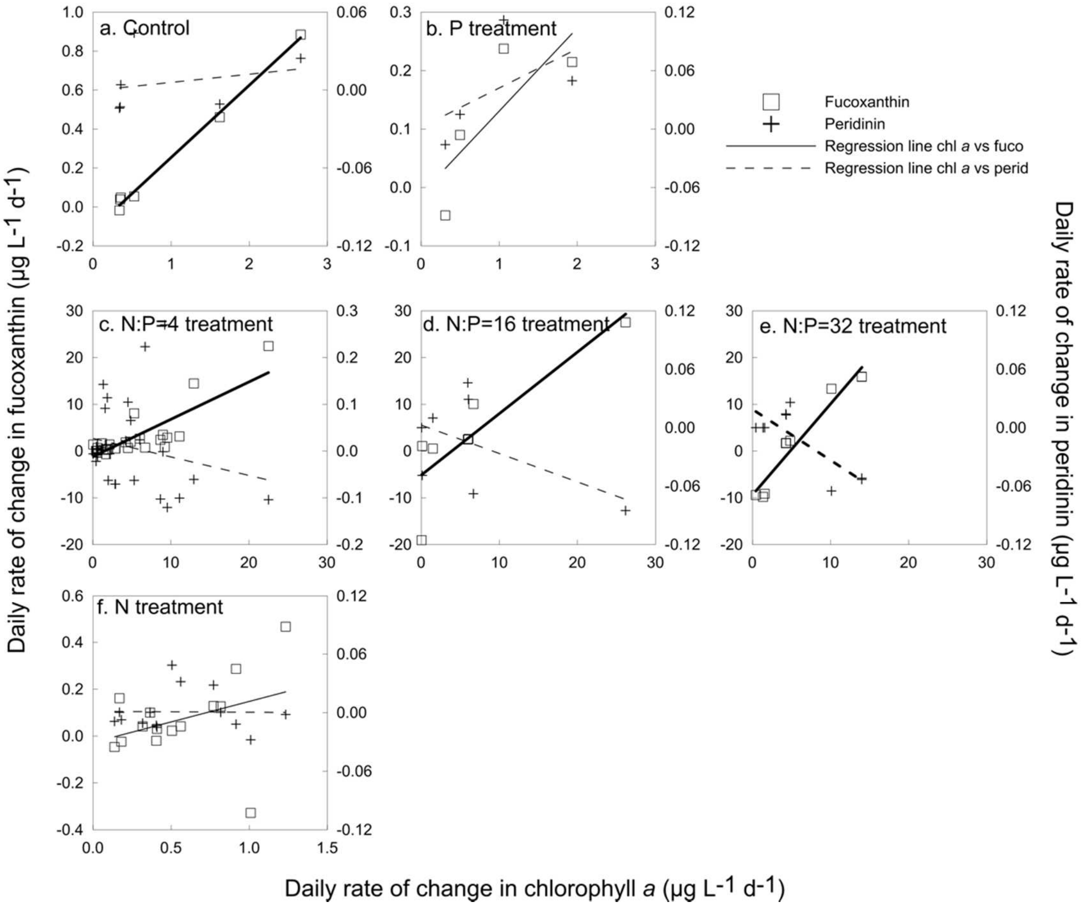

Figure 3.8 Comparisons of regressions of the daily rate of change in fucoxanthin (open squares, solid line; Y-axis is on the left) and peridinin (crosses, dashed line; Y-axis is on the right) (µg L^{-1} d^{-1}) as a function of the daily rate of change in chlorophyll *a* (µg L^{-1} d^{-1}) for data compiled from all mesocosm samples. Both daily-pulse and single-pulse treatments were included. Regressions that were significant ($p<0.05$) are shown with a bold line.

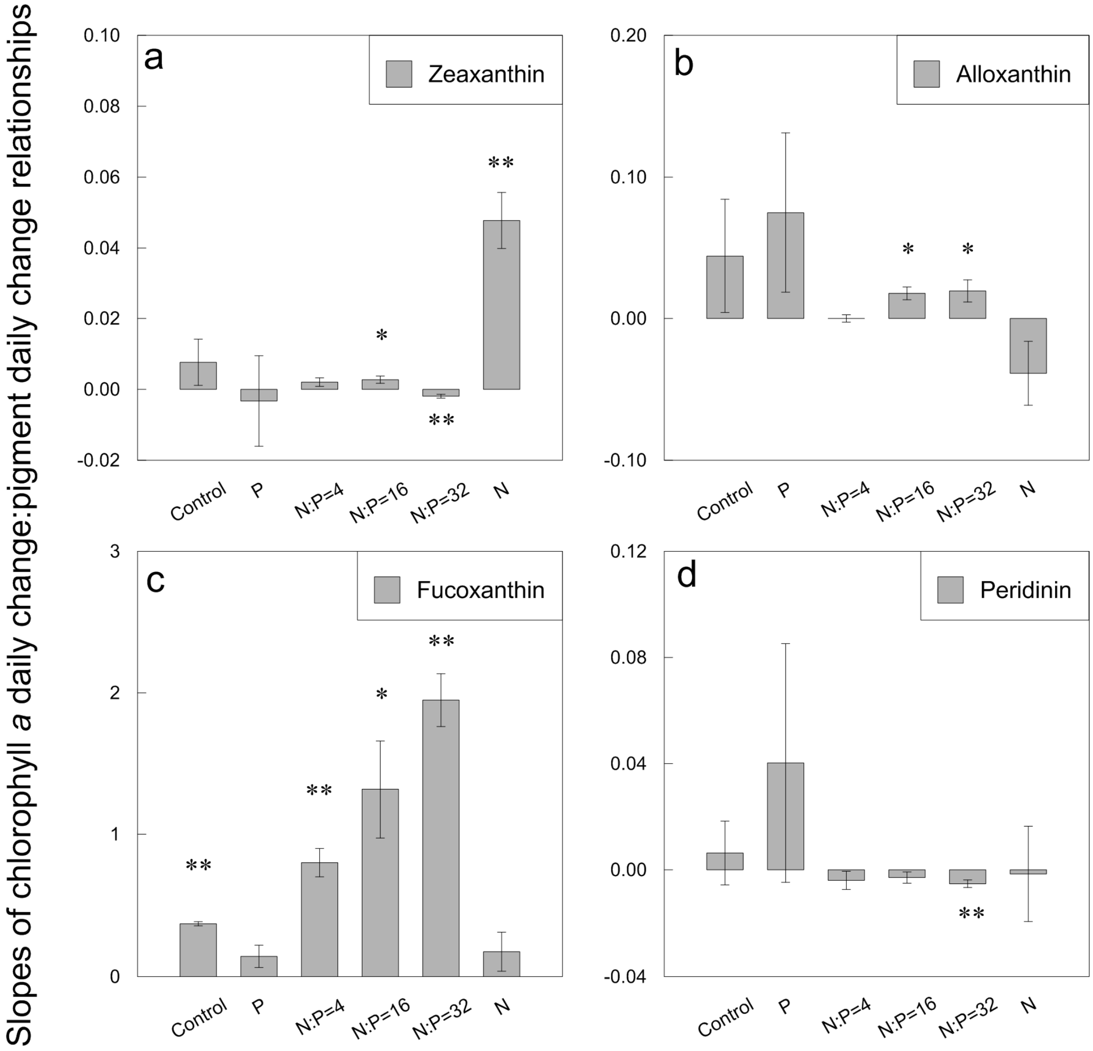

Figure 3.9 Comparisons of the slopes for the regressions of the daily rates of change in signature pigments and the rates of change in chlorophyll *a* for mesocosm treatments indicated. Both daily-pulse and single-pulse treatments were included. Data are given as means ± 1 SE. Values of R^2 that were significant at $p<0.01$ (**) and $p<0.05$ (*) are indicated.

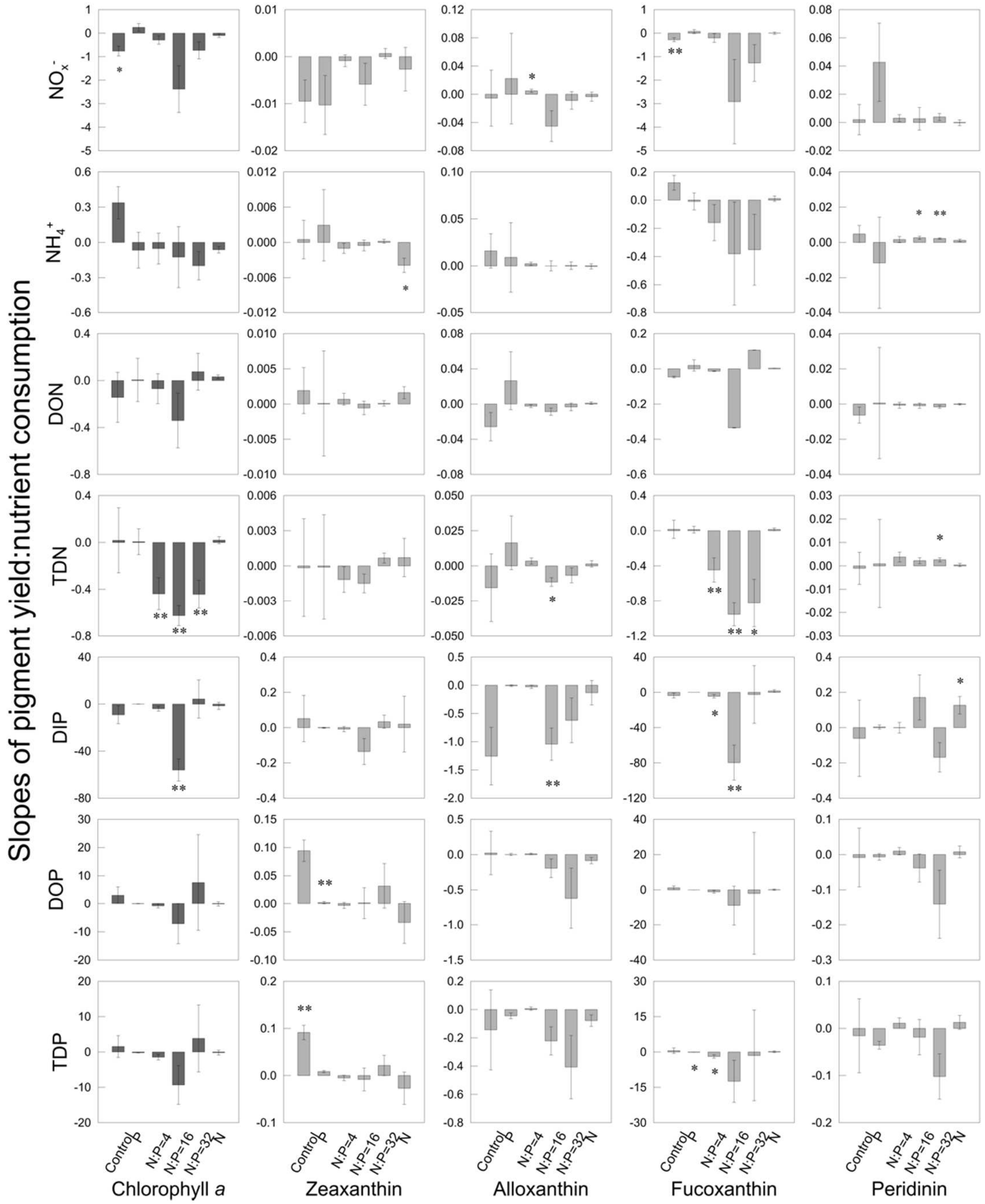

Figure 3.10 Comparisons of the slopes for the regressions of the daily rates of change in chlorophyll *a* and signature pigments and the rates of change in chlorophyll *a* for mesocosm treatments indicated. Only single-pulse treatments were included. Data are given as means ± 1 SE. Values of R^2 that were significant at $p < 0.01$ (**) and $p < 0.05$ (*) are indicated.

Chapter 4 : A Statistical Model to Quantify the Effects of Freshwater Discharge and Nutrient Loading on Phytoplankton Biomass and Assemblage Composition in Semi-Enclosed Lagoon Systems in Florida Bay

Abstract

A statistical box model was developed to gain insights about how altered water flow and nutrient concentrations affect phytoplankton biomass and assemblage taxa, cyanobacteria and diatoms in the saline West Lake Chain system in the southern Everglades region of northern Florida Bay. Freshwater drains from the Everglades into the upper West Lake, then to downstream, Long Lake, and then to northern Florida Bay at Garfield Bight. Relationships between nutrients and phytoplankton in the model were parameterized based on previously established relationships from field measurements in different salinity regimes. Model simulations with altered flow indicated that higher freshwater discharge from the Everglades would affect the three lakes differently. Increased flow without changes in nutrients in the model decreased salinity and nutrients due to dilution. Phytoplankton biomass, and the functional groups picocyanobacteria and diatoms, all sustained reductions in West Lake; biomass and picocyanobacteria decreased in Long Lake while diatoms increased; and Garfield Bight had a high potential to generate algal blooms. Increasing nutrient loads from the Everglades in the model resulted in different effects depending on the nutrient. Most notable was the potential impact from a 5-fold modeled increase in dissolved organic nitrogen (DON), which resulted in a tripling of picocyanobacteria

in West Lake. The model can be used as a research tool to help understand the effect of the ongoing restoration of flow in the Everglades on the nutrient regime and phytoplankton biomass and assemblage composition in the lake systems and in northern Florida Bay.

Introduction

The Comprehensive Everglades Restoration Plan (CERP) was initiated to shift Florida Bay to more closely resemble its previous ecological state. As the dominant primary producers in the Florida Bay system, seagrasses sustained a catastrophic decline in the late 1980s that affected about 30% of that community (Robblee et al., 1991; Hall et al., 1999). This decline was followed by further ecological degradation as a result of frequent and prolonged picocyanobacteria, *Synechococcus* spp., blooms (Phlips et al., 1999; Glibert et al., 2004; 2009; Berry et al., 2015) and higher-trophic-level mortalities (Robblee et al., 1991; Fourqurean & Robblee, 1999; Peterson et al., 2006). Hypersalinity has been considered to be one major contributing factor to the seagrass loss (McIvor et al., 1994; Zieman et al., 1999), but confounding effects due to nutrient changes have also been shown to play important roles in these changes (Boesch et al., 1993; Lapointe & Barile, 2004). To lower the salinity levels, CERP was designed to direct water south through the Everglades and into Florida Bay.

The C-111 Spreader Canal Western Features project, a component of CERP, began operations in 2012. The C-111 project was implemented to retain and increase freshwater flow within the primary drainage system into northern Florida Bay, Taylor Slough. The general goal of the ongoing restoration program is to shift the salinity regime by changing the timing and distribution of freshwater flow. Yet, the increase in water flow will also likely change the quantity and quality of nutrients discharged to Florida Bay, leading to unknown impacts on phytoplankton and ultimately seagrass and ecosystem health. Therefore, it is important to understand the combined effects of nutrients and freshwater flow on phytoplankton, its biomass and community composition.

Northern Florida Bay is particularly sensitive to changes in salinity and nutrients. As it lies at the interface between Florida Bay and the Everglades, this region should be the first to respond to the changes from the C-111 project. Also, this region has many quasi-Intermittent Closed Open Lakes and Lagoons (ICOLLs) (Suzuki et al., 1998; Frankovich et al., 2011; Maher et al., 2011). They are not true ICOLLs in the sense that they are not completely physically separated from the Bay, but they function as ICOLLs, receiving limited, or even reversed, flow in the dry season. ICOLLs often act as sinks of land-derived nutrients because of their restricted water exchange, and they tend to be rich with organic matter and nutrients (Kjerfve & Magill, 1989). They are considered to be highly productive ecosystems and thus have the potential to become eutrophic with increased nutrient enrichment (Suzuki et al., 1998; Menendez & Comin, 2000).

This study focused on one of the quasi-ICOLLS in northern Florida Bay (Fig. 4.1). The West Lake Chain (WLC) is typically planktonic-dominated and nutrient- rich, with high chlorophyll *a* (chl *a*) concentrations but low coverage of submersed aquatic vegetation (SAV) (Frankovich et al., 2011; Shangguan et al. submitted a). The WLC receives freshwater mainly from Taylor Slough, draining from the upper West Lake

(WL) to the downstream lake, Long Lake (LL) and then to northern Florida Bay at Garfield Bight (GB). A shift toward increasing freshwater flow, decreased salinity, decreased total dissolved nitrogen (TDN – all forms), but increased total dissolved phosphorus (TDP – all forms) in northern Florida Bay, was found following the first phase of C-111 implementation (Chapter 2; Shangguan et al. submitted a). In contrast to the expectation that the WLC lakes would experience increased eutrophication following CERP implementation, however, there also was a ~50% decline in overall phytoplankton biomass, and a shift to smaller sized cells, picocyanobacteria. These lakes were eutrophic before CERP implementation.

Salinity and nutrients can shape phytoplankton biomass and assemblage composition in multiple ways. As previously documented (Chapter 2; Shangguan et al. submitted a), both before and after CERP implementation, those lakes having salinity lower than 20, referred to as low salinity (LS) lakes herein, are generally located in the upstream and tended to have higher nutrient concentrations and higher chlorophyll *a* (chl *a*), while the lakes having salinity higher than 20, herein high salinity (HS), generally located more downstream, tend to have lower nutrient concentrations and lower chl *a* (Chapter 2; Shangguan et al. submitted a). The phytoplankton community of the LS and HS lakes responded somewhat differently to nutrient availability. In the LS lakes, the proportion of picocyanobacteria was positively correlated with dissolved organic nitrogen (DON), and the proportion of diatoms was positively correlated with NO_x^- (NO_3^-+NO_2^-). In the HS lakes and embayments, the proportion of picocyanobacteria was positively correlated with DON, whereas the diatom proportion was negatively correlated with DON (Chapter

2; Shangguan et al. submitted a). These relationships were further confirmed in mesocosm experiments conducted in the adjacent eastern Florida Bay (Chapter 3; Shangguan submitted b). In those experiments, a high N:P ratio and high proportion of chemically-reduced forms of N, NH_4^+ and DON, were both related to a higher proportion of picocyanobacteria. At the other end of the spectrum, low N:P and a high proportion of oxidized form of N, NO_x^-, were related to the diatom-dominant communities.

There is considerable interest in developing predictive capability of both seagrass and phytoplankton community composition in relation to changing salinity and nutrients in Florida Bay. The Seagrass Ecological Assessment and Community Organization Model (SEACOM) was developed for a dynamic numerical simulation of the seagrass community in representing basins of all major areas of Florida Bay (Fig. 4.2) (Madden & McDonald, 2010; Madden, 2014). In this model, the growth of seagrass is characterized by primary factors, including light sufficiency, nutrient sufficiency, salinity, sulfide concentration and temperature relative to optimal or saturating requirements (Fig. 4.2). The primary factors are affected by the secondary factors including some influence from phytoplankton such as the light attenuation from dense phytoplankton blooms that can reduce the photosynthetically active radiation at the seagrass leaf surface, and the autochthonous and allochthonous nutrients that are assimilated or recycled by the phytoplankton (Fig. 4.2). However, the current SEACOM model has limited capability for phytoplankton prediction. Thus, development of a phytoplankton module that can predict both phytoplankton abundance and its major functional groups within the SEACOM framework has become an important goal.

The purpose of this chapter is to present initial results of ecosystem modeling directed at understanding relationships among freshwater discharge, nutrient loading, and phytoplankton biomass and community composition in the WLC system of Florida Bay. The structure and calibration of a phytoplankton ecosystem model is first described, and then phytoplankton responses to modeled changes in flow and nutrients input are interpreted.

Methods

Site description

Florida Bay is a shallow coastal lagoon approximately 2,200 km^2 in area with average depth approximately 1.5 m (Fig. 4.1). It is connected to the Everglades wetland system at the northern boundary. Water exchange with surroundings is restricted. Connection with the Atlantic Ocean is inhibited by the Florida Keys and reef tract at the eastern and southern boundaries (Boyer et al., 2006; Madden et al., 2009). The Bay exchanges with the Gulf of Mexico at the open western border, via diurnal tides and currents. Hydrological circulation within the Bay itself is limited, as many shoals and shallow basins restrict water transport (Lee et al., 2002). The Bay has an average annual temperature of 25^{o}C and two distinct meteorological seasons: a November-April dry season and a May-October rainy season when 75% of annual precipitation occurs (Duever et al., 1994). Freshwater input from the Everglades is minimal during the dry season but considerable, through sheet flow, in the wet season (Lee et al., 2007).

The WLC is located in northern Florida Bay, a transition area connecting the Everglades and the Florida Bay. These interconnected, saline lakes receive freshwater flow from Taylor Slough, a major drainage system in south Florida. Within the lake chain system, flow is hydrologically connected, but the lakes are effectively separated, however, by narrow mangrove creeks and small, shallow (<50 cm) ponds. Therefore, each lake displays distinct salinity, nutrients, and phytoplankton characteristics. Typically, from the upper lake to the lower lakes or embayments, WL to LL, and LL to GB, salinity increases while nutrients and phytoplankton biomass decreases.

General model description

An ecological model was constructed to explore the effect of increasing freshwater flow and altered nutrient loading from the Everglades on phytoplankton biomass and community composition in the WLC. State variables included water mass, 5 forms of nutrients (DON, NH_4^+, NO_x^-, dissolved organic phosphorus - DOP, and dissolved inorganic phosphorus - DIP), phytoplankton chl *a* (CHL), and two generally abundant phytoplankton groups, picocyanobacteria (CYANO) and diatoms (DIA). These two groups were selected because they have shown clear differences in responses to nutrients. The model was run with varying freshwater flow and nutrient inputs from the Everglades to test the sensitivity of nutrients and phytoplankton in the WLC lakes. The model was built and run using Stella 9.0.3 (leesystems.com), solved by second-order Runge-Kutta numerical integration.

As derived from direct observations from field measurements, the phytoplankton community responded different to nutrient availability depending on salinity. A salinity of 20 was used to differentiate between the LS and HS system responses (Chapter 2; Shangguan et al. submitted a) and thus, salt (salt mass in each lake and salt mass flow between lakes) is included as an important parameter in this model. The model was designed to show how-phytoplankton communities could respond to changing nutrients in LS and HS regions. Using DON as an example, the water, salt, and nutrient change through the lakes in the model are given in Fig. 4.3, and the selected parameters are listed in Table 4.1. All abbreviations are summarized in Table 4.2.

The main equations that connected the water mass and the salt mass, the water mass and the nutrient masses, and water flow between the upper and lower lakes, exemplified here between WL and LL, are:

$$\text{Salt Flow W-G} = \text{WM Flow} \times \text{Salinity in WL} = \frac{\text{WM Flow} \times \text{Salt in WL}}{\text{WM in WL}} \quad (1)$$

$$\text{DON Flow W-L} = \text{WM Flow} \times \text{DON Conc in WL} = \frac{\text{WM FLow} \times \text{DON in WL}}{\text{WM in WL}} \quad (2)$$

The surface areas (A) of WL, LL, and GB are, respectively, 8.3×10^6, $1. 5 \times 10^6$, and 6×10^6 m^2, thus the initial Water Mass (WM) of WL, LL, and GB were estimated to be 1.25×10^{10}, 2.3×10^9, and 9×10^9 kg, respectively, using the estimated average water depth of 1.5 m. The observed average salinity in WL, LL, and GB were 13, 19, and 35, respectively (Chapter 2). The initial salinities of each lake were assumed to be 10, 20, and 30, thus the salt mass estimates for WL, LL, and GB were 1.25×10^8, 4.6×10^7, and 2.7×10^8 kg, respectively. Since seawater intrusion from northern Florida Bay was not illustrated in the model, a salt pulse was added from Day 270 to Day 365 each year to simulate the dry season. The salt pulse masses included were 3.4×10^5, 3.1×10^5, and 5×10^5 kg $day^{-1}$for WL, LL, and GB respectively (Fig. 4.3). The WLC

WM flow from the Everglades to WL, WL to LL, LL to GB, GB to the northern Florida Bay was assumed to be the same.

Box model

The water source in each lake included the water inflow from upstream, precipitation, water-column recycling, and groundwater. The water outflow included evaporation and flow to the next lower lake. Input into WL was from the Everglades, while inflow to the lower lakes was solely considered to have come from the respective upper lakes. The WM flow, precipitation and evaporation were all based on unpublished data of Price (Dr. René M. Price, School of Environment, Art, and Society, Florida International University, pers. comm.), assuming that the wet season extends from May 1st to October 31st and the dry season covers the remainder of the year (Days 1 – 120 and 305 – 365). Thus:

$$\text{WM flow} = 6.7 \times 10^6 + 1.7 \times 10^7 \times \sin(0.0172 \times \text{time} + 2.064) \tag{4}$$

$$\text{Precipt in WL} = A \times (3.3 + 3.3 \times \sin(0.0172 \times \text{time} + 2.064)) \tag{5}$$

$$\text{Evap in WL} = A \times (4.2 + 2.5 \times \sin(0.0172 \times \text{time} + 2.064)) \tag{6}$$

Groundwater discharge is relatively patchy but does occur at some locations, particularly in WL, based on Price (pers. comm.). Only the groundwater discharge at WL was estimated, using a set of random numbers from 0.8 to 1 mm, thus:

$$\text{Ground WM in WL} = A \times \text{random}(0.8, 1) \tag{7}$$

The major exogenous nutrient input sources for the lakes in northern Florida Bay included the upper lakes, atmosphere, and groundwater. As a shallow and subtropical lagoon system, the rate of regeneration of nutrients in the system is relatively high, both within the plankton and from the sediment resuspension (Yarbro & Carlson, 2008; Gardner & McCarthy, 2009; Glibert et al., 2010). Nutrient sinks in this system were defined to include phytoplankton, SAV, and nutrient output to the Florida Bay.

The intial nutrients in each lake, nutrient inputs, and benthic fluxes were estimated from a range of sources to parameterize the model (Table 4.3). Initial nutrient concentrations in each lake were based on direct measurements (Chapter 2; Shangguan et al. submitted a). Estimated total nitrogen (TN), dissolved inorganic nitrogen (DIN), and total phosphorus (TP) concentrations from Taylor Slough/C-111 Canal to Florida Bay were derived from Rudnick et al. (1999). Atmospheric deposition is an important nutrient source particularly for DIN, and was estimated from Boyer and Keller (2007), as was annual precipitation based on measurements at Miami Beach (https://rainfall.weatherdb.com/l/66/Miami-Florida). An estimate of the groundwater source was derived from Boyer and Keller (2007), but the data were used with caution because well-water nutrients can be affected by biogeochemical processes and advective flow through surficial sediments (Huettel & Gust, 1992), thus lowering the concentrations (Table 4.3).

Several other assumptions guided model development, particularly for nutrients. It was assumed that TN equals TDN, TP equals TDP, and that NH_4^+ and NO_3^- represent 75% and 25%, respectively, of the DIN pool, and dissolved organic phosphorus (DOP) and dissolved inorganic phosphorus (DIP) represent 67% and 33% respectively, of the TDP pool, based on literature and direct measurements (Chapter 2; Shangguan et al. submitted a). Dissolved organic matter (DOM) is an important nutrient source for phytoplankton in Florida Bay (Glibert et al., 2004; Heil et al., 2007). Limited data are available on the bioavailability of the dissolved organic matter pool and rates of DON flux from the sediments. Studies on sediment fluxes suggested that the DIN sediment fluxes were generally low but highly variable (Cornwell & Owens, 2003; Owens et al., in review) and, thus, were assumed to be zero unless otherwise noted. It had previously been shown that when the temperature is above 30°C and dissolved oxygen decreases below 0.2 mg/L, rates of nitrification and denitification are inhibited by low oxygen availability, and the NH_4^+ flux correspondingly can increase substantially (Rudnick et al., 2001). Temperatures higher than 30°C are infrequently observed; therefore, a relatively low recycling rate from the sediment was assumed (Table 4.3). Sediment release of P is also typically low to negligible, based on average rates previously measured in Florida Bay (Boyer & Keller, 2007). Therefore, for recycling parameters, it was assumed that 1 μg L^{-1} chl *a* phytoplankon was converted to 1 μmol L^{-1} particulate nitrogen (PN) and 0.125 μmol L^{-1} particulate phosphorus (PP) (Geider et al., 1997). Furthermore, it was assumed that 20% of PN was recycled to reduced forms of N, DON and NH_4^+, 10% for each, and that 20% of PP was recycled to DOP (Jang & Baglama, 2005). For the phytoplankton consumption terms, it was estimated that 90%, 0.75%, and 0.25% of PN was from DON, NH_4^+, NO_x^-, respectively, and that 67% and 33% PP was from DOP and DIP, respectively, assuming that the phytoplankton consumed each nutrient form proportionately as given.

Phytoplankton biomass and assemblage prediction

This model used phytoplankton biomass and community composition data previously reported from field measurements (Chapter 2; Shangguan et al. submitted a). Total phytoplankton biomass (CHL) was derived from chl *a* values, and CYANO and DIA abundances were derived from measurements of signature pigments using high-performance liquid chromatography (HPLC; Van Heukelem & Thomas, 2001). Zeaxanthin was considered as an indicator of picocyanobacteria, and fucoxanthin as an indicator of diatoms (Jeffrey & Wright, 1994; Jeffrey & Vesk, 1997; Glibert et al., 2004). The relationships between CHL, CYANO, DIA and nutrients, DON, NH_4^+, NO_x^-, DIP and DOP in the two different salinity groups were analyzed using multiple linear regression analysis with SAS software (Fig. 4.4). These relationships, for the initial condition that does not include new water flow ("no flush") from the Everglades due to CERP, were described as follows:

In the LS stations,

$$CHL = -19 + 0.49 \times DON + 0.65 \times NH_4^+ - 0.68 \times NO_x^- - 5.1 \times DOP - 5.1 \times DIP \quad (8)$$

$(n = 40, R^2 = 0.57, P < 0.01)$

$$CYANO = (-0.33 + 0.15 \times \ln DON - 0.033 \times \ln (NH_4^+ + NO_x^-)/DIP + 0.030 \times \ln (DON + NH_4^+)/ NO_x^-) \times CHL \quad (9)$$

$(n = 40, R^2 = 0.31, P < 0.01)$

$$DIA = (0.15 - 0.030 \times \ln DON + 0.020 \times \ln (NH_4^+ + NO_x^-)/DIP - 0.0050 \times \ln (DON + NH_4^+)/ NO_x^-) \times CHL \quad (10)$$

$(n = 40, R^2 = 0.48, P < 0.01)$

In the HS stations,

$$CHL = -16 + 0.23 \times DON + 0.35 \times NH_4^+ + 0.47 \times NO_x^- - 8.5 \times DOP + 25 \times DIP \quad (11)$$

$(n = 52, R^2 = 0.50, P < 0.01)$

$$\mathrm{CYANO} = (-0.13 + 0.0024 \times \mathrm{DON} + 0.00059 \times (\mathrm{DON}+\mathrm{NH_4^+}+ \mathrm{NO_x^-})/(\mathrm{DOP}+\mathrm{DIP}) + 0.000019 \times (\mathrm{DON}+ \mathrm{NH_4^+})/ \mathrm{NO_x^-}) \times \mathrm{CHL} \quad (12)$$

$(n = 52, R^2 = 0.34, P < 0.01)$

$$\mathrm{DIA} = (0.26 - 0.00091 \times \mathrm{DON} - 0.00019 \times (\mathrm{DON} + \mathrm{NH_4^+} + \mathrm{NO_x^-})/(\mathrm{DOP}+\mathrm{DIP}) - 0.000023 \times (\mathrm{DON}+ \mathrm{NH_4^+})/ \mathrm{NO_x^-}) \times \mathrm{CHL} \quad (13)$$

$(n = 52, R^2 = 0.27, P < 0.01)$

With the water flow from upper lakes to the lower lakes, the output of the phytoplankton to the downstream lakes and the input from the upstream lakes occurs. The equations below show an example from West Lake in the “flush” condition:

CHL in WL = CHL no flush in WL × (WM in WL – WM Flow) / WM in WL (14)

CYANO in WL = CYANO no flush in WL × (WM in WL – WM Flow) / WM in WL (15)

DIA in WL = DIA no flush in WL × (WM in WL – WM Flow) / WM in WL (16)

<u>Model calibration and runs</u>

As previously mentioned, data from field studies during 2010-2014 were used to calibrate the model (Chapter 2; Shangguan et al. submitted a). Due to the lack of information on the water budget and uncertainty on the groundwater input, an Unknown Input was added to the water mass in LL and GB to force water mass conservation in each lake (Fig. 4.3). The biogeochemical parameters and water flow between each lake were all dynamically calculated to force the model to be stable for ten years. The model was then run with a simulation time of 365 days and a time step (dt) of 24 h. To simulate the C-111 project and its associated increasing freshwater discharge, sensitivity analysis was first conducted at 5 WM flow levels, factors of 1, 2, 3, 4, and 5 × equation 4 (mode 1). Next, the model was run with the WM flow unchanged but the concentration of nutrients, including DON, NH_4^+, NO_x^-, DIP and DOP from the Everglades shifting, with factors of 1, 2, 3, 4, and 5 (mode 2). Changes were assessed for each lake in salinity, nutrients, and phytoplankton, both as total biomass (CHL) and composition (CYANO and DIA, based on signature pigment concentrations).

Results and Discussions

The goal of the model is to have a tool that will realistically estimate of phytoplankton biomass and composition changes with altered flow and nutrients. It was thus of interest to establish how salinity changes with variable discharge, allowing different phytoplankton responses based on whether the responses fell in the high or low salinity response ranges.

Model runs with increasing Everglades discharge

The model successfully simulated most of the annual pattern of salinity measured in the field in each lake (Fig. 4.5). When the WM flow factor was 1, i.e. the initial scenario with no additional freshwater input, salinity slightly declined from January to April, peaked from April to September, and decreased again from September to October, reflecting the seasonal patterns of the Everglades discharge, precipitation, and evaporation. From October through the end of the year, however, the model showed an increasing trend in salinity, countering field observations due to the forced salt pulse in the model. The average modeled salinities in WL, LL, and GB were 9.5, 17.4, and 29.4, respectively. As for model output in terms of salinity groupings, WL was categorized in the LS category for the entire year (365 days); LL was in the LS category for 352 days; and GB was in HS category for the entire year.

With higher simulated freshwater discharge, the salinities in WL, LL, and GB all decreased, with more substantial changes in LL and GB than in WL (Fig. 4.5). Declines in salinities of 5.7, 12.7, and 15.2 on Day 365 were observed in WL, LL, and GB, respectively, when a 5-fold discharge was implemented in the model. The higher the freshwater input, the greater the decrease in salinity over 365 days. This trend altered the LS/HS groupings and, thus, the nutrient and phytoplankton relationships in each lake.

When only freshwater input was changed in the model runs, the relative proportions of all N forms in the three lakes did not change appreciably with changes in discharge (Fig. 4.6). The initial concentrations of DON and NH_4^+ were about 50-fold and 2-fold higher, respectively, than initial NO_x^-, and NO_x^- typically showed different behavior than DON and NH_4^+. When an increased freshwater discharge was imposed in the model, DON and NH_4^+ all decreased in the 3 lakes. In the most extreme scenario of a 5-fold discharge increase, by Day 365, DON concentrations declined by 16%, 17%, and 8%, and NH_4^+ concentrations declined by 17%, 17%, and 14% in WL, LL, and GB, respectively. NO_x^- decreased 4% in WL, but increased by 10% and 30% respectively, in LL and GB (Fig. 4.6). The differing trends in each form of N may have been related to the nutrient exchange among the lakes. While the DON and NH_4^+ is more likely to be flushed to the northern Florida Bay, the NO_x^- from the upstream is more likely to remain in LL and GB.

Relatively large reductions in P were noted in response to simulated increasing freshwater discharge in the model (Fig. 4.6). As the limiting nutrient in this system, P, whether as DOP or DIP, was < 0.6 μM. Concentrations of DOP and DIP were higher in WL than in LL and GB, and higher in LL than in GB. In addition, reductions in P concentrations in WL were more substantial than in LL which, in turn, were more substantial than those in GB. Concentrations of DOP were reduced 48%, 37%, and 17%, respectively, in the 3 lakes. The decline in DIP was less, with reductions of 38%, 32%, and 18% in WL, LL, and GB, respectively. Thus, both the forms and proportions of N and P changed with altered discharge from the upper to the lower lakes and northern Florida Bay.

The CHL and phytoplankton taxa variables, CYANO and DIA, were functions of salinity and different nutrient forms. As the salinity in LL was, by definition, different in the LS and HS sites, the shift in the phytoplankton variables was large (Fig. 4.7). In the initial scenario, CHL in WL and GB was in a dynamic pattern that followed a trigonometric function, ranging from 32.8 to 37.7 μg L^{-1} and 9.2 to 10.7 μg L^{-1} respectively, whereas CHL in LL averaged 36.2 μg L^{-1} in LS category and 15.2 μg L^{-1} in HS category. Similarly, CYANO in WL and GB ranged from 6.4 to 7.7 μg L^{-1} and from 0.9 to 1.1 μg L^{-1}, respectively. CYANO in LL in the LS category ranged from 7.6 to 8.7 μg L^{-1} and, in the HS category CYANO was 1.9 μg L^{-1}. DIA ranged from 0.85 to 0.9 μg L^{-1} and 0.63 to 0.72 μg L^{-1}, respectively, in WL and GB. In LL, DIA was ~1.3 μg L^{-1} in the LS category and ~0.9 μg L^{-1} in the HS category.

The phytoplankton assemblage showed interesting changes with increasing freshwater discharge in model simulations (Fig. 4.7). In the increasing freshwater scenario, changes in WL were relatively small, so WL remained in the LS category. Typically, the higher the flow, the lower the CHL, CYANO and DIA values in the model. In scenario 5 (discharge from the Everglades 5-fold of the initial), the CHL, CYANO, and DIA, respectively, were 33.8, 6.7, and 0.9 μg L^{-1} in WL by Day 365. In LL, increasing the freshwater flow by factors ranging from 2 to 5 depressed salinity for the entire year. Higher discharge generally reduced CHL and CYANO. In the scenario of a 5-fold initial freshwater discharge, CHL declined to 26.6 μg L^{-1} and CYANO to 4.7 μg L^{-1} by Day 365. The pattern was opposite for DIA in LL; diatom abundance was predicted to increase to 1.5 μg L^{-1} in the 5-fold discharge scenario by Day 365. In GB, as salinities declined to < 20 in the scenarios of 4-fold and 5-fold increases in discharge, substantial increases were predicted in phytoplankton CHL, CYANO and DIA. CHL, CYANO, and DIA reached 27.8, 5.7, and 1.9 μg L^{-1}, respectively, when the salinity alteration was first imposed.

Model runs with increasing nutrients

Simulations were run to explore how changes in nutrients alone, without changes in freshwater flow, could affect assemblage composition. Modeled increases in DON from the Everglades to the WLC system altered nutrients and phytoplankton differently in each lake (Fig. 4.8). In the scenario of a 5-fold DON increase from the Everglades, the most pronounced effect occurred in WL, where the water-column DON concentration doubled, but that in LL and GB sustained 34% and 8% increases, respectively. In response, the model predicted that in WL, CHL increased from 34 μg L^{-1} on Day 0 to 74 μg L^{-1} on Day 365. A ~3-fold increase was predicted in CYANO, and a 1.4 –fold increase in DIA. More muted phytoplankton responses were predicted for LL. Since the salinity was not changed, the phytoplankton biomass and assemblage in the increasing DON input scenario was little changed from the initial condition. When DON was simulated to increase by a factor of 5, CHL in LL increased to 54 µg L^{-1} in the LS group and declined to 24 µg L^{-1} in the HS group, about 1.6 times higher than responses to the factor 1 condition. Similarly, in LL with a 5-fold increase in DON imposed, CYANO reached a maximum of 13 µg L^{-1}, about 1.5 times higher than the CYANO maximum in the initial scenario, and then plummeted to 4.3 µg L^{-1} in day 365, still more than double the CYANO abundance in the initial scenario. In LL under the 5-fold increase scenario, DIA peaked at 1.7 µg L^{-1} in the LS grouping, and declined to 0.9 µg L^{-1} in HS grouping. The relative changes in phytoplankton that were predicted from model simulations for GB were much less, expected since the nutrient regime was not appreciably changed. In comparison to the initial scenario, CHL, CYANO and DIA in GB were predicted to increase by only 7%, 23%, and 5%, respectively, by Day 365.

Changes in nutrients and phytoplankton from a modeled increase in NH_4^+ and NO_x^- input from the Everglades were not as obvious as those from the modeled DON condition (Fig. 4.9-4.10). Similarly as for the DON increase, the predicted increase in NH_4^+ and NO_x^- in WL was higher than those in LL and GB in response to increased NH_4^+ from the Everglades. Concentrations of NH_4^+ in WL and LL were predicted to increase by 30% and 13%, respectively, on Day 365 when NH_4^+ was increased by a factor of 5, whereas NH_4^+ declined by 1% in GB (Fig. 4.9). In response to these changes in NH_4^+, CHL, CYANO, and DIA in WL increased by only 3%, 2%, and 10%, respectively. In LL and GB, the changes in all phytoplankton parameters were

< 5%, regardless of factor increase imposed. The imposed condition of a 5-fold increase in NO_x^- loading from the Everglades elevated NH_4^+ by 27%, 20%, and 12%, respectively, in WL, LL, and GB (Fig. 4.10). When increases in NO_x^- were imposed, the changes imposed in the 5 scenarios were predicted to result in a change of < 5% for all phytoplankton parameters in all 3 lakes (Fig. 4.10).

Up to 5-fold loading increases in DOP and DIP from the Everglades caused less than a 5% change in nutrients and phytoplankton in any of the lakes (Fig. 4.11-4.12). The Everglades are a relatively small source of P (Rudnick et al., 1999; Sutula et al., 2003), as P is rapidly removed by biota (Gleason & Spackman, 1974) or abiotically retained in the Everglades wetlands (Jones & Amador, 1992; Reddy et al., 2011). This model successfully captured these dynamics, and showed the comparatively minimal effect of increased P loading from the Everglades within the simulated parameter ranges.

Conclusions

This model analysis indicates that increases in freshwater discharge from the Everglades will result in differing changes in phytoplankton biomass and community composition among the 3 WLC lakes. With higher freshwater discharge, nutrient levels other than NO_x^- consistently declined in all lakes (Fig. 4.6). Total phytoplankton, picocyanobacteria, and diatom biomass (as pigment quantities) all showed decreasing trends in WL (Fig. 4.7). This finding is consistent with phytoplankton dilution/washout due to the elevated freshwater discharge in WL that has been observed in the field (Chapter 2; Shangguan et al. submitted a).

Phytoplankton biomass and picocyanobacteria in LL decreased with simulated flow increases, compared to phytoplankton and picocyanobacteria levels under the initial discharge condition. Because of the salinity group alteration, however, diatom abundance in LL increased with the simulated increase in the Everglades discharge. In addition, the GB was shown to have the highest potential to sustain large increases in total phytoplankton and both CYANO and DIA abundance. This was the major effect detected from both the salinity shift and the flushing effect.

Model simulations also suggest that the impact on phytoplankton due to increasing DON from the Everglades would be much greater than effects of other N forms (Fig. 4.8-4.12). Increased DON loading would substantially impact WL nutrients and phytoplankton, in particular, as CYANO abundance was predicted to more-than-triple. Previous researchers have shown that picocyanobacteria generally favor chemically reduced N forms such as DON (Heil et al., 2007; Wawrik et al., 2009; Domingues et al., 2011; Glibert et al., 2016 and references therein). In addition, *Synechococcus*, the blooming picocyanobacteria in Florida Bay, is capable of using DON such as urea (Glibert et al., 2004; Boyer et al., 2006; Heil et al., 2007), and has also responded to DON in Florida Bay, as shown in experimental conditions (Glibert et al., 2004; 2009; Shangguan et al. submitted b). The effect of increasing nutrients from the Everglades is weakened and becomes less noticeable as the nutrients are moved downstream. Still, picocyanobacteria are the phytoplankton functional group that disproportionately responds. The model thus confirms that picocyanobacteria are the most likely to bloom in northern Florida Bay (Fig. 4.8).

This preliminary model, based on field data, is a practical tool for use in predicting phytoplankton biomass and community shifts based on freshwater flows and nutrient concentrations, which will continue to be altered by the ongoing restoration project in south Florida. Additional hydrological and biogeochemical data will contribute to refining the thresholds for phytoplankton blooms and habitat quality.

Table 4.1. List of model parameters and units.

Parameters	Unit
WM in WL	kg
WM Flow	kg day^{-1}
Salt pulse in WL	kg day^{-1}
Evap in WL	kg day^{-1}
Precipt in WL	kg day^{-1}
Ground WM in WL	kg day^{-1}
DON in WL	kg
DON inflow WLC	kg day^{-1}
DON Flow W-L	kg day^{-1}
Precipt DON in WL	kg day^{-1}
Sed DON in WL	kg day^{-1}
Ground DON in WL	kg day^{-1}
DON Conc in WL	kg L^{-1}
DON outflow WLC	kg day^{-1}
CHL in WLC	μg L^{-1}
CYANO in WLC	μg L^{-1}
DIA in WLC	μg L^{-1}

Table 4.2. List of abbreviation used in this chapter.

Abbreviation	Meaning
A	Surface Area
CERP	Comprehensive Everglades Restoration Plan
CHL	Chlorophyll *a*
CYANO	Cyanobacteria
DIA	Diatom
DIN	Dissolved Inorganic Nitrogen
DIP	Dissolved Inorganic Phosphorus
DON	Dissolved Organic Nitrogen
DOP	Dissolved Organic Phosphorus
Evap	Evaporation
GB	Garfield Bight
HS	High Salinity
ICOLLs	Intermittent Closed Open Lakes and Lagoons
LL	Long Lake
LS	Low Salinity
N	Nitrogen
P	Phosphorus
PN	Particulate Nitrogen
PP	Particulate Phosphorus
Precipt	Precipitation
SAV	Submersed Aquatic Vegetation
SEACOM	Seagrass Ecological Assessment and Community Organization Model
TDN	Total Dissolved Nitrogen
TDP	Total Dissolved Phosphorus
TN	Total Nitrogen
TP	Total Phosphorus
WL	West Lake
WLC	West Lake Chain
WM	Water Mass

Table 4.3. The nutrient concentrations and regeneration rate in each nutrient form, dissolved organic nitrogen (DON), NH_4, NO_3, dissolved organic phosphorus (DOP), and dissolved inorganic phosphorus (DIP) for model parameters. (Chapter 2, Shangguan et al. submitted a; Huettel & Gust, 1992; Geider et al., 1997; Rudnick et al., 1999; Jang & Baglama, 2005; Boyer & Keller, 2007)

Nutrient form	In WL (kg)	In LL (kg)	In GB (kg)	Input concentrations from Everglades (kg L^{-1})	Precipitation concentration (kg L^{-1})	Groundwater concentration (kg L^{-1})	Sediment regeneration rate (kg m^{-2} day^{-1})	Water column regeneration rate (μmol L^{-1})
DON	2.0×10^4	3.8×10^2	1.2×10^4	8.0×10^{-7}	5.6×10^{-17}	4.8×10^{-7}	0	0.1 × phytoplankton biomass
NH_4	8.8×10^2	84	5.2×10^2	2.7×10^{-8}	2.7×10^{-16}	6.2×10^{-7}	2.8×10^{-8}	0.1 × phytoplankton biomass
NO_3	4.3×10^2	30	67	9×10^{-9}	9×10^{-17}	2.1×10^{-8}	0	0
DOP	225	34	106	1.9×10^{-9}	3.1×10^{-18}	1.4×10^{-8}	4.0×10^{-12}	0.025 × phytoplankton biomass
DIP	128	20	57	9.2×10^{-10}	1.5×10^{-18}	6.9×10^{-9}	2.7×10^{-12}	0

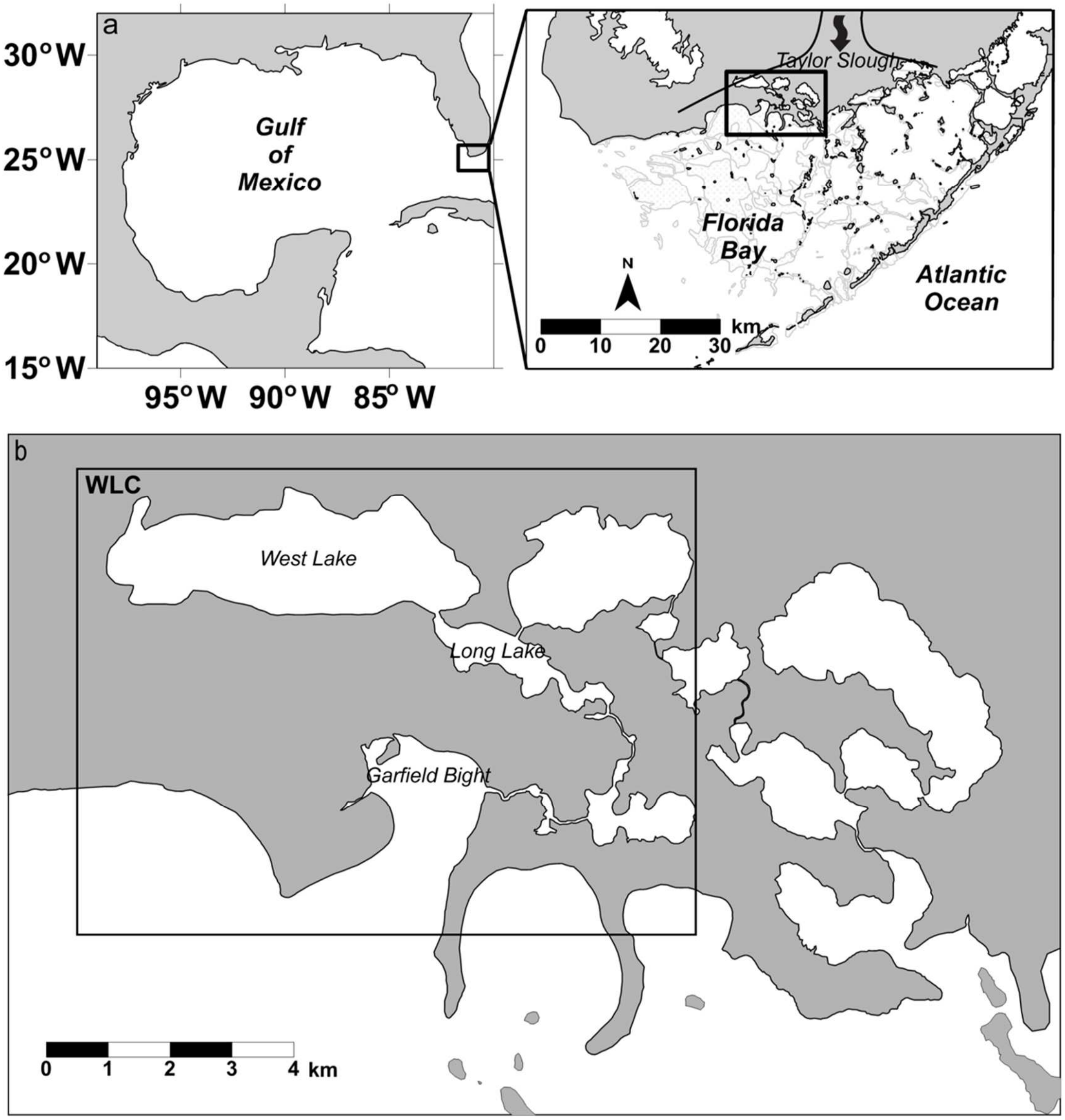

Figure 4.1 Map of Florida Bay and the study stations. (a) The general location of Florida Bay (inset map), Taylor Slough. (b) Enlargement showing the northern Florida Bay ICOLLs. The box indicates the Wet Lake Chain system, including West Lake, Long Lake, and Garfield Bight.

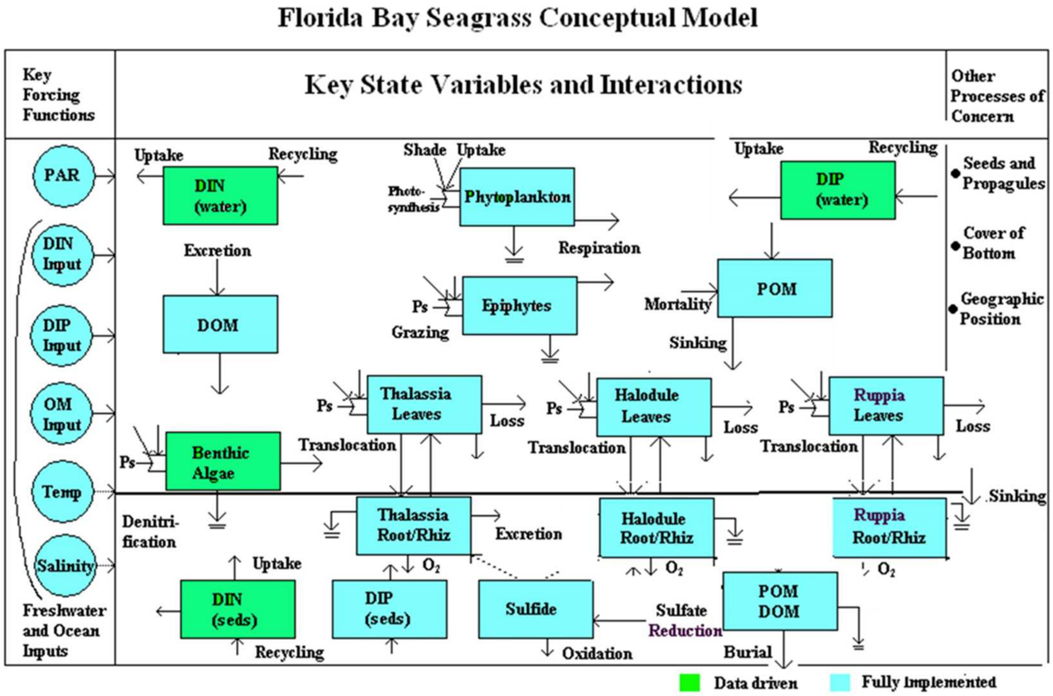

Figure 4.2 The Florida Bay Seagrass Ecological Assessment and Community Organization Model (SEACOM) showing state variables, forcing functions and interactions (reproduced from Madden & McDonald, 2009).

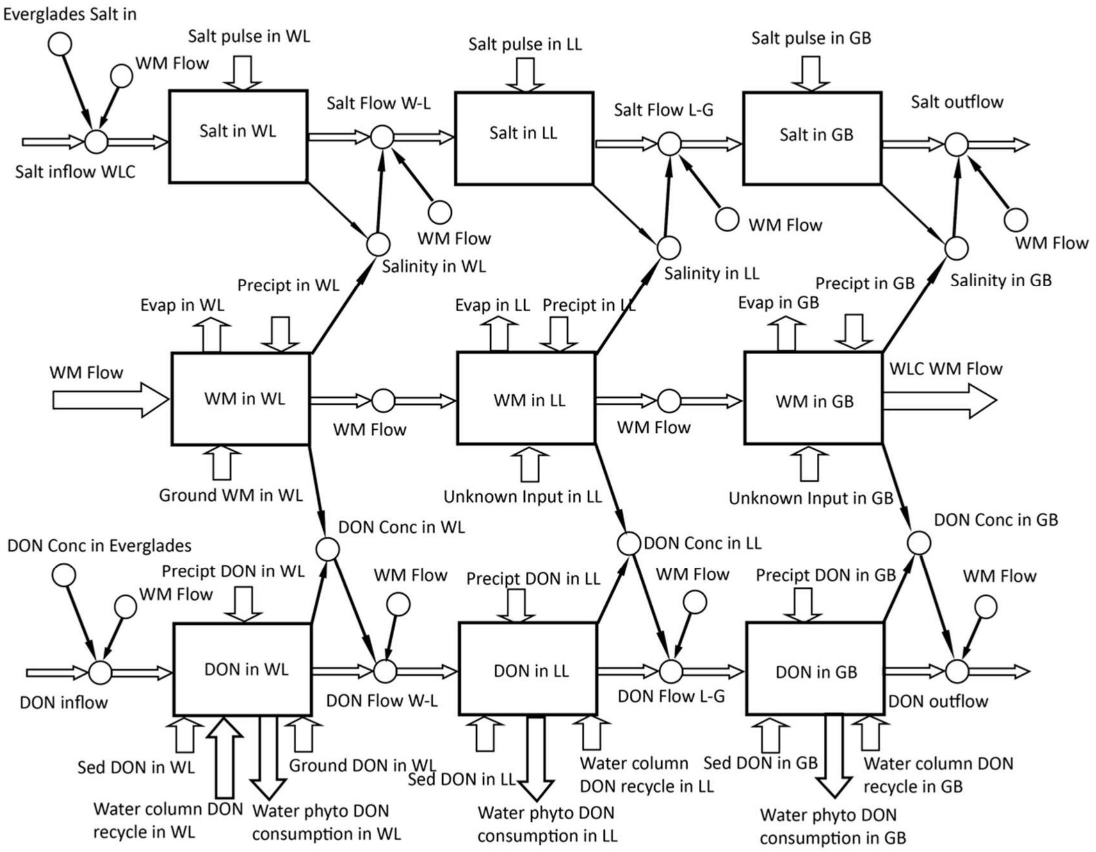

Figure 4.3 Diagram of the model showing water mass, salinity mass and nutrient mass flows from the Everglades, West Lake, Long Lake, Garfield Bight to the out of the system.

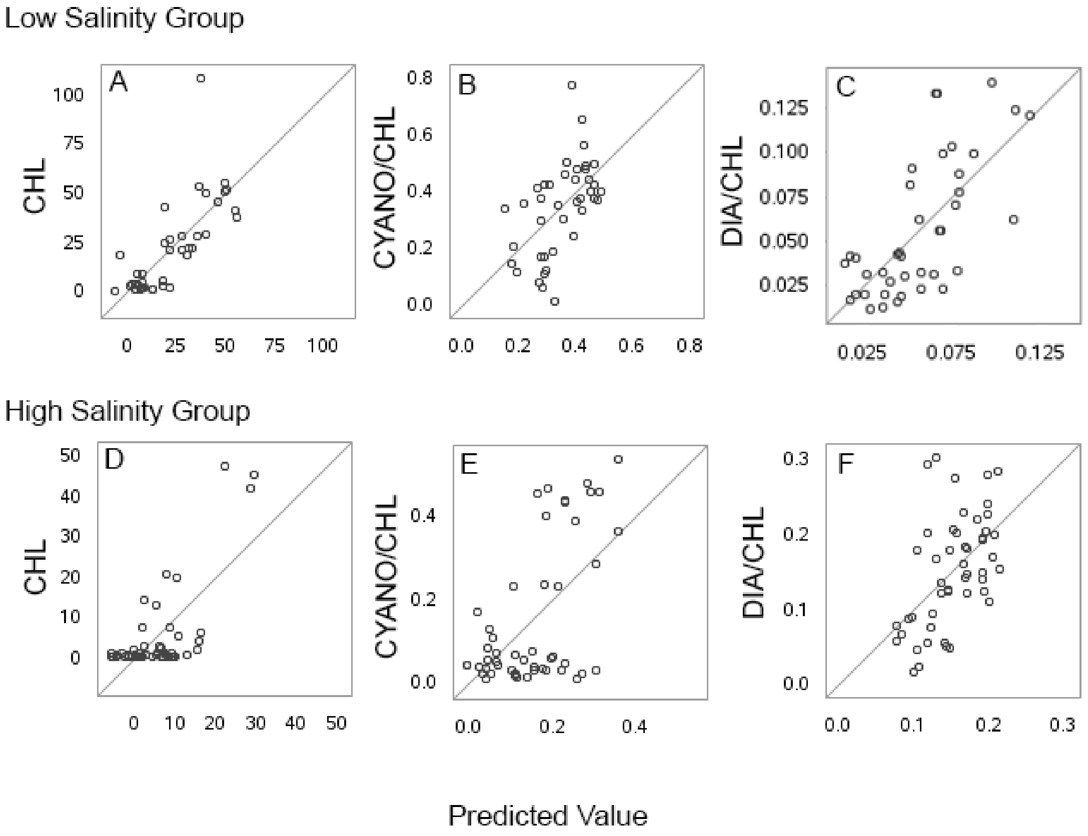

Figure 4.4 Statistics showing the predicted values from the multiple linear regression considering chlorophyll *a* (CHL), cyanobacteria/chl *a* (CYANO/CHL), diatoms/chl *a* (DIA/CHL) and nutrients (equation 8-13) and the observed values in low-salinity (LS) and high-salinity (HS) groups.

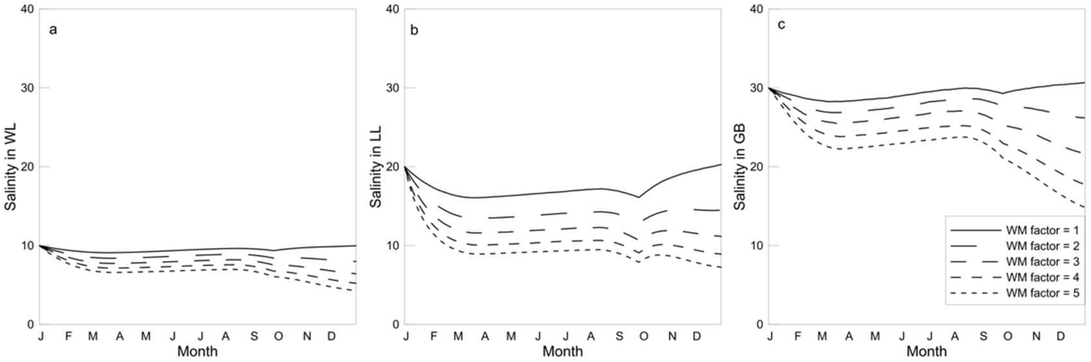

Figure 4.5 Salinity changes in response to the WM flow from the Everglades as flow is changed from a factor of 1 to 5 in West Lake (WL; a), Long Lake (LL; b), and Garfield Bight (GB; c).

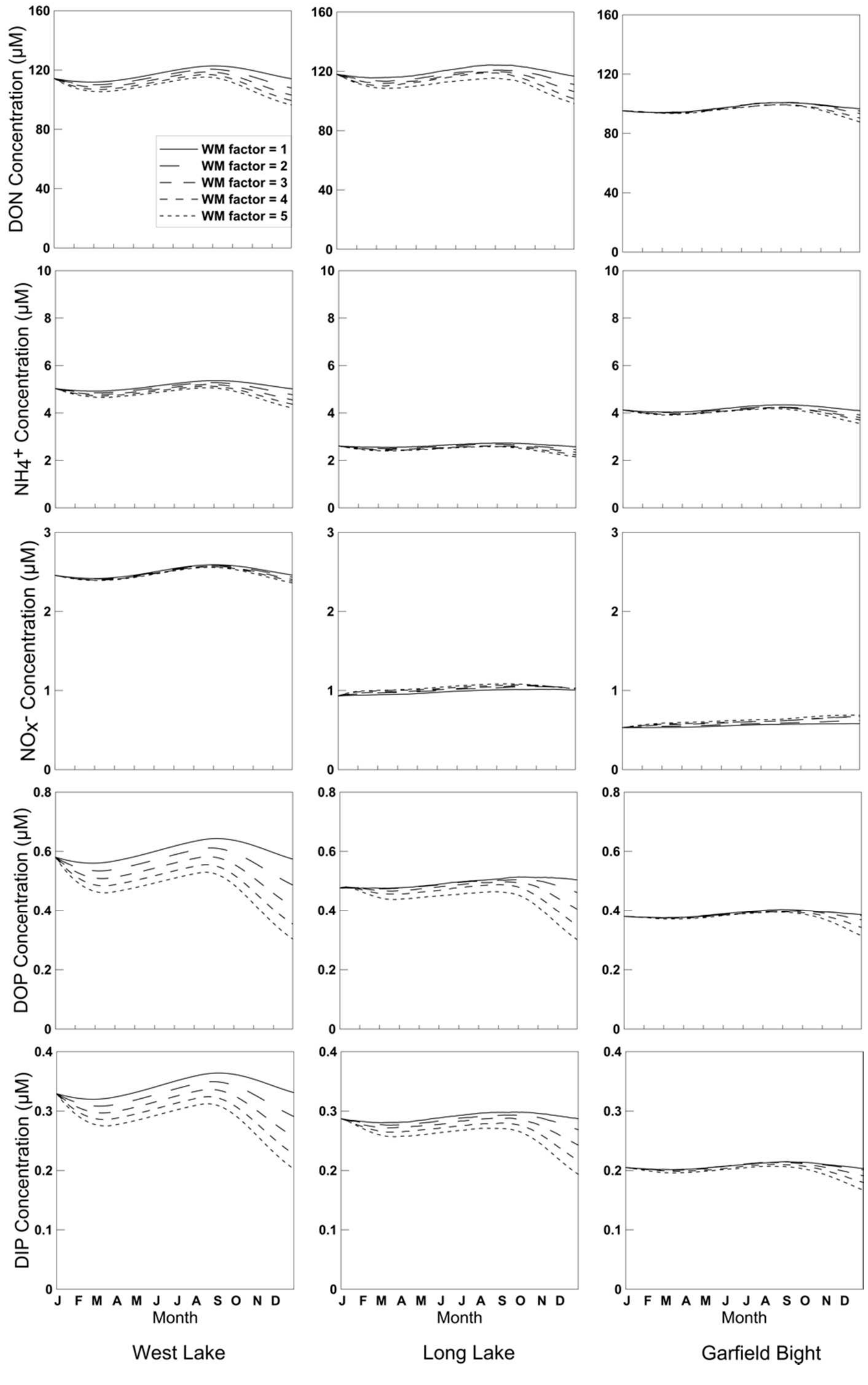

Figure 4.6 Changes in concentrations of nutrients, including dissolved organic nitrogen (DON), NH_4^+, NO_x^-, dissolved organic phosphorus (DOP), and dissolved inorganic phosphorus (DIP) in response to changes in the WM flow from the Everglades by factors of 1 to 5 in West Lake (WL), Long Lake (LL), and Garfield Bight (GB).

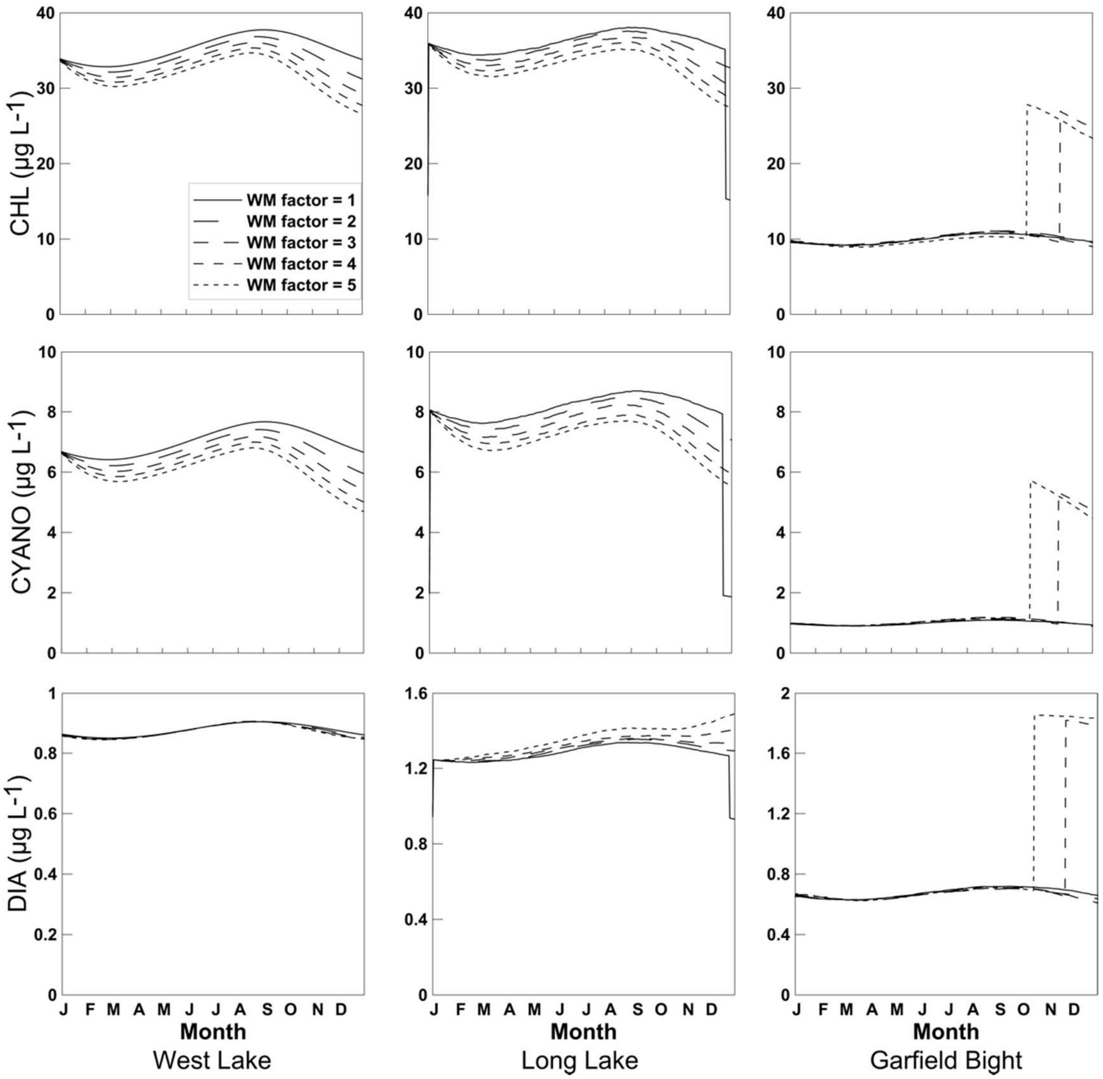

Figure 4.7 Changes in concentrations of chlorophyll *a* (CHL), cyanobacteria (CYANO), and diatoms (DIA) in response to changes in the WM flow from Everglades, altered by factors of 1 to 5 in West Lake (WL), Long Lake (LL), and Garfield Bight (GB).

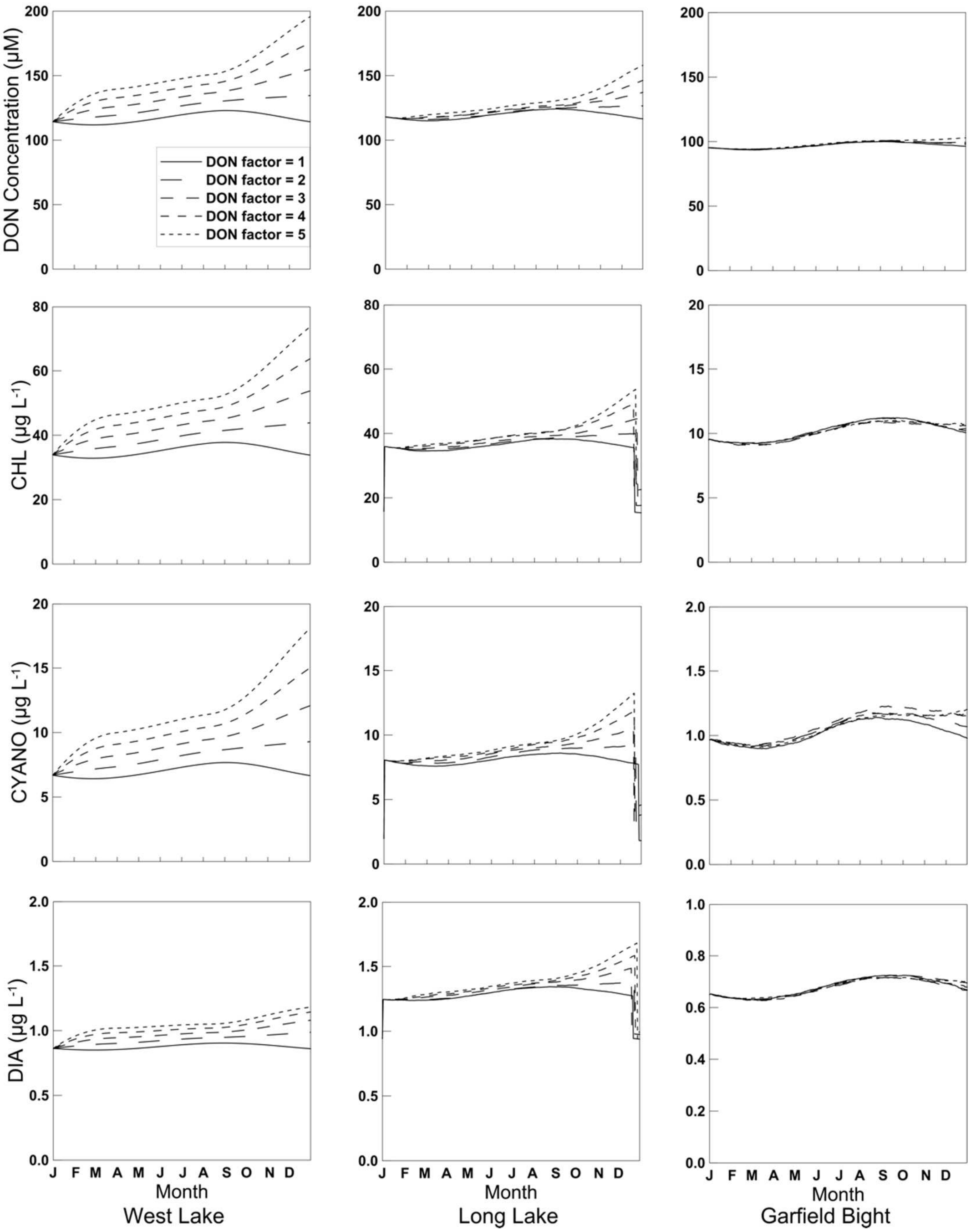

Figure 4.8 Changes in concentrations of dissolved organic nitrogen (DON), chlorophyll *a* (CHL), cyanobacteria (CYANO), and diatoms (DIA) in response to the DON loading from the Everglades, altered by factors 1 to 5 in West Lake (WL), Long Lake (LL), and Garfield Bight (GB).

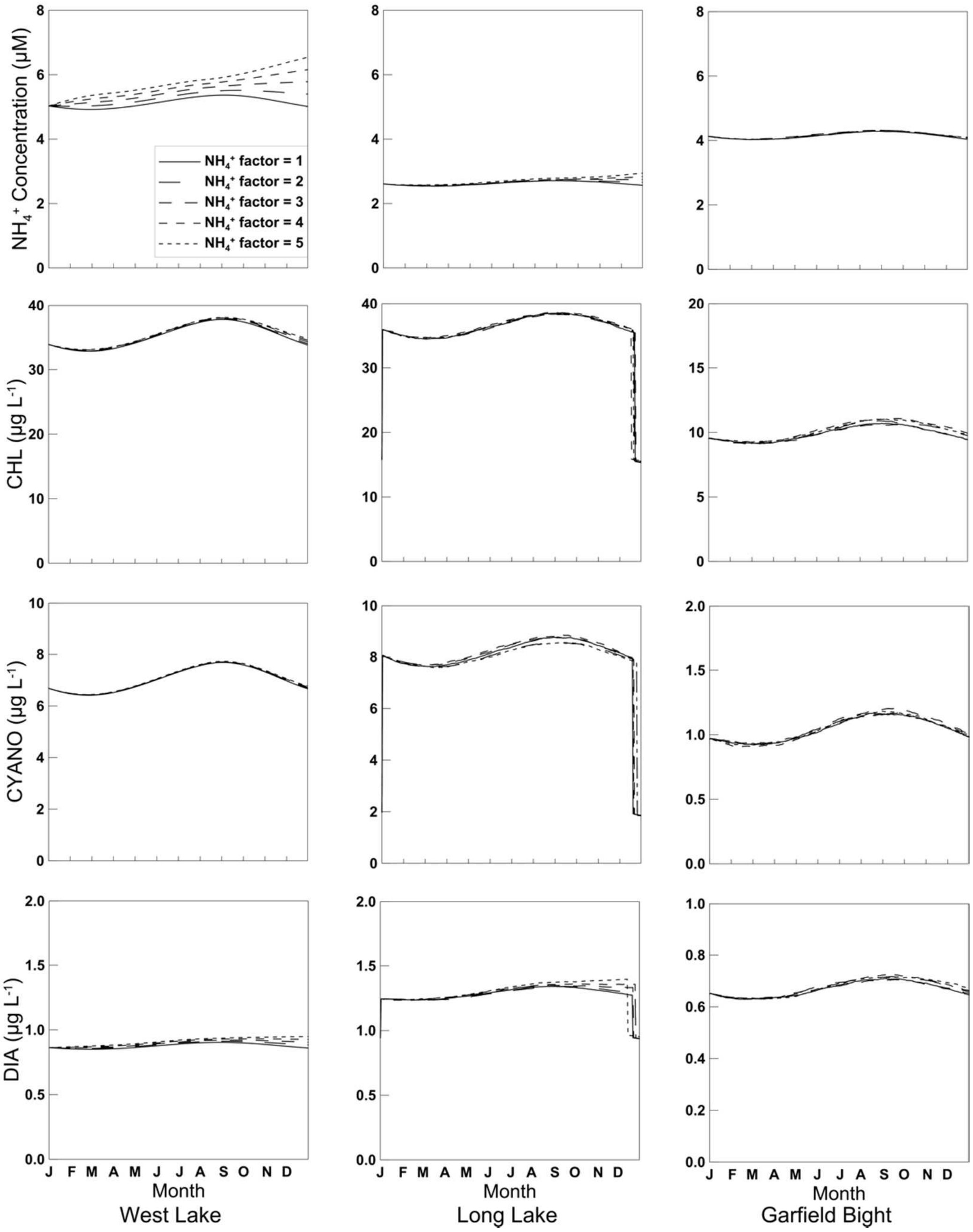

Figure 4.9 Changes in concentrations of NH$_4^+$, chlorophyll *a* (CHL), cyanobacteria (CYANO), and diatoms (DIA) in response to the NH$_4^+$ loading from Everglades, altered by factors 1 to 5 in West Lake (WL), Long Lake (LL), and Garfield Bight (GB).

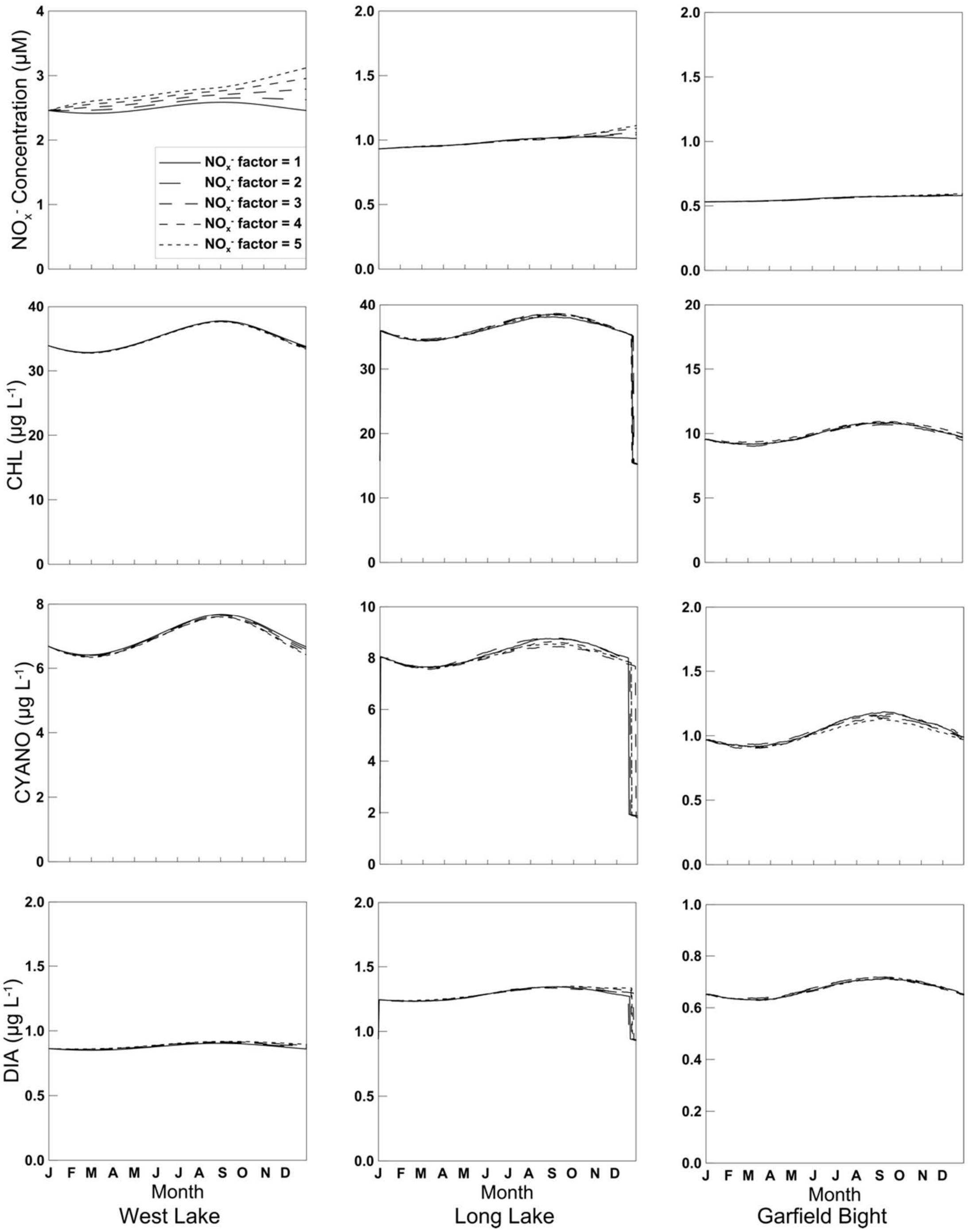

Figure 4.10 Changes in concentrations of NO_x^-, chlorophyll *a* (CHL), cyanobacteria (CYANO), and diatoms (DIA) in response to the NO_x^- loading from the Everglades, altered by factors 1 to 5 in West Lake (WL), Long Lake (LL), and Garfield Bight (GB).

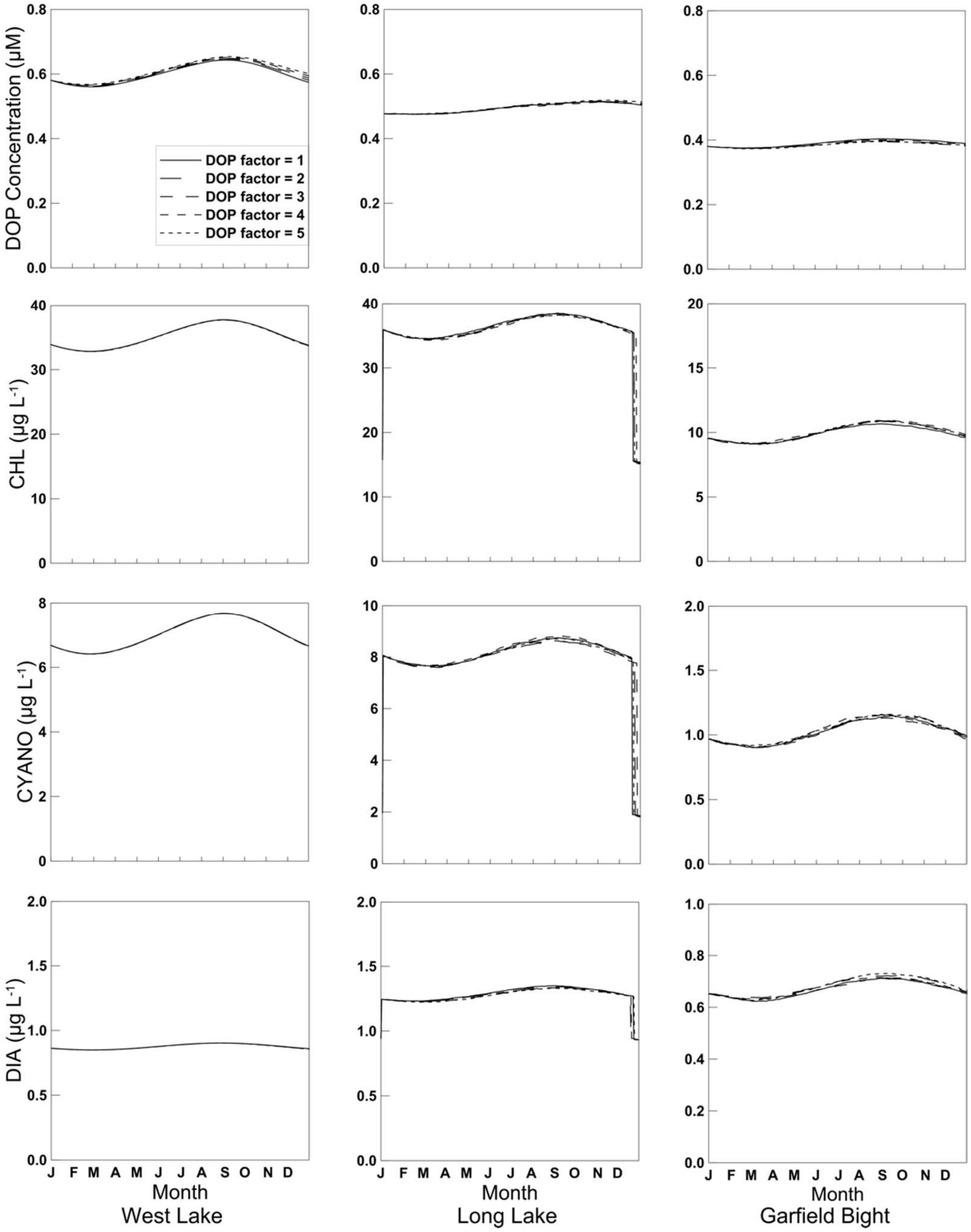

Figure 4.11 Changes in concentrations of dissolved organic phosphorus (DOP), chlorophyll *a* (CHL), cyanobacteria (CYANO), and diatoms (DIA) in response to-DOP loading from the Everglades, altered by factors 1 to 5 in West Lake (WL), Long Lake (LL), and Garfield Bight (GB).

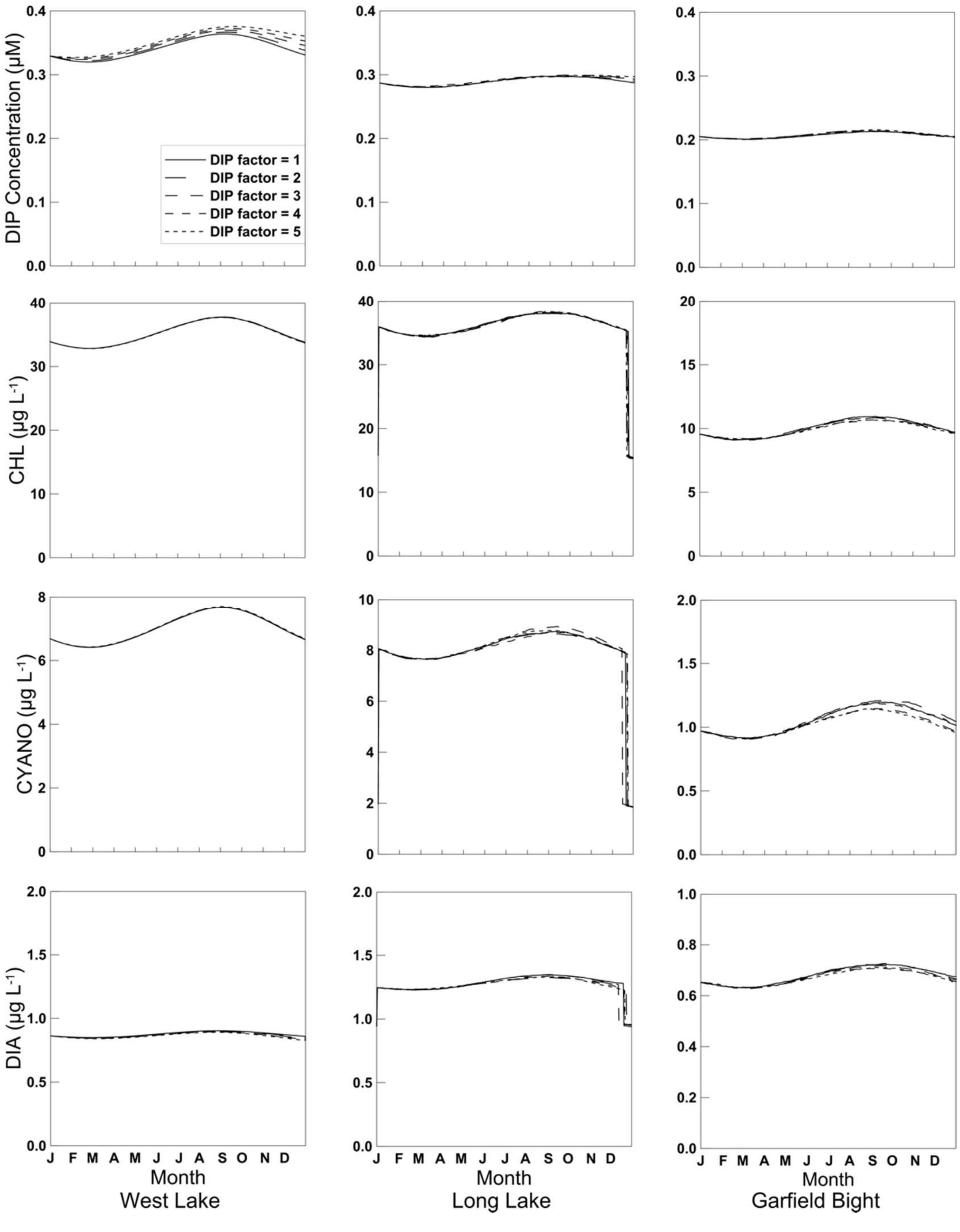

Figure 4.12 Changes in concentrations of dissolved inorganic phosphorus (DIP), chlorophyll *a* (CHL), cyanobacteria (CYANO), and diatoms (DIA) in response to the DIP loading from the Everglades, altered by factors 1 to 5 in West Lake (WL), Long Lake (LL), and Garfield Bight (GB).

Chapter 5 : Summary and Synthesis

The Comprehensive Everglades Restoration Plan (CERP) was initiated to help shift Florida Bay toward its previous ecological state. It redirects water south through the Everglades into Florida Bay, thus lowering the salinity level, Hypersalinity has been considered to be one major contributing factor to documented seagrass loss (McIvor et al., 1994; Zieman et al., 1999), but confounding effects due to nutrient changes have also been shown to play important roles in these changes (Boesch et al., 1993; Lapointe & Barile, 2004). As a component of CERP, the C-111 Spreader Canal Western Project has been designed to retain and increase freshwater flow within the primary drainage system into northern Florida Bay, Taylor Slough. However, the increase in water flow will also likely change the quantity and quality of nutrients discharged to Florida Bay, leading to unknown impacts on phytoplankton and ultimately seagrass and ecosystem health. This dissertation addressed this question from perspectives of field observations, mesocosm experiments, and a statistical box model. The work is considered below in the context of recent research to summarize the status of knowledge on nutrients and phytoplankton in the coastal lagoon system of Florida Bay, and more generally.

This dissertation focused on two lake chains referred to as quasi-Intermittent Closed Open Lakes and Lagoons (ICOLLs) in northern Florida Bay, a region that lies closest to the C-111 canal and should be the first to respond to related changes. The quasi-ICOLLs are fairly isolated from each other and each one exhibits distinctive geological and ecological characteristics. The two chains of ICOLLs have been described as being poised at different stable states (*sensu* Scheffer & Carpenter, 2003). Prior to CERP, the West ICOLL chain prior to CERP was considered to be eutrophic in a plankton-dominated state, while the second ICOLL chain was oligotrophic and maintained in a benthic-dominated state (Carpenter et al., 1999; Poor, 2010; Stanley et al., 2010).

Nutrients in these two lake chains have several general features. First, they are characterized as having proportionately more chemically reduced (organic N and ammonium, NH_4^+) rather than oxidized nitrogen (N) forms (nitrate, NO_3^-) (Glibert et al. 2004; 2010). In these lakes and Florida Bay, DON and reduced N forms are an important N form contributing to picoplankton productivity (Rudnick et al., 1999; Glibert et al., 2004; Boyer et al., 2006; Boyer & Keller, 2007; Yarbro & Carlson, 2008). Second, the two lake chains are generally considered to be phosphorus (P) limited, primarily because P is adsorbed to carbonate sediments. Sediment absorption of P contributes to total dissolved N:total dissolved P (TDN:TDP) ratios that are 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). P limitation in Florida Bay shows a west-east gradient, as the major P source is from the west, the Gulf of Mexico, whereas eastern Florida Bay has more P-adsorbing carbonate sediment (Fourqurean et al., 1992; Noe et al., 2001) and, therefore, very low P concentrations.

Picocyanobacteria are the blooming-forming-phytoplankton in this region as a result of the nutrient regime. First, they generally favor chemically-reduced forms of N (Heil et al., 2007; Wawrik et al., 2009; Domingues et al., 2011; Glibert et al., 2016 and references therein), and they can use organic nutrients such as urea for growth

(Glibert et al., 2004; Boyer et al., 2006; Heil et al., 2007; Wawrik et al., 2009). Second, some picocyanobacteria can be prevalent in P-limited environments due to their low P requirement (Van Mooy et al., 2009; Popendorf et al., 2011) and their ability to use organic P (Glibert et al., 2004; 2009).

The initiation of water diversion operations in the C-111 Spreader Canal Western Project was clearly evident in the increased water levels and reduced salinity in the ICOLLs. After C-111 project implementation, TDN and all forms of N decreased, whereas TDP and both forms of P generally increased, resulting in lower TDN:TDP and dissolved inorganic N:dissolved inorganic P (DIN:DIP) ratios in the ICOLLs. Phytoplankton response differed in each of the ICOLLs. The benthic-dominated ICOLL, Seven Palm Lake Chain (SPLC), shifted to a more planktonic state, whereas there was a major decline in plankton biomass in the previously eutrophic West Lake Chain (WLC).

Overall, these findings characterize the first phase of changes from C-111 implementation, and suggest that nutrient composition and concentration, and phytoplankton biomass and its assemblage, likely will change with increased freshwater flow. The ICOLLs may act as reactors, buffering these changes. In the WLC, phytoplankton biomass likely declined because the dilution rate exceeded maximum growth rates so that washout of cells occurred. Also, the highly contrasting character of the two ICOLL chains suggests that with increasing flow and N availability, the SPLC has-greater potential for increased phytoplankton biomass, while the WLC will probably become less eutrophic, moving phytoplankton production from the upstream ICOLLs to the downstream receiving water body.

Without the ICOLLs, there would be higher potential for phytoplankton blooms in Florida Bay with increased flow and its associated nutrient changes.

The mesocosm study consisted of five experiments, and demonstrated relationships between nutrients and phytoplankton assemblages that could be quantified. Chlorophyll *a* tripled in the +P treatments and increased 10-fold in the +N+P treatments, but did not increase appreciably in the +N treatments. Typically, the +N+P treatments, particularly the $+NO_3^-$+P at a +N:P molar ratio of 32, promoted high concentrations of fucoxanthin (generally indicative of diatoms) relative to chl *a*. While chl *a* did not increase substantially in the +N alone treatments, there was a change in the phytoplankton assemblage. The +N treatments, especially N 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 chl *a* increased, whereas the relative concentrations of zeaxanthin and peridinin (generally indicative of photosynthetic dinoflagellates) declined. These results are generally consistent with the published literature (Dyhrman & Ruttenberg, 2006; Heil et al., 2007; Wawrik et al., 2009; Domingues et al., 2011; Glibert et al., 2016 and references therein).

Based on this study, control of N (especially chemically reduced forms such as NH_4^+) as well as P is recommended for the waterbodies in this region. While substantial increases in biomass were not observed following N enrichment, the results support the premise that a substantial change in biodiversity may occur via an increase in the relative proportion of picocyanobacteria (Finkel et al., 2009; Dugdale et al., 2013; Glibert et al., 2014b; Glibert et al., 2016). Different phytoplankton groups responded to pulsed additions of nutrients when there were residual or even apparently saturating nutrients in the water column at the beginning of the experiments. These results counter the conventional argument that nutrients at saturating concentrations have no regulatory control on phytoplankton assemblage (sensu Reynolds, 1999), a premise that has also been challenged based on physiological data (Glibert et al., 2013; 2016).

The statistical box model was based on relationships measured in the field in different salinity regimes, and enabled more quantitative insights about how altered water flow and nutrient concentrations may be expected to affect phytoplankton biomass and abundance of cyanobacteria and diatoms in the West Lake Chain system. Model simulations indicate that higher freshwater discharge from the Everglades would differentially affect the 3 lakes of this ICOLL chain. Increased flow without changes in nutrients resulted in decreased salinity and lower nutrient concentrations, due to dilution. Under such conditions, phytoplankton biomass as well as the picocyanobacteria and diatom functional groups sustained reductions in the upper lake, West Lake, and phytoplankton biomass and picocyanobacteria decreased and diatoms increased in the lower lake, Long Lake, but in the lower receiving embayment, Garfield Bight, phytoplankton biomass increased, illustrating the potential for algal blooms in this sub-region.

The conclusions of this dissertation are relevant to the dynamics of other coastal lagoon ecosystems, which share common features of shallowness, long residence time, high regeneration rate, and intrinsic sensitivity to allochthonous nutrient inputs.

These findings also provide a key insight for water quality management: As mentioned previously, control of both N and P coming from the Everglades is suggested to avoid algal blooms in this region.

Considering future studies and directions for future research, there is much yet to be understood in terms of phytoplankton responses to changes in nutrients in this region of Florida. The flow of water directly into Taylor Slough is projected to double, adding as much as 2.5×10^{10} L (6.5 billion gallons) more freshwater per year, with the aim of promoting seagrass recovery. This work suggests that a nutrient regime shift will be a likely outcome in both WLC and SPLC, with increased freshwater flow and the associated potentials of algal blooms also expected in Florida Bay. Thus, long-term control of land-based N (especially chemically-reduced forms) and P is highly recommended. More studies are also needed to strengthen understanding about how specific phytoplankton groups – including individual harmful phytoplankton species of interest – respond to changing nutrient conditions. Such data will continue to inform the box model, and can contribute additional parameters. Meshing this nutrient-phytoplankton model with the Florida Bay Seagrass Ecological Assessment and Community Organization Model (Madden & McDonald, 2009), will refine predictions about the thresholds of nutrient loads that promote noxious phytoplankton blooms, as well as predictions about seagrass coverage and, thus, the habitat quality of these important and sensitive regions of Florida Bay.

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